Laboratory Diagnosis and Epidemiology of Bacterial Sexually Transmitted Diseases

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Doctor of Science

The University of Edinburgh

1996
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ACKNOWLEDGEMENTS

My introduction to the microbiology of sexually transmissible diseases and hence the work presented in this thesis is totally dependent on the late Professor R R Gillies (died 1983), Department of Microbiology and Immunology, Queens University of Belfast and Professor B P Marmion, recently retired as Director of the Division of Virology, Institute of Medical and Veterinary Science, Adelaide. Dr Gillies as Reader in Bacteriology with responsibility for the Diagnostic Bacteriology Service and Professor Marmion who held the Robert Irvine Chair of Bacteriology (1968-1978) were responsible for my appointment in 1973 as Clinical Lecturer in the Department of Bacteriology (later renamed the Department of Medical Microbiology). During these early years they were a valuable source of advice and encouragement.

Much of the work in this thesis would not have been possible without the collaboration of the medical and nursing staff in the Department of Genitourinary Medicine in the Royal infirmary - however, I am particularly indebted to the interest, enthusiasm, and support of Dr D H H Robertson, Head of the University Department of Sexually Transmitted Diseases (later renamed Genitourinary Medicine) from the time I joined the University in 1973 until his retirement in 1990, and to his successor Dr A McMillan, who served as a registrar and senior registrar in the Department of Genitourinary Medicine during my early career. I am also grateful to Dr Jonathan Ross for his interest in my gonococcal typing work and for
providing the detailed clinical correlation as part of his MD thesis on the epidemiology of gonococcal infection in Scotland.

I am also indebted to the technical colleagues who have worked with me in the Sexually Transmitted Diseases Diagnostic Laboratory over the years - in particular to the late Clark Henrichsen (died 1986) who worked with me between 1973 and 1977 and to Alex Moyes who joined me in the STD Diagnostic Laboratory in 1988.

Thanks are also extended to the following PhD students who enabled me to explore wider aspects of the subject than possible within the strict remit of a service laboratory commitment: Sam Sarafian (graduated 1981 - sadly died 1995), Katherine Reid (graduated 1985) and Diane Coghill (graduated 1988). I am also indebted to: Mrs Joan McElhinney who, since 1990, has provided secretarial support including the maintenance of positive syphilis and gonococcal databases; and Professor Sebastian Amyes for encouragement and advice throughout the preparation of the thesis.

Finally, I acknowledge the support and interest of my wife Maggie and son Neil and thank them for their patience throughout the many times that I have worked at home on some aspect of the microbiology of Sexually Transmitted Diseases when I should have been doing other things!
DECLARATION

Of the 118 papers presented in this thesis I was first, or sole, author of fifty one.


Thirty four papers were first-authored by either research students, Medical Laboratory Scientific Officers, or junior medical staff, but were the direct result of research that I had initiated and supervised all aspects of laboratory work, usually including data analysis, and had a very significant in-put into writing.


Thirty three papers were collaborative, often with more senior authors although, in the majority of these cases, I had a significant in-put into the design, analysis and writing up.

Publications Numbered: 2, 5, 6, 10, 12, 13, 14, 18, 19, 20, 21, 26, 32, 44, 46, 49, 54, 55, 57, 58, 59, 63, 65, 73, 80, 90, 91, 93, 104, 105, 106, 112, 114.
This thesis brings together 118 published studies on the microbiology of sexually transmitted diseases resulting from work performed in the University of Edinburgh Department of Medical Microbiology between 1973 and 1995. The main aim of these studies was to improve microbiological aspects of the diagnosis and management of syphilis and gonorrhoea.

The earliest publication on syphilis serology was the first to recommend the use of a specific treponemal antigen test, the Treponema pallidum haemagglutination assay (TPHA) for routine screening. As a result of this study a screening schedule comprising the Venereal Diseases Research Laboratory (VDRL) and TPHA tests was introduced into routine practice in late 1973. Soon the same screening schedule was widely adopted in the United Kingdom and Europe. Appreciating the importance of computerisation and automation I validated and standardised a prototype commercial enzyme immunoassay (EIA) as a single serological screening test and demonstrated that this gave a performance comparable to screening with the VDRL and TPHA tests while being suitable for automation and electronic report generation. Screening for syphilis by EIA is now becoming widespread throughout Europe. Because false positive EIA reactions may also show reactivity in the FTA-abs test, immunoblotting was evaluated as a confirmatory test. The possibility of syphilis reactivation and loss of treponemal markers in patients co-infected with HIV were also studied. Reviews and contributions to books have dealt with screening and confirmatory strategies for syphilis diagnosis and have highlighted the prozone phenomenon as a cause of false negative reactions.
Improvements in culture and identification methods for *Neisseria gonorrhoeae* are described in the early studies on gonococcal infection and included the successful modification of a rapid carbohydrate utilisation test (RCUT) to detect pre-formed enzyme rather than depend on slow and unsatisfactory conventional growth dependent systems. The development of a simple modified formulation of New York City Medium termed MNYC medium substantially improved the rapidity and reliability of the cultural diagnosis of gonorrhoea. MNYC medium was introduced into routine practice in February 1977 with the result that the number of diagnostic tests in women could be reduced from three to two making a significant saving in clinical and laboratory resources. Evaluation of a range of commercial pre-poured media in 1994 demonstrated that none performed as well as the "in-house" medium prepared to the 1976 formula. These basic improvements in the cultural diagnosis of gonorrhoea laid the foundation for further studies: to determine the utility of various identification methods including immunological identification of gonococci by polyclonal and monoclonal antibodies; to explore non-cultural diagnosis based on the detection of gonococcal components by various methods (the limulus lysate assay, genetic transformation, an indirect sandwich EIA, and a dot-blot immunoassay); and to define the classes of immunoglobulin reactive with *N. gonorrhoeae*. Other studies defined the spectrum of anatomical colonisation by gonococcal and non-gonococcal neisseriae and applied gonococcal serogrouping, monoclonal antibody serotyping, auxotyping, and plasmid analysis to study the diversity of gonococcal strains and their correlation with geographical diversity, temporal change, sexual orientation, clinical features, antibiotic susceptibility, and reliability of identification by monoclonal antibodies. As a result of the work on gonococcal infection, in 1992 my
laboratory became the Scottish *Neisseria gonorrhoeae* Reference Laboratory thus allowing me to monitor various aspects of gonococcal infection within Scotland. Several publications relating to this aspect of my work are included in the thesis.
INTRODUCTION

Sexually transmitted diseases have long been a major communicable disease problem on a global scale. The extent of their morbidity and mortality is now known to range from uncomplicated local genital infection to the lethal consequences of the acquired immune deficiency syndrome (AIDS). Between these extremes lies their significant effect on infertility resulting from pelvic inflammatory disease, the health and well-being of the foetus and neonate, and the possible viral aetiology of genital malignancy, particularly cervical carcinoma. However, when I commenced these studies in the Department of Bacteriology, Edinburgh University Medical School and the Royal Infirmary, in January 1973 the human immune deficiency virus was unknown and the traditional venereal diseases, gonorrhoea and syphilis, the two most important of the three diseases legally defined as venereal diseases in the UK, were much more common than they are today. Nevertheless, now as then, in relation to syphilis, and to a lesser extent in the case of gonorrhoea, the social and medical implications are so serious that in any clinic dealing with sexually transmissible diseases the two main objectives in every case are:

1. To detect or exclude early syphilis and to trace contacts
2. To detect or exclude a gonococcal infection.

A third objective is to make specific clinical and microbiological diagnoses in a wide variety of lesser conditions.

The satisfactory fulfilment of these objectives is dependent on close collaboration between medical microbiologists and clinicians engaged in the diagnosis and management of sexually transmitted disease. Furthermore, our increasing ability to
offer specific and effective treatment to patients increasingly obliges us to make a definitive diagnosis as promptly as possible. Therefore a joint laboratory and clinical approach is essential to make improvements in diagnosis, treatment and epidemiological surveillance that form the mainstay of individual patient management while at the same time having a significant impact on the overall control and prevention of infection.

The main aim of the studies presented in this thesis was to improve the microbiological aspects of the two important objectives outlined above and to establish collaborative links with the Department of Sexually Transmitted Diseases (now the Department of Genitourinary Medicine). Although the list of publications and copies of the papers are included in chronological order various aspects of the work were pursued in parallel and for this reason the Commentary considers the relevance of the published studies under the following main headings:

1. Syphilis serology

2. *Neisseria gonorrhoeae* and related organisms
   2a Culture and identification methods
   2b Non-cultural diagnosis
   2c Neisserial ecology and macro-epidemiology
   2d Epidemiological typing and clinical associations
   2e Reviews and contributions to books

3. Other sexually transmissible infections, authorship and editorship of books
COMMENTARY ON PUBLICATIONS LISTED

Section 1: Syphilis serology

The social and medical implications of syphilis are so serious that a clinical diagnosis must always be confirmed by laboratory tests. Since the causative organism, *Treponema pallidum*, cannot be cultured *in vitro*, these tests usually depend upon demonstrating antibodies in serum.

In January 1973 the serological diagnosis of syphilis, in the Microbiological Service Laboratories of the Royal Infirmary Edinburgh and the United Kingdom generally, relied upon the widespread use of non-specific screening tests such as the cardiolipin Wassermann reaction (CWR) and the Venereal Diseases Research Laboratory (VDRL) slide test in combination with the Reiter protein complement fixation (RPCF) test.

In the service laboratory of the Royal Infirmary, approximately 15,000 specimens of blood were received annually with a request to detect or exclude syphilis. The performance of the above three tests on such a large number of specimens was laborious and demanding on technician time. The lack of sensitivity, however, with regard to the detection of latent syphilis was a more serious shortcoming in the screening schedule used routinely at that time. Also, since a specific confirmatory test for treponemal infection was not in use in our own hospital laboratory, every specimen giving a positive or equivocal result in any one of the three screening tests had to be sent to a reference laboratory, either at Newcastle or Manchester, where a specific confirmatory test such as the *Treponema pallidum* immobilization (TPI) test
or the Fluorescent treponemal antibody absorbed (FTA-abs) test could be carried out.

These confirmatory tests were necessary in order to ascertain whether the positive results were in fact due to treponemal infection or to a biological false positive reaction*. The necessity of sending specimens to a reference laboratory often led to delay in making a definitive diagnosis, thus aggravating the difficulties always present in this sensitive area of patient management.

The *Treponema pallidum* haemagglutination assay as a specific serological screening test

Between 1965 and 1970 a new test for syphilis, the *Treponema pallidum* haemagglutination assay (TPHA), was being developed in both Denmark and Japan. Early work on the TPHA test had shown that it was simple to perform, reproducible, had a wide spectrum of reactivity in the different stages of syphilis, and possessed a specificity and sensitivity comparable with the accepted reference tests such as the TPI and FTA-abs. In spite of these features which were determined in specialised laboratories the TPHA test had not yet (by January 1973) been employed as a routine screening procedure. The first two papers submitted 1,2 assessed the suitability of the TPHA test as a screening procedure for syphilis in a serology laboratory serving the general hospital population, an ante-natal clinic and a sexually transmitted diseases clinic.

*As a result of the widespread nature of cardiolipin, antibodies reactive with it are occasionally found in the serum of healthy individuals (less than 1% of the population) or patients without any clinical evidence of syphilis. These reactions are termed Biological False Positive (BFP) and individuals showing such changes are referred to as biological false positive reactors.*
As a result of these investigations a screening schedule comprising the VDRL and TPHA tests was introduced into routine practice in late 1973. The FTA-abs was introduced as a confirmatory test for any specimen giving positive or equivocal results in either of the screening tests. These early publications were the first to recommend the use of a specific treponemal antigen test for routine screening.

However, over the next few years the same screening schedule was adopted in the majority of diagnostic laboratories in the United Kingdom and many throughout Europe. This screening schedule improved the efficiency and reliability of the laboratory diagnosis of syphilis and in so doing also improved the management of patients at the clinical level.

The expertise developed in serological testing for syphilis led to an invitation to test a panel of sera collected from over 2,000 Ethiopian women and to advise on the interpretation and significance of the results.

Further research into syphilis serology was not undertaken for some years for the following reasons:

(a) the system described above worked well and was in fact used in the author's laboratory until 1988

(b) to tackle more basic problems in syphilis serology would require access to virulent Treponema pallidum. Maintenance of these organisms depends on intra-testicular inoculation and weekly passage in rabbits. Apart from ethical considerations this is a costly and extremely time-consuming process which brings some risk to investigators and is normally restricted to a few reference centres

(c) there were other important areas such as the culture and identification of Neisseria gonorrhoeae that merited study (see Section 2).
Enzyme immunoassay using anti-treponemal IgG as a specific serological screening test

Between 1973 and the latter part of the 1980's there had been many fundamental changes in laboratory practice. Two of these, the development and application of enzyme immunoassay (EIA) for serological diagnosis, particularly of viral infections, and the development of laboratory computer systems were ideally suited to syphilis serology.

In 1989 we published the first evaluation of a new EIA test to detect anti-treponemal IgG. This study was performed in the late developmental stages of the test at the request of Mercia Diagnostics, Guildford, UK and was responsible for setting the "cut-off" value for the test. We set parameters for the dilution of serum to be tested and the cut-off value of the antibody index (ratio of test serum to a low titre positive control) that resulted in a performance comparable with that provided by a combination of the VDRL test and the TPHA. The potential for automation made the Captia Syphilis G test (as it became known later) an attractive screening protocol, particularly in larger centres, and we introduced it as a single screening test in 1988.

Other workers (Lefevre J, Bertrand M, Bauriad R. J Clin Microbiol 1990; 28: 1704-1707) confirmed the overall high sensitivity of EIA (98.3%). However, because the test was of lower sensitivity in untreated primary infection (82%) it was suggested that an EIA to detect anti-treponemal IgG was more suited to confirmatory testing than to screening. In view of these findings we reviewed the performance of the VDRL and TPHA as a combined screen for syphilis to provide a baseline for assessing screening by anti-treponemal IgG EIA. Of the 44 primary cases reviewed the VDRL detected 32 (73%), the TPHA 31 (71%) and the
combination of tests 37 (84%): this was not significantly different from the reported sensitivity of 82% for EIA. We concluded that in the case of primary syphilis there was no evidence to suggest that the Captia Syphilis G test which detects anti-treponemal IgG was significantly less sensitive than the combination of VDRL and TPHA tests: this view is supported in the literature reviewing "in-house" and other anti-treponemal IgG EIA's. Provided that clinicians are made aware of the "seronegative window" that may exist for one to two weeks during early primary infection, maintain a high level of clinical suspicion and have the facility to request additional tests, such as specific IgM or FTA-abs, in cases of suspect primary infection, then there are many practical advantages and benefits in screening with a single test that lends itself readily to automation and computerised report generation, thus overcoming problems of subjective interpretation and transcriptional errors.

False positive reactions in EIA and limitations of the FTA-abs confirmatory test
Apart from high sensitivity the above studies 61,84 demonstrated a specificity of over 99% for the Captia Syphilis G EIA. As false positive EIA reactions were not associated with conventional BFP reactions we assessed the role of other STD's in producing false positive reactions in the Captia Syphilis G EIA 71. No individual STD was associated with false positive reactions although false reactivity was more common in an STD Clinic population than in pregnant women. Despite a low positive predictive value (because of the low prevalence of treponemal infection) EIA was comparable to traditional screening methods with regard to sensitivity and specificity but is potentially cheaper when large numbers of samples are processed, particularly by automated systems.
Although of high overall specificity the introduction of EIA as a screening schedule had an effect on the reliability of the FTA-abs as a confirmatory test. Comparing four commercial FTA-abs test kits we showed that there was a wide variation in test performance: the agreement between all four kits was 63% for treponemal, and 59% for non-treponemal, sera. Discrepancies with treponemal sera were associated with low levels of antibody characterised by a TPHA titre ≤ 160 and a negative VDRL test. Discrepancies with non-treponemal sera were significantly associated with false reactivity on screening with EIA. With all four kits it was necessary to interpret equivocal reactions as positive in order to obtain a high negative predictive value and avoid categorising sera falsely as non-treponemal. This means that the recommendation of CDC to abolish the borderline FTA-abs report is not suitable for laboratories testing sera that have been screened by treponemal tests. As discussed under confirmatory strategies in the review entitled "Syphilis: new diagnostic directions", when EIA is used as the treponemal screening test the TPHA makes a good alternative to the FTA-abs.

Immunoblotting as a confirmatory test for syphilis

Because of the association between unexplained reactivity in the FTA-abs test and EIA, the general decrease in specificity of the FTA-abs resulting from the need to include borderline reports when sera are selected by screening with a treponemal test we advocated the need for a commercial immunoblotting kit and were the first group to evaluate such a prototype test. The Western blot test gave a sensitivity of 99.1% (113/114) and a specificity of 88.2% (15/17) when indeterminate reactivity (reactivity in one band only) was scored positive and 98.2% (112/114) and 100%
(17/17) when indeterminate reactivity was scored negative. As the non-treponemal sera contained sera that were falsely reactive in either the FTA-abs or the EIA our results supported a role for a commercial Western blot test in the confirmatory diagnosis of syphilis. Further evaluation is required, however, in order to confirm that the criteria for designating a positive result will ensure optimum sensitivity and specificity.

Screening with non-treponemal tests and the prozone phenomenon

The above studies highlighted many of the differences in screening strategies in the UK and Europe, where treponemal tests are used for screening, compared with the United States where non-treponemal tests are still used for screening. Following an article from the United States warning that false negative reactions due to the prozone phenomenon (excess of antibody preventing agglutination) were associated with HIV infection we highlighted the broader issue of screening with non-treponemal tests and described the occurrence of the prozone phenomenon in two cases of neurosyphilis in non-HIV infected men\textsuperscript{96}. This view was supported by an earlier study that showed that the prozone reaction could occur in up to 10\% of cases of early syphilis with high TPHA titres\textsuperscript{84}.

Interactions between syphilis and HIV

Reactivation:- Following reports from the United States on the reactivation of syphilis in patients with HIV, the VDRL, TPHA and FTA-abs tests were used to examine the extent of reactivation of syphilis in a group of HIV-positive patients who had been treated previously for syphilis\textsuperscript{65}. In only one case, probable reactivation of
syphilis, as judged by rising titres in the VDRL test, was noted. Although we concluded that reactivation of syphilis is uncommon, we recommended that regular serological testing should be undertaken in HIV patients who have previously been treated for syphilis.

Markers of past syphilis in HIV infection:- Because of the interactions of syphilis and HIV it is important that new tests for syphilis are evaluated for sensitivity and specificity in HIV-infected patients. The aim of this publication was to ensure that the anti-treponemal IgG EIA: (i) is as sensitive as other treponemal tests as a marker of past syphilis in HIV-infected patients and (ii) specificity is not compromised in HIV-infected patients without syphilis as a result of polyclonal B cell activation. In patients with a past history of syphilis each treponemal test (EIA-IgG), TPHA, and FTA-abs gave a lower sensitivity (82%, 86%, 79%) in the HIV-positive group than in the HIV-negative group (97%) but the difference was significant only in the case of the FTA-abs test. Lower sensitivity in the FTA-abs than in other treponemal tests is in keeping with our previous findings. In the HIV-positive patients 11% (3/28) were negative in all three treponemal tests while 25% (7/28) were negative in at least one treponemal test. The specificity of EIA was similar in HIV-positive and HIV-negative patients. Because HIV-infected patients may lose markers to one or more treponemal antibody tests, we concluded that the exclusion of past treponemal infection is more reliable if such patients are evaluated using several different tests.

Reviews and contributions to books
The diagnosis of syphilis was initially reviewed in detail in Chapter 4 of publication while the aetiology, transmissibility and epidemiology of the infection is considered in
Chapter 3 of the same publication: these reviews were updated in Chapters 8 and 7 of the 2nd edition of the textbook\textsuperscript{57}. Chapters on the diagnosis of syphilis have also been contributed to other major textbooks\textsuperscript{62} (updated in reference\textsuperscript{116}),\textsuperscript{66,89} and current developments in syphilis serology including screening and confirmatory strategies, interactions with HIV infection, and application of new diagnostic developments such as recombinant antigens and PCR covered in an invited editorial review\textsuperscript{82}.

**Conclusion**

From the early 1970's when the laboratory diagnosis of syphilis in Edinburgh relied on screening with non-specific tests we have now reached the stage where screening involves a single semi-automated EIA test the results of which are read objectively by a plate reader linked to a computer which, after appropriate validation, can generate a report in the patient file. We also perform a panel of confirmatory tests and act as a reference centre. The low level of syphilis in the 1990's makes confirmatory testing less cost-effective and we are becoming increasingly widely used as a reference centre by other laboratories throughout Scotland, and also a few from England. Usage of EIA for screening for syphilis infection has increased markedly over the past few years and work is on-going to determine the most suitable test for confirming the treponemal nature of EIA reactors as well as monitoring the activity of different EIA's including tests that use recombinant antigens. Over the next few years we intend developing further links with the serology laboratory in Adelaide\textsuperscript{103} to look at methods such as PCR to detect \textit{T. pallidum} in genital and non-genital ulcers.
Section 2: *Neisseria gonorrhoeae* and related organisms

2a: Culture and identification methods

In the early 1970's once the new syphilis screening schedule had been shown to be highly effective in routine practice I turned my attention to improvements in the cultural diagnosis of gonococcal infection.

Microbiological tests are mandatory in making a diagnosis of gonorrhoea. Because of the short incubation period and high infectivity, rapid and accurate diagnosis followed by immediate effective treatment is the mainstay of control of infection within the community. Although a presumptive diagnosis of gonococcal infection may be made by examination of Gram-stained smears whilst the patient is at the clinic, this must be supplemented and confirmed by culture if the maximum number of positive results is to be obtained: Gram-staining of urethral and cervical secretions in women and urethral secretions in men will detect approximately 55% and 95% of infected patients respectively. Cultures are obligatory in the diagnosis of rectal, oral, disseminated, and asymptomatic infections in both sexes. They are also essential in determining antibiotic sensitivities and for evaluating treatment. When medico-legal issues may be involved proof of diagnosis by culture and the rapid carbohydrate utilization test, for example, is essential.

At the start of my investigations in early 1975, culture and identification of *Neisseria gonorrhoeae* depended upon growth on Thayer Martin* medium and demonstration

* The cultural diagnosis of gonorrhoea was greatly improved by the introduction of the selective medium of Thayer and Martin in 1966; the antibiotics (vancomycin, colistin and nystatin) present in the medium prevent other flora, which may be present at the sampling site, from overgrowing any gonococci present.
of the ability of the isolated organism to utilise glucose but not maltose when inoculated onto a solid medium containing the appropriate carbohydrate. To confirm or establish a diagnosis in this way by the isolation of oxidase-positive, Gram negative diplococci and biochemical proof of identity normally took up to three or four days. Delay was often due to the failure to demonstrate acid production from glucose only. A more rapid and reliable specific diagnosis followed by immediate treatment would be helpful in the control of the disease.

Improvements in routine culture and identification procedures and their contribution to patient management

The first improvement in the rapidity and the reliability of the laboratory diagnosis of gonorrhoea was the successful modification of a rapid carbohydrate utilisation test (RCUT) to detect pre-formed enzyme. The RCUT and delayed immunofluorescence were introduced together as routine identification procedures in 1976. The RCUT system was modified by Oxoid Limited, Basingstoke for commercial production using paper discs impregnated with the appropriate carbohydrates and buffer.

The RCUT was later modified to allow the acidometric detection of beta lactamase as part of the routine identification procedure. Penicillinase (beta-lactamase)-producing N. gonorrhoeae (PPNG) were first detected in the United States and England in 1976; by October 1977, 15 countries had reported 397 infections caused by these strains to the World Health Organization. As a result of routine screening with the newly developed method, as far as is known, the first penicillinase-producing isolate in Scotland was detected in August 1977: the infection had been acquired in West Africa and there were no secondary contacts in
the United Kingdom. In Edinburgh during the years 1978-80 a total of 3163 episodes of gonococcal infection were confirmed by culture, and of these only four (0.13%) were due to PPNG strains. Therefore in terms of laboratory practice routine surveillance may seem relatively unrewarding. Under such circumstances the highest standards of laboratory practice and quality control are imperative. In view of this we examined the comparative sensitivity of various test methods to detect beta-lactamase activity. We showed that the chromogenic cephalosporin method was the most sensitive, followed by the beta-lactamase strip, the acidometric method (as used in the RCUT), and lastly the iodometric method. In spite of the differences in sensitivity none of the methods gave a negative reaction with any of the test isolates. Because of its convenience we retained the acidometric method for screening all primary cultures - however, as a safeguard against missing a PPNG we also instituted a policy of testing all isolates against a 10 unit penicillin disk: isolates with a zone size \( \leq 20 \) mm are tested by the chromogenic cephalosporin assay. This protocol also enables the detection of beta-lactamase producing colonies in a mixed culture where the majority of colonies may be non-beta-lactamase producers.

An increase in PPNG isolates over the next few years influenced clinic treatment policy in an effort to minimise the risk of spread of PPNG. It soon became the practice not to use penicillin as the first choice of treatment in patients who may have acquired their infection in the Far East or West Africa.

On introducing delayed immunofluorescence and the RCUT for the routine identification of gonococci it was found that slow growth on Thayer Martin medium became the limiting factor in the speed of diagnosis. A medium designated modified New York City (MNYC) medium was developed. In a comparative trial with
Thayer Martin medium, which was in use at the time, MNYC medium substantially improved the efficiency and rapidity of the cultural diagnosis of gonorrhoea. MNYC medium was introduced in February 1977 for the routine isolation of gonococci. The formulation described for MNYC medium was subsequently produced commercially by Oxoid Limited, Basingstoke, UK.

Once the efficiency of MNYC medium had been established it proved possible to increase the speed of diagnosis substantially by carrying out the RCUT and penicillinase test directly from the primary isolation plate. We were also able to show that the introduction of MNYC medium made a significant contribution to improved patient management. The high incidence of gonorrhoea in the late 1970's imposed a considerable burden on both clinician and bacteriologist involved in the diagnosis of sexually transmissible diseases. When MNYC medium was used the increased reliability and rapidity of cultural diagnosis improved individual patient management considerably. The reliability of MNYC medium meant that the number of diagnostic tests in women could be reduced from three to two making a significant saving in clinical and laboratory resources.

Diagnosis of gonorrhoea outwith GUM clinics is often less than optimal. In collaboration with colleagues in GUM and Dr Smith in the chlamydial laboratory we undertook a study designed to develop liaison between the departments of GUM and Gynaecology in the management of women with suspected pelvic inflammatory disease (PID), both by improving diagnostic methods and also by demonstrating the value of contact tracing and examination of sexual partners. *Chlamydia trachomatis* alone was detected in 21 of 165 patients, *N. gonorrhoeae* alone in 5, and both organisms in 6 patients, giving a total of 32 (19%) patients in whom an STD
was diagnosed. It was concluded that the combination of an endocervical swab placed in Amies transport medium for gonococcal isolation on MNYC medium and an endocervical slide for immunofluorescent detection of chlamydiae proved to be a simple and accurate method of screening for these infections. Of 16 sexual contacts traced, three cases of gonorrhoea and nine cases of chlamydial infection were diagnosed. None of the men had any symptoms of urethritis highlighting the importance of male asymptomatic infection in the development of PID in their female partners: normally women would be contact traced as a result of their male partner seeking medical attention for urethritis.

Because of cost and workload screening for chlamydial infection was usually restricted to contacts of men with urethritis. A further study supported the view that asymptomatic chlamydial infection in men is important in the spread of infection.

We screened 216 women who attended a student health centre with vaginal discharge and who were not contacts of men with urethritis: C. trachomatis was isolated from 5% of the women but none yielded gonococci. It was concluded that if chlamydial screening is restricted only to those patients who give a history of sexual contact with symptomatic men then some cases will be missed and pelvic inflammatory disease may result.

In collaboration with Dr A McMillan, then a Consultant Physician in Genitourinary Medicine at Glasgow, a study was undertaken to define the pattern of infection with N.gonorrhoeae in males who had had homosexual contact. We showed that it is insufficient to test only the urethra of a homosexual man who has gonococcal urethritis, because the rectum and pharynx are often infected but only detected after repeat cultures.
I was also able to demonstrate that the methods developed for gonococci could be applied to related organisms\textsuperscript{15,46}. The rapid and reliable characterisation of meningococcal isolates from primary cultures on MNYC medium\textsuperscript{15} could potentially improve the cost-effectiveness and rapidity of meningococcal carrier surveys. A collaborative study on the characterisation of \textit{B. catarrhalis} and its differentiation from \textit{Neisseria} spp\textsuperscript{46} was undertaken in collaboration with bacteriological colleagues from the Respiratory Bacteriology Unit at the City Hospital in Edinburgh. At this time (mid 1980's) there was an increasing awareness of the importance of \textit{Branhamella (Moraxella) catarrhalis} as a respiratory pathogen and the need to differentiate it from non-pathogenic \textit{Neisseria} spp. Using simple laboratory media, DNase, and superoxol tests, it was possible to identify \textit{B. catarrhalis} and to distinguish it from pathogenic and non-pathogenic \textit{Neisseria} spp.

Once the improved routine culture and identification procedures were in place we had a yardstick against which to compare new diagnostic tests, evaluate non-cultural detection, and pursue developments in the macro and micro-epidemiology of gonococcal infection.

\textbf{Immunological identification of gonococci by polyclonal and monoclonal antibodies}

Although immunofluorescence could be used to identify gonococci from primary cultures within 30 minutes or so the test requires expensive equipment and skilled personnel. When the Phadebact Gonococcus test, a rapid slide coagglutination (CoA) test for the immunological confirmation of \textit{N. gonorrhoeae}, was introduced by Pharmacia it was shown that identification by CoA from primary cultures on MNYC medium provided a very rapid, simple, and efficient system for the cultural diagnosis
of ano-genital gonorrhoea in women and urethral gonorrhoea in men. The advent of CoA reagents based on monoclonal antibodies to gonococcal protein I overcame the specificity problems of polyclonal reagents. This kit (GONO GEN, New Horizons Diagnostic Company) was one of the first to use monoclonal antibody CoA reagents and was based on a single panel of antibodies against protein I. Although specificity was improved sensitivity was poorer, presumably due to a limitation of the monoclonal antibodies in the pool.

The Phadebact Monoclonal test (Pharmacia, Uppsala, Sweden) which was introduced later used separate monoclonal antibody pools to detect isolates bearing protein IA and IB epitopes and resulted in higher sensitivity whilst maintaining absolute specificity: the test had a specificity of 100% (286/286) and a sensitivity of 99.7% (1077/1080). There were only three non-reactive strains among the 1080 gonococcal isolates tested and these were shown to be epidemiologically linked and were of the very unusual serovar IB-17. A major benefit resulting from the high specificity of the Phadebact monoclonal test is the dual isolation of gonococci and meningococci from the throat while the use of two separate panels of monoclonal antibodies allows the detection of mixed infections with both IA and IB serotype strains. A direct immunofluorescence test based on monoclonal antibodies (Syva MicroTrak) was also shown to be 100% specific but the single panel of antibodies failed to react with the rare IB-24 serovar.

Other identification methods for gonococci

Because of the limitations of immunological identification with polyclonal antibody reagents described above, and prior to the advent of monoclonal antibodies, the
superoxol test (catalase test with 30% hydrogen peroxide) was evaluated as a simple means of differentiating gonococci from related species of organisms that were cultured from a variety of anatomical sites. When this evaluation was almost complete Dr John Tapsall from Sydney, New South Wales visited my laboratory and it transpired that he had also been looking at the superoxol test. We therefore decided to publish our results jointly41: we concluded that an isolate was almost certainly not a gonococcus if it gave a negative superoxol test result. Unfortunately a positive superoxol test was not restricted to gonococci. We also examined the identification of neisseriae based on enzyme profiles determined with chromogenic substrates in 198648 and again in 199492 following the development of newer kits. We found that there were several mis-identifications, particularly when isolates were tested from primary cultures, and concluded that these methods were not sufficiently reliable for the culture confirmation of gonococci and meningococci.

Absolute sensitivity and specificity of gonococcal identification was finally achieved with a test that depends on nucleic acid technology89. The AccuProbe test (Gene-Probe Inc) uses a chemiluminescent labelled single stranded DNA probe that is complementary to gonococcal rRNA. The range of gonococci detected by AccuProbe included isolates of the rare serovars, IB-17 which had been shown previously to be non-reactive in Phadebact Monoclonal GC test69 and IB-24 previously shown to be non-reactive in the Syva MicroTrak fluorescent test56. We are extending this work to evaluate the test using organisms from primary cultures and to detect gonococci directly from patient exudates.
**Studies relating to antibiotic susceptibility and treatment**

The development of improved culture and identification methods enabled us to participate in an evaluation of the new 4-quinolone, ciprofloxacin as a single dose treatment for gonorrhoea. This study showed that single dose oral ciprofloxacin was a safe and effective treatment for gonococcal urethritis and proctitis in men (including PPNG infection) and also gave good results in treating pharyngeal infection. These early findings were confirmed in studies by others and a 500 mg oral dose of ciprofloxacin is now first line treatment for men with rectal or pharyngeal infection. In spite of the lack of an increase in clinically significant penicillin resistance in Edinburgh between 1990 and 1992 the proportion of cases that were treated with ciprofloxacin increased over the same period because it is the treatment of choice for male rectal infections. As homosexually acquired gonorrhoea accounts for more than half of all gonorrhoea in Edinburgh this makes ciprofloxacin the most widely used antibiotic treatment.

Ciprofloxacin resistance although now common in Japan and parts of South East Asia remains rare in this country. However, strains associated with failure following treatment by fluoroquinolones may be imported to the UK. In conjunction with colleagues from Glasgow we described three PPNG strains which were non-typable with the Genetic monoclonal antibody panel but were of the rare serovar AvBx with the Pharmacia panel. The source of these isolates which had a ciprofloxacin MIC (minimum inhibitory concentration) of 0.06 mg/L was Spain: the majority of Scottish isolates have an MIC $\leq 0.004$ mg/L.

Following the occurrence of two unrelated cases of ciprofloxacin resistant gonococci amongst 25 gonococcal isolates referred from Fife during the first six months of 1995
we reviewed the prevalence of ciprofloxacin resistant gonococci in Scotland. The overall prevalence of ciprofloxacin resistance (defined by an MIC > 0.05 mg/L) was low in Scotland and was found in only 1.3% (25/1960) of all isolates tested between 1991 and 1994. The actual level of ciprofloxacin resistance also tended to be low: the MIC's of the 25 strains were 0.064 mg/L (4 strains); 0.125 mg/L (6 strains) 0.25 mg/L (7 strains); 0.50 mg/L (7 strains), and 2.0 mg/L (1 strain).

Ciprofloxacin resistance was associated with serovar IB-1 and with PPNG isolates: 8.5% (11/30) IB-1 isolates were resistant and 7 of these were PPNG. One of the Fife isolates, a serovar IB-1 PPNG strain demonstrated a high level of ciprofloxacin resistance while the other, a non-PPNG serovar IB-3 strain, had an MIC of 0.125 mg/L: the source of both infections was outwith the UK. These cases highlight the importance of importation of ciprofloxacin resistant strains which should be taken into account in selection of therapy for patients who may have acquired their infection outwith the UK.

The E test (AB Biodisk, Solna, Sweden) is a new simple method for determining minimum inhibitory (MIC) values which is particularly attractive to smaller laboratories. Prior to our evaluation of 52 PPNG by this method very few PPNG had been included in comparative studies. Although chromosomally mediated penicillin resistant isolates were readily detected by E test we found that not all PPNG were classified as resistant and several isolates had MIC values below 1 mg/L. We concluded that it is important that isolates with an E test penicillin MIC ≥ 0.19 mg/L should be checked by a specific test for beta-lactamase production.

As a result of on-going national surveillance through the Scottish *Neisseria gonorrhoeae* Reference Laboratory we were able to demonstrate the spontaneous
loss of a penicillinase-producing plasmid during natural transmission within a small cluster of PPNG infection in Central Scotland during 1992\textsuperscript{106}. All of the PPNG involved in the outbreak were of the serovar combination 1B-1/Bopst, of the non-requiring auxotype, had a ciprofloxacin MIC of 0.06 mg/L and contained 2.6, 3.05 and 24.5 MDa plasmids. All of the non-penicillinase isolates epidemiologically linked with the cluster were also of the serovar combination 1B-1/Bopst, of the non-requiring auxotype, and had a ciprofloxacin MIC of 0.06 mg/L: there were no other 1B-1/Bopst strains isolated in Scotland during 1992. Without detailed typing data the non-PPNG isolates would not be recognised as part of the same cluster of infection and contact tracing would be less effective.

**Evaluation of commercial pre-poured media**

This evaluation resulted from a decision within the Microbiology Directorate to replace "in-house" media with commercial pre-poured plates provided that the latter gave an equally high standard of performance. As there were no comparative \textit{in vitro} evaluations of commercially available pre-poured media we decided to examine 14 commercial pre-poured plates from four major manufacturers\textsuperscript{117}. None of the commercial pre-poured media performed as well as the "in-house" MNYC medium described earlier and used since 1978. Apart from the in-house medium only three pre-poured media grew all of the gonococci tested. However, the inhibition of unwanted organisms was 71.8%, 80.3% and 85.9% compared with 94.4% for the "in-house" medium. We found that failure to support the growth of gonococci correlated with serogroup IA isolates, vancomycin rather than lincomycin in the antibiotic cocktail, the arginine, hypoxanthine, uracil (AHU) auxotype and
concentrations of blood below 10%. Two particular serovars, IA-5 and IA-21 showed higher failure rates than other IA serovars. The routine use of gonococcal selective media that fail to grow these isolates could have a significant impact on the proportion of infections caused by these serovars as a result of failure to detect them. We concluded that manufacturers are providing too great a selection of media that perform inadequately and recommended guidelines for a rational restriction in the variety of selective media that are produced which should result in more effective gonococcal selective media.

Storage and repeated recovery of *N. gonorrhoeae*

Due to the fastidious nature of *N. gonorrhoeae* a simple, inexpensive and efficient system for the storage and recovery of clinical isolates and quality control strains is required for good laboratory practice, in research and for epidemiological studies. Methods such as lyophilisation and freezing in liquid nitrogen have many disadvantages including inconvenience. The Pro-Lab Microbank is a simple and convenient commercial storage system in which coloured beads are coated with a suspension of organisms in cryopreservative fluid and stored in a small cryovial at -70°C. As this system had not been evaluated for gonococci we tested 100 gonococcal isolates, representing eight protein IA serovars and 14 protein IB serovars\(^\text{107}\). At monthly intervals, over a two year period, a bead from each cryovial was removed and cultured on MNYC medium. The overall recovery rate was excellent at 98.6% (2365/2400), the 35 failures representing 13 separate isolates. Failures were associated with certain minor serovars such as IA-16, IB-15 and IB-25. We concluded that the Pro-Lab Microbank system provides a highly effective and
convenient system for storage of *N. gonorrhoeae*, particularly when multiple retrieval is required. These findings may suggest that the transmissibility/viability of minor serovars may be lower than that of the common serovars and could be a significant factor in the epidemiology of gonococcal infection.

**Conclusion**

In the early 1970's the laboratory diagnosis of gonorrhoea in Edinburgh was characterised by slow growth on a basic selective medium and confirmation by conventional sugar utilisation tests. The developments described above lead to a highly enriched selective medium providing rapid growth of gonococci as well as rapid identification by immunological and biochemical methods. These developments not only improved patient management but enabled the reliable evaluation of other identification methods including nucleic acid probes. The reliable culture and identification system was also a pre-requisite to the development and evaluation of non-cultural methods described in the next section.
2b: Non-cultural diagnosis

In the mid to late 1970's it was considered that a highly sensitive and specific serological test would be of value in the control of gonorrhoea and that the need for genital examinations might be reduced: this was the background to studies on the antibody response to gonococcal infection as well as the search for a suitable non-cultural diagnostic method based on antigen detection. These studies on antibody response and detection of antigen for diagnosing gonococcal infection were aided greatly by the increased reliability of cultural diagnosis established in earlier studies.

Detection of serum antibody.

Although the gonococcal complement fixation test (GCFT) is the only test which has been used to any extent in routine diagnosis the nature of the antigen was undefined and often relied on a mixture of strains. We evaluated the GCFT with whole cell antigen prepared from a strain of *N. gonorrhoeae* (S9) considered to possess antigenic features that might be quantitatively or qualitatively specific to species of *N. gonorrhoeae*. Our results with the GCFT compared favourably with those obtained using more modern serological tests but it was likely that cross-reaction with *N. meningitidis* was responsible for some of the unexplained positive reactions in the patients in whom there was no evidence of gonorrhoea.

By using gonococcal pili as antigen in an enzyme-linked immunosorbent assay (ELISA) it proved possible to eliminate cross-reactions due to meningococci but sensitivity remained poor. Although the performance characteristics of the test were
not considered suitable for diagnosis the test could be of value in epidemiological studies.

Detection of gonococcal components

The detection of gonococcal components in the secretions of infected patients provides a more direct approach than the detection of antibody. Such a method, independent of the viability of the gonococcus, would overcome the transport-associated problems evident in many localities. Moreover, a rapid method independent of culture might be used for 'immediate' diagnosis at the clinic. A variety of non-cultural methods were investigated including the limulus lysate assay, genetic transformation, an indirect sandwich enzyme-linked immunosorbent assay, and a dot-blot immunoassay. A comparative study of antigen detection methods was also made in parallel with with the quantitative microbiological investigation of cervical and vaginal washings. This study was important in that it established that the mean number of viable gonococci in cervical aspirates was $1 \times 10^6$ cfu/ml with a range from $5 \times 10^3$ to $8 \times 10^6$ cfu/ml; the corresponding values for vaginal aspirates were $8.4 \times 10^4$ cfu/ml with a range from $1 \times 10^4$ to $1 \times 10^6$ cfu/ml.

Classes of immunoglobulin reactive with *Neisseria gonorrhoeae*

In parallel with the development of serological methods suitable for large scale screening programmes I collaborated with Dr A McMillan on an immunofluorescence study to define the classes of immunoglobulin reactive with *N. gonorrhoeae* in the serum and secretions of patients with naturally acquired uncomplicated gonorrhoea. These studies included temporal aspects of the serum response in men and women.
the demonstration that anti-gonococcal IgA and IgG were the most frequently found classes of immunoglobulin in cervical secretions\textsuperscript{14} and urethral secretions of men\textsuperscript{12}. An antibody response to infection in the oropharynx was also demonstrated in most patients with infection at these surfaces\textsuperscript{29}. A high proportion of homosexual patients with anorectal gonorrhoea but no evidence of pharyngeal infection had antibody against the gonococcus in the saliva suggesting that these men may have had pharyngeal gonorrhoea which was not detected.

Detection of anti-gonococcal IgA in cervical secretions was also evaluated as a diagnostic test. Unfortunately, the sensitivity of such a diagnostic test was no better than that of Gram-staining of secretions\textsuperscript{19}. The expense and the laboriousness of the test militate against its use as a routine diagnostic procedure. The lower rate (72\%) of detection of antibody in this study compared with 95\% in our earlier report\textsuperscript{14} probably reflects the different sampling method. The mean IgA concentration in the secretions eluted from swabs was less than half of that found in secretions obtained by aspiration. The collection of secretions by aspiration is however, sometimes difficult, cumbersome, and not suitable for routine use in a busy clinic.

Conclusion

Although this work increased our understanding of the antibody response to gonococcal infection and defined the numbers of gonococci that are present in natural infection we were unable to produce an antibody or antigen detection test of sufficiently high sensitivity and specificity to be of value in routine diagnosis. Others have likewise been largely unsuccessful in this area and as stated by the WHO in the "Sexually Transmitted Diseases Diagnostic Initiative: a Briefing Document"
dated 14 July 1994 the development of simple, rapid, accurate, inexpensive, and stable diagnostic tests for the detection of markers (eg antigens, antibodies, nucleic acids, enzymes or metabolites) of symptomatic and asymptomatic infections due to *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Haemophilus ducreyi*, and other STD's is a priority research area. The development of nucleic acid probe technology and the PCR reaction has enabled the production of sensitive non-cultural detection methods for gonococci. Unfortunately the nature of the technology and their expense precludes their use in developing countries. However, it is likely that they will gain widespread use in developed countries.
2c: Neisserial ecology and macro-epidemiology

Non-gonococcal neisseriae from ano-genital sites

In the course of my studies on gonococcal infection it became clear that the methodology developed for detecting gonococcal infection enabled me to explore the broader topic of neisserial infection and contribute to our knowledge on the macro-epidemiology of gonococci and their interaction with other micro-organisms such as meningococci and certain oral streptococci. The methodology developed for gonococci facilitated the accurate identification of non-gonococcal neisseriae from the genital tract and anal canal which can be encountered when screening for gonorrhoea\textsuperscript{5,23,85}. Before we reported the isolation of \textit{N. lactamica} from a vaginal swab specimen\textsuperscript{23} there had been only one previous report of the isolation of this organism from a genital site. The importance of non-gonococcal neisseriae in relation to the predictive value of immunological tests was reviewed in Chapter 3 of the textbook "Immunological diagnosis of sexually transmitted diseases\textsuperscript{52}. These studies reinforced the view that the full biochemical identification of oxidase-positive Gram negative diplococci growing on selective medium is necessary in order to avoid the serious social and medico-legal consequences that could result from a mis-diagnosis of gonorrhoea

Individual susceptibility to neisserial infection

We also demonstrated an association of throat-carriage of meningococci and ano-genital gonorrhoea\textsuperscript{16}. Meningococci were isolated from the pharynx 3.4 times more often from men and 2.0 times more often from women with genital gonorrhoea
than from those without while gonococci were isolated from a genital site 2.7 times more often in men and 1.8 times more often in women who also carried meningococci in their pharynx than those who did not. The factors responsible for the above association are unknown but it could be that there are specific host factors which increase the susceptibility of certain individuals to neisserial colonisation, or alternatively, that the more frequent acquisition of both organisms is related to behavioural factors. We postulated that if these results on the carriage of neisseriae merely reflect the association of intimate behaviour with the exchange of flora then oropharyngeal carriage of other 'marker' organisms should show a similar correlation with genital gonorrhoea. Although we undertook a study using beta-haemolytic streptococci as 'marker' organism there was a statistically significant association between non-groupable streptococci and gonorrhoea but not between groupable beta-haemolytic streptococci and gonorrhoea. Unfortunately, the question of whether or not the association of genital gonorrhoea and throat carriage of meningococci is the result of individual susceptibility to neisserial infection or is attributable to behavioural factors cannot be answered from these results.

In collaboration with Dr Ron Fallon, Director of the Scottish Meningococcus Reference Laboratory, we demonstrated that the association between ano-genital gonorrhoea and pharyngeal colonisation with meningococci held for groupable but not non-groupable meningococci. These results can be interpreted as lending support to a behavioural association rather than an individual susceptibility: viz colonisation with groupable meningococci results predominantly from direct salivary spread leading to a higher level of colonisation in "high risk" patients by virtue of their greater degree of mouth-to-mouth contact. In contrast, if non-groupable
meningococci are spread primarily via droplet spray then most individuals will be exposed to a similar extent.

**Neisserial colonisation of the pharynx**

Our first detailed study of the spectrum of neisserial colonisation of the pharynx and the importance of a course of treatment in eradicating pharyngeal gonorrhoea was published in 1983\(^{37}\) at which time the prevalence of pharyngeal gonorrhoea was 4.3% for all men, 11% for homosexual men and 7.9% for women. We also noted that the observed coexistence of gonococci and meningococci in the pharynx (0.39%) was significantly lower than the theoretically expected value (1.7%). After the introduction of the Phadebact Monoclonal GC Test in 1985 it was easier to identify gonococci in the presence of meningococci and this resulted in an actual dual isolation rate of 2.5% which was not significantly different from the theoretical rate of 2.9%\(^{60}\). The clinical and microbiological significance of pharyngeal gonorrhoea increased dramatically during the latter half of the 1980's when the average level for the period 1986-1991 was 16.8% in women, 8.9% in heterosexual men and 35.7% in homosexual men\(^{100}\).

**Correlation between sexual orientation and phenotype of infecting strain**

Following the development of a serogrouping system in Sweden we obtained the appropriate strains from colleagues at the Karolinska Institute and prepared our own polyclonal antibody coagglutination reagents for use in serogrouping studies. Using these reagents we were able to confirm the preliminary Swedish report of a correlation between serogroup WII and rectal infection in men as well as confirming
the association for homosexually acquired urethral and pharyngeal infection. In a further study which re-confirmed the correlation between serogroup WII and homosexually acquired infection we were also able to show that the proportion of serogroup WII infections in heterosexual men showed temporal variation, that there was no correlation between serogroup and site of infection or symptoms, and perhaps most importantly that there was concordance in the serogroups isolated from contact pairs.

We also demonstrated a correlation between the Mtr phenotype with reduced cell envelope permeability with homosexually acquired WII isolates but not with heterosexually acquired WII isolates: this suggested (and was later confirmed with monoclonal antibody typing - see next section) that serogroup WII isolates from homosexual men were different from those from heterosexual patients.

Serogrouping was also used to demonstrate that the positive correlation between C. trachomatis infection and serogroup WI gonococcal infection reported from Sweden did not hold for women in Edinburgh.

Rectal gonorrhoea as an indicator of unsafe sexual behaviour

As a result of the on-going collaboration with the Department of Genitourinary Medicine in Edinburgh we were able to highlight an increase in gonorrhoea (including rectal gonorrhoea) and syphilis in homosexual men in Edinburgh during 1990. At face value this observation had obvious implications relating to health education, the avoidance of anal intercourse and the adherence to safer sexual practices. However, others had challenged the tenet that male gonorrhoea reflects unsafe sexual behaviour (Tomlinson et al, Lancet 1991; 337: 501) and our microbiological findings lend some
support to this. On reviewing the neisserial colonisation of ano-genital sites in homosexual men for the period 1986 to 1990 we found that *N. gonorrhoeae* and *N. meningitidis* was isolated from the throats of 29% and 30% of patients, respectively while rectal gonorrhoea was seen in 41% of patients and rectal carriage of *N. meningitidis* in 9%. These data support the hypothesis that oro (or at least salivary)-anal contact is likely to spread gonorrhoea: clearly if *N. meningitidis* can become established in the rectums of 9% of patients then the gonococcus with a predilection for the columnar epithelial cells of the ano-genital tract may be transferred with even greater frequency. However, as acquisition of infection by one route in one individual does not exclude acquisition by another route in another individual it would seem prudent to maintain the view that rectal gonorrhoea is an indicator of unsafe sexual behaviour.

**Cases of gonorrhoea seen at GUM represent the main pool of infection**

An audit of cases of gonorrhoea within the Edinburgh area showed that 91% of infections were seen at the GUM clinic confirming that the molecular epidemiology of gonococcal isolates from GUM patients is representative of the main pool of gonococcal infection.

**Conclusion**

The studies to define the spectrum of anatomical colonisation with pathogenic neisseriae in sexually active individuals highlighted the importance of extra-genital gonococcal infection and ano-genital meningococcal infection. The extent of these occurrences have major implications for the reliability of identification methods, the
efficacy of certain treatment regimens, and the assessment of 'risk' behaviour in relation to the possible acquisition of HIV infection. The introduction of serogrouping demonstrated that certain strains of gonococci were associated with homosexually acquired infection and stimulated the introduction and application of monoclonal antibody and other typing systems to enable a more detailed study of strain related epidemiological and clinical associations.
2d: Epidemiological typing and clinical associations

A rapid, inexpensive typing system for gonococci would be of value in epidemiological studies, for distinguishing treatment-failures from cases of reinfection, and possibly in the early recognition of antibiotic resistant isolates or strains that might have special pathogenic features such as those associated with disseminated infection. In the mid-1970s when I started working on gonococci there was no suitable epidemiological typing system for *N. gonorrhoeae*. In a joint study with Dr Caroline Blackwell of the Department of Medical Microbiology who was interested in bacterial lipopolysaccharide we investigated the use of pyocines for typing gonococci\(^{10}\). Although we were able to divide a variety of gonococcal strains into 15 fairly evenly distributed sensitivity patterns this system did not gain universal acceptance. Work on gonococcal typing was set aside for some years as work on cultural and non-cultural diagnosis was given a higher priority.

Serotyping with monoclonal antibodies

The development of a serogrouping system in Sweden was soon followed by a serotyping system based on monoclonal antibodies against gonococcal protein I which occurs in two mutually exclusive forms A and B: serogroup WI strains corresponded to isolates containing protein IA and WII/III isolates to protein IB. In 1985 I made a research visit to Dr Solgun Bygdeman's laboratory at the Karolinska Institute in Stockholm for training in serotyping with monoclonal antibody coagglutination reagents. On my return to Edinburgh I applied this methodology to study the micro-epidemiology of gonococcal infection and characterise certain clinical associations.
In our first publication using serotyping\textsuperscript{47} we employed two separate panels of monoclonal antibodies developed by Pharmacia in Sweden (Ph panel) and Genetic Systems in the USA (GS panel) to type 357 clinical isolates. The Ph reagents identified four WI serovars (serovariants) and 21 WII/III serovars, whereas the GS reagents identified 10 WI serovars and 18 WII/III serovars. By combining the results obtained with each panel, 15 Ph/GS WI serovars and 33 Ph/WII/III serovars were recognised. The serovar patterns for men and women were very similar, except for one serovar combination (Back/Bropyt) that was ten times more common in isolates from men than from women. In a further study\textsuperscript{50} we extended this work and confirmed our earlier hypothesis that serogroup WII isolates from homosexual men were different from WII isolates acquired heterosexually by demonstrating that the serovar combination termed Back/Bropyt predominated in homosexual men accounting for 69\% of homosexually acquired infections - the same combination was found in only 1\% of heterosexual men and was absent from women. These findings suggested that serovar determination could prove valuable as an indicator of homosexually acquired infection. We also identified a GS serovar termed Bajk which correlated with rectal infection in women\textsuperscript{53}.

As part of an extended surveillance serotyping of all gonococcal strains isolated in Edinburgh was introduced as a routine. During the period 1986-90, 32 different serovars were associated with 175 homosexually acquired infections\textsuperscript{74}. There was a dynamic temporal change in the dominant serovars with a continual influx of new strains some of which become established in the community but most of which appeared only transiently. The number of infections caused by minor serovars correlated with rates of infection\textsuperscript{72}. During the same period there were 44 different
serovars associated with 1356 episodes of heterosexually acquired infection\(^8\).

Again we found similar dynamic and temporal changes in the incidence of certain serovars. The serovars observed however differed markedly from those seen in homosexual men.

As most studies assume that the isolated serovar remains stable during natural transmission we examined the reliability of serotyping by examining isolates from known contact pairs\(^8\): the overall rate of discordant transmission episodes was 12% but was significantly higher in the case of IB serovars (19%) than IA serovars (3%).

We concluded that serotyping should be combined with other methods such as auxotyping for detailed microbiological studies involving partner notification.

The A/S (auxotype/serovar) classification system

As part of Alex Moyes' M Phil thesis we examined various aspects of gonococcal typing. These studies included an analysis of the various monoclonal antibody typing panels\(^7\) as well as the added discrimination provided by lectin agglutination\(^7\) and lectin agglutination plus auxotyping\(^6\). As a result of these studies we moved from typing with the Swedish panel of monoclonal antibodies and a descriptive serovar nomenclature to the standard American panel of monoclonal antibodies and a numerical nomenclature. The use of the American serotyping system in combination with auxotyping allows the characterisation of isolates by the standard A/S classification system recommended by CDC at Atlanta, Georgia and facilitates epidemiological comparisons between diverse geographical regions. Lectin agglutination is a useful adjunct to the standard A/S classification and can further divide many of the common A/S classes for micro-epidemiological purposes.
Clinical correlation and serovar specific immunity

Collaborative studies with Dr Jonathan Ross showed that serovar IA-2 infections tended to be less symptomatic than average but infections with the minor serovars tend to produce more symptoms than average. Serovar IA-2 was more infectious than other serovars as assessed by the likelihood of infecting sexual contacts. It was concluded that infectivity and presence of symptoms play a role in determining the prevalence of individual serovars within a population but probably in combination with other factors. A more detailed study of 508 episodes of infection and using multivariate logistic regression showed the following significant associations:

- asymptomatic infection was more likely to occur in infection with serovar IB-1 and was less likely for infections caused by minor serovars;
- homosexual acquisition of infection was associated with IB-2 infections and significantly less likely in IA-2 infections; and minor serovars were less likely to cause infections in men and IB-3 infections were less likely to affect the endocervix.

As it had been suggested that serovar specific immunity could have an influence on the prevalence of serovars within a community (Plummer, F. A. et al - Journal of Clinical Investigation 1989;83: 1472-1476) we examined 508 episodes of gonococcal infection diagnosed in Edinburgh with respect to their initial and subsequent serovar as a surrogate marker of serovar specific immunity. There were 22 patients with two or more infections over a four year period but there was no significant difference in the prevalence of serovars isolated following a repeat infection compared with those without repeat infections. We concluded that there was no evidence of serovar specific immunity in our population. However, it remains possible that serovar specific immunity may have an influence on the serovars circulating in
populations with a higher prevalence of gonorrhoea and more frequent repeat infections.

**Geographical variation and national monitoring**

We also performed a detailed analysis of gonococcal infections in Aberdeen\textsuperscript{94} and Glasgow\textsuperscript{108}. In contrast to other areas of Scotland Aberdeen has a high prevalence of gonorrhoea, the majority of strains belong to serovar IA-2 (as opposed to the diversity of strains seen in other Scottish cities), and a low rate of homosexually acquired infections. In Glasgow serovar IB-2 and IB-6 strains were isolated significantly more often in homosexual patients while serovar IB-17 was associated with lack of symptoms in male heterosexuals. In a comparative study between Edinburgh and Newcastle we showed that serovar IB-1 was the prevalent homosexually acquired serovar in Edinburgh during the study period while serovar IB-6 was the prevalent homosexually acquired serovar in Newcastle\textsuperscript{98}. Although homosexually acquired infections are associated with certain serovar IB isolates we found that serovar IA-6 which is relatively rare in the UK was associated with 21\% of homosexually acquired infections reported in Edinburgh in 1993\textsuperscript{95}. There was some epidemiological evidence to suggest that a bisexual male imported this strain from the Far East where IA-6 infections are common.

The serotyping studies performed in Edinburgh resulted in requests from other geographical areas to type their gonococcal populations. The first comparative survey of Scottish isolates included 869 strains isolated in 1988 in Edinburgh, Greater Glasgow, Fife and Dundee\textsuperscript{68}. Using two monoclonal antibody panels a total of 11 IA and 47 IB serovars were recognised and marked geographical differences
were demonstrated within both major and minor serovars. The majority of PPNG strains (71%) were due to serotype IA strains. During 1989 547 episodes of infection were isolated from the geographical areas described above. The decrease in the overall prevalence of gonorrhoea was reflected in a decrease in the number and diversity of serovars circulating in the gonococcal population. There was also a slight decrease in PPNG. However, three PPNG strains which were epidemiologically linked with Spain showed decreased susceptibility to ciprofloxacin. The standard Genetic Systems monoclonal antibody panel and numerical serovar nomenclature as recommended by CDC Atlanta was introduced for the analysis of 544 Scottish gonococci isolated during 1990. Six IA and 16 IB serovars were found among the Scottish isolates: global studies had defined 24 IA and 32 IB serovars. Serovar IB-6 isolates were more common in Lothian than in other areas and were associated with homosexually acquired infection whereas serovar IB-17 which is negative in the Phadebact Monoclonal GC test was found only in Glasgow. This isolate which is associated with asymptomatic urethral infection in men persisted in Glasgow until 1992. The level of CMRNG remained low and no ciprofloxacin resistant isolates were found. In contrast to 1988 when the majority of PPNG strains were IA isolates the majority of the 1990 PPNG were IB isolates. The diverse pattern of PPNG isolates revealed by typing suggests occasional importation and subsequent spread rather than major indigenous acquisition and spread of PPNG. On monitoring 558 isolates in 1991 we found that 10 (2.7%) had a ciprofloxacin MIC of > 0.008 mg/L on screening: all of the 1990 isolates had ciprofloxacin MICs of ≤ 0.008 mg/L. On extended testing of the ten isolates only two had MICs > 0.05 mg/L (both 0.012 mg/L), the level at which treatment failure may occur.
Strains received during 1991 also included two isolates from Glasgow of a previously unrecognised serovar. These were sent to CDC Atlanta for confirmation of the monoclonal antibody reaction pattern and were accorded the new serovar number IA-25. There were three isolates of serovar IA-25 in Lothian in 1992 and it has not been found since then. The number of IA serovars recognised worldwide has remained at 25 since 1991.

In 1992 my laboratory was recognised and centrally funded by the Scottish Office as the Scottish Neisseria gonorrhoeae Reference Laboratory. Monitoring of isolates during 1992, 1993 and 1994 demonstrated an increase in chromosomally mediated resistant N. gonorrhoeae (CMRNG) -defined by a penicillin MIC ≥ 1.0 mg/L. The number of isolates tested between 1988 and 1995 and the prevalence of PPNG and CMRNG are summarised in Figure.
Chromosomally mediated clinical resistance to penicillin, defined by an MIC $\geq 2.0$ mg/L and associated with less than 90% efficacy of single dose treatment, increased from 1.3% to 5.8% during the same period. Although the data for 1995 is provisional it suggests that the decline in gonorrhoea seen in recent years has been reversed. Ciprofloxacin resistance remained low in 1994 at 1.5%.

During 1994 auxotyping was introduced enabling further subdivision of serovars and allowing a more detailed comparison with other geographical areas. For example the seven IA serovars encountered represented 14 A/S classes while the 13 IB serovars represented 34 A/S classes. Within Lothian IB-2/NR strains tended to be homosexually acquired whereas IB2/PAU strains were heterosexually acquired. IB-6/NR isolates also tended to be homosexually acquired whereas IB-6/PAU strains were heterosexually acquired. The finer discrimination resulting from A/S classification allows the recognition of geographical associations within serovars, eg although there were eleven IB-8 strains these represented three different foci of infection with IB-8/PAU found only in Forth Valley, IB-8/NR in Glasgow and IB-8/P in Lothian.

Conclusion

The implementation of monoclonal antibody serotyping and auxotyping facilitated detailed epidemiological evaluations: these studies demonstrated that the circulating gonococcal strains undergo marked geographical and temporal variation. Several clinical and behavioural associations have also been discovered with particular serovars correlating with homosexually acquired infection, symptomatic versus asymptomatic infection, infectivity, and antibiotic resistance. At the level of
management of the individual patient typing has proved valuable in differentiating treatment failure from re-infection and has also been used in medico-legal situations involving sexual abuse. By applying detailed standardised typing of virtually all of the gonococci isolated in Scotland we are able to perform meaningful surveillance of antibiotic resistance and to compare the prevalence of particular types with their prevalence in other areas of the world. These studies have also laid the groundwork for the application of molecular typing methods such as restriction fragment length polymorphism based on restriction endonuclease analysis and pulsed field gel electrophoresis. Isolates typed by standard methods have been supplied to Mr G Abbas of the Department of Medical Microbiology at the University of Aberdeen to develop this methodology.
2e: Reviews and contributions to books

In addition to the original publications described above, the genus *Neisseria* has been reviewed in several editions of a standard microbiology textbook. The system developed in Edinburgh was the subject of an early review while the broader aspects of recent advances in routine procedures for the laboratory diagnosis of gonorrhoea have also been reviewed. The aetiology, pathogenesis and clinical features of gonorrhoea and laboratory and clinical practice are discussed in Chapters 10 and 11 respectively of publication: these reviews were updated in Chapters 14 and 15 of the 2nd edition of the textbook. The immunological diagnosis of gonococcal infection has also been reviewed.
Section 3: Other sexually transmissible infections, authorship and editorship of books

Studies on other sexually transmissible diseases included the isolation of metronidazole resistant *Trichomonas vaginalis*\(^1\)\(^8\), and the isolation and significance of *Ureaplasma urealyticum* in men\(^2\)\(^6\) and women\(^27,31\) attending an STD clinic. In collaboration with colleagues in the University Departments of Surgery and Urology we applied the expertise developed in these studies to examine the role of *U. urealyticum* in infertile men\(^32,44\). In collaboration with Unipath Ltd (Bedford, England) we helped develop a prototype rapid immunodiffusion test for *Chlamydia trachomatis* to a suitable commercial test format\(^7\)\(^6\).

In conjunction with Dr D H Robertson and Dr A Mcmillan I shared the authorship of a major textbook entitled "Clinical Practice in Sexually Transmissible Diseases" which was first published in 1980\(^21\). This text was translated into Spanish in 1984 (Enfermedades de transmision sexual - Diagnostico, tratamiento, prevencion y repercusion social, Revision de la edicion espanola, J. Peyri Rey, Ediciones Doyma, Barcelona) and a second edition published in 1989\(^57\). I also co-authored the chapter on "Treponematoses and sexually transmissible diseases" in Forfar and Arneil's Textbook of Paediatrics\(^8\)\(^0\).

As a result of my work on monoclonal antibody tests and publications on STDs I was invited by the publisher Marcel Dekker to edit a book entitled "Immunological Diagnosis of Sexually Transmitted Disease"\(^6\)\(^5\). My responsibilities as editor were to specify the overall content, the format for each chapter, and to recruit authors for each chapter. I invited my colleague Dr A Mcmillan to act as co-editor and we selected a team of internationally renowned contributors to cover those area where the monoclonal antibody technology was most advanced and where the development
of monoclonal antibodies might be most helpful: 26 contributors were responsible for 17 chapters covering 500 pages.

I was also invited by Kluwer Academic Publishers to act as Editor-in-Chief for the new publication "The Immunoassay Kit Directory Series B: Infectious Diseases" with responsibility to organise and define the content. I organised the directory into three distinct parts: each part was published individually and compared immunoassay kits using a set of defined criteria: Part 1 of the Directory dealt with Genitourinary Infections, Part 2 Respiratory Infections and Part 3 Enteric and Other Infections. As well as acting as Editor-in-Chief, I co-edited the issue on Genitourinary Infections and co-authored the introductory article on immunoassays. A chapter dealing with immunological and serological methods in the diagnosis of microbial infections was also contributed to a standard text.
LIST OF PUBLICATIONS SUBMITTED (CHRONOLOGICAL ORDER)


PUBLICATIONS (COPIES)
Treponema pallidum haemagglutination test as a screening procedure for the diagnosis of syphilis

BY
H. YOUNG, C. HENRICHSN AND D. H. H. ROBERTSON

Reprinted from British Journal of Venereal Diseases, Vol. 50, No. 5, October, 1974
**Treponema pallidum** haemagglutination test as a screening procedure for the diagnosis of syphilis

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AND  
D. H. H. ROBERTSON  
Department of Sexually-Transmitted Diseases, The Royal Infirmary of Edinburgh

The serological diagnosis of syphilis relies upon the widespread use of non-specific screening tests, such as the cardiolipin Wassermann reaction (CWR) and the Venereal Diseases Research Laboratory (VDRL) slide test, in combination with the Reiter protein complement-fixation (RPCF) test (British Cooperative Clinical Group, 1972). Where necessary, the fluorescent treponemal antibody-absorbed (FTA-ABS) or *Treponema pallidum* immobilization (TPI) tests can be used for verification of treponemal infection.

The *T. pallidum* haemagglutination (TPHA) test developed by Rathlev (1965, 1967) and modified by Tomizawa (Tomizawa and Kasamatsu, 1966; Tomizawa, Kasamatsu, and Yamaya, 1969) appears to possess characteristics of both a screening and a verification test. It is simple to perform and reproducible, and has a wide spectrum of reactivity in the different stages of syphilis (Tringali, 1970) and a specificity and sensitivity comparable with the FTA-ABS and TPI tests (Garner, Backhouse, Daskalopoulos, and Walsh, 1972; Johnston, 1972; O'Neill, Warner, and Nicol, 1973). In spite of these features, the TPHA test has not yet been widely used as a routine screening procedure.

This paper assesses the suitability of the TPHA test as a screening test for syphilis in a serology laboratory serving the general hospital population, an antenatal clinic, and a Sexually-Transmitted Diseases Department.

**Material and methods**

**CLINICAL MATERIAL**

A total of 7,312 sera, derived from the following sources in the Royal Infirmary Edinburgh (RIE), were examined over a 6-month period:

- Sexually-Transmitted Diseases Department (STDD) 3,533
- Antenatal Clinic (ANC) 1,824
- General Hospital Population (GHP) 1,955

**SEROLOGICAL TESTS**

All specimens were screened by the VDRL slide test, RPCF, and TPHA tests. The FTA-ABS test was carried out on all specimens reactive in any of the screening tests.

A modification of the Whitechapel technique (ACP Broadsheet 41, 1969) was used for the RPCF test (1:1 MHD of complement and a fixation period of 30 min. at room temperature followed by 30 min. at 37°C.). Standard techniques were used for the VDRL slide test (Harris, Rosenberg, and Reidel, 1946) and the FTA-ABS test (Hunter, Deacon, and Meyer, 1964).

**TPHA TEST**

This was carried out by a modification of the micro-method described by the suppliers of the reagents (Fujizoki Pharmaceutical Company, Tokyo, Japan). Each kit comprised lyophilized test cells (antigen coated) and control cells, absorbing diluent to remove non-specific reactions, and a positive control serum. Test and control sera were re-constituted and diluted 1 in 6-5 as recommended by the manufacturer. Positive and negative control sera were included in each batch of screening tests and the positive serum titrated whenever any quantitative tests were performed.

(a) **Screening tests**

Sera were inactivated by heating at 56°C. for 30 min. and a 1 in 20 dilution of serum was made by adding 10 µl. serum to 190 µl. absorbing diluent. Samples were allowed to absorb for 30 min. at room temperature, after which time 25-µl. aliquots were transferred to U-type microtitre plates. Test cells (75 µl.) were then added to each sample to give a final serum dilution of 1 in 80. Plates were examined after 4 hrs incubation at 25°C. A final reading was made after overnight incubation.
Results were recorded as follows:

Negative A smooth ring or button of cells.
Positive A diffuse carpet or a thin ring of cells with marked agglutination.
Weak positive A slightly enlarged ring of cells with peripheral agglutination.
Doubtful An appearance intermediate between weak positive and negative (an enlarged ring of cells surrounded by a rough margin.)

Specimens showing positive, weak positive, and doubtful reactions were tested quantitatively as follows:

(b) Quantitative test for positive reactors
Serums were absorbed as described previously and doubling dilutions prepared, in 25-μl volumes in diluent, over the range 1 in 20 to 1 in 1,280. Test cells (75 μl.) were then added to give final serum dilutions from 1 in 80 to 1 in 5,120. Each specimen was also tested against control cells at a final serum dilution of 1 in 80. After incubation, the titre was read as the highest dilution giving definite agglutination.

(c) Quantitative test for weak positive and doubtful reactors
These were titrated over a range of serum dilutions from 1 in 40 to 1 in 320 by preparing an initial serum dilution of 1 in 10 in diluent. Samples were also tested against control cells at a 1 in 40 serum dilution. Specimens showing slight agglutination at 1 in 40 but doubtful agglutination or none at 1 in 80 were regarded as negative, while specimens showing strong agglutination at 1 in 40 and weak agglutination at 1 in 80 were reported as 'very weak positive' (VWP). In all other cases the titre was read as described above.

Samples showing agglutination of control cells were reported as 'Non-specific agglutination—test invalid'.

All the 35 doubtful reactors by the screening test were classified as negative when tested as described above. FTA-ABS tests on these sera were also negative.

Results
335 of the 7,312 sera examined were reactive in one or more of the screening tests; this represented 251 of the 6,500 patients examined in the course of the survey. An examination of the clinical records showed that 256 of the 335 reactive sera were from 185 cases of syphilis diagnosed by accepted clinical and serological criteria (including the TPI test in some cases).

Table I shows the number of specimens detected by each test and combination of tests, the agreement of the tests with the FTA-ABS, and their efficiency in detecting the 256 sera from cases of syphilis. The 287 sera detected by the TPHA test did not include the 35 doubtful reactors by the screening test which were classed as negative after the quantitative test.

From Table I it is clear that the TPHA test was significantly more sensitive than either the VDRL or RPCF tests. A combination of the VDRL and TPHA tests detected all 256 sera from cases of syphilis.

A summarized analysis of clinical data in relation to laboratory results is shown in Table II. Full clinical information was not available for certain patients presenting at various out-patient departments associated with the hospital and for a small number of defaulters; these are grouped as cases with 'insufficient information'.

All specimens which were positive in all of the three screening tests were also FTA-ABS positive. Cases of untreated syphilis in this group were mainly of late syphilis (late syphilis, twelve sera; early syphilis, three sera). Sera found to be positive in both the VDRL and TPHA test and negative in the RPCF test also showed 100 per cent. agreement with the FTA-ABS test and there were no cases of untreated syphilis in this group.

The TPHA test failed to detect ten sera from patients with syphilis which were positive in the FTA-ABS. Eight of the sera were from cases of untreated primary syphilis (two were VDRL and RPCF positive and six were positive in the VDRL only) and two were specimens from a case of treated latent syphilis (positive in the VDRL only). The

<table>
<thead>
<tr>
<th>TABLE I Effectiveness of individual tests or combination of tests as a screen for the detection of syphilis. Results from 7,312 sera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of sera detected by each test or combination of tests</strong></td>
</tr>
<tr>
<td><strong>Test(s)</strong></td>
</tr>
<tr>
<td>TPHA</td>
</tr>
<tr>
<td>VDRL</td>
</tr>
<tr>
<td>RPCF</td>
</tr>
<tr>
<td>TPHA, VDRL, RPCF</td>
</tr>
<tr>
<td>TPHA, VDRL</td>
</tr>
<tr>
<td>TPHA, RPCF</td>
</tr>
<tr>
<td>RPCF, VDRL</td>
</tr>
</tbody>
</table>
Laboratory result | No. of sera positive | Agreement with FTA-ABS | Known syphilis | No clinical evidence or history of syphilis | Insufficient information |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>VDRL</td>
<td>RPCF</td>
<td>TPHA</td>
<td>Number FTA-ABS positive</td>
<td>Percentage</td>
<td>Total</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>134</td>
<td>134</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>49</td>
<td>49</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>14</td>
<td>13</td>
<td>92.8</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>90</td>
<td>56</td>
<td>62.2</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>38</td>
<td>9</td>
<td>23.7</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>1</td>
<td>14.3</td>
</tr>
</tbody>
</table>

VDRL, therefore, detected all sera from patients with syphilis which were negative by the TPHA whereas the RPCF detected only two of these.

Ninety sera positive in the TPHA but negative in the VDRL and RPCF tests: 56 were FTA-ABS positive and 34 negative

56 Sera positive in the TPHA and FTA-ABS tests

45 of these sera were from cases of diagnosed syphilis (37 from patients with treated syphilis and eight from cases of untreated latent syphilis).

Of the remaining eleven specimens, five were from cases attending the STDD for treatment for gonorrhoea. There was insufficient clinical data to evaluate the remaining six sera.

34 Sera positive in the TPHA test but negative in the FTA-ABS test

These sera were derived from 23 patients. In five of these patients there was a reliable history in keeping with the interpretation that the positive TPHA reaction was due to antibody against T. pallidum; these cases are summarized in Table III. In eight patients, seven of whom had attended the STDD, there was neither clinical evidence, nor a reliable history of syphilis (Table IV); sera from five of these patients gave a reciprocal TPHA titre of 160 or more on at least one occasion but repeat specimens were negative. The remaining ten patients (STDD 4; ANC 3; GHP 3), all in the ‘very weak positive’ category, attended once only and could not therefore be fully evaluated.

Non-specific agglutination

Four sera agglutinated both test and control cells. These sera were negative in the VDRL, RPCF, and FTA-ABS tests. This represented 0.05 per cent. of all sera tested.

The pattern of agglutination of the antigen-coated cells by these non-specific sera was found to differ from the normal agglutination pattern (Figure). The reason this for distinctive settling pattern is obscure but these sera could invariably be distinguished from genuine reactors in the routine screen in the absence of control cells.

Discussion

On the basis of the results presented, we conclude that the VDRL and TPHA tests together provide a very effective screen for treponemal infection. Both tests are simple to perform and their activity is complementary. The VDRL test is more sensitive than the TPHA in the detection of very early syphilis.

Table III: Summary of five cases in which antibody against T. pallidum was detectable by the TPHA test but not by the FTA-ABS or other tests

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yrs)</th>
<th>Area attended</th>
<th>TPHA results*</th>
<th>Clinical state</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>STDD</td>
<td>VWP; 80</td>
<td>Primary syphilis treated 5 years previously</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>STDD</td>
<td>VWP; 80</td>
<td>Primary syphilis treated 7 years previously</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>STDD</td>
<td>80; negative; VWP</td>
<td>Primary syphilis treated 24 years previously</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1</td>
<td>STDD</td>
<td>VWP; 160</td>
<td>Transplacental antibody from serologically positive mother treated with penicillin before and during this pregnancy</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>GHP</td>
<td>VWP</td>
<td>Neurosyphilis</td>
</tr>
</tbody>
</table>

*TPHA results are expressed as the reciprocal of the final serum dilution showing definite agglutination. VWP = very weak positive.
TABLE IV  A summary of TPHA results and clinical findings in eight patients in whom there was no evidence of syphilis

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Area</th>
<th>TPHA results (time in days from first test)</th>
<th>Clinical data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>M</td>
<td>STDD</td>
<td>640</td>
<td>Negative (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>160</td>
<td>(25) Negative (67)</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>F</td>
<td>STDD</td>
<td>160</td>
<td>(25) Negative (67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>320</td>
<td>(5) Negative (166)</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>M</td>
<td>STDD</td>
<td>80</td>
<td>Negative (81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>160</td>
<td>(4) Negative (49)</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>M</td>
<td>STDD</td>
<td>160</td>
<td>(4) Negative (49)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td>VWP (84)</td>
</tr>
<tr>
<td>5</td>
<td>43</td>
<td>M</td>
<td>STDD</td>
<td>160</td>
<td>(25) VWP (35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td>VWP (49)</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>M</td>
<td>STDD</td>
<td>80</td>
<td>Negative (77)</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>M</td>
<td>STDD</td>
<td>160</td>
<td>Negative (77)</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>F</td>
<td>ANC</td>
<td></td>
<td>10 weeks pregnant at first visit</td>
</tr>
</tbody>
</table>

with or without a detectable chancre, while the TPHA is more sensitive and specific than the VDRL in the detection of latent or late syphilis whether treated or untreated.

Similar conclusions regarding the value of the TPHA were reached by Johnston (1972) and Sequeira and Eldridge (1973). In contrast to these findings Luger and Spendlingwimmer (1973) found the TPHA more sensitive than either the VDRL or the FTA-ABS test in untreated primary syphilis. It is of interest to note that Luger and Spendlingwimmer performed their TPHA tests without previous inactivation of sera.

The RPCF test would appear to be grossly undersensitive, detecting only 58.2 per cent. of the known syphilitic sera. Similar results were quoted in a review by Sparling (1971).

**FIGURE**  Enlarged area (magnification ×3) of a routine screening plate. Each well contains antigen-coated cells and patient's serum.

N = negative reaction
P = positive reaction
NSA = non-specific agglutination

In the case of the NSA, the cells have settled into a thick coarse ring with a fairly large diameter, but very little peripheral agglutination is observed. In contrast to this, the positive serum shows a thin ring of cells with marked peripheral agglutination.
The specificity of the TPHA test in our survey was 99.7 per cent, based on accepted clinical and serological criteria. 21 sera positive in the TPHA test only were from patients who had no history or clinical evidence of syphilis (Table II). However, five of these sera were also reactive in the FTA-ABS test.

Garner, Backhouse, Daskalopoulos, and Walsh (1973), in contrast to our findings and their previous work (Garner and others, 1972), found 11.3 per cent. of 274 biological false positive sera showed false positive TPHA reactions. From a survey of only 306 sera, Blum, Ellner, McCarthy, and Papachristos (1973) calculated that the TPHA test gave a false positive rate of 8.8 per cent. The reagents used in the latter study, unlike the previous one, were not supplied by the Fujizoki Company.

Sequeira and Eldridge (1973), however, using an alternative TPHA technique in which sensitized fowl erythrocytes are used in place of sheep erythrocytes, reported a specificity similar to ours (99.8 per cent.). Buist, Pertile, and Morris (1973) also found the TPHA test to be highly specific, giving less than 1 per cent. false positive results, while O'Neill and others (1973) reported a false positive rate of 1 in 4,000 in the general hospital population.

A transient positive TPHA reaction was given by the sera of five patients; four of these attended the STDD, three with non-gonococcal urethritis. The transient TPHA activity in this group could be due to antibody formed against an organism with a common antigen, perhaps commensal treponemes of the genital mucosa. In this context, it is of interest that three out of seven patients positive in the RPCF test only were cases of gonorrhoea; one patient having had the infection six times.

Thus one reason for the transient positive TPHA results could be the failure of the absorbing diluent to remove group-specific antibody. Sorbent will sometimes fail to remove all group treponemal antibody in the FTA-ABS test (Király, Jobbágy, and Kováts, 1967). Another reason for false positive TPHA results could be a failure of the control cells to show agglutination in the presence of heterophil antibody. Cox, Logan, and Stout (1971) reported that three of one-hundred sera from patients with infectious mononucleosis gave false positive TPHA reactions and did not agglutinate the control, unsensitized, erythrocytes. There was one such specimen in our survey. The rate of non-specific agglutination in our tests, less than 1 per thousand specimens, was relatively low compared to a rate of approximately 5 per thousand reported by Garner and others, (1972).

From our experience with the TPHA test we conclude that the main value of the test is for screening purposes. The screening schedule should also include a cardiolipin antigen test, such as the VDRL, to improve the detection of early syphilis. The FTA-ABS test should be retained as a confirmatory test for sera with equivocal results and in special cases, e.g. suspected early syphilis. It must be recognized, however, that sera positive in the TPHA but negative in the FTA-ABS test may represent previous T. pallidum infection.

Summary

During a 6-month period, 7,312 sera were screened by the VDRL, RPCF and TPHA tests. Sera reactive in any of these tests were then examined by the FTA-ABS test. The sensitivity and specificity of each test was calculated on the basis of accepted clinical and serological criteria.

The TPHA test had a specificity of 99.7 per cent. and, except in cases of untreated primary syphilis, was the most sensitive of the 3 screening tests detecting 96.1 per cent. of known syphilitic sera; the VDRL detected 74.2 per cent. and the RPCF only 58.2 per cent. All cases of untreated primary syphilis, negative in the TPHA were detected by the VDRL test. 23 patients whose sera were reactive in the TPHA test but negative in all other tests including the FTA-ABS are discussed; 5 of these had a reliable history of syphilis.

The TPHA test and VDRL slide test were found to be a good combination for screening. However, the FTA-ABS test is still required to resolve cases which give equivocal results.

References


BUIST, D. G. P., PERTILE, R., and MORRIS, G. J. (1973) Pathology, 5, 249


Le test d'hémaglutination du *Treponema pallidum* dans les épreuves de dépistage en vue du diagnostic de la syphilis

**SOMMAIRE**

Pendant une période de 6 mois, 7,312 sérums furent systématiquement soumis aux épreuves VDRL, RPCF et TPHA. Tous les sérums réactifs à l'un de ces tests furent examinés à l'épreuve FTA-ABS. La sensibilité et la spécificité de chaque épreuve fut établie sur la base des critères acceptés en clinique et en sérologie.

Le TPHA montra une spécificité de 99,7 pour cent, détectant, sauf en cas de syphilis primaire non traitée, 96,1 pour cent des sérums syphilitiques connus et se montrant la plus sensibles des trois épreuves de diagnostic; le VDRL reconnut 74,2 pour cent des cas et le RPCF seulement 58,2 pour cent. Tous les cas de syphilis primaire non traités, négatifs avec le TPHA, furent détectés par le VDRL, 23 malades, dont le sérum était positif au TPHA mais négatif à tous les autres tests, dont le FTA-ABS, sont discutés; 5 d'entre eux avaient des antécédents valables de syphilis.

Pour le dépistage, le TPHA et le VDRL sur lame constituent une bonne association. Cependant, l'épreuve FTA-ABS est toujours nécessaire pour résoudre les cas où les résultats sont équivoques.
Clinical value of the *Treponema pallidum* haemagglutination test

BY

D. H. H. Robertson, A. McMillan, H. Young and C. Henrichsen

Reprinted from British Journal of Venereal Diseases, Vol. 51, No. 2, April, 1975

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British Journal of Venereal Diseases

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LONDON

BRITISH MEDICAL ASSOCIATION

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Clinical value of the *Treponema pallidum* haemagglutination test

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And

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The haemagglutination test, utilizing pathogenic *Treponema pallidum*, for the serological investigation of syphilis, was developed by Rathlev (1967) and provides the clinician with a test of high specificity and sensitivity. Commercially available reagents from Japan have been evaluated clinically by Uete, Fukazawa, Ogi, and Takeuchi (1971) and a similar haemagglutination test, using fowl erythrocytes, has been investigated by Sequeira and Eldridge (1973). Their results have confirmed that the haemagglutination test combines high sensitivity with high specificity in treated and untreated syphilis, except in some cases of early syphilis. With the late appearance of the antibody and its persistence after treatment, the *Treponema pallidum* haemagglutination test (TPHA) appears to share the advantages and, to a lesser extent, the disadvantages of the *T. pallidum* immobilization test.

This report is based on the results of an application of the TPHA test, using the commercial reagents from Japan, to the serological investigation of patients attending the Sexually-Transmitted Disease (STD) Department of the Royal Infirmary of Edinburgh (R.I.E.) during the first part of 1973 (Young, Henrichsen, and Robertson, 1974). In all cases showing positive reactions, even very weakly positive reactions, the fluorescent treponemal antibody absorbed (FTA-ABS) test was carried out.

### Patients investigated

From February 20 to May 8, 1973, a total of 1,129 individual patients (697 males and 432 females) attended the STD Department at the R.I.E. As part of the investigations on these patients, serological tests to exclude syphilis were performed. In addition the same tests were carried out on 65 patients treated previously for syphilis (with penicillin).

### Methods

The serological tests comprised the venereal disease research laboratory (VDRL) slide test and the Reiter protein complement-fixation (RPCF) test in addition to the TPHA test using the commercial reagents supplied by the Fuji-zoki Company, Tokyo, Japan. The FTA-ABS test was available when necessary. The methods used, including that in the quantitative TPHA test, were the same as described previously (Young, Henrichsen, and Robertson, 1974).

### Results

**Patients attending the STD department**

1. **Cases of syphilis discovered by VDRL and/or RPCF test and confirmed by positive FTA-ABS or by the finding of *T. pallidum* in a genital sore by darkground examination**

   Of the 1,129 patients, six cases of syphilis (Table I) were detected by the VDRL test and two of these were found to have a positive RPCF test. In two of the primary cases of syphilis the TPHA test was negative and in another female patient under surveillance after contact with a patient with early syphilis, the TPHA test did not become positive before changes were detected by the FTA-ABS and the VDRL test (Case B2).

   2. **Cases of syphilis discovered by the TPHA test and confirmed by the FTA-ABS but not detected by the VDRL and/or the RPCF test**

      Of the 1,129 patients, a further six cases of syphilis belonged to this category (Table II). These cases would not have been discovered by the VDRL or RPCF tests. Three of the six patients were merchant seamen; they admitted previous sexually-transmitted disease such as gonorrhoea, venereal warts, or non-specific urethritis, but denied a previous history of syphilis.

   3. **Cases discovered by the TPHA test but negative by the FTA-ABS, VDRL and RPCF tests**

      Of the 1,129 patients examined, there were seven whose TPHA reaction was positive without other evidence of previous or developing syphilis. The TPHA reaction in relation to these patients is dealt with in detail in our earlier report (Young and others, 1974).
Table I: Cases discovered by the VDRL and/or RPCF tests and confirmed by a positive FTA-ABS or by the finding of T. pallidum in a genital sore

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>VDRL (Reciprocal of titre)</th>
<th>RPCF (Reciprocal of titre)</th>
<th>TPHA (Reciprocal of titre)</th>
<th>FTA-ABS</th>
<th>Form of syphilis</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>M</td>
<td>27</td>
<td>Positive (2)</td>
<td>Negative</td>
<td>Positive (2,560)</td>
<td>Positive</td>
<td>Primary</td>
<td>DG negative for T. pallidum</td>
</tr>
<tr>
<td>A2</td>
<td>M</td>
<td>38</td>
<td>Positive (4)</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Primary</td>
<td>DG positive for T. pallidum</td>
</tr>
<tr>
<td>A3</td>
<td>M</td>
<td>35</td>
<td>Positive (64)</td>
<td>Positive (160)</td>
<td>Positive</td>
<td>Positive</td>
<td>Primary</td>
<td>DG positive for T. pallidum</td>
</tr>
<tr>
<td>A4</td>
<td>M</td>
<td>56</td>
<td>Positive (2)</td>
<td>Negative</td>
<td>Positive (80)</td>
<td>Positive</td>
<td>Late</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>F</td>
<td>63</td>
<td>Positive (Weak serum)</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Early</td>
<td>Sexual contact of patient with T. pallidum in genital sore</td>
</tr>
<tr>
<td>B2</td>
<td>F</td>
<td>42</td>
<td>Positive (10)</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DG = Dark-ground examination of serum from lesion.

Table II: Cases discovered by the TPHA test and confirmed by the FTA-ABS test but not detected by VDRL and/or RPCF tests

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Diagnosis</th>
<th>TPHA (Reciprocal of titre)</th>
<th>FTA-ABS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A5</td>
<td>M</td>
<td>63</td>
<td>Congenital syphilis first treated with arsenicals and bismuth 49 yrs ago and penicillin 12 yrs ago</td>
<td>Positive (320)</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>M</td>
<td>25</td>
<td>Latent syphilis in seaman with history of other sexually-transmitted disease</td>
<td>Positive (80)</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>M</td>
<td>26</td>
<td>Latent syphilis in seaman with history of other sexually-transmitted disease</td>
<td>Positive (640)</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>A8</td>
<td>M</td>
<td>34</td>
<td>Latent syphilis in seaman with history of other sexually-transmitted disease</td>
<td>Positive (160)</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td>M</td>
<td>69</td>
<td>Latent syphilis with previous risks of other sexually-transmitted disease</td>
<td>Positive (160)</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>M</td>
<td>34</td>
<td>Probably latent syphilis</td>
<td>Very weak positive</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Patient defaulted</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients Treated Previously for Syphilis

In none of the 25 cases of penicillin-treated early syphilis (nine seropositive primary, seven secondary, and nine early latent) had the TPHA test become negative. Even in cases examined 20 years or more after treatment, the TPHA remained positive in all cases. The titres found in the tests are indicated in Table III.

Similarly, in none of the forty cases of penicillin-treated late syphilis (28 latent, one cardiovascular, eight neurosyphilis, and three congenital) was the TPHA negative after treatment (Table IV). Among the forty cases of late syphilis a titre of 1/640 was found in nineteen.

Discussion

In 1,129 individual patients attending the STD Department, a diagnosis of syphilis, confirmed in all by the FTA-ABS test, was made in twelve (1 per cent.). In six of these patients (0.5 per cent.) syphilis was discovered by the TPHA test and would not have been detected by the VDRL and/or RPCF tests. The TPHA test, therefore, doubled the detection rate of syphilis.

In one patient with a healing primary sore, the TPHA was positive. In two other cases of primary syphilis the TPHA rest was negative and in one patient examined during the incubation period the VDRL and FTA-ABS tests became positive before any changes were detected in the TPHA.

The TPHA had not become negative in any of the cases of treated syphilis. There was considerable variation in the TPHA titre in cases of treated syphilis. Statistically there was no significant relationship (P > 0.1) between the titre found and the length of the interval after treatment for late syphilis, and there were insufficient data to enable
conclusions to be drawn about the relationship in early cases. More information is required concerning the TPHA titre in untreated syphilis.

The TPHA test is of undoubted value in the detection of syphilis beyond the very early stage. Unexplained positive reactions can occur, however, so that positive results in the TPHA only should be confirmed by another specific test such as the FTA-ABS before a firm diagnosis is made.

In screening for early syphilis the VDRL test is very important and should be repeated over a period of at least 3 months. In special cases, such as in contacts of infectious syphilis, the FTA-ABS test should be carried out. The normal practice of making dark-ground examinations in patients with lesions is, of course, also essential.

Summary
In 1,129 patients attending the Department for Sexually Transmitted Diseases, the serum was examined by three screening tests (VDRL slide, RPCF, and TPHA) and twelve cases of syphilis (1 per cent. of patients attending the clinic) were discovered.

Six of these patients were considered to have latent syphilis (5 acquired, 1 congenital) and were detected only by the TPHA; all six cases were confirmed by the FTA-ABS.

The TPHA failed to detect three of the remaining six cases (2 primary and 1 very early, the latter in a contact of a patient with primary syphilis). All six cases were, however, detected by the VDRL.

In seven cases, the TPHA was positive in the absence of other evidence of present or previous syphilis. In these cases the FTA-ABS was also negative. The clinical application of the TPHA test in the detection of syphilis is discussed.

We wish to thank Dr. R. J. Prescott, Department of Social Medicine, University of Edinburgh, for his help in making the statistical evaluation.
Une estimation de la valeur clinique du test d'hémaglutination du tréponème pâle

SOMMAIRE

Les sérum de 1129 consultants du département des maladies sexuellement transmises furent examinés par trois épreuves de triage (VDRL sur lame, RPCF et TPHA) et l'on découvrit 12 cas de syphilis (1 pour cent des consultants de la clinique).

Six de ces malades furent considérés comme atteints de syphilis latente (5 acquises, 1 congénitale) et ne furent reconnues que par le TPHA; tous les six cas furent confirmés par le FTA-ABS.

Le TPHA ne reconnut pas trois des six cas restants (2 primaires, 1 très précoce, le dernier étant un contact d'un malade atteint de syphilis primaire). Tous ces six cas furent cependant détectés par le VDRL.

Dans sept cas, le TPHA fut positif en l'absence d'autres preuves de syphilis actuelle ou antérieure. Dans ces cas, le FTA-ABS fut négatif également. On discute de l'application de l'épreuve TPHA dans la détection de la syphilis.
Rapid carbohydrate utilization test for the identification of *Neisseria gonorrhoeae*

BY

H. YOUNG, I. C. PATERSON, AND D. R. MCDONALD


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Rapid carbohydrate utilization test for the identification of Neisseria gonorrhoeae

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Department of Bacteriology, University Medical School, Edinburgh, EH8 9AG

Summary
A rapid carbohydrate utilization test for the identification of N. gonorrhoeae was investigated, with reference to its use in a routine diagnostic laboratory. The rapid test was shown to give accurate results in agreement with those of a conventional serum-free sugar medium. Because of the shorter time taken for the confirmation of an isolate, and several other advantages, it is proposed that the rapid test is an extremely useful alternative to conventional sugar tests.

Immunofluorescence was also used to identify isolates of N. gonorrhoeae and the rapid carbohydrate utilization test was found to assist in differentiating between N. gonorrhoeae and N. meningitidis when equivocal or negative immunofluorescence results were obtained.

Introduction
Although a presumptive diagnosis of gonococcal infection may be made by examination of Gram-stained smears whilst the patient is at the clinic, this must be supplemented and confirmed by culture if the maximum number of positive results is to be obtained (Jephcott, Morton, and Turner, 1974). Confirmation of the diagnosis by the isolation of oxidase-positive, Gram-negative diplococci and biochemical proof of identity, normally takes 3 to 4 days and the delay is sometimes due to the failure to demonstrate acid production from glucose only.

A more rapid confirmatory diagnosis followed by immediate treatment would be helpful in the control of the disease. Consequently, immunofluorescence techniques (Hare, 1974) and a rapid fermentation test (RFT) (Kellogg and Turner, 1973) have been investigated.

Kellogg and Turner (1973) reported that the RFT was suitable for the confirmation of identity of Neisseria gonorrhoeae from either primary isolation media or purification media. In the RFT, pre-formed enzyme is measured by adding a suspension of the overnight growth of the suspect organism to a buffered (non-nutrient) solution containing the sugar to be tested and a pH indicator. Since the test measures pre-formed enzyme, it has the advantage of being independent of growth. In contrast, in the conventional technique, a serum-free agar medium containing the appropriate carbohydrate is inoculated with the test organism (Flynn and Waitkins, 1972). A positive reaction is dependent on adequate growth of the test organism and this may take up to 48 hrs. In addition, several strains of N. gonorrhoeae grow very poorly on the test medium containing maltose, making it impossible to score a valid negative result in the absence of adequate growth.

Unfortunately, as reported by Brown (1974), several laboratories were unable to obtain results comparable to those described by Kellogg and Turner (1973). In view of these conflicting reports, we decided to investigate a rapid carbohydrate utilization test (RCUT), in parallel with the conventional sugar technique and immunofluorescence, and to determine its value in a routine diagnostic laboratory.

Material and methods
Bacterial strains
The best conditions for the RCUT were established using stock strains of N. gonorrhoeae and N. meningitidis. These were N. gonorrhoeae strain 9, a gift from Dr. D. S. Kellogg, Center for Disease Control, Atlanta, Georgia, and N. gonorrhoeae strains 1, 2, 3, 6, and N. meningitidis groups A, B, C, D, E, 29E, W-135, X, and Z, originally obtained from the Neisseria Repository, Berkeley, California, and kindly made available by Professor B. P. Marmion of our Department. Cultures of commensal Neisseria species were provided by Dr. Margaret Calder, Bacteriology Laboratory, City Hospital, Edinburgh.

Specimens
To assess the test in routine use, specimens were obtained from patients attending the Department for Sexually Transmitted Diseases, The Royal Infirmary of Edinburgh. Material for culture, normally from the urethra, cervix,
and rectum in female cases and from the urethra in male cases, was plated directly on to Columbia agar base (Ellner, Stoesself, Drakeford, and Vasi, 1966) enriched with 10 per cent. heated human blood; 80 per cent. of the concentrations of vancomycin, colistin, and nystatin recommended by Thayer and Martin (1966) were incorporated. The cultures were processed in a different section of the laboratory as part of the normal diagnostic work; this included sugar utilization tests using a serum-free medium (Flynn and Waitkins, 1972). Any isolates of Gram-negative diplococci were then passed to us for investigation by the RCUT and immunofluorescence techniques outlined below.

**Rapid carbohydrate utilization test (RCUT)**

Buffer-salt solution 5-2 mM K$_2$HPO$_4$/KH$_2$PO$_4$ buffer containing 0-8 per cent. KCl and 0-1 per cent. phenol red, pH 7-1-7-15. This solution, which is a modification of those of Kellogg and Turner (1973) and Brown (1974), was made by mixing the following solutions: 40 ml. 0-1 M-K$_2$HPO$_4$, 12 ml. 0-1 M-KH$_2$PO$_4$, 100 ml. 8 per cent. (w/v) KCl, 10 ml. 1 per cent. (w/v) aqueous phenol red, and 838 ml. sterile distilled water. The solution was dispensed in 20-ml. amounts in Universal bottles and stored at —20°C.

Sugar solutions 100 ml. of 10 per cent. (w/v) stock solutions of glucose, maltose, sucrose, fructose, and lactose were prepared using sterile distilled water and stored at —20°C. in 4-ml. amounts. Several batches of maltose contained excessive amounts of glucose which produced false positive results. Extra pure maltose (BDH Chemicals Ltd., Poole, England) gave satisfactory results and was used throughout this study. Each week one bottle of buffer-salt solution and one bottle of each sugar was thawed and stored at 4°C.

**Cultures** Presumptive positive growth from the primary isolation plate was inoculated on to Difco GC base supplemented with L-glutamine, thiamine pyrophosphate, ferric nitrate, and glucose as described by Kellogg, Peacock, Deacon, Brown, and Pirkle (1963), except that the glucose concentration was reduced from 0-4 to 0-2 per cent. Plates were incubated overnight at 37°C. in an atmosphere containing 10 per cent. CO$_2$. After overnight incubation, each culture was checked for purity by Gram-staining and the oxidase reaction was performed by flooding a small area of the surface of the plate with a 1-0 per cent. (w/v) solution of tetramethyl-p-phenylene-diamine dihydrochloride.

In addition, several of the stock strains of pathogenic Neisseriae were inoculated on to Thayer-Martin medium and incubated as above before the RCUT was performed on the overnight growth.

**Setting up the RCUT** Four tubes (70×10 mm.) are required for each culture to be tested. Using a Finn pipette* 20 ml. 10 per cent. (w/v) glucose, maltose, and sucrose were added to individual tubes followed by 100 ml. buffer-salt solution. A thick suspension of overnight growth was made in 300 ml. of the buffer-salt solution, mixed well with a Pasteur pipette, and 30 ml. were transferred to each sugar-containing tube. The tubes were then shaken and incubated at 37°C. in a waterbath. Cultures giving weak or no fluorescence on the primary isolation plate were tested against fructose and lactose in addition to glucose, maltose, and sucrose. Normally a reading could be made after 30 to 60 min., but incubation was continued for 3 hrs before a definitive reading was made:

- Positive—yellow (occasionally, yellow-orange)
- Negative—red.

**Immunofluorescence** At the time of inoculating the plate for the RCUT, an extremely light suspension of the suspect colony from the primary isolation plate was made by emulsifying part of the colony in 200 ml. distilled water; 10 ml. of the suspension were immediately transferred to a spot on a 12-well multi-spot slide* and allowed to dry in air. Stock cultures of N. gonorrhoeae and N. meningitidis were also tested on each slide. Fluorescin-labelled antigonococcal conjugate† 10 μl. was added to each spot and the slide incubated in a moist chamber at 37°C. for 10 min. The slide was then washed in phosphate-buffered saline (Bacto FA, Difco) and distilled water; 5 min in each. The dried slides were mounted in buffered glycerol (FA mounting fluid, Difco) and examined under ultraviolet light using a Zeiss RA microscope and a x 100 objective.

**Results** All stock strains of pathogenic Neisseriae and clinical isolates of commensal Neisseria species gave the correct carbohydrate utilization pattern with the RCUT. The results for the commensal Neisseria species were also confirmed using conventional serum-free sugar medium.

Table I shows the source of the 97 isolates from 48 male and 32 female patients, the results obtained by the RCUT and immunofluorescence, and the identity of each isolate as determined by conventional techniques. Ninety of the isolates were identified as N. gonorrhoeae and seven as N. meningitidis. The RCUT gave the correct carbohydrate utilization pattern in each case. Immunofluorescence results also showed high agreement with both the RCUT and conventional techniques. Only four of the ninety strains subsequently identified as N. gonorrhoeae gave a doubtful reaction on first examination; one of these was a 24-hr culture which, even after subculture, always gave a doubtful or very weak fluorescence, while the remaining three were 72-hr cultures, all of which gave good fluorescence when 24-hr subcultures were tested.

A direct comparison of the time taken for complete identification by immunofluorescence, the RCUT, and conventional methods is shown in Table II.

All the isolates from the 28 cases shown in Table II were N. gonorrhoeae, 60-7 per cent. of which were

* Buckley Membranes, 24 Clifton Road, Amersham, Buckinghamshire
† Difco Laboratories, U.K. Division, P.O. Box 14B, Central Avenue, East Molesley, Surrey
TABLE I Results of immunofluorescence and rapid carbohydrate utilization tests on 97 isolates

<table>
<thead>
<tr>
<th>Patients investigated</th>
<th>Isolates examined</th>
<th>Fluorescence</th>
<th>RCUT</th>
<th>Identity by conventional methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Site</td>
<td>Number</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Male</td>
<td>Urethra</td>
<td>44</td>
<td>40 (4 doubtful)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Throat</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Mid-stream specimen of urine</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Urethra</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>20</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Throat</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Bartholin’s gland</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G = glucose, M = maltose, S = sucrose, F = fructose, L = lactose.
+ = acid production − = no acid production

TABLE II Comparison of time taken for complete identification by immunofluorescence and/or rapid carbohydrate utilization and conventional methods in 28 cases

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>Number of cases identified by Immunofluorescence</th>
<th>RCUT</th>
<th>Conventional techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>17</td>
<td>60.7</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>11</td>
<td>39.3</td>
<td>16</td>
</tr>
<tr>
<td>72</td>
<td>0</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>96</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

identified at 24 hrs using immunofluorescence; the remaining 39-3 per cent. were identified at 48 hrs. In 57-2 per cent. of the total cases, or sixteen of the seventeen cases positive at 24 hrs, an RCUT result at 48 hrs confirmed the fluorescence result. One subculture was contaminated with Gram-positive cocci and had to be freshly subcultured before the RCUT was carried out at 72 hrs. By 72 hrs all the isolates had been identified by a combination of immunofluorescence and the RCUT compared with 78-6 per cent. identified at 72 hrs by conventional techniques.

Discussion

Our investigation confirms that a rapid carbohydrate utilization technique for the identification of N. gonorrhoeae can give reliable results in a much shorter time than the conventional technique. The majority of strains gave positive reactions in 30 to 60 min., although incubation was always continued for 3 hrs before a definitive reading was made. In the RCUT system described, the danger of early reading is minimized since strains of N. meningitidis invariably gave a positive reaction with maltose quicker than with glucose.

In pilot experiments the buffer solutions described by Kellogg and Turner (1973) and Brown (1974) were found to be unsatisfactory. When we used thick suspensions in order to obtain a rapid result there was a tendency for the maltose tube to give a positive reaction, and when we used a thin suspension changes in the glucose tube were slower and sometimes incomplete. We overcame these problems by increasing the molarity of the buffering system and the proportion of the basic salt. These changes, along with an increase in the amount of phenol red indicator, gave rapid, easy to read, reproducible, results.

When Thayer-Martin medium was used to subculture strains before testing, we found that the growth was difficult to remove from the surface of the medium and did not form a uniform suspension, even after being mixed with a Pasteur pipette. The enzyme activity of the organisms appeared to be lower when they were grown on Thayer-Martin medium, and the colour change was slower and less pronounced than when they were grown on the supplemented Difco GC base. Possibly the glucose in the latter medium has the effect of increasing the carbohydrate metabolizing activity of the cells by enzyme induction. Therefore, in contrast to the findings of Kellogg and Turner (1973), we consider that the amount of growth normally obtained on Thayer-Martin primary isolation plates would be unsuitable as a source of material for the RCUT.
In laboratories where identification of *N. gonorrhoeae* is undertaken by immunofluorescence there is still a need for carbohydrate utilization tests, since there have been reports of cross-reaction between *N. gonorrhoeae* and *N. meningitidis* in immunofluorescence techniques (Reyn, 1969; Hare, 1974). Also, *N. meningitidis* may be implicated in urogenital infection (Beck, Fluker, and Platt, 1974) and the incidence of pharyngeal gonococcal infection continues to increase in certain countries (Wallin, 1975), so that it is important for epidemiological and clinical purposes to distinguish these organisms carefully.

The application of the RCUT to Gram-negative diplococci giving weak fluorescence or none provides a means of detecting gonococci antigenically distinct from those used in the preparation of the anti-gonococcal conjugate and a safeguard against cross-reaction with *N. meningitidis*.

We conclude that the RCUT can speed the confirmation of an isolate as *N. gonorrhoeae*. As pointed out by Kellogg and Turner (1973), the reagents for the test are easily prepared and can be stored for several months at —20°C. in contrast to the limited shelf-life of conventional media. The obvious danger inherent in inoculating sugar-test media from a selective medium does not apply to the RCUT. The RCUT, as described here, is thus a useful alternative to the conventional sugar method.

We should like to acknowledge the co-operation of the staff of the University Bacteriology Laboratory, The Royal Infirmary, Edinburgh.

Thanks are also extended to Dr. R. R. Gillies for valuable advice during the preparation of this paper and to Mrs. Joan Collins for typing the manuscript.

References


Amotrophic lateral sclerosis (A.L.S.) is a slowly progressive disorder of the nervous system of unknown cause that begins in late adulthood. Oldstone et al. 7 have reported evidence for anti-antibody complexes in the serum and kidney of A.L.S. patients, a finding providing possible indirect evidence for persistent viral infection. We tested the sera of ten patients with A.L.S. for L.C.A., postulating that the presence of L.C.A. might indicate a viral association for the disease. However, none of the sera contained L.C.A.

Department of Neurology
Section of Rheumatology
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BLOOD LEAD IN MENTALLY RETARDED CHILDREN

Sir,—I was puzzled by the scatter diagram used by Dr Moore and his colleagues (April 2, p. 717) to illustrate a relationship between water-lead and blood-lead levels in normal and mentally-retarded children. The regression line shown does not appear to be the least-squares regression line since less than a third of the scatter points lie below the line. The remainder lie above the line, and are generally further away from it.

Department of Clinical Epidemiology
Royal Free Hospital
London NW3 2BN

SIR,—We read with interest the paper by Dr Moore and his colleagues. We are concerned, however, at the statistical analysis of the data presented in table 1. Our calculations, using the same grouped data that Moore et al. used, yield values for chi-squared of 5.58 (A) and 3.46 (B), neither of which reaches accepted standards of statistical significance. These values are considerably lower than those presented by Moore et al., a finding which casts some doubt on the principal conclusion of their paper.

Department of Pharmacology
St. Thomas's Hospital Medical School
London SE1 7EH

These letters have been shown to Dr Moore and his colleagues, whose reply follows.—Ed. L.

Sir,—The regression line on which Miss Beresford comments was calculated from a least-squares regression program. The analysis, however, gave a greater percentage weighting to the higher water leads because of the greater probability that increased water leads would have a greater influence on whole-body lead burden and, therefore, on blood-lead than would the lower water-lead values, which probably contribute little to the total blood-lead concentration in proportion to the quantity of lead taken in food. If one carries out a simple regression on a small calculator program with equal weighting, the resulting line is similar to the one published.

The equation of this line is:

\[ \text{Blood-lead} = 0.93 + 0.12 \times \text{water lead} \]

Thus, although the intercept on the y axis is increased, the gradient of the line remains well within the standard deviation of the published regression line (0.14 ± 0.07). This figure is virtually in agreement with the published figure, which is less

G. W. BISSET
S. E. SMITH

SCREENING NEISSERIA FOR PENICILLINASE PRODUCTION

Sir,—It is important to monitor the possible spread of penicillinase-producing gonococci and to include meningococci and other Neisseria spp. in this surveillance. A discussion held during the joint meeting of the British Society of Antimicrobial Chemotherapy and the Scottish Branch of the British Society for the Study of Infection, in Edinburgh, on April 1 and 2, and led by Prof. I. Phillips and Prof. J. D. Williams of London, made it clear that such monitoring should not be based on routine minimum inhibitory concentration (M.I.C.) determinations.

I have modified the penicillinase assay of Constance Phillips and her colleagues, 2 which depends on the colour change of a pH indicator when acid is produced from the splitting of penicillin to penicilloic acid, to incorporate it into the rapid carbohydrate utilisation test 1 for the identification of N. gonorrhoeae. Ampicillin, which is more sensitive to TEM β-lactamase, is used instead of buffered penicillin G. The test is done in a buffered solution so that strains can be tested directly from medium containing carbohydrate.

The following strains of penicillinase-producing gonococci were kindly supplied by Dr A. E. Wilkinson, Venerable Diseases Reference Laboratory, Whitechapel, London: 76/454, 75/592, AP/672, 76/849, 76/1249, 3254, 6988. The first five of these strains had been sent to Dr Wilkinson by Dr G. C. Turner, Public Health Laboratory, Fazakerley Hospital, Liverpool, and all may be the same strain. Dr Turner also kindly sent me the penicillinase producing strain which he now thinks was the strain that caused a single outbreak and affected a relatively small area of Liverpool from February to December, 1976.

All of the neisseriae isolated in 1977 from the department of venereology, Edinburgh Royal Infirmary, were also tested (168 N. gonorrhoeae, 60 N. meningitidis, and 5 N. lactamica).

Ampicillin is dissolved in buffer-salt solution (5.2 mmol/l phosphate buffer containing 0.9% potassium chloride and 0.01% phenol-red, pH 7.1 to 7.15) to a concentration of 250 mg/ml: the solution is dispensed in 100 µl volumes and stored at −20°C.

Five tubes are required for each gonococcal isolate to test

Hereditary Multi-infarct Dementia

Sir,—To our knowledge the concept of "multi-infarct dementia" has not yet been applied to a well-defined hereditary disease affecting the brain. We describe here the clinical, genetic, and morphological features of a previously unrecognized type of multi-infarct dementia affecting young adults of both sexes. In five cases belonging to the same family the diagnosis is certain, in another three it is probable.

The disease begins acutely in previously healthy people, the age of onset showing little variation (range 29-38). Four patients survived for between 10 and 15 years, but one patient had a severe cerebral hemorrhage 5 months after onset of the disease, possibly due to anticoagulant therapy.

In the early stage there are recurrent attacks separated by longer or shorter periods of remission. The prevailing symptoms are neurological, including pyramidal tract, bulbar, and cerebellar symptoms. Later on mental symptoms develop, leading to an organic psychosis and ending with severe deterioration in mental functioning.

The five fully confirmed cases (three males) appeared in three generations without interruption. The disease has been transmitted from female to female, from female to male, and from male to male. None of them was born of a consanguineous marriage. The family history thus indicates a disease transmitted by a simple dominant, autosomal gene.

Necropsy of three siblings and a second-generation descendant of one of them revealed a remarkably uniform macroscopic picture of the cerebral changes, consisting in multiple small cystic infarctions, notably in the central grey and white matter and pons, and pronounced central and cortical atrophy caused by occlusive disease of small intracerebral and lepto meningeal vessels. The vascular changes, which we consider to represent primary manifestations of a disease process leading to multiple infarcts of the brain, were largely confined to small arteries and arterioles of the pia-arachnoid, cerebral white matter, basal ganglia, thalamus, and pons. A wide variety of vascular changes were encountered. In accordance with a current view of arterial lesions and arteriolar sclerosis? they may be described as a series of consecutive stages in the evolution of the same vascular disease process: hyaline degeneration of the intima type was considered as an early change, while hyaline degeneration of the collagenous type and fibroblastic proliferation of the intima with narrowing of the vascular lumen can be regarded as a later stage of the vascular disease process. There was no cerebral vascular amyloidosis. Changes of small arteries also occurred in spinal roots and in some vessels, particularly the spleen. Thus this may be a generalised vascular disease preferentially affecting the brain and mostly lacking symptoms and signs in any other organ.

The fully-blown neuropsychiatric syndrome fits in with the multiple local lesions found within the central nervous system, and these lesions, together with the central and cortical atrophy, seem responsible for the severe dementia. The neuropathic findings, the course of the disease, and the histopathological findings rule out Pick and Alzheimer diseases and other presenile mental disorders.

The cerebral vascular lesion could, we think, be due to an immune disorder or to a metabolic disturbance of the vascular connective tissue. Although there is no evidence of genetic factors in the development of immune disorders of the connective-tissue system, autoimmunity cannot be excluded as a primary factor in such diseases in this group. The disease may be activated by an environmental agent such as infection or by a simple hypersensitivity reaction in persons with a genetic pre-disposition to the diseases. 

PATRICK SOURANDER

PARKINSONISM ASSOCIATED WITH CHRONIC INHALATION OF CARBON TETRACHLORIDE

Sir,—Exposure to toxic agents such as manganese, carbon monoxide, and carbon disulphide may produce parkinsonism. We have seen a patient whose parkinsonian syndrome developed after chronic inhalation of carbon tetrachloride (CCl4). Some 20 kg of CCl4 slowly leaked through a faulty evaporator and contaminated the laboratory of this 40-year-old chemist. He was exposed to CCl4 vapour for about 3 months. Shortly afterwards rapidly progressive akinesia, rigidity, and rest tremor developed. The parkinsonian features became severe within a few months but were unaccompanied by other neurological signs and responded to levodopa. There was no history of encephalitis, head injury, or administration of drugs and no family history of parkinsonism. Biochemical and metabolic investigations were negative, and there was no evidence of hepatic or renal involvement.

Neurological manifestations of CCl4 intoxication include encephalomyelopathy, cerebral hemorrhages, cerebellar degeneration, neuropathy, and optic atrophy. Chronic administration to rats produces neuronal loss and astrocytosis in the corpus striatum. Poisoning with other carbonic compounds such as carbon monoxide and carbon disulphide may cause parkinsonism. These data, taken together with the patient's age, the lack of history of encephalitis, and the timing of the exposure to the toxic compound, suggest that chronic inhalation of CCl4 caused this extrapyramidal disorder.

Department of Neurology, Hadassah University Hospital, Jerusalem, Israel.

E. MELAMED

S. LAVY
Isolation of *Neisseria meningitidis* and *Neisseria catarrhalis* from the genitourinary tract and anal canal

BY

CAROLINE BLACKWELL, HUGH YOUNG, AND SHEILA S. R. BAIN

Isolation of *Neisseria meningitidis* and *Neisseria catarrhalis* from the genitourinary tract and anal canal

CAROLINE BLACKWELL, HUGH YOUNG, AND SHEILA S. R. BAIN*

From the Department of Bacteriology, University of Edinburgh, and the University Department of Venereology, Edinburgh Royal Infirmary

SUMMARY During a 12-month period 285 isolates of *Neisseria* species, other than *Neisseria gonorrhoeae*, were cultured from patients attending the Department of Venereology, Royal Infirmary, Edinburgh. There were eight patients in whom genitourinary or rectal isolates of *Neisseria meningitidis* or *Neisseria catarrhalis* were found. Differences between data from our series and from previous reports are discussed with particular regard to sites routinely cultured in female patients. We also report in vitro inhibition of *N. gonorrhoeae* by an isolate of *N. meningitidis* cultured from the cervix of a patient.

**Introduction**

*Neisseria* species other than *Neisseria gonorrhoeae* have been found to cause symptomatic infections of the genitourinary tract and the anal canal. A report of an increase in incidence of isolation as well as a brief review of the literature on *Neisseria meningitidis* infections of these sites has been recently published (Givan *et al*., 1977). In the present study we isolated 285 non-gonococcal species of *Neisseria*. Among these there were four *N. meningitidis* isolates and five *Neisseria catarrhalis* isolates from genitourinary or rectal sites. Our findings for *N. meningitidis* differ from those previously reported—in particular, the number of male patients in the series from whom the organism was isolated, overt signs of infection, autoinoculation from the nasopharynx as a source of the organism. The possibility of inhibitory substances produced by *N. meningitidis* preventing infection by *N. gonorrhoeae* in one patient is discussed.

**Materials and methods**

**Patients**

The patients in this study attended the Department of Venereology, Edinburgh Royal Infirmary between 1 July 1976 and 30 June 1977. During this period there were approximately 2000 women and 2500 men.

In men, if a urethral discharge was present, or if there was presumptive evidence of contact with gonorrhoea, a Gram-stained film of urethral discharge was examined microscopically and material inoculated directly on to culture plates. In the case of homosexuals, material from the urethra, rectum and pharynx was cultured routinely, and if negative on the first occasion, repeated twice at weekly intervals.

In women, Gram-stained films of material from the urethra and cervix, and cultures from the urethra, cervix, rectum and, if indicated, from the pharynx were taken. Generally, if the first cultures were negative for *N. gonorrhoeae*, these were repeated twice at weekly intervals.

**Culture and identification**

During the first six months of the study cultures were made on Thayer-Martin medium containing the antibiotics vancomycin (4·0 μg/ml), colistin (6·0 μg/ml), and nystatin (10·0 μg/ml). During 1977, cultures were made on modified New York City Medium (Young, 1978) containing the antibiotics lincomycin (1·0 μg/ml), colistin (6·0 μg/ml), amphotericin B (1·0 μg/ml), and trimethoprim lactate (6·5 μg/ml).

 Cultures were incubated at 36°C in a carbon dioxide enriched (10%) atmosphere. After incubation for 24 hours, plates were examined and any suspect colonies tested by the oxidase reaction and Gram's stain. Oxidase positive Gram-negative diplococci
were identified by the rapid carbohydrate utilisation test and direct immunofluorescence using Difco fluorescein-labelled anti-gonococcal conjugate as described by Young et al. (1976). Negative cultures were re-examined after 48 hours of incubation.

Demonstration of the inhibitory substance from N. meningitidis was by means of the soft agar method for bacteriocin production (Fredericq, 1957).

Results

During the 12-month period from 1 July 1976 to 30 June 1977 approximately 4500 patients attended the Department of Venereology, Royal Infirmary, Edinburgh. There were 350 specimens of N. gonorrhoeae isolated from the 2000 female patients and 480 specimens isolated from the 2500 male patients.

The Neisseria isolates, other than N. gonorrhoeae, from these patients are summarised in Table 1.

<table>
<thead>
<tr>
<th>Neisseria</th>
<th>Total no. of isolates</th>
<th>Throat</th>
<th>Genito-urinary tract</th>
<th>Rectal isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. meningitidis</td>
<td>250</td>
<td>246</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>23</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N. catarrhalis</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>N. flava</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N. perflava</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

N. meningitidis was the species most often isolated (250); among these there were two from the anal canal and two from the genitourinary tract. We found N. lactamica (23), N. flava (3), and N. perflava (2) only in throat cultures. Of the seven N. catarrhalis, two were from throat cultures, one was from the genitourinary tract, and four from the anal canal. There were four patients from whom N. meningitidis was isolated. The information available for each patient is summarised in Table 2. The only additional systemic complaints from Patient 1 were a loss in weight of 7 lb (3.175 kg), dysuria, and frequency of micturition. N. gonorrhoeae were not isolated at this time nor on the three subsequent sets of cultures. She had had a regular consort for the past two years and the only previous history of a sexually transmitted disease (STD) was warts in 1975 and genital candidosis in 1974. A second rectal isolate was from Patient 2 who had attended the clinic four months previously, at which time N. meningitidis was isolated from the throat. The only systemic complaint was eczema on face, trunk, and limbs and there was no previous history of STD. Patient 3, a bisexual male with no previous history of STD, had attended the clinic two months earlier. At that time N. meningitidis was isolated from the throat but no Neisseria had been found in cultures of rectum or urethra.

The only patient in the series from which we isolated N. meningitidis and, later, N. gonorrhoeae was Patient 4. She was seen in the clinic as a gonorrhoea contact; her husband had become infected from an extramarital consort. Microscopical examination of material from the cervix revealed large numbers of polymorphonuclear leucocytes with intracellular Gram-negative Diplococci. She was treated with co-trimoxazole as there was a possibility of penicillin allergy. The culture of cervical material grew only N. meningitidis and no gonococci were isolated from any other site. A month later gonococci were cultured from the cervix and she was treated with another course of co-trimoxazole. Disc sensitivity tests revealed both the previously isolated meningococcus and the gonococcus to be sensitive to trimethoprim-sulphamethoxazole (disc containing 23.5 µg sulphamethoxazole, 1.5 µg trimethoprim). At the time it was serotyped, the meningococcus was

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Site of isolation</th>
<th>Throat culture</th>
<th>Serotype</th>
<th>Reason for attending STD clinic</th>
<th>Diagnosis, treatment, and follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>Rectum</td>
<td>—</td>
<td>B</td>
<td>Warts</td>
<td>Cautery and liquid nitrogen for warts; 3 subsequent sets of cultures negative</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>Rectum</td>
<td>Not done</td>
<td>B</td>
<td>Frequency of micturition; warts</td>
<td>Pedophyllin and cautery for warts; no follow-up cultures subsequent to isolation of N. meningitidis from anal canal</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Urethra</td>
<td>Not done</td>
<td>NT</td>
<td>Syphilis contact</td>
<td>No treatment; no follow-up cultures</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>Cervix</td>
<td>—</td>
<td>NT</td>
<td>Gonorrhoea contact</td>
<td>Asymptomatic gonorrhoea (only N. meningitidis cultured); co-trimoxazole 5 days; 2 subsequent sets of cultures negative; N. gonorrhoeae cultured 3 times during next 3 months</td>
</tr>
</tbody>
</table>

— = no Neisseria isolated
NT = non sero-typable N. meningitidis
Isolation of Neisseria meningitidis and Neisseria catarrhalis from the genitourinary tract and anal canal

43

Further tested and found to grow on 0.1 µg/ml sulfadiazine but not on 1 µg/ml. She denied any sexual contact with her husband during this period. The soft agar method for demonstrating sensitivity to bacteriocins showed the meningococcus elaborated a substance which greatly inhibited the gonococci from Patient 4 and several other N. gonorrhoeae isolates from different sources. It was later discovered that the gonococcal infection was probably not a treatment failure or inhibition by the meningococcal substance but infection from an extramarital contact. There was no systemic upset in Patient 4, but her extramarital contact and his wife developed systemic infections and arthritis.

There were four female patients from whom N. catarrhalis was isolated (Table 3). None of these had any systemic upset and no gonococci were cultured at follow-up visits.

Discussion

Givan et al. (1977) have reviewed case reports of isolation of N. meningitidis from the genitourinary tract and anal canal. Our data from the past 12 months provide a somewhat different view. These differences may be owing to routine culturing of urethra, cervix, and rectum of all female patients. Previous reports mention only cervical cultures of women, with additional blood or joint cultures from two systemic infections (Keys et al., 1971; Morgan et al., 1976). Other series contain a predominance of men. 58 of the 84 patients in the literature; in our study three of the four patients from whom we isolated N. meningitidis and all of the patients from whom we isolated N. catarrhalis were women.

There were no overt symptoms of these meningococci causing infection in any of the patients, again in contrast to the findings of others. The main reason for attending the Department of Venereology was genital warts (four out of eight patients). Three were either syphilis or gonorrhoea contacts, and the fourth was referred for follow-up of a gonococcal infection. There was no evidence of rectal infections with N. meningitidis associated with systemic infection. Currently there is no evidence for treating rectal carriers, but follow-up cultures to monitor any spread to the urethra or the cervix may be of value.

It has been suggested (Givan et al., 1977) that there may be autoinoculation of these areas from the nasopharynx. In their series all six of the rectal carriers from whom there were throat cultures had meningococcus in the pharynx. In our series, none of the patients from whom throat cultures were taken was carrying N. meningitidis in the nasopharynx. It is also interesting to note that meningococci were not isolated from either contact of Patient 4.

The significance of the inhibitory effect of N. meningitidis from Patient 4 on N. gonorrhoeae is difficult to assess. A similar instance of apparent protection has been reported for a male patient with asymptomatic meningococcal urethritis (Volk and Kraus, 1973). The role of this inhibitory effect in preventing infection from her initial exposure is not clear. The meningococcus would probably have been eliminated by the first co-trimoxazole treatment before her second exposure and subsequent infection with N. gonorrhoeae.

These observations underline the necessity of correct and full identification of all neisserial isolates, even when a selective medium is used. These non-gonococcal Neisseria represent approximately 2% of the anogenital isolates in women and 0.2% of those from men.

This work is part of a project supported by a grant from the Scottish Home and Health Department, Medical Research Committee.

Serotyping of meningococci and sulfadiazine sensitivity testing was kindly carried out by Dr R. J. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Glasgow.

Table 3. Isolation of N. catarrhalis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Site of isolation</th>
<th>Throat culture</th>
<th>Reason for attending STD clinic</th>
<th>Diagnosis, treatment, and follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>F</td>
<td>Rectum</td>
<td>—</td>
<td>Gonorrhoea contact</td>
<td>No treatment; 3 subsequent sets of cultures negative</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Rectum</td>
<td>Not done</td>
<td>Warts</td>
<td>Podophyllin for warts; 3 subsequent sets of cultures negative</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Rectum</td>
<td>Not done</td>
<td>Follow-up treatment for gonorrhoea</td>
<td>Non-gonococcal vaginal discharge; nystatin for discharge</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>Urethra Rectum</td>
<td>Not done</td>
<td>Warts</td>
<td>Podophyllin for warts; 4 subsequent sets of cultures negative</td>
</tr>
</tbody>
</table>

— no Neisseria isolated
References


Gonorrhea in the Homosexual Man:
Frequency of Infection by Culture Site

ALEXANDER McMILLAN, MB, CHB, MRCP, AND HUGH YOUNG, PHD

The aims of this study were to determine the frequencies of infection with Neisseria gonorrhoeae at various sites in homosexual men who were attending clinics for treatment of sexually transmitted diseases in central Scotland and to appraise the diagnostic tests used. Specimens for culture were taken from the urethra, pharynx, and anorectum of every homosexual man in the study. When the first cultures of pharyngeal and rectal specimens were negative, these cultures were repeated twice at weekly intervals. The urethra was infected in 169 (60.8%), the anorectum in 114 (41.0%), and the pharynx in 23 (8.3%) of 278 patients who had gonorrhea. By reliance on only one set of tests, eight (7.0%) of 114 patients who had rectal gonorrhoea and six (26.1%) of 23 patients with pharyngeal infection would have been missed. The results indicate the importance of obtaining specimens for culture from all sites that might possibly be infected, regardless of the symptoms.

GONORRHEA is common in homosexual men. In one study,1 about 20% of homosexual patients attending a clinic for the management of sexually transmitted diseases were found to be infected with Neisseria gonorrhoeae. Infections of both the anorectum2 and the pharynx3 are often asymptomatic; thus, the asymptomatic carrier represents an important public health problem. Although the natural history of untreated rectal and pharyngeal gonorrhea has not been elucidated fully, it is clear that the patient himself is at risk from the development of local or systemic complications.2,4 Therefore, reliable detection of infection at these sites is important.

The purpose of the present work was to determine the prevalences of infection at sites affected by N. gonorrhoeae in the homosexual man. The diagnostic tests that are available for identification of infected individuals are reviewed.

Materials and Methods

Patients

All male patients with gonorrhea attending the clinic of the Department of Sexually Transmitted Diseases at the Glasgow and Edinburgh Royal Infirmary who gave histories of homosexual contact were studied. Data were collected from cases of patients attending the clinic between November 1976 and January 1978. During this period records of 208 men in Glasgow and 70 men in Edinburgh were reviewed.

Laboratory Studies

Specimens for culture were obtained from the pharynx, urethra, and anorectum of every patient, regardless of whether he had symptoms referable to these areas.

When a urethral discharge was present, a gram-stained smear was examined microscopically. When there was no evidence of urethritis, a gram-stained smear was not prepared, but material for culture was obtained by insertion of a plastic inoculating loop (Nunc Products, Kamstrup, Denmark) into the anterior urethra to a distance of about 3 cm and gentle scraping of the walls of the urethra.

Material from the anal canal and rectum was obtained under direct vision by use of a proctoscope lubricated with K–Y jelly (Johnson and Johnson, Slough, U. K.). Gram-stained smears of material obtained from the anorectums of 81 homosexual men

Received for publication on May 3, 1978, and in revised form on July 24, 1978.

We are grateful to Dr. D. H. H. Robertson, Department of Venereology, Royal Infirmary of Edinburgh, for permission to study patients under his care.

Reprint requests: Dr. Alexander McMillan, Department of Sexually Transmitted Diseases, 67 Black Street, Glasgow G4 OEF, Scotland, United Kingdom.

From the Department of Sexually Transmitted Diseases, Greater Glasgow Health Board, and The Department of Bacteriology, University of Edinburgh Medical School, Edinburgh, Scotland, United Kingdom.
attending consecutively between June and December 1977 were examined.

Pharyngeal specimens were taken by gentle rolling of a cotton wool-tipped applicator stick over the tonsils or tonsillar beds and the posterior pharyngeal wall.

Men diagnosed as having urethral gonorrhea on the basis of microscopic examination were treated at the first clinic visits.

Patients were reexamined seven days after the initial attendance. When the results of the first set of diagnostic tests were negative, pharyngeal and rectal cultures were repeated twice at weekly intervals.

In Edinburgh material was inoculated directly onto modified New York City (MNX) medium, and inoculated plates were held at 36°C until transfer to the laboratory (mean interval, 2.1 hr; range, 30 min to 3 hr). Specimens from the Glasgow clinic were taken on charcoal-impregnated swabs and sent to the laboratory in Stuart’s transport medium; the mean interval between collection of specimens and inoculation onto culture medium was 3.2 hr (range, 1–6 hr). The culture medium used in Glasgow was Columbia heated blood agar (Oxoid, Ltd., Basingstoke, Hants, U. K.) containing vancomycin (2.5 μg/ml), trimethoprim (3.0 μg/ml), and polymyxin (15 units/ml). In both centers the identity of suspected colonies of *N. gonorrhoeae* was confirmed by fluorescent antibody testing and sugar utilization reactions.

**Results**

The various sites affected in 278 homosexual men with gonorrhea are shown in tables 1 and 2. The urethra was infected in 169 (60.8%), the anorectum in 114 (41%), and the pharynx in 23 (8.3%) of 278 patients. Of the 169 patients who had urethral gonorrhoea, 23 (13.6%) concomitant infection of the anorectum and/or pharynx.

**TABLE 1. Distribution of Sites Infected with Neisseria gonorrhoeae in 278 Homosexual Men with Gonorrhea**

<table>
<thead>
<tr>
<th>Site(s)</th>
<th>Glasgow (208 Cultures)</th>
<th>Edinburgh (70 Cultures)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra alone</td>
<td>113 (54.3)</td>
<td>33 (47.1)</td>
</tr>
<tr>
<td>Anorectum alone</td>
<td>65 (31.3)</td>
<td>26 (37.1)</td>
</tr>
<tr>
<td>Pharynx alone</td>
<td>9 (4.3)</td>
<td>6 (8.6)</td>
</tr>
<tr>
<td>Urethra and anorectum</td>
<td>15 (7.2)</td>
<td>3 (4.3)</td>
</tr>
<tr>
<td>Urethra and pharynx</td>
<td>2 (1.0)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Pharynx and anorectum</td>
<td>2 (1.0)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td>Urethra, pharynx, and anorectum</td>
<td>2 (1.0)</td>
<td>—</td>
</tr>
</tbody>
</table>

Anorectal gonorrhoea was diagnosed by culture in 114 cases and was associated with infection at other sites in 23 (20.2%). Bacteriologic examination of material obtained at the initial visits showed infection in 106 (93.0%) of the 114 cases of patients with anorectal infection. Cultures repeated one and two weeks after the initial attendance identified another eight infected men. Six of the latter patients (four from the Glasgow clinic and two from the Edinburgh clinic) had positive cultures of material obtained at the second visits, and two (both from the Glasgow clinic) had positive cultures of material obtained at the third visits. In all eight cases the infection was limited to the anorectum, and there was no history of sexual contact between clinic attendances.

*Neisseria gonorrhoeae* was isolated from the pharynx in 23 cases; infection was confined to this site in 15. In 17 (73.9%) of these 23 cases the disease was diagnosed by cultures taken at the first clinic visits. Six infected patients were identified by cultures taken at the second (three men; two in Glasgow, one in Edinburgh) and third attendances (three men, all in Glasgow). In all six cases only the pharynx was infected, and apparently there had been no sexual contact between visits. None of the patients who had pharyngeal gonorrhoea had symptoms referable to the throat.

**TABLE 2. Rates of Isolation of Neisseria gonorrhoeae from the Anorectum and Pharynx on the First, Second, and Third Visits of 114 Men with Anorectal Gonorrhoea and 23 Men with Pharyngeal Infection**

<table>
<thead>
<tr>
<th>Visit</th>
<th>Anorectum Isolated from Indicated Site</th>
<th>Pharynx Isolated from Indicated Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) with <em>N. gonorrhoeae (both)</em></td>
<td>No. (%) with <em>N. gonorrhoeae (both)</em></td>
</tr>
<tr>
<td>First</td>
<td>106 (93.0)</td>
<td>17 (73.9)</td>
</tr>
<tr>
<td>Second</td>
<td>6 (5.3)</td>
<td>3 (13.0)</td>
</tr>
<tr>
<td>Third</td>
<td>2 (1.7)</td>
<td>3 (13.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>114 (100)</td>
<td>23 (100)</td>
</tr>
</tbody>
</table>

*Neisseria gonorrhoeae* was cultured from specimens.
obtained from 47 of 81 homosexual men who attended consecutively over a seven-month period whose gram-stained smears were examined microscopically (table 4), and gram-negative diplococci were seen within polymorphonuclear leukocytes from 27 (58%) of these men. Gram-stained smears from another three men had a similar appearance, but N. gonorrhoeae was not isolated on culture. These three cases are not included in the total number of cases of anorectal infection.

*Neisseria meningitidis* was isolated from the anorectums of four homosexual men, each of whom attended the Edinburgh clinic, and from the urethra of another man. These men had no evidence of concomitant infection with *N. gonorrhoeae*.

**Discussion**

Gonorrhea affecting the urethra, anorectum, and pharynx in the male homosexual is well recognized.6 The purpose of the present study was to define the pattern of infection with *N. gonorrhoeae* in homosexual men attending clinics for treatment of sexually transmitted diseases, and to appraise diagnostic requirements.

**TABLE 3. Symptoms Referable to the Anal Region in Male Homosexual and Heterosexual Patients**

<table>
<thead>
<tr>
<th>Group (No. of Patients)</th>
<th>Mean Age in Years (Range)</th>
<th>No. of Men with Symptoms Indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexuals with anorectal gonorrhea (60)</td>
<td>24.4 (15–56)</td>
<td>No Symptom Pruritus Ani Anorectal Bleeding Anal Discharge Pain in Anal Region</td>
</tr>
<tr>
<td>Homosexuals with no cultural evidence of gonorrhea (60)</td>
<td>23.7 (15–59)</td>
<td>42 6 2 8 0</td>
</tr>
<tr>
<td>Heterosexuals with urethral gonorrhea (60)</td>
<td>25.1 (16–49)</td>
<td>40 13 3 3 1</td>
</tr>
<tr>
<td>Heterosexuals with no evidence of gonorrhea attending for other reasons (60)</td>
<td>22.8 (17–61)</td>
<td>43 12 6 0 1</td>
</tr>
</tbody>
</table>

Different culture media were used in the two centers, but we have not attempted to draw conclusions regarding the comparative efficacies of these media.

The urethra was infected in almost two-thirds of all cases of homosexual men with gonorrhea. There was, however, concomitant infection of the pharynx and/or anorectum in >10% of the cases. All but one of these men had symptoms of urethral gonorrhea that prompted their attendance at the clinics.

It has been shown that microscopic examination of urethral material is an insensitive means of diagnosing gonorrhea in the male patient without urethral discharge,2 and in this series urethral specimens from men without symptoms of urethritis were only cultured.

Anorectal gonorrhea was also common in the men studied. These patients and those with pharyngeal infections were attending the clinics because they had symptoms of urethritis or were contacts of persons known to have gonorrhea, or for routine examination. Less than a third of men who had anorectal infection had symptoms referable to the anal region. This observation has been reported by others.2 Anal discharge was significantly more prevalent in homosexual than in heterosexual men, although there appeared to be no significant difference between the symptoms of infected and noninfected individuals. The number of patients studied may have been too small, or the reluctance of some men to discuss symptoms in the anogenital region may have led to an underestimate of the incidence of symptomatic anorectal infection. Certainly one cannot rule out gonorrhea as a cause of anal symptoms.

Although gonococcal infection of the rectum may produce mild to severe proctitis,2 no attempt was made to grade the severity of this infection on the basis of proctoscopic findings. It has been shown that
there is considerable variation among observers in describing changes in the appearance of the rectal mucosa.\textsuperscript{8}

The limited value of examination of gram-stained smears of material obtained by proctoscopy is clear. Less than 60\% of culturally-proven cases were diagnosed correctly on the basis of such smears. Organisms morphologically resembling \textit{N. gonorrhoeae} were observed in gram-stained smears of rectal exudate from three patients who attended the Glasgow clinic, but neither \textit{N. gonorrhoeae} nor other species of \textit{Neisseria} were isolated on culture. The nature of the organisms that were observed is uncertain; however, it is possible that they were gonococci that did not survive in transit to the laboratory. Coliform bacteria may grow in Stuart's transport medium and may inhibit growth of \textit{N. gonorrhoeae}.\textsuperscript{9}

Other species of \textit{Neisseria} may be found in the anorectum\textsuperscript{10}; \textit{N. meningitidis} was isolated from anorectal specimens from four of our patients. In homosexual men, it is important to confirm, preferably by sugar utilization tests, the identity of any \textit{Neisseria} isolated from the pharynx or anorectum.

Most of the rectal infections (93\%) were diagnosed as a result of cultures of specimens taken at the patients' first clinic attendances. Significant numbers of infected men were identified at subsequent visits. The latter were contacts of men known to have gonorrhea, and there was no history of sexual contact between clinic visits. It would seem advisable, particularly for known contacts, not to rely on one set of tests to exclude infection. One, or preferably, two, further diagnostic tests should be performed. (This also holds true for gonorrhea in women: about 10\% of cases may not be identified when only one set of diagnostic tests is relied upon [A. McMillan, unpublished observation].)

Although it was used as a lubricant for proctoscopic examination in this study, K-Y jelly may inhibit growth of \textit{N. gonorrhoeae},\textsuperscript{11} and its use should probably be avoided. When proctoscopy is considered advisable, perhaps an anorectal specimen for culture should be taken before the instrument is passed.

Although the number of patients in this study who had pharyngeal gonorrhea was small, the usually asymptomatic nature of infection in this area was demonstrated. Infection was confined to the pharynx in two-thirds of these patients. Three infected men were diagnosed at the second and three at the third attendances at the clinic. The difficulties involved in identifying pharyngeal infection are well recognized.\textsuperscript{12}

It is possible that the gonococcus is localized in the tonsillar crypts and is shed intermittently into the lumen of the pharynx. The intermittent presence of organisms on the surface mucosa may account for some of the difficulties entailed in diagnosis.

In the United Kingdom, oral administration of a single dose of one of several antimicrobial drugs is the current treatment of choice for uncomplicated urethral gonorrhea in men.\textsuperscript{13} However, it is recognized that single-dose treatment of pharyngeal and male rectal infections may not always be satisfactory,\textsuperscript{12,14} although Fiumara\textsuperscript{15} has reported excellent results in the treatment of rectal gonorrhea with spectinomycin. Clearly it is insufficient to test only the urethra of a homosexual man who has gonococcal urethritis because the rectum and pharynx often are infected. Cultures from these sites should be repeated after treatment for determination of its efficacy.

The male carrier of pharyngeal and rectal gonorrhea can be identified satisfactorily only by culture of material from these sites. Absence of symptoms must not dissuade the clinician from performing the appropriate tests. Not only is the infected patient a hazard to the community, he himself is at risk of developing disseminated infection.\textsuperscript{4}

In the absence of a satisfactory serologic test for gonorrhea, bacteriologic investigations are mandatory to diagnose infection. The results of the present study clearly demonstrate the need to obtain specimens for culture from all potentially infected sites, and show that one set of tests of material from the anorectum and pharynx may fail to identify a significant proportion of infected patients. The performance of repeated cultures depends on patient cooperation, financial resources, and laboratory facilities.

Although the default rate among homosexual patients attending these clinics is low, it is recognized that, particularly in larger conurbations, the default rate is higher, and it may be difficult, if not impossible, to persuade a patient to attend for repeated testing. When a patient known to be a contact of a man with gonorrhea is expected to be unreliable in attending the clinic, it may be advisable to treat him before the results of laboratory tests are available.

Results of this study indicate that all physicians who deal with the medical problems of the homosexual patient must be aware of the pattern of distribution of \textit{N. gonorrhoeae} in the body and of the limitations of the diagnostic techniques that are available at present.

References

Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium

BY

H. YOUNG

Reprinted from British Journal of Venereal Diseases, Vol. 54, No. 1, February, 1978
Cultural diagnosis of gonorrhoea with modified New York City (MNYC) medium

H. YOUNG

From the Department of Bacteriology, University Medical School, Edinburgh

SUMMARY A simply prepared modified New York City medium, designated MNYC was compared with Thayer Martin (TM) medium for the cultural diagnosis of gonorrhoea. MNYC medium contained lincomycin, commercial gonococcal base and lysed whole blood, whereas the original New York City medium contained fresh horse plasma and haemoglobin solution, a basal medium prepared from basic ingredients and vancomycin. Using MNYC medium gonococci were cultured from 96-1% of men and 100% of women with gonorrhoea (positive film and/or culture) compared with only 77-6% and 69% respectively using TM medium. There were no patients positive by culture on TM medium but negative by culture on MNYC medium. The proportion of men with positive films but negative culture was reduced from 17-1% on TM medium to 3-9% on MNYC medium. There were no women with positive films but negative cultures on MNYC medium compared with 19% on TM medium. MNYC medium is recommended as a simply prepared and highly efficient medium for the cultural diagnosis of gonorrhoea.

Introduction

Although microscopical examination is important in making a presumptive diagnosis of gonorrhoea so allowing immediate treatment, cultures are obligatory in the diagnosis of rectal, oral, disseminated, and asymptomatic infections in both sexes. They are also essential in determining antibiotic sensitivities and for evaluating treatment.

The selective medium of Thayer and Martin (1966) is widely used in many laboratories and has increased the number of positive isolates from all sites, but it has proved particularly valuable in isolating the gonococcus from heavily contaminated sites such as the rectum (Roepstorff and Hammarström, 1966). Unfortunately, at least 3% of gonococcal strains are sensitive to vancomycin at a concentration of 3 μg/ml (Reyn, 1969) and are therefore unlikely to be detected when Thayer Martin (TM) medium, containing vancomycin 5 μg/ml, is used. Also, isolates tend to grow slowly on TM medium and produce small colonies.

On introducing delayed immunofluorescence for the routine identification of gonococci we found that slow growth on TM medium became the limiting factor in the speed of cultural diagnosis. Faur et al. (1973a, b) described a new selective medium, designated NYC (New York City) medium, which provided luxuriant growth of pathogenic neisseriae after incubation for 24 hours. NYC medium essentially consists of a proteose peptone-corn starch agar buffered base to which is added a haemoglobin solution prepared from fresh horse erythrocytes, with horse plasma, yeast dialysate, glucose and the antibiotics—vancomycin, colistin, amphotericin B, and trimethoprim lactate.

The aim of this investigation was to compare culture results obtained using our standard TM medium with those obtained using a simply prepared modification of NYC medium.

Material and methods

MEDIUM

TM medium comprised lab m Columbia agar base (London Analytical and Bacteriology Media Ltd., 50 Mark Lane, London) supplemented with 10% (by vol.) heated (56°C for 60 min) human blood and the antibiotics vancomycin (4 μg/ml), colistin (6 μg/ml), and nystatin (10 μg/ml).

MNYC (Modified New York City) medium A comprised Difco gonococcal base enriched with

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Received for publication 26 July 1977
10% (by vol.) defibrinated horse blood (Wellcome Ltd) lysed with 0.5% (by vol.) saponin, 2.5% (by vol.) yeast dialysate prepared as described by Faur et al. (1973a), 0.1% (by vol.) glucose, lincomycin (0.5 μg/ml), colistin (6 μg/ml), amphotericin B (1.0 μg/ml), and trimethoprim lactate (6.5 μg/ml).

MNYC medium B was prepared as above but with lincomycin at a concentration of 1.0 μg/ml.

MNYC medium C was prepared as medium B but contained 10% (by vol.) human blood, lysed with 0.5% (by vol.) saponin, in place of defibrinated horse blood.

In the first part of the trial 'split' plates were made by pouring 10 ml of MNYC medium A into one half and 10 ml of TM medium into the other half of diametrically partitioned and vented Petri plates.

In the second part of the trial split plates contained MNYC medium B and MNYC medium C.

**Determination of minimum inhibitory concentration (MIC) to vancomycin and lincomycin**

At the time of isolation, a suspension of each gonococcal isolate was made in skimmed milk (10%), quick frozen with a mixture of solid carbon dioxide and acetone and stored at −20°C. Later, strains were recovered and the MICs to vancomycin and lincomycin determined using sensitivity test agar (Oxoid Ltd) supplemented with 10% lysed human blood: vancomycin and lincomycin were added at concentrations of 2, 4, 8, 16, and 32 μg/ml.

**Patients**

The patients in this study attended the Department of Venereology, Edinburgh Royal Infirmary, between 15 November 1976 and 21 February 1977.

In men, if a urethral discharge was present, or if there was presumptive evidence of contact with gonorrhoea, a Gram-stained smear of urethral discharge was examined microscopically and material inoculated directly on to culture plates as described below. In the case of homosexuals, material from the urethra, rectum and pharynx was cultured routinely, and, if negative on the first occasion, cultures were repeated twice at weekly intervals.

In women, Gram-stained smears of material from the urethra and cervix, and cultures from the urethra, cervix, rectum and, if indicated, from the pharynx, were taken. If the first cultures for *N. gonorrhoeae* were negative, these were generally repeated twice at weekly intervals.

In the first part of the study, 15 November until 31 December 1976, all specimens for *N. gonorrhoeae*, including tests of cure when required, were plated directly on to both TM medium and MNYC medium A; the order of inoculation of the medium alternated with each patient. During this period there were 256 women yielding 2124 cultures and 422 men yielding 600 cultures.

In the second part of the study, 13 January until 21 February 1977, when cultures were made on MNYC medium B and MNYC medium C there were 287 women and 417 men.

**Incubation and Identification**

After inoculation, plates were held at 36°C and transferred to the laboratory within a few hours where they were incubated at 36°C in a carbon dioxide enriched (10%) atmosphere. After incubation for 24 hours, plates were examined and any suspect colonies tested for the oxidase reaction and Gram smears prepared. Oxidase positive Gram-negative diplococci were identified by the rapid carbohydrate utilisation test and by delayed immunofluorescence with Difco fluorescein-labelled anti-gonococcal conjugate as described by Young et al. (1976): all isolates of *N. gonorrhoeae* produced acid from glucose only and gave a positive fluorescent antibody test. Negative cultures were re-examined after incubation for 48 hours.

**Results**

**Studies with MNYC medium A**

This medium contained 10% horse blood and lincomycin (0.5 μg/ml).

**Female Patients**

The numbers of specimens positive for *N. gonorrhoeae* by culture on TM medium are compared with those on MNYC medium A in Table 1.

<table>
<thead>
<tr>
<th>Site</th>
<th>No. sampled</th>
<th>No. of cultures positive On TM medium</th>
<th>On MNYC medium A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra</td>
<td>683</td>
<td>16</td>
<td>29</td>
</tr>
<tr>
<td>Cervix</td>
<td>681</td>
<td>29</td>
<td>40</td>
</tr>
<tr>
<td>Rectum</td>
<td>586</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Throat</td>
<td>160</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>14*</td>
<td>4†</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>2124</td>
<td>56</td>
<td>86</td>
</tr>
</tbody>
</table>

*Bartolfin's gland (11), high vaginal swab (1), wrist pustule (1), coi (1)*

The 56 cultures positive on TM medium corresponded to 29 patients while the 86 cultures positive on MNYC medium A corresponded to 42 patients. Results of culture and microscopical examination for these patients are shown in Table 2.
Table 2  Results of microscopical examination and culture on TM medium and MNYC medium A for 42 female patients with gonorrhoea

<table>
<thead>
<tr>
<th>Pattern of results</th>
<th>Patients with gonorrhoea and pattern of results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On TM medium</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Culture+ smear+</td>
<td>20 (47.6)</td>
</tr>
<tr>
<td>Culture+ smear−</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>Culture+ smear 0</td>
<td>3 (7.1)</td>
</tr>
<tr>
<td>Culture− smear+</td>
<td>8 (19.0)</td>
</tr>
<tr>
<td>Total</td>
<td>37 (88.0)</td>
</tr>
</tbody>
</table>

+ = Positive, − = negative, 0 = no smear

Of the 13 patients with gonorrhoea detected by culture on MNYC medium A but missed by culture on TM medium, eight were smear positive, three were smear negative, and there were no corresponding smears for two patients. Smears were positive but TM cultures negative in 19.0% (8/42) of patients with gonorrhoea. There were no positive smears with negative cultures when MNYC medium A was used. Microscopical examination detected 66.7% (28/42) of the women with gonorrhoea. Excluding the five patients from whom no smears were available, microscopical examination detected 75.7% (28/37) of infected patients. A combination of microscopy and culture on TM medium detected 88% (37/42) of the positive patients whereas culture on TM medium alone detected only 69.0% (29/42).

MALE PATIENTS

The numbers of specimens positive for N. gonorrhoeae by culture on TM medium are compared with those on MNYC medium A in Table 3.

Table 3  Results of culture for Neisseria gonorrhoeae when 600 specimens from 422 male patients were plated on TM medium and MNYC medium A

<table>
<thead>
<tr>
<th>Site</th>
<th>No. sampled</th>
<th>On TM medium</th>
<th>On MNYC medium A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra</td>
<td>394</td>
<td>53</td>
<td>69</td>
</tr>
<tr>
<td>Rectum</td>
<td>43</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Throat</td>
<td>152</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>11*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>56</td>
<td>73</td>
</tr>
</tbody>
</table>

*Prostatic secretion (3), urine thread or deposit (7), eye swab (1)

As only one culture site was positive for each patient there were 56 patients with gonorrhoea detected by TM culture and 73 patients detected by culture on MNYC medium A. Results of culture and microscopical examination for these patients and three patients from whom positive smears but negative cultures were obtained are shown in Table 4.

Table 4  Results of microscopical examination and culture on TM medium and MNYC medium A for 76 male patients with gonorrhoea

<table>
<thead>
<tr>
<th>Pattern of results</th>
<th>Patients with gonorrhoea and pattern of results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On TM medium</td>
</tr>
<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Culture+ smear+</td>
<td>40 (52.6)</td>
</tr>
<tr>
<td>Culture+ smear−</td>
<td>8 (10.5)</td>
</tr>
<tr>
<td>Culture+ smear 0</td>
<td>8 (10.5)</td>
</tr>
<tr>
<td>Culture− smear+</td>
<td>13 (17.1)</td>
</tr>
<tr>
<td>Total</td>
<td>69 (90.8)</td>
</tr>
</tbody>
</table>

+ = Positive, − = negative, 0 = no smear

Of the 17 patients giving positive cultures only on MNYC medium A 13 were smear positive, two were smear negative, and in two instances no smears were available. There were no patients giving positive cultures only on TM medium. Smears were positive but cultures negative in 17.1% (13/76) of patients when TM medium was used compared with only 3.9% (3/76) using MNYC medium A. Microscopical examination detected 73.7% (56/76) of all patients with gonorrhoea. Excluding the 10 patients from whom no smears were available, microscopical examination detected 84.8% (56/66) of cases. A combination of microscopy and culture on TM medium detected 90.8% (69/76) whereas culture on TM medium alone detected only 77.6% (56/76). Culture on MNYC medium alone detected 96.1% of men with gonorrhoea.

BOTH MALE AND FEMALE PATIENTS

Rapidity of growth

Colonies of Gram-negative diplococci were present after incubation for 24 hours in 51.8% (29/56) of the cultures positive in women and 42.9% (24/56) of the cultures positive in men when TM medium was used compared with 79.1% (68/86) and 78.1% (57/73) of the cultures respectively when MNYC medium A was used.

MIC determinations

These were carried out on isolates from nine of the 40 patients positive only on MNYC medium A and 14 of the 85 patients positive on both TM and MNYC medium A. The MICs of the nine strains isolated only on MNYC medium A were: vancomycin, 8 µg/ml (three strains), 16 µg/ml (five strains), and >32 µg/ml (one strain); lincomycin, 8 µg/ml (two strains), 16 µg/ml (six strains), and >32 µg/ml (one strain). The MICs of the 14 strains positive on
both media were: vancomycin, 8 μg/ml (two strains), 16 μg/ml (seven strains), and >32 μg/ml (five strains); lincomycin, 8 μg/ml (four strains), 16 μg/ml (seven strains), and >32 μg/ml (three strains).

**Overgrowth by Proteus spp.**
Using TM medium 2.0% of all sites examined in women and 0.5% of sites in men were overgrown with *Proteus* spp. compared with 0.5% and 0.17% of sites respectively when MNYC medium A was used.

**Growth of unwanted micro-organisms**
This was more pronounced in rectal cultures plated on MNYC medium A than on TM medium. In order to lessen contamination the lincomycin concentration was increased to 1.0 μg/ml for the second part of the study. Because of the expense of horse blood, human blood was also tested in parallel.

**STUDIES WITH MNYC MEDIUM B AND MNYC MEDIUM C**
MNYC medium B contained 10% horse blood and MNYC medium C 10% human blood: both contained lincomycin (1.0 μg/ml).

**Women**
Of the 287 female patients examined, 56 gave positive cultures on both media. One additional patient was detected by culture on MNYC medium B.

**Men**
Of the 417 male patients examined 67 gave positive cultures on both media. One additional patient was detected by culture on MNYC medium B.

Growth of unwanted micro-organisms seemed to be less pronounced with lincomycin (1.0 μg/ml) in the medium. However, this was not a direct comparison with the same medium containing lincomycin (0.5 μg/ml).

**Discussion**
MNYC medium substantially improved the efficiency and rapidity of the cultural diagnosis of gonorrhoea. In men 96.1% of cases of gonorrhoea were detected by MNYC culture compared with only 77.8% by TM; the corresponding figures for women with gonorrhoea were 100% and 69.0% respectively.

Conventional TM medium has been shown to give poor results in other trials. Willcox and John (1976) found that 91.2% of 102 male patients with positive smears gave positive cultures using the Ames detection kit which contains modified TM medium, compared with 60.8% positive cultures using conventional TM medium: Ames detection kit or 'Microcult-GC' contains lincomycin, colistin, amphotericin B, and trimethoprim.

The better results with MNYC medium could be because of its superior nutritional value, the replacement of vancomycin with lincomycin, or a combination of these factors. The more rapid growth with MNYC medium demonstrates the improved nutritional state of MNYC medium: after 24 hours of incubation gonococcal colonies were present in 78.6% (125/159) of specimens positive on MNYC medium compared with only 47.3% (53/112) of specimens positive on TM medium. When the inoculum from the patient is low the improved nutrition provided by MNYC medium may be critical in allowing gonococcal growth.

Since none of the strains isolated only on MNYC medium was sensitive to vancomycin at the concentration present in TM medium (4.0 μg/ml) there is no direct support for vancomycin sensitivity as a cause of culture failure with TM medium. Nevertheless, the safety margin with lincomycin is much greater (MIC usually 16 times the concentration present in the medium) than with vancomycin (MIC usually two to four times greater than the corresponding medium concentration). This safety margin combined with the better nutritional value of MNYC medium may be particularly important in the case of small inocula.

Ødegaard et al. (1975) found that 'chocolate' (heated blood) agar medium containing lincomycin (0.5 μg/ml) in place of vancomycin (3.0 μg/ml) increased the number of samples positive for gonococci by 7%, and the number of patients with gonococcal infection by 4%; MICs were not reported in this study. These workers also found that the growth of unwanted micro-organisms was more pronounced on the medium containing lincomycin (0.5 μg/ml) in place of vancomycin (3.0 μg/ml).

Trimethoprim lactate did not completely prevent overgrowth by *Proteus* spp. but it reduced the problem considerably. It did not appear to have any inhibitory effect on gonococci since no isolates were obtained only on TM medium lacking trimethoprim.

MNYC medium was not compared directly with the NYC medium described by Faur et al. (1973a, b) and it is therefore impossible to assess the performance of the modified medium with the original. The comparison with our conventional TM medium and the very low proportion of smear-positive culture negative patients using MNYC medium suggests that the modified medium performs extremely well. The modified medium is much
obtained when the proteose peptone-epron starch agar buffered base was prepared from basic ingredients although quantitative data were not presented. The other major simplification in the MNYC medium is the use of completely lysed whole blood in place of fresh plasma and haemoglobin solution.

Faur et al. (1976) stated that if horse blood was not available cow blood could be substituted in MNYC medium, but that sheep blood was unsuitable: 92 of 522 cultures for N. gonorrhoeae were positive on both NYC media supplemented with either horse blood or cow blood, but only 85 positive cultures were obtained using sheep blood. Owing to the expense of horse blood and the small difference in the yield of positive cultures when human blood is used, this is considered to be an acceptable alternative to horse blood for the cultural diagnosis of gonorrhoea.

As a result of these findings, MNYC medium containing 10% human blood and lincomycin (1-0 μg/ml) was introduced in February 1977 for the routine isolation of gonococci.

I thank Dr D. H. H. Robertson and clinical staff of the Department of Venereology, Edinburgh Royal Infirmary for their patience during the course of the trial. Thanks are also extended to the technical staff of the diagnostic laboratory for their expert assistance and to Professor J. C. Collee and Professor B. P. Marmion for their helpful advice during the preparation of the paper.

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MACKIE & McCARTNEY

MEDICAL MICROBIOLOGY

A GUIDE TO THE LABORATORY DIAGNOSIS
AND CONTROL OF INFECTION

THIRTEENTH EDITION
VOLUME 1: MICROBIAL INFECTIONS

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CHURCHILL LIVINGSTONE
EDINBURGH LONDON AND NEW YORK 1978
PREFACE

The alteration of the title of this edition is intended to remind readers of the origins of the book and it meets suggestions made by a number of our readers. The use of the names of the original Authors will provide continuity as contemporary editorial teams change from time to time.

Professor Robert Cruickshank—whose loss all our contributors mourn—played an important part in the evolution of the book from a technical manual to a clinically-orientated text; nevertheless, its important element of technique was retained by the creation of Volume 2.

Since the last edition—the 12th—much new material has accumulated in medical microbiology, for example, in virology (hepatitis; chronic virus infections, etc.), immunology (lymphocyte function and interaction; immunogenetics, etc.); in bacteriology there have been steady advances on many fronts.

The Editors were reluctant to allow an increase in the size of the text to cope with this expansion. Instead, new information has been inserted into existing chapters by replacement of older material, so that the length of Volume 1, 13th edition, remains the same as that of the 12th edition. Eventually, however, taxonomic and other considerations will make a reordering of material unavoidable.

Professor R. R. Gillies has given up his association with the book. Professor B. P. Marmion is moving to Adelaide, South Australia; he will remain as an Editor with a particular interest in the virological aspects.

We are most grateful to those readers who have taken time to draw our attention to errors of composition, fact or emphasis, and we have often followed their advice. We are also grateful for the constant help and patience of our publishers.

June, 1978  The Editors.
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The neisseriae are Gram-negative diplococci of which the pathogenic members, meningococcus and gonococcus, are characteristically found inside the polymorphonuclear pus cells of the inflammatory exudate. Although difficult to differentiate on morphological and cultural characters, these two pathogens are associated with entirely different diseases. Neisseria meningitidis is the cause of an acute purulent meningitis, variously called epidemic cerebrospinal meningitis, cerebrospinal fever or, because of a purpuric rash which is sometimes present, ‘spotted fever’. It may also cause a subacute septicaemia with a petechial rash but without meningitis, particularly during epidemics of meningococcal meningitis. The term meningococcal infection is used to embrace these two syndromes. Neisseria gonorrhoeae is the cause of the venereal disease, gonorrhoea, a purulent infection of the mucous membrane of the urethra and also of the cervix uteri in the female; there may be rectal infection and secondary local and metastatic complications, e.g. epididymitis, salpingitis and arthritis may occur if the primary infection is not promptly treated. A purulent conjunctivitis of the newborn, ophthalmia neonatorum, and a vulvo-vaginitis in young girls also occur as primary gonococcal infections.

The non-pathogenic or potentially pathogenic members of the neisseria genus are common commensals of the upper respiratory tract, which is also the reservoir of the meningococcus. N. lactamica has been isolated frequently from the nasopharynx during meningococcal carrier surveys. It has a substantial cultural resemblance to the meningococcus but is of much lower potential pathogenicity and is rarely isolated from blood or cerebrospinal fluid (Lauer and Fisher, 1970). The classical nasopharyngeal commensals, N. subflava, N. flava, N. perflava and N. sicca are included in the umbrella species N. pharyngis. The proposal by Catlin (1970) that N. catarrhalis, a common commensal of the upper respiratory tract, should form the type species of a new genus, Branhamella, has been adopted by Cowan and Steel (1974) and Buchanan and Gibbons (1975).

**Description**

The two pathogenic neisseriae, N. meningitidis and N. gonorrhoeae are so similar in their morphological and cultural characters that they may be described together. They are Gram-negative oval cocci occurring in pairs with the apposed surfaces flat and even slightly concave (bean-shaped), and with the axes of the pair parallel, not in line as in the pneumococcus (Colour-plate 1.3) In pus from inflammatory exudates, such as cerebrospinal fluid or urethral discharge, many diplococci are found in a small proportion of the polymorphonuclear cells; extracellular cocci also occur and there may be considerable variation in the size and intensity of staining of the cocci. In films from cultures, the diplococcal arrangement is less obvious and faintly staining involucral forms are frequent in older cultures. **Growth Requirements.** Both the meningococcus and the gonococcus are exacting in their growth requirements due in part to a susceptibility to inhibitory substances in the culture medium. The addition of lysed whole blood or ascitic fluid, or both, to nutrient agar will ensure good growth of colonies from infective material. Provided incubation is done in a moist atmosphere containing 5 to 10 per cent CO₂, preferably at 35 C to 36 C. Growth is rather slow but on a good medium greyish glistening slightly convex colonies of 0.5 to 1.0 mm in diameter appear in 18 to 24 hours; aerobic incubation should, however, be continued for another 24 hours when the colonies are much larger disks (2 to 3 mm) with slightly roughened surfaces and a tendency to crenation of the margins, particularly in the gonococcus. Gonococci are divisible into four types (T1 to T4) related to colonial appearance, auto-
agglutinability and virulence as demonstrated by the induction of urethritis in human volunteers (Kellogg et al., 1968) Types 1 and 2 form small (0.4 to 0.5 mm) glistening, convex, brown colonies whose cells are autoagglutinable, virulent and possess pili; types 3 and 4 form large (1.0 to 2.0 mm) flat, unpigmented colonies whose cells lack pili and are avirulent. The precise relationship between pili, other surface antigens and virulence is unclear at present.

Colonies of both pathogenic neisseriae react positively to the oxidase test (q.v.) and quickly develop a dark purplish colour; colonies of non-pathogenic neisseriae react more slowly.

Species identification depends on carbohydrate utilization reactions; the meningococcus produces acid from glucose and maltose but not from lactose or sucrose, whereas the gonococcus produces acid from glucose only. The majority of strains of N. lactamica can grow on ordinary serum-free culture media and all produce acid from glucose, maltose and lactose but not sucrose or fructose. The non-pathogenic neisseriae which grow readily on ordinary culture media, e.g. N. pharyngis are pigmented and usually utilize sucrose and/or fructose in addition to glucose and maltose (see Vol. II, Chap. 26). N. catarrhalis (Branhamella) is non-pigmented and does not attack sugars.

**SEROLOGICAL CLASSIFICATION.** Antigenically, the meningococcus are divisible into four main serogroups named A, B, C and D. Group A is in most countries the serogroup associated with epidemics of cerebrospinal meningitis, but in recent years group A has been supplanted by groups B and C as the main epidemic types, first among service personnel and now affecting civilian populations in the U.S.A. and Canada. Of the other serogroups (i.e. X, Y, Z. 29E and W-135), W-135 is isolated most frequently in Britain, not only from carriers but also from clinical cases. The serogroup of a culture is usually determined by a slide agglutination test made with absorbed, group-specific antiserum.

The gonococci are antigenically more heterogeneous than the meningococci so that serogrouping has not proved practicable: they share certain somatic antigens with the meningococci and non-specific antibody tests with human sera may show cross-reactions with both neisseriae.

**MENINGOCOCCAL INFECTION**

**PATHOGENESIS**

The natural habitat of the meningococcus is the nasopharynx of man. Surveys of normal populations will demonstrate a carrier rate around 5 to 10 per cent. In communities in which outbreaks of cerebrospinal meningitis are occurring, the carrier rate of the epidemic strain may range from 20 per cent to 80 to 90 per cent and certain studies have shown that a sharp increase in the carrier rate of group A or other pathogenic groups of meningococci precedes the occurrence of clinical cases. However, this carrier:case ratio is variable in different outbreaks.

The route of spread of the meningococcus from the nasopharynx to the meninges is a controversial matter; the organism may either spread directly through the cribiform plate or the subarachnoid space by the perineural sheaths of the olfactory nerve; or, much more probably, it may be blood-borne. In favour of the latter route are the frequent positive blood cultures in the early stages of infection, the purpuric rash in many cases with the isolation of meningococci from the skin lesions, and the occurrence, particularly during epidemics, of meningococcal septicæmia with rash but no clinical meningitis.

The problem of main concern in pathogenesis is the occurrence of cerebrospinal meningitis among only a limited proportion of the population at risk. Recent studies have confirmed some early observations that the absence of bactericidal antibody in the blood is the factor most closely related to susceptibility to clinical infection. Evidence in support of this relationship is: (1) the age-distribution of meningococcal disease which has its highest incidence in infants and young children, from 3 months to 3 years of age, amongst whom humoral meningococcalicidal antibodies are rarely found; the analogy with haemophilus meningitis is obvious; (2) the reciprocal relationship in the appearance of these bactericidal antibodies in older children and adults with the decreasing incidence of cerebrospinal meningitis, except when it occurs in outbreaks among adults brought together for special reasons, e.g. in service training centres and, in earlier days, in ships and jails; (3) prospective studies among military recruits which
showed that whereas only 1 per cent of the total population at risk became clinically affected, 38.5 per cent of those lacking specific bactericidal antibody to meningococcus and who became infected with the epidemic group C strain developed meningococcal meningitis; and (4) patients convalescent from meningococcal infection develop typical immunoglobulins and bactericidal antibody to the infecting strains (Goldschneider, Gotschlich and Artenstein, 1969).

It is clear, nevertheless, from the relatively low incidence of meningitis in young children and the absence of meningitis in a large proportion of adults lacking specific antibody that in most persons the first infection of the nasopharynx with meningococcus leads to antibody production without the development of meningitis. Presumably non-specific defence mechanisms are generally successful in preventing infection of the blood and meninges.

**Laboratory Diagnosis**

Lumbar puncture should be done as soon as meningitis is suspected. In a case of meningococcal meningitis the spinal fluid is under pressure and is turbid in appearance due to the large number of pus cells present. In the early stages of infection the Gram-negative diplococci are present usually in considerable numbers in the purulent cerebrospinal fluid and can be recognized by microscopic examination of the centrifuged deposit. At a later stage they may be scanty and even apparently absent.

Films made from the sediment are stained by methylene blue and Gram's method (with Sandford's counterstain). In the early untreated case, Gram-negative diplococci are seen, filling a limited number of the pus cells but also extracellularly; if the organisms are scanty, they may be more easily demonstrated in the smear stained with methylene blue. Cultures are made on blood or 'chocolate' (heated blood) agar and incubated for 18 to 24 hours in an atmosphere of 5 to 10 per cent CO₂. If Gram-stained films from the resulting growth show typical Gram-negative cocci, sugar utilization tests are carried out. The isolate may be subcultured onto medium containing the test carbohydrate and an indicator as in the conventional procedure, or alternatively, performed by a rapid carbohydrate utilization technique (Young, Paterson and McDonald, 1976). The serological group may be identified by agglutination tests with the appropriate antisera.

For quick differential diagnosis, which is essential for early effective chemotherapy, microscopic examination is often sufficient. However, in the later stages of infection, or if sulphonamides have been administered, the organisms may be scanty or undetectable in the centrifuged deposit. Countercurrent immunoelectrophoresis can be used to demonstrate group-specific polysaccharide antigen in the cerebrospinal fluid of patients with meningitis due to group A and C meningococci.

In cases of suspected meningococcal septicaemia, and also in cases of meningitis, blood cultures should be carried out and subcultures made on blood agar every day for 4 to 7 days.

**Chemotherapy**

Overall, approximately 80 to 90 per cent of strains of meningococcus are sensitive to the sulphonamides but sensitivity varies considerably within groups, e.g. in 1975, 53 per cent of group A strains tested were resistant (MIC > 50 mg/ml) compared with 3 per cent of group B and 10 per cent of group C strains (see British Medical Journal, 1, 466, 1976). Because the sulphonamides diffuse readily into the cerebrospinal fluid, a sulphonamide compound e.g. sulphadiazine given orally (or intravenously in comatose patients) is the best drug for cases of meningitis due to a strain known to be sensitive. However, since more than 10 per cent of all strains show evidence of resistance to sulphonamide, these drugs should not be used alone in the treatment of meningococcal infections. Benzyl penicillin or ampicillin parenterally should be used in addition to a sulphonamide; these drugs pass from the blood through inflamed, though not through normal, meninges into the cerebrospinal fluid. Otherwise chloramphenicol may be given orally since it diffuses even more readily into the cerebrospinal fluid. Prompt chemotherapy
can be life saving although fulminating infections, particularly in infants, sometimes with haemorrhagic involvement of the adrenals (Waterhouse-Friederichsen syndrome), may, despite treatment, end fatally within 24 hours of onset.

Epidemiology

Meningococcal infections which are world-wide in distribution are notifiable in 142 countries (see World Health Organization Chronicle, 1969, 23, 48). Most cases of meningococcal meningitis tend to occur in the first six months of each year. About two-thirds of the cases occur in the first five years of life and, of these, more than half occur in infants under one. Incidence is higher in males than in females. The recorded number of meningococcal infections (mostly meningitis) in England and Wales fell from 1391 in 1950 to 293 in 1967. However, after 1967 the incidence of meningococcal meningitis increased markedly and in 1974 there were 1296 statutory notifications in England and Wales (see British Medical Journal, 1976, 1, 466). Over the last 10 years there has been a world-wide increase in meningitis. Recently, there have been large scale meningococcal epidemics in Brazil (group C in 1971 and group A in 1974) while epidemics in Finland and parts of Africa were predominately due to group A. In Britain, in 1974 and 1975, 60 per cent of cases of meningococcal meningitis were due to group B strains, 15 to 20 per cent to group C and about 10 per cent to group A.

Outbreaks have frequently occurred among young adult populations recently recruited to live together in semi-closed communities and what might have been limited epidemics in Britain were fanned into great conflagrations of cerebrospinal meningitis among troops in training in the early years of both world wars. In recent years, localized outbreaks have been a regular occurrence in some American Army base camps; the epidemic strains have been groups B or C, not group A. Intensive studies of the infection in these training centres have added much to our knowledge of the natural history of the disease. A high proportion of the recruits become meningococcus nasopharyngeal carriers and if the carrier strain is relatively avirulent, it induces bactericidal antibodies to virulent strains without causing clinical disease. On the other hand, clinical infection may occur in a high proportion of susceptible individuals who acquire the epidemic strain. The natural acquisition of immunity with increasing age from early childhood in civilian communities is therefore likely to be due to asymptomatic infection with avirulent strains.

Outbreaks of meningococcal meningitis require at least three factors: the presence in the population of a proportion of susceptible individuals who lack bactericidal antibodies to the current strains, a high transmission rate from person to person, and a virulent meningococcus. The important factors that determine virulence and communicability of the meningococcus are still not understood.

Control Measures

In the control of outbreaks, mass chemoprophylaxis with sulphadiazine given orally for 2 to 3 days proved to be very effective in reducing carrier and case rates until the emergence of sulphonamide-resistant strains of meningococcus, initially in U.S.A. and more recently in Britain and other European countries. A satisfactory alternative drug for large scale chemoprophylaxis has not yet been found. Minocycline and rifampicin have proved to be 80 to 90 per cent effective in eradicating carriage of meningococci. Unfortunately, significant and frequent vestibular reactions follow the administration of minocycline while rifampicin treatment is associated with the development of rifampicin resistant meningococcus. Rifampicin has also been criticized because of its immunosuppressive effect and the possibility of producing rifampicin resistant Mycobacterium tuberculosis.

Since resistance to meningococcal meningitis is closely related to the possession of bactericidal antibodies, whether transiently derived from the mother or actively acquired by latent infection, the possibility of inducing immunity by vaccination has been explored from time to time and has now become reality with the separation from the epidemic serogroups A and C of high molecu-
lar specific polysaccharides which have been shown to be good immunizing agents. Polysaccharide vaccines against disease caused by serogroups A and C are now licensed for use in the United States. These vaccines may be used to control outbreaks of meningococcal disease caused by serogroups A or C but routine vaccination of civilians is not recommended. The protection provided by the vaccine is group-specific and there is a compensatory increase in both carrier rates and clinical disease with group B meningococci (Artenstein and Gold, 1970). In Britain, where 60 per cent of meningococcal disease is attributable to group B strains, widespread use of such vaccines would have little effect.

GONOCOCCAL INFECTION
PATHOGENESIS

The gonococcus is a strictly human parasite and all attempts to infect animals had failed until recently when chimpanzees have been experimentally infected. Unlike the meningococcus, the gonococcus is not found in healthy carriers but only in cases of acute, chronic, or asymptomatic infection; the latter may account for 70–80 per cent of infections in women and 5–10 per cent in men. The initial infection generally affects the anterior urethra in men and the urethra and cervix uteri in women, but if untreated the infection may become chronic and/or lead to upper genital tract or systemic complications: asymptomatic infection may also lead to complicated infection. In acute urethritis the gonococcus is found in the urethral exudate on the surface of or within epithelial cells, within a proportion of polymorphonuclear leukocytes and also extracellularly.

In men the organism infects the urethra and produces a suppurative inflammation with purulent discharge. The cocci are present in large numbers in the discharge at an early stage, but later are scanty. Infection may spread to the prostate, seminal vesicles and epididymis or may invade the peri-urethral tissue, producing an inflammatory reaction, peri-urethral abscess and subsequent stricture.

In women the urethra and cervix uteri are infected, but rarely the vaginal mucosa; discharge is often scanty. Infection may extend to the vestibular glands ( Bartholinitis), the endometrium (endometritis) and Fallopian tubes (salpingitis) and even the peritoneal cavity may be invaded.

Rectal infection occurs in both men and women. It is, of course, exogenously acquired by passive male homosexuals: in the female, it usually spreads to the anus from the genital infection.

Blood invasion may result from primary gonorrhoeal infections, and arthritis and tenosynovitis may occur as complications. Although the gonococcus has on occasion been cultivated from joint fluid in arthritis, the possibility of gonococcal arthritis being an allergic manifestation must be considered. There have been recent reports of septic gonococcal dermatitis, usually associated with arthritis, arthralgia and fever. The skin lesions, varying from maculopapules to vesiculopustules are scaly, present mostly on the extremities or around joints and are more common in females than in males (Barr and Danielsson, 1971).

Gonococcal infection of the pharynx may be acquired by oro-genital contact. Such infection is most commonly found in homosexual men but is also present in women, and less frequently in heterosexual men. Infection may be asymptomatic or associated with mild sore throat, pharyngitis, or tonsillitis.

In female infants and children the gonococcus may produce a persistent vulvo-vaginitis with involvement sometimes of the rectum. Outbreaks of this infection used to occur in paediatric wards and children’s institutions, but gonococcal vulvo-vaginitis is now rare, and associated mostly with sexual offences. In newborn infants, gonococcal ophthalmia may result from direct infection at birth when the mother has gonorrhoea.

LABORATORY DIAGNOSIS

In acute infections thin evenly spread smears are made from the discharge. In men, specimens are taken from the urethral discharge: the meatus should be cleansed with sterile gauze soaked in saline solution, and specimens taken with a wire loop from within the meatus. In women specimens are taken from the urethra and
cervix uteri with a wire loop or swab and a vaginal speculum; specimens should also be taken from the rectum as a routine, and other sites such as the orifice of the greater vestibular gland may also be sampled. Rectal specimens should be taken from men homosexuals and pharyngeal specimens from both sexes whenever considered necessary.

Separate films are stained by methylene blue and Gram’s method (with neutral red or Sandford’s stain as the counter-stain). In the acute stage, both in men and women, the occurrence of the characteristic Gram-negative intracellular diplococci is strongly suggestive of gonorrhoea. However, intracellular cocci may be scanty and pleomorphic, particularly if the patient has already received treatment; or only extracellular cocci are seen.

In chronic infections, the cocci may be relatively scanty in films and difficult to identify accurately among the secondary infecting organisms which may include Gram-negative commensal diplococci. In the male the ‘morning drop’ of secretion from the urethra should be examined, or films are made from a centrifuged urinary deposit or from any discharge after prostatic massage. In the female, secretion from the cervix uteri, and not vaginal discharge, should be examined. Any vaginal discharge should, however, be examined for Trichomonas vaginalis and Candida albicans.

Recently, fluorescent techniques for the identification of gonococci in smears have come into use but this ‘on-the-spot’ diagnosis is more time consuming and technically demanding than Gram-staining and is not recommended for routine use. Fluorescent staining is most useful in the rapid identification of gonococci after incubation of the primary isolation plate: this ‘delayed’ technique is used widely as a routine procedure. The cultural diagnosis of gonorrhoea has been greatly improved by the introduction of the selective medium of Thayer and Martin (1966): the antibiotics (vancomycin, colistin and nystatin) present in the medium prevent other flora, which may be present at the sampling site from overgrowing any gonococci present.

Inoculation of material to be cultivated should, if possible, be made directly from the patient on to a suitable medium prewarmed to 37°C, and the culture should be incubated at once, or at least within an hour or two, since the gonococcus may die quickly in an adverse environment.

When direct plating and immediate incubation is impracticable several culture and transport systems are now available. The medium, usually present in a small chamber containing CO₂ or a CO₂ generating system, can be inoculated directly from the patient and transported to the laboratory, either before or after incubation. Alternatively, a swab may be sent in a straightforward transport medium, e.g. Amies modification of Stuart’s medium. Dry swabs should not be sent.

Cultures are incubated at 35°C to 36°C for 1 to 2 days in an atmosphere of 5 to 10 per cent CO₂. Identification is by means of the oxidase reaction (q.v.) followed by delayed immunofluorescence and/or carbohydrate utilization tests.

**SEROLOGY.** The gonococcal complement fixation test (GCT) may be useful in the diagnosis of chronic infections in females and for suspected gonococcal complications such as salpingitis and arthritis. However, the value of the test is limited due to cross-reactions with other neisseriae.

**CHEMOTHERAPY**

The gonococcus is ordinarily sensitive to a wide range of antimicrobial drugs but a proportion of strains have developed resistance to those drugs which have been most commonly used in therapy, viz. the sulphonamides, penicillin and streptomycin. Sulphonamides were used at first very effectively but between 1936 and 1946 the proportion of highly resistant strains increased from less than 10 per cent to 80–90 per cent, by which time penicillin became generally available and replaced sulphonamide in the treatment of gonorrhoea. Since then, when all strains were susceptible to levels of 0.03 to 0.06 units/ml of penicillin, the incidence of relatively resistant strains (MIC ≥ 0.125 units/ml) has increased to 10–20 per cent in Britain: the upper limit of resistance has rarely exceeded an MIC of 1.0 unit/ml and such strains usually respond to
Sources and Coccus contact other.

However, to exposure environment, almost is transmitted from Infection less direct very to killing gonococcus. The is drugs that penicillin. The is widely. Complicated infections have been recommended as penicillin is still the treatment. In acute infections, single-dose oral treatment, e.g. ampicillin (2g) and probenecid (1g), is highly effective and used widely. Complicated infections are normally treated by a course of injections of benzyl penicillin. The bactericidal combination of trimethoprim and sulphamethoxazole make cotrimoxazole a suitable alternative to penicillin in uncomplicated gonorrhoea. Other drugs that have been used successfully are tetracyclines and spectinomycin. Unless the sensitivity to penicillin is known before treatment is given it is prudent not to use this drug in the case of patients whose infections may have been acquired in the Far East and West Africa where B-lactamase producing strains exist. Fortunately, these strains are at present sensitive to a variety of other antibiotics such as spectinomycin, cotrimoxazole and a new cephalosporin, cephalosporin.

Epidemiology

Sources and Modes of Transmission

Infection is transmitted from person to person almost exclusively by sexual contact, so that gonorrhoea is classified as a venereal disease. The gonococcus is so exceptionally susceptible to killing by the conditions of the extra-corporeal environment, e.g. drying, cold, exposure to air, absence of nutrients, that it can only very rarely survive transmission by means less direct than the immediate transfer from the urogenital tract of one person to that of another. However, infection may be acquired by oro-genital contact and occasionally the gonococcus may survive rapid transfer of exudate on the fingers to the conjunctiva or on damp towels as in the spread of gonococcal vulvo-vaginitis in institutions.

Gonorrhoea has been increasing at an alarming rate in most countries of the world during the past decade. Its global incidence, estimated at 60 million cases in the early 1960s is now estimated at 200 million. In the U.S.A. gonorrhoea is the most frequently reported communicable disease; the rate in 1974 was 420 cases per 100,000 population compared with a rate of 1.25 per 100,000 population in Britain. The prevalence in the U.S.A. can be regarded as epidemic since the incidence rises fourfold when estimated unreported cases are included. In Britain, after a peak incidence immediately following the Second World War and relatively low levels in the early 1950s, probably as a result of effective chemotherapy, the annual returns from venereal diseases clinics in England and Wales increased from 17,550 cases in 1954 to 53,425 cases in 1970 (see Fig. 25.1); between 1970 and 1974 when there were 58,139 cases, the increase was less marked and the annual incidence varied by less than 10 per cent. These figures do not include patients treated privately or in the Armed Forces. The proportion of cases occurring in teenagers has risen to around 20 per cent with more girls than boys affected. The rate for girls under 16 years is about four times that for boys of the same age group. This sex ratio may be contrasted with that for adults, 1.8:1, males to females.

The processes of modern life which have led to earlier maturity, greater mixing of the sexes, economic freedom for the young and increasing facilities for travel tend to lead to promiscuity. Thus, factors that contribute to the mounting incidence of gonorrhoea are (a) an apparent increase in promiscuity, both heterosexual and homosexual, in a permissive society; (b) the easy availability of birth control methods and of effective chemotherapy for the infected; (c) difficulties in recognition (and therefore of treatment) in women and in passive male homosexuals; (d) greater population mobility within and between countries for cultural, commercial, touristic or military purposes; and (e) high infectiousness and short incubation which make the chain of infection difficult to break, to which may be added the development of drug resistance in the gonococcus which accentuates
the need for accurate diagnosis, effective treatment and tests for cure.

**CONTROL MEASURES**

Control measures are aimed at the early recognition and effective treatment of clinical cases, tracing of infected contacts and bringing them in for treatment, use of physical barriers during intercourse, and health education. Treatment of venereal diseases in Britain is free and confidential at ‘special clinics’ which are usually sited in the outpatient department of large general hospitals. These clinics deal with a wide range of sexually transmitted and related conditions besides venereal diseases which affect only about one-quarter of all the patients who attend. Many of the clinics have attached social workers who help to tackle the problems of young people, unmarried mothers, unstable marriages, etc. A most important function of the clinics is contact tracing, which means seeking information from the patient about possible sources or recipients of infection and, hopefully, persuading them to attend the clinic for examination and treatment. General practitioners may prefer to treat their own patients and if so, they, too, must endeavour to trace infected contacts. More health education is needed to warn the community that promiscuity carries a real risk of acquiring venereal diseases which, in women, may be easily missed and may be followed by serious sequelae. There is, for example, evidence of an increasing incidence of salpingitis which can lead to sterility.

Future developments may include greater emphasis on contact-tracing, better serological tests for the detection of symptomless infections in women, and the selective use of prophylactic vaccines.

**REFERENCES**


Identification and Penicillinase Testing of *Neisseria gonorrhoeae* from Primary Isolation Cultures on Modified New York City Medium

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Received for publication 4 November 1977

The fluorescent-antibody test, rapid carbohydrate utilization test (RCUT), and a test for penicillinase production were performed on the bacterial growth from primary cultures on modified New York City medium. Of 134 gonococcal infections in men, 88.8% were diagnosed by the fluorescent-antibody test and 70.9% by the RCUT after incubation for 24 h; the corresponding figures for 75 infections in women were 86.7 and 54.7%, respectively. After incubation for 48 h, 100% of infections were diagnosed by the fluorescent-antibody test, whereas 88.8% of infected males and 86.7% of females were also diagnosed by the RCUT. Primary isolation cultures on modified New York City medium proved suitable for determining the ability of strains to produce penicillinase by a modified RCUT procedure. The method of isolation and identification by using primary cultures from modified New York City medium is both rapid and economical. The rapidity of diagnosis provided by the RCUT makes this a very useful diagnostic method, particularly in laboratories lacking immunofluorescence equipment. Since the penicillinase production test forms part of a routine identification procedure, no extra culture media or subcultures are required. The rapidity of this test should make it of value in tracing contacts of patients infected with penicillinase-producing strains.

The rapid cultural diagnosis of gonorrhea not only benefits the individual patient but also aids control of the infection within the community (4). The time taken for diagnosis by conventional biochemical methods can be substantially reduced by using a combination of the delayed fluorescent-antibody (FA) method and a rapid carbohydrate utilization test (RCUT) (6, 10). Unfortunately, slow growth of gonococci on Thayer-Martin (TM) medium and the need to subculture isolates prior to performing the RCUT (10) limit the value of these rapid techniques. To improve further the speed of cultural diagnosis, we have modified the New York City medium described by Faur et al. (2, 3), enabling us to carry out the RCUT directly from the primary isolation plate. In an earlier trial (9), modified New York City (MNYC) medium gave a significantly higher rate of initial recovery of *Neisseria gonorrhoeae* than TM medium.

The recent emergence of penicillinase-producing strains of *N. gonorrhoeae* highlights the importance of laboratory surveillance of the penicillin sensitivity of clinical isolates (7). Penicillinase-producing gonococci can be detected simply and economically by a modification of the RCUT (8). When MNYC medium is used for primary isolation, penicillinase testing can be performed directly from the isolation plate. This paper reports our experience using MNYC medium for the rapid culture, identification, and penicillinase testing of *N. gonorrhoeae* in a routine diagnostic laboratory.

**MATERIALS AND METHODS**

**Isolation medium.** MNYC medium contained Difco GC base enriched with 10% (vol/vol) human blood lysed with 0.5% (wt/vol) saponin, 2.5% (vol/vol) yeast dialysate prepared as described by Faur et al. (3), 0.1% (wt/vol) glucose, lincomycin (1.0 μg/ml), colistin (6 μg/ml), amphotericin B (1.0 μg/ml), and trimethoprim lactate (6.5 μg/ml).

**Specimens.** Cultures were obtained from patients attending the Department of Venerology, The Royal Infirmary of Edinburgh, during April, May, and June 1977. Urethral cultures were taken routinely in all male patients, and rectal and pharyngeal cultures were also taken as a routine from homosexual men. In female patients, urethral, cervical, rectal, and, if indicated, pharyngeal cultures were made. Material was inoculated directly onto culture plates at the time of the patient examination.

**Culture and examination of plates.** After inoculation, plates were held at 36°C in a carbon dioxide-enriched (10%) atmosphere. After incubation for 24 h plates were examined, any suspect colonies were tested for.
for the oxidase reaction, and Gram smears were prepared. Presumptive gonococci (oxidase-positive gram-negative diplococci) were then identified fully and tested for penicillinase production as outlined below. Negative cultures were reexamined after incubation for 48 h. If presumptive gonococci were present, they were examined by the FA technique, and a subculture was made onto a fresh MNYC plate for carbohydrate and penicillinase tests next day.

**FA test.** The FA test was performed using Difco fluorescein-labeled anti-gonococcal conjugate as described previously (10). Each day, stock strains of *N. gonorrhoeae* and *N. meningitidis* were used as positive and negative controls, respectively.

**RCUT and penicillinase test.** The test for penicillinase production (8) depends on the color change of a pH indicator when acid is produced from the splitting of ampicillin to penicillic acid. Sodium ampicillin (Beecham Research Laboratories) was dissolved in buffer-salt solution (BSS) (5.2 mmol of phosphate buffer per liter containing 0.8% [wt/vol] potassium chloride and 0.01% phenol red, pH 7.10 to 7.15) to a concentration of 250 mg/ml; 2 ml of the solution was dispensed in 100-μl volumes and stored at −20°C for up to 2 weeks.

Five tubes (70 by 10 mm) were used for each gonococcal isolate tested; 20 μl of 10% aqueous (wt/vol) glucose, maltose, and sucrose and 20 μl of ampicillin (250 mg/ml in BSS) were added to four individual tubes, followed by 100 μl of BSS. If there was sufficient growth of presumptive gonococci on the primary isolation plate at 24 h, this was harvested using a cotton-tipped swab, and a thick suspension was made in 300 μl of BSS in the fifth tube and mixed well; 30 μl of this suspension was transferred to each sugar-containing tube and to the ampicillin tube. Tubes were shaken and incubated at 37°C in a water bath. A positive result was normally available in 30 to 60 min and a definitive reading at 3 h. A yellow color (occasionally yellow-orange) is positive; red is negative. Each day, a penicillinase-negative and penicillinase-negative strain of *N. gonorrhoeae* and a strain of *N. meningitidis* were set up as controls.

If there was insufficient growth of presumptive gonococci on the primary isolation plate at 24 h, a subculture was made onto MNYC medium, and the RCUT and penicillinase tests were carried out after overnight incubation.

### RESULTS

During the period of the study, excluding specimens requiring processing over the weekend period, there were 134 cases of gonorrhea in men and 75 cases in women. All isolates of *N. gonorrhoeae* produced acid from glucose only, were FA-test positive, and did not produce penicillinase. Tables 1 and 2 show the time taken to obtain these results in male and female patients, respectively.

From Tables 1 and 2 it is seen that the FA test was equally efficient in the rapid diagnosis of gonorrhea in men (88.8% of patients diagnosed at 24 h) and in women (86.7% in 24 h). In contrast, rapid diagnosis by the RCUT was more suitable for isolates from men than from women (70.9% and 54.7% of patients diagnosed at 24 h, respectively). After subculture and incubation of any suspect gonococci present at 24 h, there was little difference in the percentage of men and women diagnosed at 48 h by the RCUT (88.8 and 86.7% of patients, respectively). Tests for penicillinase production were always performed at the same time as the RCUT.

To determine whether differences exist in the rapidity of diagnosis for particular culture sites, the number of urethral, cervical, rectal, and fauces cultures that could be tested at 24 h was determined and is shown in Tables 3 and 4.

There was little difference in rapidity of diagnosis by the FA test in various culture sites in both male and female patients. The FA test was of particular value in the rapid identification of gonococci in rectal cultures. Positive rectal cultures in women generally produced a more rapid and abundant growth of gonococci with less growth of unwanted microorganisms than did rectal cultures in men; 96.0% of positive rectal cultures in women were positive by the FA test at 24 h, compared with only 81.8% of positive cultures in men, and the corresponding figures for the RCUT at 24 h were 68.0 and 45.5%, respectively.

### DISCUSSION

Many workers have emphasized the importance of rapid cultural diagnosis of gonorrhea (1, 4, 5). Our results with MNYC medium show that more than 90% of infections were diagnosed by immunofluorescence at 24 h. In a previous report.

#### Table 1. Time taken to establish diagnosis in 134 cases of gonorrhea in men

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of patients (%) at:</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>FA</td>
<td>119 (88.8)</td>
</tr>
<tr>
<td>RCUT</td>
<td>95 (70.9)</td>
</tr>
<tr>
<td>Penicillinase production</td>
<td>95 (70.9)</td>
</tr>
</tbody>
</table>

*a One or more isolates tested per patient.

#### Table 2. Time taken to establish diagnosis in 75 cases of gonorrhea in women

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of patients (%) at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>FA</td>
<td>65 (86.7)</td>
</tr>
<tr>
<td>RCUT</td>
<td>41 (54.7)</td>
</tr>
<tr>
<td>Penicillinase production</td>
<td>41 (54.7)</td>
</tr>
</tbody>
</table>

*a One or more isolates tested per patient.
Growth on these cultures from certain genital isolates, ered sufficient identification in the case of anogenital isolates, carbohydrate utilization tests are usually performed in the case of isolates from oral, disseminated, and conjunctival infections. Carbohydrate utilization tests are necessary to identify neisseriae giving negative or equivocal results in the FA test. The rapidity of diagnosis provided by the application of the RCUT to the MNYC isolation plate makes this a very useful diagnostic method in all laboratories but particularly in those lacking immunofluorescence facilities. To ensure reliable results, only overnight cultures should be used for the RCUT.

Primary isolation cultures on MNYC medium proved suitable for testing for penicillinase production. Since this test (8) forms part of a routine identification procedure, no extra culture media or subcultures are required, making it a simple, inexpensive, and very rapid method of testing for penicillinase production. Although none of the gonococcal strains in this study produced penicillinase, 2 months after the study was concluded a penicillinase-producing strain was detected. The test was positive after 24 h of incubation and 1 day before disk sensitivity results were known. Such a saving in time should allow rapid tracing of contacts and could make an important contribution to the control of penicillinase-producing strains.

We conclude that the above scheme of isolation, identification, and penicillinase-production testing using primary cultures from MNYC medium is rapid and economical in terms of culture media and time required for examination of plates and performance of tests. It may be particularly useful in developing countries.

ACKNOWLEDGMENTS

I thank W. King, Fellow of the Institute of Medical Laboratory Sciences, for his skilled technical assistance; D. H. Robertson and colleagues of the Department of Venerology, Edinburgh Royal Infirmary, for sending clinical specimens; and B. P. Marmion of the Department of Bacteriology for the interest expressed in this work.

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SENSITIVITY OF NEISSERIA GONORRHOEAE TO PARTIALLY PURIFIED R-TYPE PYOCINES AND A POSSIBLE APPROACH TO EPIDEMIOLOGICAL TYPING

C. Caroline Blackwell, H. Young and Isabel Anderson*

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A rapid, inexpensive typing system for Neisseria gonorrhoeae would be of use in epidemiological studies, for distinguishing failures of treatment from cases of reinfection, and in the early recognition of strains that might have special pathogenic features such as those associated with disseminated infection (Knapp and Holmes, 1975; Morello, Lerner and Bohnoff, 1976; Schoolnick, Buchanan and Holmes, 1976; Eisenstein, Lee and Sparling, 1977). Previous efforts to type gonococcal strains have involved the serological detection of antigenic differences (Glynn and Ward, 1970; Maeland, Kristoffersen and Hofstad, 1971; Apicella, 1974; Johnston, Holmes and Gotschlich, 1976; Traumont et al., 1976; Wang et al., 1977), differences in nutritional requirements (Carifo and Catlin, 1973) and variations in sensitivity to bacteriocines (Flynn and McEntegart, 1972; Walstad, Reitz and Sparling, 1974; Knapp, Falkow and Holmes, 1975; Lawton et al., 1976).

Observations on the production of pyocines and demonstration of sensitivity to these bacteriocines have been used as the basis of typing systems for Pseudomonas aeruginosa (Farmer and Herman, 1969; Govan and Gillies, 1969). Sensitivity to R-type pyocines has been reported for strains of N. gonorrhoeae (Morse et al., 1976) and the possible use of pyocines as a typing tool has been investigated (Sidberry and Sadoff, 1977). Three types of bacteriocines have been described in Ps. aeruginosa: soluble bacteriocines (S-type) similar to the colicines of Escherichia coli (Ito, Kageyama and Egami, 1970), rod-type (R-type) particles resembling defective bacteriophages (Kageyama, 1964), and the flexuous F-type bacteriocines (Govan, 1974b). The R-type and F-type pyocines were chosen for the present study because they can be sedimented by ultracentrifugation and are visible in electron micrographs; these qualities allow their partial purification and separation from the S-type pyocines and other soluble inhibitory substances produced by Ps. aeruginosa.

Using 23 pyocine preparations, Sidberry and Sadoff (1977) obtained 30 sensitivity patterns among 106 strains of N. gonorrhoeae but the majority of the strains fell into two groups and further evaluation of the method with gonococcal isolates from several sources was suggested by the authors.

Received 8 Dec. 1978; accepted 10 Jan. 1979
Materials and methods

Micro-organisms and culture media. Strains of *P. aeruginosa* producing the R-type pyocines listed in table I were obtained from Dr John Govan of this department. Kageyama (1975) classified many R-type pyocines on the basis of their spectrum of activity, and strains from groups R1, R2, R3 and R5 were specifically chosen for use in this study. The Kageyama classification for the R-type pyocines produced by these strains is given when known. Strains of *P. aeruginosa* were obtained from Dr John Govan of this department.

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**Table I**

<table>
<thead>
<tr>
<th>Kageyama classification</th>
<th>Pyocine-producing strain</th>
<th>Pyocine indicator strain</th>
</tr>
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<tbody>
<tr>
<td>R1</td>
<td>IS4</td>
<td>ISC</td>
</tr>
<tr>
<td></td>
<td>IS6</td>
<td>ISC</td>
</tr>
<tr>
<td></td>
<td>IS8</td>
<td>ISC</td>
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<tr>
<td></td>
<td>ISD</td>
<td>ISC</td>
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<tr>
<td></td>
<td>2285</td>
<td>ISC, IS6</td>
</tr>
<tr>
<td>R2</td>
<td>ISB</td>
<td>ISC</td>
</tr>
<tr>
<td></td>
<td>R21</td>
<td>IS8</td>
</tr>
<tr>
<td></td>
<td>pp430</td>
<td>IS8</td>
</tr>
<tr>
<td>R3</td>
<td>ISE</td>
<td>IS4</td>
</tr>
<tr>
<td>R4</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>R5</td>
<td>ISC</td>
<td>IS4, IS5</td>
</tr>
<tr>
<td>Unclassified</td>
<td>R205</td>
<td>ISC</td>
</tr>
</tbody>
</table>

... = Not available for examination.

United States Public Health Service, Seattle, Washington; these included prototrophic strains and strains of different auxotypes from three geographic areas: Atlanta, Indianapolis, and Seattle. All RIE organisms had been identified as *N. gonorrhoeae* on the basis of Gram stain, oxidase reaction and sugar utilisation. On arrival at our laboratory each was retested for oxidase reaction and Gram staining. Strains were stored in skimmed milk at -20°C. The gonococcal strains were maintained on either Thayer Martin medium (TM) or modified New York City medium (MNYC; Young, 1978). When needed, colony types T1, T2, T3 and T4 were selected from GC Medium (Difco) with the aid of a stereoscopic microscope with a double system of substage lighting (Jephcott and Reyn, 1971).

Sensitivities to antibiotics were determined on clear GC Medium (Difco) with the following additions per 960 ml after autoclaving: 0.5% (w.v) ferric nitrate (British Drug Houses) 10 ml, GC supplement* 20 ml, 20% (w.v) glucose 10 ml. Antibiotics were added to GC agar at the following concentrations: penicillin G (Glaxo) sodium salt: 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2 units/ml; tetracycline hydrochloride (Achromycin, Lederle Ltd): 0.05, 0.1, 0.25, 0.5, 1 µg/ml; spectinomycin hydrochloride (Trobicin, Upjohn Ltd): 2, 4, 8, 16 µg/ml; chloramphenicol (Chloromycetin, Parke-Davis Ltd): 0.1, 0.2, 0.4, 0.8, 1.6 µg/ml; lincomycin hydrochloride (Upjohn Ltd): 4, 8, 16, 32 µg/ml.

* GC supplement: L glutamine (Koch Light Chemicals) 0.5 g, cocarboxylase (British Drug Houses) 0.01 g, water to 100 ml 20%.
**Antibiotic-sensitivity testing.** Visibly turbid suspensions (approximately $1 \times 10^8$ to $3 \times 10^8$ colony-forming units/ml) of gonococci were made in NaCl 0.85% (w/v) containing soluble starch 1% (starch-saline). Portions of the suspension were placed in the wells of a Steers replicator which delivered approximately 0.0025 ml of the suspension to the antibiotic plates (Steers, Foltz and Graves, 1959). The same suspensions were also used for the assay of sensitivity to pyocines. The plates were then incubated for 48 h at 37°C with 10% CO$_2$ in a Searle Qualitemp (80 TC) CO$_2$ incubator. Minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic at which there were five or fewer colonies per spot inoculated.

**Induction and preparation of pyocines.** Pyocine preparations were obtained by a modification of the method of Govan (1974a). From an overnight nutrient-broth culture, 2 ml was added to 25 ml of sodium glutamate broth (SGB) which contained sodium glutamate 20 g, glucose (20% w/v solution) 20 ml, magnesium sulphate (MgSO$_4$ 7H$_2$O) 0.1 g, disodium hydrogen phosphate (Na$_2$HPO$_4$ 12H$_2$O) 5.63 g, potassium dihydrogen phosphate (KH$_2$PO$_4$) 0.25 g, calcium nitrate 10 mg, yeast extract 1 g, and distilled water to 1 litre. The sterile glucose in solution (Seitz filtered) was added to the cooled autoclaved medium before use (Kageyama and Egami, 1962).

The pseudomonads were incubated at 30°C in an orbital incubator (Gallenkamp) at 100 r.p.m. for 2-5 h. Mitomycin C (1-5 μg/ml) was added at this point and the cultures were incubated for another 2-5-3 h. The lysates were centrifuged at 10 000 g for 30 min. to remove bacterial cells and debris; each supernatant was decanted, shaken with chloroform 5% (v/v) and stored at 4°C. This preparation was the crude pyocine lysate.

The crude lysate (12.5 ml) was ultracentrifuged at 100 000 g for 3 h and the supernatant containing S-type pyocine activity and other soluble inhibitory substances was discarded. The pellet containing the R-type activity was resuspended in 10 ml 0.01M Tris buffer (pH 7.5) containing 0.01M MgCl$_2$6H$_2$O and 0.01M MgSO$_4$7H$_2$O (referred to hereafter as Tris buffer). This partially purified pyocine preparation was stored at 4°C.

**Assay of pyocine activity against Ps. aeruginosa and N. gonorrhoeae.** Pyocine activities of the crude lysates and the partially purified preparations were assayed by spotting doubling dilutions on nutrient-agar (NA) plates and MNYC plates and allowing the spots to dry. The NA plates were then flooded with the appropriate standard strains of Ps. aeruginosa (indicator strains) known to be sensitive to the particular test pyocine. Sensitive and resistant gonococcal strains, identified in pilot studies, were tested on MNYC.

Partially purified pyocine lysates were diluted 1 in 2 in Tris buffer and spotted with a dropper pipette calibrated to deliver 0.02 ml on plates of GC, MNYC or TM medium and allowed to dry. Overnight plate cultures of gonococci were suspended in starch-saline and used to flood the plates to produce a confluent lawn. The plates were allowed to dry and incubated overnight at 37°C aerobically with 10% CO$_2$. Some gonococci grew more slowly and were incubated for an additional 24 h. Results were recorded as $+$ = inhibition, $-$ = inhibition with some overgrowth, $-$ = no inhibition. Ps. aeruginosa indicator strains were included in each test as well as the gonococcal strains CDC486 or CDC046, which were sensitive to most pyocines tested, and strain 879 which was resistant to most pyocines tested. The sensitivity of a gonococcal strain to each pyocine was examined at least three times. Preparations were assayed regularly to monitor levels of active pyocines. Those with low titres usually gave equivocal or negative results. Table III includes representative data of pyocine titres and sensitivities of sensitive strains CDC486, CDC046 and resistant strain 879.

Serotyping of meningococci and sulphanilazine sensitivity testing were done by Dr R. J. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Glasgow.

**Results**

**Preliminary studies**

None of the F-type pyocines listed (see Methods) inhibited any of 84 gonococcal strains against which they were tested in pilot studies. Several of the R-type pyocines were able to inhibit 94.5% (104/110) of the gonococcal
strains selectively. Inhibition was observed on clear GC, TM, or MNYC or media; the darker background of the TM and MNYC plates facilitated readings and these media were less subject to contamination. Addition of 1% soluble starch or 2% bovine serum albumin to the GC plates did not affect inhibition of gonococci by the pyocines.

Comparison of sensitivities of different indicators to the test pyocines

Two types of zones of inhibition corresponding to those found for soluble (S-type) and rod-type (R-type) pyocines were observed with the gonococci. The S-type zones resembled those found with soluble bacteriocines (Fredericq, 1957) or other inhibitory substances that diffuse readily into agar away from the site of inoculation. Large zones of this type were observed with crude lysates. Typical R-type zones—smaller, discrete zones of inhibition limited to the site of inoculation (Ito et al., 1970)—were found with the partially purified ultracentrifuged preparations. Table II illustrates the differences in inhibition produced by crude lysates and the partially purified pyocines. These were titrated against the appropriate Pseudomonas indicators and two gonococcal strains, one that had appeared to be resistant to pyocines tested in the pilot studies and one that appeared to be sensitive to many of the pyocines. Ultracentrifugation separated the heavier R-type particles from the S-type pyocines, pigments, and other soluble substances that had an inhibitory effect on the gonococci. The pyocines in table II were selected as examples of this effect because the partially purified pyocines of Kageyama groups R2 and R3 did not inhibit gonococci under the conditions used in the survey but the crude lysates did. These observations are of importance when our results are compared with those of other workers.

The concentration of the gonococcal inoculum usually made little difference to the inhibition observed with a series of twofold dilutions of pyocines up to 1 in 8192. Undiluted gonococcal inocula and tenfold dilutions up to 1 in 100

<table>
<thead>
<tr>
<th>R-type pyocines from</th>
<th>Titre of the stated pyocine, preparation in tests with</th>
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<tbody>
<tr>
<td></td>
<td>Pseudomonas indicator</td>
</tr>
<tr>
<td>Kageyama group</td>
<td>Crude pyocine</td>
</tr>
<tr>
<td>R2</td>
<td>ISB</td>
</tr>
<tr>
<td>R21</td>
<td>pp430</td>
</tr>
<tr>
<td>R3</td>
<td>ISE</td>
</tr>
</tbody>
</table>

*1 = Undiluted preparation; 0 = no inhibition.
were tested. A more dilute suspension occasionally gave a clearer zone of inhibition; strains were retested with diluted suspensions when only faint reactions (±) were obtained with undiluted preparations. The partially purified pyocine preparations were diluted in twofold steps to 1 in 8192 and each dilution was spotted on to appropriate plates for assay of inhibitory activity against a known standard sensitive strain of *Ps. aeruginosa*, a sensitive gonococcal strain and a resistant gonococcal strain. The titres of inhibitory activity for the pyocines of Kageyama groups R1, R2, R3, and R5 and the pyocine that is unclassified are outlined in table III.

### Table III

Comparative sensitivities of *Ps. aeruginosa* and *N. gonorrhoeae* to partially purified pyocines

<table>
<thead>
<tr>
<th>R-type pyocines from Kageyama group</th>
<th>Titre of the partially purified pyocine preparation in tests with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ps. aeruginosa</em> indicator</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>R1 IS4</td>
<td>8192</td>
</tr>
<tr>
<td>IS6</td>
<td>8192</td>
</tr>
<tr>
<td>IS8</td>
<td>8192</td>
</tr>
<tr>
<td>2285</td>
<td>8192</td>
</tr>
<tr>
<td>ISD</td>
<td>1024</td>
</tr>
<tr>
<td>R2 ISB</td>
<td>128</td>
</tr>
<tr>
<td>pp430</td>
<td>8192</td>
</tr>
<tr>
<td>R21</td>
<td>4096</td>
</tr>
<tr>
<td>R3 ISE</td>
<td>8192</td>
</tr>
<tr>
<td>R4</td>
<td>...</td>
</tr>
<tr>
<td>R5 ISE</td>
<td>8192</td>
</tr>
<tr>
<td>Unclassified R205</td>
<td>2048</td>
</tr>
</tbody>
</table>

Footnote as in table II.

**Group R1.** Preparations of pyocines in this group were potent in tests against the sensitive *Pseudomonas* indicator strain, and less potent against sensitive gonococci strain CDC486 or CDC046, usually by three dilution tubes. There was only a slight inhibition of the “resistant” strain 879 by the undiluted strain 2285 pyocine.

**Group R2.** Although the titres for pyocines pp430 and R21 on sensitive strains of *Ps. aeruginosa* were similar to those for pyocines for group R1, the sensitivity of gonococci to these pyocines was dramatically lower and not reliably reproducible. The titre for pyocine ISB determined with *Ps. aeruginosa* for this set of tests was lower than that of pp430 or R21 and no inhibition of gonococci was noted. Slight inhibition of the sensitive gonococcal strain was observed with another preparation of pyocine ISB that had a higher titre on the sensitive *Pseudomonas* indicator, but this was only with the undiluted pyocine.
Group R3. The only example of this group, pyocine ISE, yielded results similar to those observed with pyocines of group R2—high titres on tests against the sensitive Ps. aeruginosa indicator strain but little or no reproducible activity against gonococci tested.

Group R4. Our collection did not include a member of this group.

Group R5. Results obtained for pyocine ISC were similar to those found for pyocines of group R1—high titres on sensitive Ps. aeruginosa strain and slightly lower titres for sensitive gonococci.

Unclassified. Pyocine R205 was not classifiable in any of the Kageyama groups. Its titres on sensitive strains of Pseudomonas and gonococci were similar to those found for groups R1 and R5.

At the higher dilutions of each pyocine, areas of inhibition of growth became increasingly turbid. Morse et al. (1976) made similar observations and suggested that this was evidence for a non-replicating inhibitory substance. Not all strains were sensitive at the same dilutions of the pyocines; therefore a dilution of 1 in 2 was chosen for the screening procedure. This dilution would provide a large number of pyocine particles and correspond to the dilution used by Sidberry and Sadoff (1977).

Typing of Neisseria spp. with partially purified pyocine preparations

Isolates from different sites of individual patients as well as isolates from known consorts were tested for sensitivity to pyocines and antibiotics. A typical set of results is presented in table IV. Sensitivities to penicillin G, lincomycin, tetracycline, chloramphenicol, and spectinomycin were determined for each isolate with the same culture suspension that was used for determining pyocine sensitivities. These “matched” isolates usually had very similar pyocine patterns and identical patterns of sensitivities to the above antibiotics. We observed no correlation between patterns of pyocine sensitivities and sensitivity to any particular antibiotic.

In table V pyocine-sensitivity patterns for CDC and RIE strains are compared. There was a broader distribution of strains than that found by Sidberry and Sadoff (1977). Although there were differences in numbers per pyocine type in the two groups, there were only five small patterns—F, G, I, J and M—that were found among the CDC isolates but not the RIE ones.

Table VI presents pyocine-sensitivity patterns for strains from disse infections. There were no extraordinary sensitivities, e.g., to pyocines of Kageyama groups R2 or R3. Patterns of sensitivity were not associated with particular sites of isolation, penicillin sensitivity or auxotypes. The majority of strains from each of the three geographic regions had similar patterns.

Strains of Neisseria meningitidis from patients attending the Department of Venereology were also collected and several were tested for sensitivity to pyocines. Only two of those sent for typing were serotypable; strain 1042B was of serotype W135 and strain 1055A was of type Z'. The majority were serologically nontypable but were of different pyocine types. There were two patients from whom meningococci were isolated from genital sites. Strain 330B was
### Table IV

Antibiotic and pyocine sensitivities of isolates from different sites of individual patients

<table>
<thead>
<tr>
<th>Gonococcus isolate no.</th>
<th>Site</th>
<th>Inhibition by pyocines from producer strains</th>
<th>Minimal inhibitory concentration* of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ISD 2285 IS4 1S6 1S8 R205 1SE R21 1SB 430 1SC</td>
<td>Pn Lm Cm Tc Sp</td>
</tr>
<tr>
<td>1045A</td>
<td>Urethra</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 8</td>
</tr>
<tr>
<td>1045B</td>
<td>Cervix</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 8</td>
</tr>
<tr>
<td>1047A</td>
<td>Urethra</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td>1047B</td>
<td>Cervix</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td>1048A</td>
<td>Urethra</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td>1049A</td>
<td>Urethra</td>
<td>+ - - + + - - - +</td>
<td>0.125 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td>1049B</td>
<td>Cervix</td>
<td>+ - - + + - - - +</td>
<td>0.125 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>+ - - + + - - - +</td>
<td>0.125 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td>1051A</td>
<td>Urethra</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>+ - - + + - - - +</td>
<td>0.015 &gt;32 0.8 0.1 16</td>
</tr>
<tr>
<td>1063A</td>
<td>Urethra</td>
<td>+ + + + + - - - +</td>
<td>0.015 &gt;32 0.4 0.5 16</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>+ + + + + - - - +</td>
<td>0.015 &gt;32 0.4 0.5 16</td>
</tr>
<tr>
<td>1078A</td>
<td>Urethra</td>
<td>+ + + + + - - - +</td>
<td>0.015 &gt;32 0.4 0.25 16</td>
</tr>
<tr>
<td>1078B</td>
<td>Cervix</td>
<td>+ + + + + - - - +</td>
<td>0.015 &gt;32 0.4 0.25 16</td>
</tr>
</tbody>
</table>

+ = Inhibition; — = no inhibition.

* Minimal inhibitory concentration = μg/ml except Pn = units/ml. Pn = penicillin G, Lm = lincomycin, Cm = chloramphenicol, Tc = tetracycline, Sp = spectinomycin.
### Table V

**Distribution of pyocine typing groups of *N. gonorrhoeae***

<table>
<thead>
<tr>
<th><em>N. gonorrhoeae:</em></th>
<th>Inhibition by pyocines from Kageyama group</th>
<th>Number of CDC strains (% of all CDC strains)</th>
<th>Number of RIE strains (% of all RIE strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pyocine-sensitivity pattern</td>
<td>R1, strain</td>
<td>unclassified strain</td>
<td>R2, strain</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
<td>-------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ISD</td>
<td>2285</td>
<td>IS4</td>
<td>IS6</td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>I</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>J</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>K</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L</td>
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<td>+</td>
</tr>
<tr>
<td>M</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>O</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- = No inhibition; + = inhibition; ± = inhibition with some overgrowth. CDC = Center for Disease Control; RIE = Royal Infirmary Edinburgh.
### Table VI

**Pyocine typing of gonococci from disseminated infections**

<table>
<thead>
<tr>
<th>Auxotype</th>
<th>Source</th>
<th>Strain number</th>
<th>Site of isolation</th>
<th>Penicillin MIC (units/ml)</th>
<th>Inhibition by pyocines from Kageyama group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R1, strain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ISD</td>
</tr>
<tr>
<td>Protrophic</td>
<td>Seattle</td>
<td>1071</td>
<td>Cervix</td>
<td>0.5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Indianapolis</td>
<td>6302</td>
<td>Joint</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Atlanta</td>
<td>6354</td>
<td>Cervix</td>
<td>0.125</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7422</td>
<td>Urethra</td>
<td>0.03</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7423</td>
<td>Blood</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Seattle</td>
<td>1947</td>
<td>Joint</td>
<td>1.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Indianapolis</td>
<td>6355</td>
<td>Cervix</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Atlanta</td>
<td>7416</td>
<td>Cervix</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7425</td>
<td>Blood</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7432</td>
<td>Blood</td>
<td>0.06</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>7502</td>
<td>Blood</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7503</td>
<td>Joint</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td>pro⁻</td>
<td>Seattle</td>
<td>7406</td>
<td>Cervix</td>
<td>0.015</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Indianapolis</td>
<td>1567</td>
<td>Joint</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Atlanta</td>
<td>867</td>
<td>Joint</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td>arg⁻</td>
<td>Seattle</td>
<td>879</td>
<td>Cervix</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1241</td>
<td>Throat</td>
<td>0.015</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1255</td>
<td>Joint</td>
<td>0.015</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1351</td>
<td>Cervix</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1350</td>
<td>Urethra</td>
<td>0.015</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1362</td>
<td>Cervix</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1385</td>
<td>Blood</td>
<td>0.015</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1402</td>
<td>Blood</td>
<td>0.015</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1560</td>
<td>Blood</td>
<td>0.015</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Inhibition; - = no inhibition; ± = inhibition with some overgrowth.
TABLE VII

Sensitivity of *N. meningitidis* to *R*-type pyocines

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Strain number</th>
<th>Site</th>
<th>Sulphadiazine MIC (mg/litre)</th>
<th>Inhibition by pyocines from Kageyama group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R1, strain</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ISD</td>
</tr>
<tr>
<td>W135</td>
<td>1043B</td>
<td>Throat</td>
<td>5-10</td>
<td>-</td>
</tr>
<tr>
<td>Z'</td>
<td>1055A</td>
<td>Throat</td>
<td>1-5</td>
<td>+</td>
</tr>
<tr>
<td>Nontypable</td>
<td>339B</td>
<td>Cervix</td>
<td>1-5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1056A</td>
<td>Throat</td>
<td>1-5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1057A</td>
<td>Throat</td>
<td>0-1-1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1058A</td>
<td>Throat</td>
<td>0-1-1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1071A</td>
<td>Throat</td>
<td>0-1-1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1093A</td>
<td>Throat</td>
<td>1-5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1096A</td>
<td>Throat</td>
<td>1-5</td>
<td>+</td>
</tr>
<tr>
<td>9102A</td>
<td>Cervix</td>
<td>5-10</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>B</td>
<td>Urethra</td>
<td>1-5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>Rectum</td>
<td>1-5</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>D</td>
<td>Throat</td>
<td>1-5</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>

+ = Inhibition; - = no inhibition; ± = inhibition with some overgrowth.
found in a cervical culture and strains 9102 A, B, C, and D from cervix, urethra, rectum, and throat respectively. The genital isolates from these two patients were non-typable serologically and had different pyocine types. Three of the four isolates from patient 9102 had similar patterns of pyocine sensitivity and similar sensitivities to sulphadiazine (1–5 mg/ml). Strain 9102A was less sensitive to sulphadiazine (5–10 mg/ml) than the other three isolates and had a different pyocine-sensitive pattern (table VII). Further studies are in progress with a larger group of meningococci from different serological groups and sources.

**Discussion**

These results are additional evidence that tests for sensitivity of gonococci to R-type pyocines may provide a rapid, simple, inexpensive method for typing *N. gonorrhoeae* (Sidberry and Sadoff, 1977). Isolates from consorts or from different body sites of individual patients could generally be identified as being similar strains. Patterns of antibiotic sensitivities for these matched isolates agreed with the findings for pyocine sensitivities. Sensitivity to pyocines appears to be a stable characteristic. A fresh local isolate and a strain from a disseminated infection, both subcultured twice a week for over a year, have maintained their original patterns of sensitivity to the pyocines tested.

Preliminary results with organisms from several sources (CDC collection) and local isolates indicated that there is a broader distribution among the pyocine sensitivity types of gonococci observed than that found by Sidberry and Sadoff (1977) with their isolates from limited geographical locations. Using 23 pyocine lysates they found 30 types; but a majority of their strains 48% (51/106) fell into one group and 18% (19/106) into a second group. We found 15 types in tests with 11 pyocines and there are several more types to be defined with the additional pyocines being used in our present studies.

Table VIII presents an analysis of our results compared with those of Sidberry and Sadoff (1977). The analysis is based on the schematic representation of pyocine receptors in *P. aeruginosa* proposed by Kageyama (1975). It was observed in our study that if a gonococcal strain was sensitive to any of the pyocines, it was sensitive to ISC, a pyocine of Kageyama group R5; the gonococcal strains in both studies were most often sensitive to members of this group. Fewer strains were inhibited by pyocines of group R1 and the proportions of strains inhibited by this group of pyocines were similar in both studies. Our results differed in that we were unable to detect reproducible inhibition by R2 and R3 pyocines among the gonococcal strains tested. Crude lysates of R2 and R3 pyocines produced large S-type zones of inhibition on the “sensitive” (CDC486) and “resistant” (879) standard strains of gonococci, but the ultracentrifuged preparations did not inhibit either strain. Because the preparations used in Sidberry and Sadoff’s studies were equivalent to our crude lysates, some of the inhibition by R2 and R3 pyocines that they observed with a few gonococcal strains may have been due to the presence of material other than the R-type pyocines.
Table VIII

Inhibition of *N. gonorrhoeae* by pyocines of Kageyama groups R1-R5; comparison of results with those of Sidberry and Sadoff (1977)

| Percentage of *N. gonorrhoeae* isolates of indicated strain inhibited by pyocines of Kageyama group |
|--------------------------------------------------|--------------------------------------------------|
| R3* | R4* | R2* | R1* | R5* |
| I = 9.4 | H = 21.8 | D = 0.9 | K = 74 | A = 100 |
| F = 2.8 | L = 1.9 | B = 100 |
| J = 16.4 | M = 74.5 | C = 100 |
| U = . . . | N = 76.8 | E = 99.1 |
| | O = 71.7 | G = 99.1 |
| | | |
| (Sidberry and Sadoff, 1977) | T = 61.4 |

* Arranged in the order of the receptor sites on the lipopolysaccharide fraction of *Ps. aeruginosa* suggested by Kageyama (1975); the receptor for R5 is nearest to and that for R3 is furthest from the cytoplasm.

The receptor site for the R-type pyocines is in the lipopolysaccharide (LPS) (LPS) of *Pseudomonas* (Ikeda and Egami, 1973; Govan, 1974a; Koval and Meadow, 1977) and a similar location has been suggested for *Neisseria* (Sidberry and Sadoff, 1977). Table VIII is based on Kageyama’s (1975) proposed pyocine receptor sites for his groups R1–R5 in the LPS of *Pseudomonas*. The receptor for R5 is in the portion of the LPS nearest the cytoplasm. The other receptors, in order towards the most distal portions of the LPS, are R1, R2, R4, R3. The “R” numbers correspond to sites thought to be essential for sensitivity to each pyocine; e.g., mutants lacking the receptor for R3 were resistant to pyocines of the R3 group but sensitive to those of groups R1, R2, R4, and R5. If the receptors for R-type pyocines in *Pseudomonas* and in gonococci are in the LPS, this analysis suggests a similarity in the “deeper” portions of the LPS of *Neisseria* and *Pseudomonas*. Other workers have also found that colony types T1 and T4 of the same strain showed no difference in sensitivity to the pyocine R-type 611, 131 (Morse et al., 1976), indicating that pili probably do not form part of the receptor sites for the pyocines examined. The receptors for F-type pyocines are not found in the LPS fractions of *Pseudomonas* (Govan, 1974b) and, significantly, we did not find any inhibition of gonococci with the 10 F-type pyocines tested.

Gonococci from disseminated infections did not have unique pyocine sensitivities differing from those found for strains from localised infections.
The sensitivity of *Salmonella typhimurium* to some bacteriophages depends on the presence of receptor sites in the O repeat units of the LPS. Mutants that have lost these outer portions, rough or R-mutants, are less virulent than the smooth wild type. They are also insensitive to bacteriophages that attack the wild type because they lack the receptor (Rapin and Kalckar, 1971). If there were a unique portion in the outer LPS of the strains from disseminated gonococcal infection that resulted in serum resistance, or increased resistance to phagocytosis, it might be detectable with pyocines. Sensitivity was anticipated to pyocines of Kageyama groups R2 and/or R3, but no reproducible sensitivities were observed.

These findings are encouraging although further investigation with an emphasis on purification, standardisation and storage of the pyocine preparations used at present is clearly needed. Testing of other pyocines of the various Kageyama groups would be of value. Partially purified preparations of R2, R3 and R4 pyocines from other sources need to be examined to determine whether there are LPS receptors for these groups or whether the inhibitory effects reported by Sidberry and Sadoff (1977) were due to material other than the R-type pyocines. Pyocines that are "unclassified" by the Kageyama scheme may be useful because we have evidence for the selective inhibition of gonococci by pyocine R205 in the present study. In addition, selection of gonococcal indicator strains will be necessary.

Preliminary experiments indicate that this system may be able to differentiate strains of *Neisseria meningitidis* that are nongroupable by present serological methods.

**Summary**

Strains of *Neisseria gonorrhoeae* from a variety of sources were examined for sensitivity to 11 partially purified R-type pyocines from *Pseudomonas aeruginosa*. Selective inhibition of gonococci by pyocines of Kageyama groups R1 and R5 was observed. "Matched isolates", those from consorts or different body sites of individual patients, usually had very similar pyocine-sensitivity patterns and identical sensitivities to five antibiotics tested. This study included local isolates, strains from diverse geographic regions, and strains from disseminated gonococcal infections. It also proposed a relationship between pyocine-receptor sites in the lipopolysaccharide of *Ps. aeruginosa* and *N. gonorrhoeae*. Topics needing further evaluation are discussed.

We are grateful to Anne Lynch and Sheena Tuach for technical assistance and to Professor J. G. Collee and Dr J. R. W. Govan for advice in preparation of the manuscript. We also thank Professor B. P. Marmion for his encouragement and support of the project. This investigation was supported by grant K/MRS/50/C22 from the Biomedical Research Committee, Scottish Home and Health Department.

**References**


PYOCINE TYPING OF GONOCOCCI


Reprinted from *Medical Laboratory Sciences*

(1979) Volume 36, 275-281
Modern trends in the laboratory diagnosis of gonorrhoea*

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(Received August 1978)

The present status of laboratory diagnosis of gonorrhoea is reviewed. Methods of immediate diagnosis, transport of specimens to the laboratory, developments in selective media, and rapid identification techniques are discussed. The importance of laboratory surveillance of antibiotic sensitivity in order to detect \( \beta \)-lactamase-producing strains is highlighted. The culture and identification scheme used in Edinburgh is outlined and discussed. Limitations of gonococcal serology are noted and preliminary results of research using an enzyme linked immunosorbent assay (ELISA) to detect gonorrhoea in women are presented.

Introduction

The world incidence of gonorrhoea has increased dramatically in recent years. The World Health Organization (WHO) have estimated that on a global scale there were 60 million new cases of gonorrhoea per year in the early 1960s, and by 1974 this had risen to 200 million.\(^1\) This increase has created a considerable demand for simple, rapid, reliable and accurate diagnostic techniques. Also, in the light of modern methods of travel, rapid diagnosis of the disease has become of vital importance in controlling its spread throughout any community.

Obviously the most suitable test is one which can be performed whilst the patient is still present, thus providing an opportunity for immediate treatment. The Gram stain is the most commonly used immediate diagnostic method and, whilst it will detect up to 90% of males from whom urethral cultures will subsequently yield \textit{Neisseria gonorrhoeae}, its value is less in the female, where Gram staining of urethral and cervical secretions will detect only 60–65% of patients who give positive cultures. However, the Gram stained smear has an important role to play in immediate diagnosis. It is cheap, can be performed with a simple light microscope, and does not require highly trained personnel to achieve reasonable results.

It was hoped that the introduction of specific fluorescent antibody (FA) staining\(^2\) of secretions direct from the patient would provide more specific and more sensitive diagnosis than Gram staining, but these hopes have not been fulfilled.\(^3\) Whilst the specificity of FA staining depends on the character of the conjugate, its sensitivity depends upon the number of organisms present, and the distribution and depth of the specimen on the slide: it also requires a fluorescence microscope and more highly trained personnel. In a short comparative trial between FA and Gram staining performed in Edinburgh,\(^4\) the increase


0308-3616/79/030275+07 $01.00/0 © 1979 The Institute of Medical Laboratory Sciences
in sensitivity was small and any benefit in immediate diagnosis outweighed by the increased time required to make a reliable examination of such smears.

**Cultural Methods**

Since even the most efficient staining will not detect all cases of gonorrhoea, specimens from patients should also be cultured in order to provide a definitive diagnosis. Table 1 summarizes certain aspects of the culture and identification of *N. gonorrhoeae*. Unless the clinic is adjacent to the laboratory a transport medium will be required, and this can either be a non-nutrient holding medium (such as Amies' modification of Stuart's medium) or a selective growth transport system (such as Transgrow). Although Amies' medium allows better survival of gonococci than Stuart's medium, there is still a significant loss of cultures after 48 h.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Method</th>
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<tbody>
<tr>
<td>Transport</td>
<td>Direct plating</td>
</tr>
<tr>
<td></td>
<td>Amies transport medium</td>
</tr>
<tr>
<td></td>
<td>Transgrow etc.</td>
</tr>
<tr>
<td>Culture Medium</td>
<td>Non-selective: ‘chocolate’ agar</td>
</tr>
<tr>
<td></td>
<td>Selective: Thayer-Martin</td>
</tr>
<tr>
<td></td>
<td>(Vancomycin, colistin and nystatin)</td>
</tr>
<tr>
<td></td>
<td>Modified New York City</td>
</tr>
<tr>
<td></td>
<td>(lincomycin, colistin, amphotericin and trimethoprim)</td>
</tr>
<tr>
<td>Identification</td>
<td>Oxidase</td>
</tr>
<tr>
<td></td>
<td>Gram-smear</td>
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<td></td>
<td>Delayed immunofluorescence</td>
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<tr>
<td></td>
<td>Carbohydrate utilization</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>Disc</td>
</tr>
<tr>
<td>Sensitivities</td>
<td>Plate MIG</td>
</tr>
<tr>
<td></td>
<td>Test for β-lactamase</td>
</tr>
</tbody>
</table>

Originally, Transgrow medium gave satisfactory results for up to 96 h transportation, but different batches of commercially prepared media were found to give variable results. A recent development of this technique uses a plastic bag in which a biological environment is maintained using CO₂ generating tablets. This medium is intended both for transport and primary culture, and results so far are encouraging. A more recent variant of the technique uses squares of dehydrated medium in a plastic container, which are rehydrated with distilled water before use and then sealed in a pouch with a CO₂ tablet. Primary recognition of organisms is obtained using ‘oxidase’ strips. Such a technique, if available cheaply, could be of great value in screening large populations – particularly in developing countries where culture facilities and trained personnel may be lacking.

Obviously, the best results will be obtained when the specimen can be inoculated directly, at the bedside, on to a selective culture medium and incubated immediately at 36.5°C in an atmosphere containing 5–10% CO₂ and 65% humidity.

Media used for the culture of gonococci from clinical specimens are enriched non-selective media such as ‘chocolate agar’, and selective media such as Thayer-Martin (TM) medium and New York City (NYC) medium. Basic chocolate agar contains protein hydrolysate and certain growth supplements and, since it does not contain any antibiotics, it will grow a wide selection of organisms, making identification of Gram negative diplococci difficult.
The Thayer-Martin selective medium, formulated to grow only gonococci and meningococci, has increased the number of positive isolations from all sites and has also proved of particular value in isolating gonococci from heavily contaminated sites such as the rectum and pharynx. The antibiotics in the medium (vancomycin, colistin and nystatin) prevent other flora at the sampling site from overgrowing any gonococci present. Trimethoprim may also be added to prevent the spreading of Proteus species.

In 1973 a new selective medium, designated New York City – NYC – medium, was described which provided a luxuriant growth of pathogenic neisseriae after only 24 h incubation. Recently, culture results were compared in this laboratory using our standard TM medium and a simply prepared modification of NYC medium designated MNYC. MNYC medium is made with a commercial GC base, whereas the original NYC base was prepared from basic ingredients. Instead of enriching the base with horse plasma and haemoglobin solution prepared from fresh horse erythrocytes, 10% (v/v) human group O blood lysed with 0.5% saponin was used. The glucose content was reduced from 0.5% in the original NYC medium to 0.1% in MNYC medium, to permit carbohydrate utilization and penicillinase testing direct from the primary isolation plate.

Both TM and MNYC media incorporate commercially prepared GC agar base but, whereas TM medium uses heated blood, MNYC medium uses saponin-lysed blood. In addition, MNYC medium contains 2.5% (v/v) yeast dialysate and 0.1% glucose. The antibiotics used are also different (with the exception of colistin). In MNYC medium, lincomycin is used in place of vancomycin because it is less inhibitory to gonococci, and amphotericin is used in place of nystatin since it is more inhibitory to yeasts. Trimethoprim is also added to MNYC medium to reduce Proteus overgrowth.

In the trial of MNYC and TM media, TM medium detected 56 cases of gonorrhoea in men compared with 73 cases detected by MNYC medium, and 29 female cases compared with 42 by MNYC. On these results the MNYC medium was superior to TM medium and was introduced in this laboratory as routine diagnostic medium in 1977, with the results shown in Table 2.

Using MNYC medium the percentage of smear positive cases unconfirmed by culture was reduced by 12%, while the number of patients with negative smears but detected by culture increased by 11%.

Apart from increasing the total number of culture-positive cases, the use of MNYC medium resulted in the detection of a larger proportion of infected patients at their first visit to the clinic: using MNYC medium 98% of cases were detected at the first visit compared with only 89% when TM medium was used. Therefore, with MNYC medium

<table>
<thead>
<tr>
<th></th>
<th>1976 TM medium</th>
<th>1977 MNYC medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases of gonorrhoea in women diagnosed by microbiological tests</td>
<td>400 100</td>
<td>468 100</td>
</tr>
<tr>
<td>Culture positive/smear positive</td>
<td>236 59</td>
<td>280 60</td>
</tr>
<tr>
<td>Culture positive/smear negative</td>
<td>82 21</td>
<td>151 32</td>
</tr>
<tr>
<td>Culture positive/no smear</td>
<td>29 7</td>
<td>31 7</td>
</tr>
<tr>
<td>Culture negative/smear positive</td>
<td>53 13</td>
<td>6 1</td>
</tr>
</tbody>
</table>
only 2% of positive cases are not being detected at the first test, helping to reduce the reservoir of untreated cases in the population.

Rapid Identification Techniques

Once the gonococcus has been grown in the laboratory it must be positively identified. Ten years ago it often took up to one week to provide a positive report: nowadays, due to developments in selective media and rapid identification techniques, a positive identification can be made after 24 h. Delayed immunofluorescence and the rapid carbohydrate utilization test have an important part to play in rapid diagnosis.

Delayed immunofluorescence involves the making of a light suspension of a presumptive gonococcal colony (oxidase positive Gram-negative cocci) and drying it on to a glass slide. When stained with specific fluorescein-conjugated antibody and viewed under ultraviolet light the gonococci fluoresce bright green, whilst other Neisseria species do not fluoresce but may appear a dull green. This test has the advantage that it can be performed when very little growth is present on the plate. Although the FA test is very rapid, sugar utilization tests are required to identify those strains giving negative FA reactions. It is also prudent to confirm the identity of all throat isolates of gonococci.

The rapid carbohydrate utilization test (RCUT) measures a preformed enzyme and has many advantages over conventional sugar utilization tests. Most important is the saving in time, since the RCUT does not require overnight incubation; and the test can also be easily modified to investigate β-lactamase activity.

Routine Procedures

The scheme of culture and identification used in this laboratory (Fig. 1) is as follows:

Specimens are inoculated onto selective medium (MNYC) directly at the bedside, held in a CO₂ incubator, and sent to the laboratory for incubation within 4 h. The following day (day 1) the plates are examined for oxidase positive Gram negative cocci. Although a presumptive diagnosis of gonorrhoea can be made on the basis of such organisms growing on selective medium this is not entirely reliable, even in the case of genital isolates.

All cultures showing oxidase positive Gram negative cocci are then tested with fluorescent antibody and, if there is sufficient growth, also tested by RCUT and screened for penicillinase production. This test is performed using five tubes (70 × 10 mm): 20 µl of 10% (w/v) aqueous glucose, maltose, sucrose and ampicillin (250 mg/cm² in buffer salt solution) are added to four individual tubes, followed by 100 µl of buffer salt solution (BSS), comprising 5.2 mM K₂HPO₄/KH₂PO₄ buffer containing 0.8% KCl and 0.01% phenol red indicator, pH 7.1 to 7.15. If there is sufficient growth of presumptive gonococci on the primary isolation plate at 24 h this is harvested using a cotton wool swab and a thick suspension made in 300 µl BSS, in the fifth tube. Thirty µl of this suspension is added to each of the sugar tubes and to the ampicillin tube, and the tubes are shaken and incubated at 37°C in a waterbath. A positive result is normally available in 30–60 min and a definitive reading within 3 h. A yellow colour (occasionally yellow-orange) indicates a positive reaction; red is negative.

If there is insufficient growth at 24 h for RCUT, fluorescence can still be carried out but the primary isolate must be subcultured for RCUT and penicillinase testing the next day. The addition of two further tubes containing lactose and fructose enables this test to be used for the rapid identification of all Neisseria species: this is carried out in the case of FA negative and non-genital isolates of Gram negative cocci. The sugar solutions used must be especially pure as (for example) traces of glucose present in many commercial preparations of maltose can result in the mis-identification of gonococci as meningococci. All isolates of
Day 0  Specimen inoculated on to MNYC at bedside

overnight incubation (36.5°C; 10% CO₂)

Day 1 (24 h)  oxidase +
GNC  
Delayed FA

Report to clinician

RCUT  G M S Amp.  RCUT  G M S L F Amp.  (For identification of other Neisseria species.)

Day 2 (48 h)  Sensitivities

Fig. 1. Culture and identification scheme. Key: +, positive; –, negative; GNC, Gram negative cocci; G, glucose; M, maltose; S, sucrose; L, lactose; F, fructose; Amp., ampicillin.

gonococci are subcultured on to sensitivity test agar containing 10% (v/v) lysed human blood and antibiotic discs added for determination of sensitivity on day 2.

Cultures which are negative at 24 h are re-incubated for a further 24 h before reporting as such.

Using the above scheme the clinician should receive a positive report after 24 h since, even if there is insufficient growth for RCUT, a positive result can still be obtained on the basis of the FA test. There is no need to delay the report for antibiotic sensitivity since a standard regime (ampicillin and probenicid) is used by the clinic and penicillin resistance is rare. Antibiotic sensitivities are, of course, reported in all cases of treatment failure. It is important that antibiotic sensitivity tests – particularly of penicillin, are carried out as a routine as any increasing antibiotic resistance, as well as strains that may be totally resistant and likely to produce β-lactamase, will be demonstrated.

As outlined above, the RCUT now includes a tube containing ampicillin to detect β-lactamase activity. The number of reported isolates of β-lactamase-producing gonococci is increasing rapidly. At present such strains are not a problem in the United Kingdom but, because of their prevalence in other parts of the world, modern travel could easily result in their introduction into the country and constant screening for β-lactamase production is therefore of the utmost importance. By employing the method of detection described above all isolates of gonococci can easily be screened. Other methods of detecting β-lactamase are available, including the use of chromogenic cephalosporin, iodine-starch paper strips, and the seeding of cultures with penicillin-sensitive organisms.

Using the above isolation and identification scheme 89% of infected males and 87% of infected females can be reported as positive after 24 h on the basis of fluorescence, and more than half of these will also have been confirmed by RCUT and screened for penicillinase production. After 48 h all infected patients will have been reported as positive by fluorescence.
Although there have been considerable improvements in the cultural diagnosis of gonorrhoea, reliable detection or exclusion of infection by culture relies upon repeated testing of multiple sites, and this places a considerable demand on clinician, patient, and laboratory. A urethral culture will obviously detect most male cases, though the rectum and throat should also be cultured in the case of homosexuals. In females a cervical culture will similarly detect most, but not all, cases and here the urethra, rectum and, where indicated, throat should also be cultured. Screening with a single cervical culture would have detected 78% of cases in 1976 when TM medium was used, whereas 90% would have been detected in 1977 using MNYC medium. A single high vaginal swab will detect some 20% fewer cases than a cervical swab, and is not a suitable specimen for detecting or excluding gonococcal infection. The need exists for a simple, inexpensive, rapid, sensitive, and specific serological test which could be applied for routine screening particularly for asymptomatic female carriers, who form a large reservoir of infection within the community.

Serological Methods

In recent years, a number of serological tests have been described. These vary widely in sensitivity and all give some non-specific reactions owing to cross-reaction with other Neisseria species due to shared group antigens. These tests include complement fixation with various antigens, both whole cell and specific cell fractions, haemagglutination, immunofluorescence, and radioimmunoassay. At present, the only serological test in clinical use is gonococcal complement fixation (GCFT) – a test notoriously insensitive and non-specific, and not recommended for the detection or exclusion of uncomplicated gonococcal infection.

Recently the possibility of using Enzyme linked immunosorbent assay (ELISA) as a test has been investigated in this laboratory. ELISA depends on two assumptions: (i) that an antibody (Ab) or antigen (Ag) can be linked to an insoluble carrier surface and still retain its activity, and (ii) that an enzyme marker can be attached to an Ab or Ag with retention of both immunological and enzyme activity. In practice, both assumptions hold true. ELISA is similar to solid phase radioimmunoassay (RIA), the principle in both cases being the same, but whereas RIA uses a radio-labelled antiglobulin, ELISA uses an enzyme-linked antiglobulin. This has advantages of safer handling and fewer storage problems. In addition there is no need for an (expensive) radiation counter.

Most commonly, ELISA is used for the detection of antibody. In this technique an antigen is linked to a polystyrene surface – either a plastic microtitre plate or a tube. The sensitized surface ‘captures’ the corresponding antibody from the test serum, an enzyme linked antiglobulin attaches to the Ag complex, and this is detected by the enzyme changing the colour of an added substrate.

An ELISA technique has been investigated in this laboratory for the detection of gonorrhoea in women. Gonococcal pili, purified by the method of Robertson, Vincent and Ward, were used as antigen. The use of pili is not without problems since the yield of pure pili is very small (approximately 1 mg per 10 g wet weight of gonococci). The pili are coated on to the surface of 10 × 55 mm polystyrene tubes and incubated with patient’s serum. Alkaline phosphatase-conjugated anti-human IgG is used to estimate antibody binding to gonococci. (This reagent is available commercially, along with its corresponding substrate). The resulting colour change, from clear to yellow-green, is measured on a spectrophotometer at 400 nm. If the coating antigen is in excess the resulting optical density is directly proportional to the quantity of antibody present in the serum.

Pili were chosen as a source of antigen since the antibody response to pili should be specific, and hence decrease non-specific cross-reactions with group antigens. To date, results show no cross reactivity with serum from patients with other neisserial infections,
although a false positive rate of around 5% has been noted: these were patients with no history, nor clinical or laboratory evidence of gonorrhoea, though all in a "high risk" group. The false negative rate (at around 32%) was higher. It is possible that many of these patients had a recently acquired infection and their IgG response had not risen to a detectable level. This theory is supported by the fact that the majority of patients with false negative ELISA results but positive gonococcal cultures were ELISA-positive when retested four weeks later. Such patients are not a problem, since by their second attendance they have been diagnosed and treated. One problem common to most serological tests is that the result does not necessarily indicate a current infection and interpretation of the results in the light of clinical information and patient history is essential.

In conclusion, the false positive rate of 5% which has been found with ELISA is not a serious problem. If the technique could be made more sensitive it could prove of value in screening patients, those with positive ELISA results being referred for full cultural investigation. In terms of sensitivity it is possible that, since the antigen comprised pili from a single strain, it failed to detect antibody produced in patients infected with antigenically different strains. This possibility still requires investigation.

Thus, whilst serology does not negate the need for culture in gonococcal diagnosis, its use as a method of mass screening would help in controlling gonorrhoea in the population. Results to date indicate that ELISA may well provide the test that is so urgently required.

References
4. Unpublished data.
7. See Ref. 5.
13. See Ref. 12.
Antibodies to *Neisseria gonorrhoeae*: A Study of the Urethral Exudates of 232 Men

Alexander McMillan, Gillian McNeillage, and Hugh Young

The results of a study of the antibodies that react with *Neisseria gonorrhoeae* in the urethral exudates of 232 men who attended a clinic of the Department of Sexually Transmitted Diseases at Glasgow, Scotland, are presented. Of 132 men with gonorrhea, antibodies to the gonococcus were identified by use of an indirect immunofluorescent antibody test in 129 (98%); IgA antibody was found in all 129 (98%), IgG antibody in 119 (90%), and IgM antibody in 64 (49%). IgA antibody to the gonococcus was found in the secretions of only one (1%) of another 100 patients who did not have gonococcal urethritis (50 had nongonococcal urethritis and 50 had no evidence of urethritis). IgG antibody was found in 13 (26%) of the 50 men with nongonococcal urethritis and in none of the 50 men who had no evidence of urethritis. When treatment of gonorrhea was successful, the titers of IgA and IgM antibody declined rapidly and were generally undetectable within 14 days; the titers of IgG declined more slowly and could still be detected 28 days after treatment.

The immunological response to infection with *Neisseria gonorrhoeae* has been studied most often by the investigation of changes in serum antibodies that occur as a result of contact with a variety of gonococcal antigens [1-4]. The humoral antibody response that is detectable in the genital secretions has been examined by the use of precipitation reactions, HA tests, and indirect immunofluorescence techniques [5-7]. Kearns et al. [7] detected IgA antibodies to the gonococcus in the urethral exudates of more than four-fifths of men who were diagnosed as having gonococcal urethritis and further demonstrated that these antibodies were chiefly secretory IgA. Similarly, O'Reilly et al. [8] showed, in their study of women infected with *N. gonorrhoeae*, that specific IgA was present in cervicovaginal secretions.

The aims of the present study were to define further the method of production of secretory antibodies in males with gonorrhea and to evaluate the influence of treatment on this immunological response.

Materials and Methods

Patients. Two hundred thirty-two men attending a clinic at the Department of Sexually Transmitted Diseases in Glasgow participated in this study. We obtained a medical history from each patient, which included details of past infection with *N. gonorrhoeae*, whether his contact was a person known to be infected, and the duration of infection. Patients who had had antibiotic treatment in the month before attendance were not studied.

We took urethral cultures for *N. gonorrhoeae* from all patients, and, when there was a history of orogenital and/or anal intercourse, we obtained oropharyngeal and/or rectal cultures. The specimens were taken on charcoal-impregnated swabs and sent to the laboratory in Stuart's transport medium. The culture medium used was Columbia blood agar (Oxoid Ltd., Basingstoke, U.K.), which contains vancomycin (2.5 μg/ml), trimethoprim (3.0 μg/ml), and polymyxin (15 units/ml). The oxidase reaction, gram staining, and sugar utilization tests were used to confirm the identity of suspected colonies of *N. gonorrhoeae*.

A Gram-stained smear of urethral material was examined microscopically only if there was a urethral discharge. Patients with urethritis were treated on their first visit to the clinic. We obtained urethral cultures from patients who had
gonorrhea seven days after treatment, and again if symptoms recurred.

Collection of material for antibody studies. About 30 μl of exudate was collected from each patient who had a urethral discharge by the use of a disposable plastic inoculating loop (Nunc Products, Kamstrup, Denmark). The exudate was then suspended in 1 ml of sterile phosphate-buffered saline (PBS), pH 7.4. When a patient had no urethral discharge, secretions were collected by urethral washings. A 2-ml syringe that was attached to a sterile polythene tube (chromatography tubing; Pharmacia Fine Chemicals, Uppsala, Sweden) was used to instill 1 ml of PBS into the anterior urethra. After a wait of 1 min for equilibration, the washings were collected by drainage into a bijou bottle. In the determination of titers, this material was referred to as undiluted secretion. One drop of 0.1% NaN₃ in PBS was added to all secretions, which were stored at −20°C until required.

Scrum was separated from blood, which had been collected at the same time as the secretions, and was stored at −20°C without the addition of any preservative.

Secretions and sera were collected at the time of each patient’s initial visit. We obtained additional secretions and sera from men with gonorrhea seven, 14, and 28 days after treatment.

Immunoglobulin estimations. The concentrations of IgA and IgG were estimated in diluted secretions from 60 men with gonorrhea who attended the clinic consecutively, from 31 men with nongonococcal urethritis (NGU), and from 35 men with no evidence of urethritis. Commercially available, low-level immunodiffusion plates (Hoescht Pharmaceuticals, Hounslow, Middlesex, U.K.) were used in the estimation of the immunoglobulin concentrations by a secretory IgA standard, which was prepared from colostrum [9], and a serum IgG standard. IgM could not be estimated by radial immunodiffusion.

Antigens used in the indirect immunofluorescent antibody (IFA) test. Strain 9 of N. gonorrhoeae, as described by O'Reilly et al. [10], was used as a gonococcal antigen, for these workers demonstrated that this organism shares antigenic features with other strains of gonococci, but not with other species of Neisseria. Secretions from one man with epididymitis were also tested against the strain isolated from the urethra. Urethral secretions from 12 infected and 16 uninfected men who attended the clinic consecutively were examined for the presence of antibody to other Neisseria species (Neisseria lactamica, Neisseria perflava, Neisseria catarrhalis, and Neisseria meningitidis), Escherichia coli, Streptococcus faecalis, Staphylococcus aureus, and Staphylococcus albus.

Individual colonies of organisms that were grown on solid culture medium were suspended in PBS; the resulting suspension was diluted until it showed only slight turbidity. Aliquots were stored at −20°C.

Indirect IFA test. The indirect IFA test was performed as described by McMillan et al. [11]. Doubling dilutions of the secretions, previously centrifuged at 750 g for 10 min, were prepared and layered onto the bacterial preparation. After these preparations were incubated in a moist chamber at 37°C for 30 min and were washed twice in PBS for 10 min, fluorescein-labeled sheep antiserum to human IgM, IgA, or IgG (Wellcome Reagents Ltd., Beckenham, U.K.) diluted at 1:16 was added; the slides were maintained at 37°C for 30 min. The preparations were washed and mounted in buffered glycerol (Difco Laboratories, West Moseley, U.K.) and were examined with a Zeiss Large Universal Microscope (Zeiss, London, U.K.).

Fluorescence was graded according to the scheme used by Welch and O'Reilly [12]: 4+ = brilliant fluorescence of all organisms in the field; 3+ = well-defined fluorescence of all organisms; 2+ = low-intensity but definite fluorescence of at least 75% of organisms; and 1+ = low-intensity fluorescence of occasional organisms. Preparations that showed no or only faint fluorescence were graded unreactive.

For our study, only tests that showed ≥2+ fluorescence were considered positive. The titer was taken as the reciprocal of the highest dilution that gave a result of 2+.

All slides were read by an observer who was unaware of the diagnosis of the patients from whom secretions had been obtained.

Absorption with antiserum to human secretory component. Secretions were incubated with equal volumes of rabbit antiserum to human secretory component (Hoescht Pharmaceuticals) for 1 hr at 37°C and then overnight at 4°C. The solu-
tions were centrifuged at 2,000 g for 20 min; doubling dilutions were then prepared, and these were used in the IFA test. As a control experiment, secretions were incubated with normal rabbit serum and treated as described above.

**Statistical methods.** Student's t-test was used for comparison of mean immunoglobulin concentrations in patients with gonorrhea, those with NGU, and those with no urethritis. The same test was used for analysis of the difference between the mean ratio of antibody titer to concentration (T/C ratio) of IgA and IgG in the secretions of men who had previously been infected and the T/C ratio in secretions of those who had not been infected. The significance of qualitative differences in antigonococcal immunoglobulins at various times was tested by use of binomial probabilities.

**Results**

Of the 232 men investigated, urethral gonorrhea was diagnosed in 132. (Both gram smear and culture were positive in 130 cases; the culture alone was positive in two.) Three of these men were asymptomatic contacts of females with proven gonorrhea: they had no signs of urethritis even when they were examined after they had not passed urine for 8 hr. Both *N. gonorrhoeae* and *Chlamydia trachomatis* were isolated from the urethra of another man who had acute epididymitis. Fifty patients had NGU, and 50 had no evidence of urethritis but presented either with other conditions, such as warts or infestation with *Phthirius pubis*, or for routine examination.

**Immunoglobulin concentrations in secretions.**

Table 1 shows the mean concentration of IgA and IgG in the urethral secretions. There was no significant difference between the mean concentration of IgA in the secretions of men with gonorrhea and that in secretions of men with NGU (*P > 0.45*) or between that in men with gonorrhea and that in men with no clinical evidence of urethritis (*P > 0.45*). Although there was no significant difference between the mean concentration of IgG in patients with gonorrhea and that in those with NGU (*P > 0.3*), the mean concentration of IgG in the secretions of men with no urethritis was significantly lower (*P < 0.05*).

The concentration of IgA varied considerably from patient to patient, but the concentration of IgA for each patient did not change greatly (± 0.4 mg/100 ml) from week to week within 28 days of treatment. There was, however, a progressive decrease in the concentration of IgG.

**IFA test.** Of 132 men with gonorrhea, 129 (98%) had a detectable antibody response. Figure 1 illustrates the distribution of antibody according to types of immunoglobulins and the duration of infection.

Of 132 infected men, IgA antibody to *N. gonorrhoeae* was found in 129 (98%), IgM in 64 (49%), and IgG in 119 (90%). IgG and IgA were also detected in the secretions of each of the three asymptomatic infected contacts. IgM, IgA, and IgG antibodies to both the infecting strain and strain 9 of *N. gonorrhoeae* were found in the secretions of the patient with epididymitis.

IgA appeared within a few days of infection—it was detected in each of four men infected for one to three days and it was still detectable at 28 days.

**Table 1. Concentrations of IgA and IgG in the urethral secretions of patients with gonorrhea, of patients with nongonococcal urethritis (NGU), and of patients with no evidence of urethritis.**

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Mean (range) concentration of IgA, mg/100 ml</th>
<th>Mean (range) concentration of IgG, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>With gonorrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (60)</td>
<td>3.0 (&lt;1.2—4.4)</td>
<td>2.8 (&lt;0.5—4.8)</td>
</tr>
<tr>
<td>7 days after treatment (25)</td>
<td>2.7 (&lt;1.1—3.9)</td>
<td>1.7 (&lt;0.5—2.1)</td>
</tr>
<tr>
<td>14 days after treatment (22)</td>
<td>2.8 (&lt;1.3—3.7)</td>
<td>1.6 (&lt;0.5—2.0)</td>
</tr>
<tr>
<td>28 days after treatment (18)</td>
<td>2.6 (&lt;1.0—3.3)</td>
<td>1.2 (&lt;0.5—2.0)</td>
</tr>
<tr>
<td>With NGU (51)</td>
<td>2.9 (&lt;0.9—4.2)</td>
<td>2.7 (1.1—4.0)</td>
</tr>
<tr>
<td>With no clinical evidence of urethritis (33)*</td>
<td>2.4 (&lt;0.5—3.1)</td>
<td>1.7 (&lt;0.5—3.5)</td>
</tr>
</tbody>
</table>

*Cultures for Neisseria gonorrhoeae for these patients were negative.*
IgM was detected in 62 (57%) of 108 men who had been infected for $\leq 14$ days but in only two (8%) of 24 men who had been infected for $> 14$ days. This difference is statistically significant ($P < 0.05$).

IgG was detected in undiluted secretions of only 13 (26%) of 50 men with NGU, and IgA was found in only one of these men. In patients who had no evidence of urethritis and whose urethral cultures for Neisseria gonorrhoeae were negative, no antibodies were detected.

Thirty-three (25%) of the 132 men with gonorrhea had been treated in the past for gonorrhea—the mean interval between previous and present infections was 28 months (range, three months to seven years). There was no qualitative difference between the classes of immunoglobulins produced during a first infection and those produced during a repeat infection.

The T/C ratio, calculated as described by Waldman et al. [13], was used for quantitative comparison of the antibodies in men who had previously been infected with those in men who had not been infected before. Of the 60 patients with gonorrhea in whom the concentrations of IgA and IgG had been determined, 24 had been previously infected. The mean T/C ratio for IgA of previously uninfected patients was 2.22, and it was 1.89 in those patients who had had a prior infection. There was no significant difference between these ratios ($P > 0.3$). Similarly, there was no significant difference between the mean T/C ratios for these two groups of patients with respect to IgG (T/C, 1.74 in men with no history of past infection and 1.81 in those who had previously been infected; $P > 0.5$).

**Sequential studies following treatment.** Table 2 shows how rapidly the level of IgA decreased after treatment for the gonorrhea. Of the 48 patients who were examined 28 days after treatment, none had detectable IgA in his urethral secretions. The level of IgG declined more slowly: this antibody was still detectable in the secretions of 40 (85%) of these men 28 days after treatment. In addition, IgM could not be detected in the secretions of 70 men who were examined seven days after successful treatment.

In a period of seven days, there were no changes
Antibodies to *N. gonorrhoeae*

Table 2. Mean log titers of IgA and IgG in the urethral secretions of 132 men infected with *Neisseria gonorrhoeae* before and after they were successfully treated for gonorrhea.

<table>
<thead>
<tr>
<th>Interval from time of treatment (days)</th>
<th>No. of patients</th>
<th>Mean log titer of antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td>0</td>
<td>132</td>
<td>0.632</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>0.249</td>
</tr>
<tr>
<td>14</td>
<td>45</td>
<td>0.054</td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>0.000</td>
</tr>
<tr>
<td>28</td>
<td>48</td>
<td>0.000</td>
</tr>
</tbody>
</table>

in the distribution of antibody class or in the titer in the secretions of three men who did not respond to initial treatment.

Figure 2 illustrates the classes of antibodies to the gonococcus that were found in the urethral secretions of a man who was reininfected (twice) over an interval of 120 days.

Results of absorption of secretions with rabbit antiserum to human secretory component. Secretions from 15 men with gonorrhea were absorbed with normal rabbit serum and with antiserum to human secretory component. Treatment of the secretions with rabbit antiserum to human secretory component produced a fourfold decrease in the titer of IgA compared with the titer in secretions that were treated with normal rabbit serum.

Specificity of antibodies to *N. gonorrhoeae*. Examinations of the urethral exudates of 28 men (12 with gonorrhea and 16 uninfected) revealed the following results. IgA antibody to *N. meningitidis* was detected in the secretions of three of the 12 infected patients (the response was to group B in three men, to group E in two, to group 29E in one, and to group Z in two), and IgA antibody to *N. lactamica* in 11 of the 12 men. IgA antibody to the other groups of *N. meningitidis* that were tested or to *N. perflava* or *N. catarrhalis* was not detected.

IgG antibody to *N. meningitidis* was detected in the secretions of eight of the 12 infected patients (the response was to group B in three men,
to group E in two, to group 29E in one, and to group Z in two), that to N. lactamica in four of the 12, and that to N. catarrhalis in seven of the 12.

The 16 uninfected patients had no IgA antibody response to any species of Neisseria, but 10 of the 16 did have an IgG response to N. meningitidis, eight to N. lactamica, eight to N. catarrhalis, and 15 to N. perflava.

The urethral secretions of these 28 men had no antibody response to E. coli, S. faecalis, S. albus, and S. aureus.

Discussion

The finding of an IgA antibody that reacts with N. gonorrhoeae in the urethral secretions of 98% of men with gonorrhea is higher than the percentage reported by Kearns et al. [7], who detected this antibody in the secretions of 83% of infected men (29 of 35). That the IgA titer in the IFA test could be reduced by prior absorption with antiserum to secretory component suggests that the secretory IgA molecules are the ones that are chiefly involved in this reaction.

IgA antibody to the gonococcus appeared in the urethral secretions of these patients within a few days of acquisition of infection. In four cases in which infection had been present for less than four days, only this antibody was detected. Thus, this antibody appears in the serum early in infection, but, unlike titers of secretory IgA, titers decline when the infection has been present for more than 14 days [11].

IgG antibody to the gonococcus appeared in the urethral exudates slightly later than IgA—there was a significantly greater chance of detecting this antibody when the infection had been present for more than seven days. The source of this antibody is not yet known, but it is possible that the majority of IgG in the secretions is derived from non-specific transudation through inflamed mucous membranes, a mechanism similar to that producing increased levels of IgG in whole saliva in periodontitis [14]. This mechanism may also explain the finding of low levels of IgG antibody to the gonococcus in the secretions of patients with NGU, as natural antibody of this class can be detected in the sera of uninfected patients [1].

Most of the IgM antibody, which was detected in about 50% of the infected men, was produced during the first 14 days of infection.

There was no difference in the clinical presentation of the three men who had no detectable antibody to the gonococcus. The total concentrations of IgA and IgG in the secretions in each case exceeded 3.5 mg/dl. A possible explanation may be that antibody produced against the infecting strain did not cross-react with strain 9.

When the treatment for gonorrhea was successful, in most cases IgM and IgA antibodies to the gonococcus could not be detected in the secretions within two weeks of treatment. A more gradual decline in IgG reactivity was observed, as this antibody was detected in > 75% of the men 28 days after treatment. No decline in antibody activity was noted in three patients who were regarded as therapeutic failures.

The pattern of rapid appearance of IgA antibody to the gonococcus in the secretions after acquisition of infection, followed by the rapid decline in antibody activity after successful treatment, may be a means by which to assess a patient's response to therapy.

Three men in the present study were asymptomatic contacts of women with gonorrhea. There was no apparent difference between the antibody distribution in the secretions of these men and that in the secretions of those who had symptoms. Tramont [15] demonstrated lack of specific IgA antibody to homologous strains of the organism from two women with pelvic inflammatory disease, although secretions from one woman had IgA antibody reactive with strain 9. It was postulated that ascending infection might result from lack of a strain-specific immune response. The patient with acute epididymitis in our study, however, had detectable IgM, IgA, and IgG antibody to both the infecting strain and strain 9 of N. gonorrhoeae. It is possible that concomitant infection with C. trachomatis was important in the pathogenesis of epididymitis in this man [16].

The presence of IgA antibody to the gonococcus, which appeared in response to strain 9 of N. gonorrhoeae, in the secretions of patients was a reliable indicator of gonococcal infection. However, IgA antibody did cross-react, to a major extent, with N. lactamica and, to a lesser extent, with N. meningitidis when these organisms were used as antigen. (Cross-reaction with N. lactamica may
Antibodies to N. gonorrhoeae

be partly explained by the similar sugar composition of the lipopolysaccharide core oligosaccharides [17].

References

Serum immunoglobulin response in uncomplicated gonorrhoea

BY

ALEXANDER McMILLAN, GILLIAN McNEILLAGE, HUGH YOUNG AND SHEILA R. BAIN

Reprinted from British Journal of Venereal Diseases, Vol. 55, No. 1, February, 1979
pp. 5-9

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Serum immunoglobulin response in uncomplicated gonorrhoea

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From the Department of Sexually Transmitted Diseases, Glasgow, and the Departments of †Venereology and §Bacteriology, University of Edinburgh, Scotland

SUMMARY Sera from 225 men and 140 women were examined by an indirect immunofluorescent antibody technique for antibody reactive with Neisseria gonorrhoeae. Antigonnococcal IgM was demonstrated at a titre of >16 in about 45% of infected, but in only 3% of non-infected, patients. Most of this antibody occurred in sera of patients who had been infected for less than 14 days. Antibody of the IgA class was found at a titre of >16 in over half the infected, but in none of the non-infected, patients. IgG antibody reactive with the gonococcus was found in each infected patient at a titre of >16 but in only 8% of controls. The mean log titre of this antibody was significantly higher in patients who had been infected for more than seven days than in those whose infection was of shorter duration.

Introduction

Although gonorrhoea is an infection usually confined to the mucosal surfaces of the body, a systemic antibody response may be demonstrated by various methods such as complement-fixation tests, haemagglutination reactions, and indirect immunofluorescent antibody techniques (Ratnatunga, 1971; Ward and Glynn, 1972; Welch and O'Reilly, 1973). The classes of antibody involved in this response have not, however, been clearly defined other than under experimental conditions (Cohen et al., 1969).

It is the purpose of this paper to record the classes of immunoglobulins reactive with Neisseria gonorrhoeae which were found in the serum of patients with naturally acquired uncomplicated infection and to examine the effect of treatment.

Material and method

PATIENTS

Sera were obtained from patients (225 men and 140 women) attending the department of venereology at the Black Street Clinic, Glasgow.

Urethral gonorrhoea in men was diagnosed by microscopical examination of a Gram-stained smear

and confirmed by culture on selective medium

The identity of suspected colonies was confirmed by direct immunofluorescence and sugar fermentation reactions. Material from the urethra was taken for culture from all the male patients and where there had been homosexual contact specimens from the oropharynx and anorectal area were also taken for culture.

Gram-stained smears of urethral and cervical secretions were examined and culture specimens taken from these sites and from the oropharynx and anorectum in all female patients. Gonococcal infection was excluded only if three sets of cultures taken at weekly intervals from these sites gave negative results.

Neither those patients who had had antimicrobial treatment within the six weeks preceding attendance at the clinic nor those with complicated infection were included in the study.

ANTIGEN PREPARATIONS

As gonococcal antigen, strain 9 of N. gonorrhoeae as described by O'Reilly et al. (1973) was used (kindly supplied by Dr D. S. Kellogg, US Center for Disease Control, Atlanta), as it had been shown to contain antigenic characteristics which were common to a variety of gonococcal strains but not shared by other neisseriae. The organism was cultured on a selective medium (Young, 1978). Cultures of Neisseria meningitidis, Neisseria periavai, Neisseria lactamica, and Neisseria catarrhalis for use in
control studies were identified by fermentation reactions. They were obtained from stock cultures held in the Department of Microbiology at the University of Edinburgh.

Colonies were suspended in phosphate-buffered saline (PBS), pH 7.4, and the suspensions diluted until the fluid was faintly turbid. Aliquots were stored at −20°C until required.

A drop of suspension was placed in each well of a Multidot slide (Hendley, Essex, UK) and dried by incubation at 37°C for 10 minutes.

**INDIRECT IMMUNOFLORESCENT TEST**
A standard indirect immunofluorescent technique (IF test) was used (Johnson et al., 1978). Fluorescein-conjugated sheep anti-human IgA, IgG, and IgM sera were obtained from commercial sources (Wellcome Reagents, UK) and their specificity confirmed by gel immunodiffusion.

Doubling dilutions of sera, previously inactivated by heating at 56°C for 30 minutes, were prepared and layered on to the prepared slides. After the slides had been incubated at 37°C for 30 minutes and washed in PBS, conjugated antiserum at a dilution of 1/16 was added, and the slides maintained at 37°C for 30 minutes. The slides were then thoroughly washed in PBS and mounted in buffered glycerol (Difco).

Preparations were examined with a Zeiss microscope (Large Universal). After being scanned with a low power objective detail was examined with a ×100 oil immersion lens.

Fluorescence was graded according to the system used by Welch and O'Reilly (1973) as follows: 4+ indicated brilliant fluorescence of all organisms; 3+, well-defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

Statistical comparisons were made by the method of binomial probabilities and Student's t test.

**Results**

**UNTREATED PATIENTS**

Tables 1 and 2 show the results obtained in the indirect immunofluorescent antibody test applied to the serum from infected and non-infected men and women.

**IgM**

A titre of ≥16 of IgM reactive with *N. gonorrhoeae* was found in 44.8% (56/125) of men with untreated gonorrhoea but in only 3% (3/100) of non-infected men (p<0.001 by the method of binomial probabilities). Similarly in 45.7% (32/70) of infected women and in 2.9% (2/70) of non-infected women antigonococcal antibody of this class was found at a titre of ≥16 (p<0.001).

| Table 1 Immunoglobulin classes of antibody against *Neisseria gonorrhoeae* in sera from men with untreated gonorrhoea and from non-infected patients |
|---|---|---|---|---|---|---|---|---|---|
| Patients | No. of sera | IgM titre | 8 | 16 | 32 | 64 | 128 |
| Infected | 1-7 days | 52 | 19 | 19 | 9 | 5 | 0 |
| 8-14 days | 45 | 25 | 11 | 8 | 1 | 0 |
| 15-21 days | 14 | 11 | 3 | 0 | 0 | 0 |
| >29 days | 12 | 12 | 0 | 0 | 0 | 0 |
| Non-infected | 100 | 97 | 3 | 0 | 0 | 0 |

| Table 2 Immunoglobulin classes of antibody against *Neisseria gonorrhoeae* in sera from women with untreated gonorrhoea and from non-infected patients |
|---|---|---|---|---|---|---|---|---|---|
| Patients | No. of sera | IgM titre | 8 | 16 | 32 | 64 | 128 |
| Infected | 1-7 days | 15 | 4 | 5 | 6 | 0 | 0 |
| 8-14 days | 24 | 7 | 7 | 8 | 2 | 0 |
| 15-21 days | 19 | 15 | 4 | 7 | 2 | 0 |
| 22-28 days | 6 | 6 | 0 | 0 | 0 | 0 |
| >29 days | 6 | 6 | 0 | 0 | 0 | 0 |
| Non-infected | 70 | 68 | 2 | 0 | 0 | 0 |
In 54.6% (53/97) of men who had been infected for 14 days or less but in 10.7% (3/28) who had been infected for longer the titre of antigonococcal IgM exceeded 8 (p<0.05). The titre of IgM antibody was >16 in 71.8% (28/39) of women infected for less than 14 days but in only 12.9% (4/31) of women whose infection was of a longer duration.

IgA

Antibody of the IgA class reactive with the gonococcus was found at a titre of >16 in 51.2% (64/125) of men and in 55.7% (39/70) of women. Patients who were not infected (100 men and 70 women) had serum titres of <8 (p<0.001).

When the infection had been present for 14 days or less antigonococcal IgA was found (at a titre of >16) in the serum of 62.9% (61/97) of men but in only 10.7% (3/28) of men who had been infected for longer (p<0.05). Similarly, IgA antibody was found at a titre of >16 (p<0.05) in 74.3% (29/39) of women who had had gonorrhoea for 14 days or less but in only 32.3% (10/31) of women whose infection was of longer duration.

IgG

Antibody of the IgG class reactive with the gonococcus was found at a titre of >16 in the serum of all the 125 (100%) men with untreated gonorrhoea but in only 8% (8/100) of men who were not infected (p<0.001). In women, this antibody was detected at a titre of >16 in the serum of 98.6% (69/70) of infected patients and in 5.7% (4/70) of non-infected patients (p<0.001).

The arithmetic mean of the log titre of IgG reactive with N. gonorrhoeae in the serum of men infected for seven days or less was 1.7830 and in patients who had been infected longer it was 2.0289. This is a statistically significant difference (p<0.001 by Student's t test). Similarly, the arithmetic mean log titre of IgG antibody in women was 1.4249 and 2.4903 in the serum of women infected for seven days or less and for eight days or more respectively (p<0.001).

Naturally occurring IgA reactive with other neisseriae

Table 3 shows the results obtained in the indirect immunofluorescent antibody test with monospecific IgA when applied to sera diluted to 1/16 from patients with gonorrhoea and from non-infected controls.

**EFFECT OF TREATMENT ON CLASSES OF ANTIBODY**

Table 4 shows the arithmetic mean log titre of antigonococcal antibodies before and at intervals after treatment. The decline in titre of antibodies of the IgM and IgA classes is shown as well as the much more gradual fall in IgG titre.

**Discussion**

Although indirect immunofluorescent antibody techniques using various antigen preparations have been evaluated as diagnostic tests (Welch and O'Reilly, 1973; Rodas and Ronald, 1974), there have been few studies correlating the distribution

---

**Table 3 Results of the indirect immunofluorescent antibody test with monospecific anti-human IgA on sera of patients with gonorrhoea and of non-infected controls**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Patients' case no. (and sex)</th>
<th>Infected</th>
<th>Non-Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. gonorrhoeae</td>
<td>4+ (M) 4+ (M) 4+ (M) 4+ (F) 4+ (M) 4+ (M)</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>group A</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>group B</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>group C</td>
<td>2+ 2+ 2+ 2+ 2+ 2+</td>
<td>— — — 1+ — —</td>
<td>— — 2+ 1+ — —</td>
</tr>
<tr>
<td>group D</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>group E</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>group 29E</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>group W135</td>
<td>— 1+ 1+ 1+ 1+ 1+</td>
<td>— — 1+ 1+ — —</td>
<td>— 1+ 1+ 1+ 2+ 1+</td>
</tr>
<tr>
<td>group X</td>
<td>— — — 1+ 1+ 1+</td>
<td>— — — 1+ — —</td>
<td>— — — 1+ 1+ 2+</td>
</tr>
<tr>
<td>group Z</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
<td>— — — — — —</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>2+ 2+ 2+ 2+ 2+ 2+</td>
<td>1+ 2+ 2+ 2+ 2+ 2+</td>
<td>1+ 1+ 1+ 2+ 1+ 2+</td>
</tr>
<tr>
<td>N. perflava</td>
<td>1+ 1+ 1+ 1+ 1+ 1+</td>
<td>2+ 2+ 2+ 2+ 2+ 2+</td>
<td>1+ 1+ 1+ 1+ 1+ 1+</td>
</tr>
<tr>
<td>N. catarrhalis</td>
<td>1+ 1+ 1+ 1+ 1+ 1+</td>
<td>2+ 2+ 2+ 2+ 2+ 2+</td>
<td>1+ 1+ 1+ 1+ 1+ 1+</td>
</tr>
</tbody>
</table>

1+ Occasional fluorescing organisms
2+ Low intensity fluorescence of all organisms
3+ Well-defined fluorescence of all organisms
4+ Brilliant fluorescence of all organisms
of antigonococcal antibodies within the immunoglobulin classes with the duration of infection.

In the present study antibody of the IgM class reactive with N. gonorrhoeae was detected at a titre of >16 in 45% of infected men and in 46% of infected women before treatment. The results obtained in women were similar to those recorded by Wilkinson (1975), who found this class of antigonococcal antibody at a titre of >16 in 43% of infected patients. He found similar antibody in only 32% of infected men; the duration of infection was not recorded.

IgM antibody was detectable in the serum of more than half of the men and of two-thirds of the women with untreated gonorrhoea who had been infected for less than two weeks. When infection was of longer duration this antibody was less commonly found at the lowest dilution of serum examined. This early IgM response was observed under experimental conditions by Cohen et al. (1969), who also showed that even without treatment those subjects who developed antigonococcal IgM had titres of this antibody which gradually fell over a period of about four months. Few patients in the present study who had been infected for more than 14 days had titres of antibody of >16. Cohen et al. (1969), however, used inocula of organisms which were much larger than those which would be acquired in natural infection, and this may partly explain the more gradual decline in IgM activity which they had observed.

The antigonococcal IgA response to infection was similar to that of IgM. Antibody of this class reactive with N. gonorrhoeae was found in the serum of about two-thirds of men and of three-quarters of women who had been infected for less than 14 days. When the infection was of longer duration only about 11% of men and 32% of women had this antibody in the serum at a dilution of 1/16 or greater. Similar findings of a rapid decline in serum IgA antibody activity within two to three weeks of acquisition of infection were reported by Cohen et al. (1969). The relationship between production of antigonococcal secretory IgA in the mucous membranes and the detection of serum IgA antibody will be dealt with elsewhere.

No naturally occurring IgA reactive with N. gonorrhoeae was detected, although natural antibody against N. perflava and N. catarrhalis was found in infected and non-infected patients as was, less commonly, antibody against N. lactamica and N. meningitidis, groups D, W135, and X.

Antigonococcal antibody of the IgG class was found in the serum at a dilution of 1/16 or greater of all infected men and of almost all infected women. The presumed false-positive rates were 8% and 6% respectively for men and women. In one study, where heat-labile antigen and antihuman IgG were used in a fluorescent antibody test, the false-positive rate was lower as was the sensitivity (Gaafar and D'Arcangelis, 1976).

IgG antibody activity increased throughout the first week of infection, so that by the second week most patients had antibody at high titres (>64). No obvious decline in antibody activity occurred in the subsequent two to three weeks. These results agree with those obtained by Cohen et al. (1969).

Successful treatment of infection in both sexes resulted in a rapid decline in antigonococcal IgM and IgA activity. A much more gradual decline in IgG antibody activity occurred. This presumably reflects differences in the half-life of the antibody classes (IgM, five days; IgA, six days; and IgG, 22 days [Tomasi, 1976]). This gradual decline in IgG antibody activity makes interpretation of diagnostic immunofluorescent test results difficult if the patient has recently been treated for gonorrhoea.

This research project was supported by a grant from the Scottish Home and Health Department (Research Grant no. K/MRS/36/C38), whose financial assistance is gratefully acknowledged.

References


Serum immunoglobulin response in uncomplicated gonorrhoea


Secretory antibody response of the cervix to infection with *Neisseria gonorrhoeae*

BY

A. McMillan, G. McNeillage, H. Young, and S. R. R. Bain

Reprinted from British Journal of Venereal Diseases, Vol. 55, No. 4, August, 1979
pp. 265-270
Secretary antibody response of the cervix to infection with *Neisseria gonorrhoeae*

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SUMMARY Cervical secretions from 157 women were examined for antibody against *Neisseria gonorrhoeae* by an indirect immunofluorescent antibody test. Antigonnococcal antibody was detected in 73 (97%) of 75 infected women, being of the IgG class in 73 (97%), of the IgA class in 71 (95%), and of the IgM class in 29 (39%). IgM antibody was nearly always associated with infections of less than 15 days' duration. Immunoglobulin G, reactive with *N. gonorrhoeae*, was found in 23 (33%) of 70 non-infected women; of these, 19 had non-gonococcal cervicitis. Neither IgA nor IgM antibodies were detected in these women. Antigonnococcal IgA and IgG was found in each of 12 women who had no evidence of infection but were contacts of infected men. Successful treatment resulted in a rapid decline in IgA antibody activity but a more gradual decrease in IgG reactivity.

**Introduction**

Although there has been considerable interest recently in the local immune system of the female reproductive tract there have been few reports relating to infection with *Neisseria gonorrhoeae* (Tapchaisri and Sirisinha, 1976; Tramont, 1977). Antibody-containing plasma cells are found principally in the lamina propria of the endocervix, there being relatively few cells in the endometrium or uterine tubes (Rebello et al., 1975). Immunoglobulin A is the predominant antibody class elaborated by these cells, whose numbers in the endocervix have been shown to increase during infection (Chipperfield and Evans, 1972).

Quantitatively, IgG is the principal immunoglobulin detectable in the cervical secretions (Tjokronegoro and Sirisinha, 1975), the ratio of the concentration of IgG to that of secretory IgA being about 2:1. Ogra and Ogra (1973) demonstrated, however, that the antibody response of the lower reproductive tract to topically applied inactivated poliovirus was associated with IgA, and most studies of the immune response of the genital tract to infection have mainly concerned this class of antibody (Waldman et al., 1971; Ackers et al., 1975; O'Reilly et al., 1976).

This paper reports our findings on the local immune response to infection with *N. gonorrhoeae*.

**Materials and methods**

**PATIENTS AND DIAGNOSTIC TECHNIQUES**

One hundred and fifty seven women were investigated; 92 attended the Department of Sexually Transmitted Diseases at the Black Street clinic, Glasgow, and 65 attended the Department of Venereology at the Royal Infirmary, Edinburgh.

At both centres a Gram-stained smear of material from the urethra and cervix was examined, and culture specimens were taken from these sites and from the anorectum and oropharynx. Infection was excluded only if three sets of culture specimens taken at weekly intervals from these sites gave negative results.

Material for culture from patients attending the Glasgow clinic was taken on charcoal-impregnated swabs and transported to the laboratory in Stuart's transport medium. The culture medium used was Columbia blood agar (Oxoid) containing vancomycin (2·5 µg per ml), trimethoprim (3·0 µg per ml), and polymyxin (15 units per ml). The mean interval between specimen collection and inoculation on the culture medium was nine hours (range 4-16 hours).

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Received for publication 30 November 1978
In the Edinburgh clinic, modified New York City (MNYC) medium (Young, 1978) was inoculated directly and held at 36°C until transfer to the laboratory (mean time interval 2-1 hours, range 30 minutes to 3 hours).

At both centres the identity of suspected colonies of \textit{N. gonorrhoeae} was confirmed by the oxidase reaction, sugar utilisation tests, and direct immunofluorescence.

Patients were divided into three groups.

\textbf{Group 1}

This consisted of 75 women from whom \textit{N. gonorrhoeae} was cultured from at least one site. The mean age of this group was 23-4 years, and the mean number of sexual partners within the preceding three months was 1-6. Eight women had previously been infected with \textit{N. gonorrhoeae}, the mean interval between the presenting and recent infection being 2-7 years (range three months to six years). As a contraceptive method, 58 used an oral oestrogen-progestogen preparation, three had an intrauterine device fitted, and 14 took no contraceptive precautions.

At the time of the initial visit 39 women were in the first half of the menstrual cycle and 36 in the second half.

Two women had acute Bartholinitis and three acute salpingitis. Patients with uncomplicated gonorrhoea were treated in Glasgow with minocycline in a single dose of 300 mg, and in Edinburgh with ampicillin (2 g) and probenecid (1 g) given orally as a singe dose. Complicated infection was treated with doxycycline given orally in a dosage of 100 mg every eight hours for 14 days.

Efficacy of treatment was assessed by microbiological examination of material obtained from the urethra, cervix, rectum, and, if indicated, oropharynx. Cure was assumed only if three sets of tests performed at weekly intervals gave negative results.

No treatment failures were observed during the period of the study.

\textbf{Group 2}

Seventy women (34 in Glasgow and 36 in Edinburgh) who had no evidence of gonorrhoea were studied. These women were not known sexual contacts of infected men, had no past history of infection, and had not received antimicrobial therapy within the preceding three months.

The mean age of this group was 23-6 years and the mean number of sexual partners within the preceding three months was 1-8. Fifty-two used oral contraception, two had been fitted with an intrauterine device, and 16 took no contraceptive precautions.

Thirty-seven women were in the first half of the menstrual cycle at their initial visit and 33 in the second half of the cycle.

Twenty-two women had clinical evidence of cervicitis; the cervix was congested and tender on palpation, and there was a marked purulent or mucopurulent discharge from the os.

\textbf{Group 3}

Twelve women attended (seven in Glasgow and five in Edinburgh) as named sexual contacts of men with culturally proved urethral gonorrhoea. There was, however, no evidence of gonococcal infection. These women had not received antimicrobial treatment within the preceding three months.

\textbf{COLLECTION OF SPECIMENS}

Cervical secretions were obtained by gentle aspiration through a sterile polythene capillary tube (chromatography column tubing, internal diameter 1-0 mm, obtained from Pharmacia Fine Chemicals, Upplands, Sweden) attached to a 5-ml syringe containing 1 ml of sterile physiological saline. Secretions in the tubing were ejected into a sterile container and a drop of 0·1% sodium azide in saline added. Specimens obviously contaminated with blood or giving a positive reaction with Haemastix strips (Ames Co. Ltd, Slough, Bucks) were discarded. The diluted secretions were centrifuged at 400 × g for 20 minutes, and the supernatant stored at −20°C until required.

Cervical secretions were obtained from each patient at the initial clinic visit and, in the case of those infected with gonorrhoea, at seven, 14, and 28 days following treatment. In addition to the initial specimen, secretions from non-infected patients were again sampled 14 days later.

Serum from each patient was obtained at the same time as the cervical secretions.

\textbf{QUANTITATION OF SECRETORY IgA AND IgG}

The concentrations of these immunoglobulins were estimated by radial immunodiffusion using commercially available low-level plates (Hoescht Pharmaceuticals, Hounslow, Middlesex). A secretory IgA standard, prepared from colostrum (Samson \textit{et al.}, 1973) and kindly provided by Dr Brian McClelland (Blood Transfusion Service, Royal Infirmary, Edinburgh) was used in estimating IgA, and a serum IgG standard was used in the determination of IgG.

\textbf{GONOCOCCAL ANTIGENS}

Strain 9 of \textit{N. gonorrhoeae} as described by O'Reilly
et al. (1973), and kindly supplied by Dr D. S. Kellogg (Center for Disease Control, Atlanta, Georgia, USA) and cultured on MNYC medium was used as gonococcal antigen. In addition, secretions from eight women with gonorrhoea were tested against the homologous strain of the organism.

To test the specificity of the antibody reactivity, secretions were tested against Neisseria meningitidis, Neisseria perflava, Neisseria catarrhalis, Neisseria lactamica, Staphylococcus aureus, Staphylococcus albus, Streptococcus faecalis, and Escherichia coli, cultures of these organisms being obtained from the stock collection of cultures at the Department of Bacteriology at the University of Edinburgh Medical School.

**INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST**

The performance of the IFA test has previously been described (McMillan et al., 1979). Doubling dilutions of secretions were tested against strain 9 of *N. gonorrhoeae*.

The system of grading fluorescence described by Welch and O'Reily (1973) was used: 4+ indicates brilliant fluorescence of all organisms; 3+, well-defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms: and +, occasional fluorescing organisms.

For the purpose of this study 'undiluted secretions' refers to the supernatant fluid obtained after centrifugation of the suspension of cervical aspirate in saline. The titre was taken as the reciprocal of the highest dilution giving a 2+ result.

**ADSORPTION OF SECRETIONS WITH RABBIT ANTI-HUMAN SECRETORY COMPONENT**

Secretions were adsorbed with serum from a rabbit immunised against human secretory component (Hoescht Pharmaceuticals, UK) or with normal rabbit serum, as described by O'Reily et al. (1976).

**Results**

**IMMUNOGLOBULIN CONCENTRATIONS**

The concentrations of total secretory IgA and IgG in the undiluted secretions are shown in Table 1. There was considerable variation in the concentration of each immunoglobulin from patient to patient, but there was no significant difference between the mean concentrations of each group of patients.

**IMMUNOGLOBULIN CLASSES OF ANTIBODY REACTIVE WITH N. GONORRHOEAE**

**Untreated infected patients**

The results obtained in the IFA test, using strain 9 of *N. gonorrhoeae* as antigen, are shown in the Figure.

**Table 1: Concentrations of secretory IgA and IgG in the secretions aspirated from the cervix of women with untreated and treated gonorrhoea, with non-gonococcal cervicitis, and with no clinical evidence of cervicitis**

<table>
<thead>
<tr>
<th>Category of patient</th>
<th>No. of patients</th>
<th>Concentration of immunoglobulin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>28</td>
<td>2.41</td>
</tr>
<tr>
<td>7 days after treatment</td>
<td>29</td>
<td>2.01</td>
</tr>
<tr>
<td>14 days after treatment</td>
<td>30</td>
<td>2.31</td>
</tr>
<tr>
<td>28 days after treatment</td>
<td>28</td>
<td>2.21</td>
</tr>
<tr>
<td>Non-gonococcal cervicitis</td>
<td>19</td>
<td>2.31</td>
</tr>
<tr>
<td>No clinical evidence of cervicitis</td>
<td>29</td>
<td>2.01</td>
</tr>
</tbody>
</table>

Antibody reactive with *N. gonorrhoeae* was detected in the cervical secretions of 73 (97%) of 75 women with gonorrhoea. In 71 (95%) of these patients the antibody was of the IgA class.

Cervical secretions from 10 women with untreated gonorrhoea were absorbed with anti-human secretory component. In each case, this resulted in a threefold, or greater, reduction in titre of IgA antibody activity as detected in the immunofluorescent test.

Antigonalococcal IgM was found in 29 (39%) of the 75 infected women. This antibody was detected in the cervical secretions of 27% (59%) of the 46 women who had been infected for 14 days or less, but in only two (7%) of 29 who had been infected longer. This difference is statistically significant (p<0.05 by the method of binomial probabilities).

Immunoglobulin G reactive with *N. gonorrhoeae* was detected in 73 (97%) infected women, being the only class of antigonalococcal antibody found in two patients.

In addition to examining the secretions using strain 9 as antigen, specimens from eight infected women, attending consecutively, were tested against the infecting strain of the organism. No difference in immunoglobulin class or in antibody titre was observed in the IFA test between secretions tested against strain 9 and those tested against the infecting strain.

There was no qualitative difference in the antibodies detected in the secretions between women who had previously been infected and those who had not, but the small number of patients precluded a quantitative comparison.

Of the three women with acute salpingitis and the two with Bartholinitis, antibody of the IgA and IgG classes reactive with the gonococcus (strain 9 and homologous strain) was detected in the cervical secretions of each patient, but no antigonalococcal IgM was detectable.
A. McMillan, G. McNeillage, H. Young, and S. S. R. Bain

Nongonococcal cervicitis

Untreated gonorrhoea (75)

No clinical evidence of cervicitis (48)

<table>
<thead>
<tr>
<th>Duration of infection (days)</th>
<th>IgM-IgA-IgG</th>
<th>IgA-IgG</th>
<th>IgA-IgG+IgM</th>
<th>IgG only</th>
<th>No antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-14</td>
<td>32</td>
<td>32</td>
<td>2</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>15-21</td>
<td>16</td>
<td>16</td>
<td>3</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>22-28</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>29</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>19</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure Immunoglobulin classes of antibody reactive with N. gonorrhoea in cervical secretions.

Non-infected women

Antibody of the IgG class reactive with the gonococcus was demonstrated at a titre of 1/2 or less in the secretions of 19 (86%) of 22 women with nongonococcal cervicitis, but in only four (8%) of 48 who had no clinical evidence of cervicitis. Antigonococcal antibody of the IgM or IgA class was not detected in the secretions of this group of women.

Treated patients

The rapid decline in the mean log titre of antigonococcal IgA following successful treatment is shown in Table 2. The mean log titre of IgG antibody declined more gradually.

Within seven days of successful treatment, antigonococcal IgM could not be detected in the cervical secretions of any of the 54 women who attended at this time. This antibody had been detected in 26 of these patients.

Named sexual contacts not found to be infected

Antibody of the IgA and IgG classes reactive with N. gonorrhoea was detectable in the cervical secretions of each of the 12 patients studied. This antibody was again found when the IFA test was repeated on secretions obtained on two further occasions within the following three weeks. No antigonococcal IgM was found in the cervical secretions of these women.

SPECIFICITY OF ANTIBODIES AGAINST N. GONORRHOEAE

The results obtained when cervical secretions from five infected women were examined for antibody against other species of Neisseria are given in Table 3. No antibody against these organisms was detectable in the secretions from six non-infected women using monospecific anti-human IgA.

Antibody of the IgG class reactive with N. meningitidis group B was found in the secretions of two non-infected women, of group C in two, of group D in one, of group E in two, of group X in one, and of group Z in three. This antibody of this class was also found against N. lactamica in five of six of these women, against N. catarrhalis in each of the six, and against N. perflava in four.

Antibody against Staph. aureus, Staph. albus, Strept. faecalis, and E. coli was not detectable in the secretions from these infected and non-infected women.
Antibody against Neisseria species in the cervical secretions of five women infected with N. gonorrhoeae

<table>
<thead>
<tr>
<th>Species of Neisseria</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. gonorrhoeae</td>
<td>IgA 4+</td>
<td>IgA 3+</td>
<td>IgA 3+</td>
<td>IgA 4+</td>
<td>IgA 3+</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group A</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group B</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group D</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group E</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group 29 E</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group W135</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group Y</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Group Z</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N. catarrhalis</td>
<td>2+</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N. perflava</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Results of IFA test

*Fluorescence graded as follows: 4+ = brilliant fluorescence of all organisms in a microscopic field; 3+ = well defined fluorescence; 2+ = low intensity fluorescence of all organisms; + = occasional fluorescing organisms; — = no fluorescence.

Discussion

In this study antigonococcal antibody was detected in the cervical secretions of 97% of women with untreated gonorrhoea. Antigonococcal antibody reactivity was associated with both IgG and IgA regardless of the duration of the infection. IgM antibody was, however, detectable mainly during the first two weeks of infections, thereby resembling the serum antibody response previously reported (McMillan et al., 1979). It is difficult to determine whether this class of antibody is derived from serum or is locally produced; certainly IgM-containing plasma cells may be found in the endocervix (Chipperfield and Evans, 1972).

Tapchaisri and Sirisinha (1976), using strain F62T1 of N. gonorrhoeae as antigen, found antibody in the secretions of about 60% of infected women. They further demonstrated that almost all antibody activity was associated with IgG, antigonococcal IgA and IgM being found in only 10% and 5% of patients respectively.

The increased sensitivity noted in the present study may reflect the choice of antigen in the IFA test. Strain 9 of N. gonorrhoeae has been shown to share antigenic features with other strains of the organism (O’Reilly et al., 1973).

The threefold or greater reduction in titre of antigonococcal IgA after absorption with anti-human secretory component suggests that most of this antibody is locally produced.

Although antibody of the IgA or IgM classes reactive with N. gonorrhoeae was not detected in the cervical secretions of women who had no evidence of gonorrhoea, and who were not known contacts of infected men, antigonococcal IgG was found at a low titre in just under 40% (26/70) of these patients. In 86% of patients with clinical evidence of cervicitis, this antibody was detectable and was probably derived from transudation of natural serum IgG through inflamed mucous membranes. Similar findings were recorded by Tapchaisri and Sirisinha (1976).

Tramont (1977) found IgA reactive with strain 9 of the gonococcus in one of two women who had acute pelvic inflammatory disease but no specific IgA antibody against the infecting strain. Antibody of the IgG class against strain 9 was detected in each of three women in the present study who had acute gonococcal salpingitis. It is difficult to be certain that N. gonorrhoeae was the aetiologic agent in these women, as other organisms such as Chlamydia trachomatis may be present concomitantly (Mardh et al., 1977).

The rapid decline in IgA antibody activity in the secretions following successful treatment of gonorrhoea had previously been observed in a smaller series of patients by O’Reilly et al. (1976). Antigonococcal IgG persisted in the secretions of most patients for at least one month after treatment, resembling the serum IgG response (McMillan et al., 1979).

It was of considerable interest to note that antigonococcal antibody could be detected in the secretions of 12 women who were known sexual contacts of infected men but from whom N. gonorrhoeae could not be isolated. There was no history of antimicrobial therapy within the preceding three months, and none of the patients was in an occupation with ready access to such drugs. It is possible that these patients had been infected, but their intrinsic immunity had prevented colonisation by the organism. It is well recognised that only a proportion, up to about 70%, of female sexual
contacts of men with urethral gonorrhoea will be found to be infected (Wallin, 1974).

This project was supported by a grant from the Biomedical Research Committee of the Scottish Home and Health Department (Grant No. K/MRS/36/C38) whose financial assistance is gratefully acknowledged.

References


Identification of *Neisseria meningitidis* from primary isolation cultures

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Summary

Throat cultures were taken from 251 patients attending a Department of Venereology. Isolates of *Neisseria meningitidis* and other pathogenic neisseriae were identified by performing a rapid carbohydrate utilization test with the growth from primary isolation cultures on modified New York City (MNYC) medium. A simple colorimetric test for β-lactamase production was done at the same time.

The carrier-rate of meningococci was 47.4 per cent (119/251) while 2.8 per cent (7/251) and 2.4 per cent (6/251) of the patients harboured *N. lactamica* and *N. gonorrhoeae* respectively. Of the meningococcal isolates 70.6 per cent (84/119) were identified directly from the primary isolation culture after incubation for 24 hours.

It is suggested that identification from primary isolation cultures, with the possible refinement of sero-typing by incorporation of anti-serum in the culture plates, could improve the cost-effectiveness and rapidity of meningococcal carrier surveys.

Introduction

Major epidemics of meningococcal meningitis have occurred in some countries in recent years and there have been local outbreaks in the United Kingdom (*British Medical Journal*, 1976). In the United States of America there has been considerable interest in preventive vaccination programmes (*United States Center for Disease Control*, 1976). Under these circumstances, procedures for the large-scale identification of asymptomatic meningococcal carriers merit appraisal. Van Peenen, Suiter, Mandel and Mitchell (1966) noted that the selective nature of Thayer Martin medium (Thayer and Martin, 1964) substantially reduced the laboratory workload in meningococcal carrier surveys. However, isolates must still be subcultured for identification by sugar utilization tests.

This paper describes a system for the identification of meningococci directly from primary isolation cultures.

Materials and methods

Specimens

Throat cultures were obtained from 251 patients, mainly gonorrhoea con-
tacts, attending the Department of Venereology, The Royal Infirmary, Edinburgh. Material was inoculated directly from the patient on to culture plates.

**Isolation medium**

This was Modified New York City (MNYC) medium (Young, 1978). It contains Difco GC base enriched with 10 per cent (v/v) human blood lysed with 0.5 per cent (w/v) saponin, 2.5 per cent (v/v) yeast dialysate prepared as described by Faur, Weisburd, Wilson and May (1973), 0.1 per cent (w/v) glucose, lincomycin (1.0 μg/ml), colistin (6 μg/ml), amphotericin B (1.0 μg/ml) and trimethoprim lactate (6.5 μg/ml).

**Culture and identification of neisseriae**

After seeding the medium at the clinic, plates were held at 36°C in a carbon dioxide-enriched (10 per cent) aerobic atmosphere and transferred to the laboratory within four hours where they were incubated under similar conditions for 18–24 hours. Plates were then examined and suspect colonies were tested for the oxidase reaction, and Gram smears prepared. Any oxidase-positive Gram-negative cocci were examined by the rapid carbohydrate utilization test (RCUT) (Young, Paterson and McDonald, 1976) and a colorimetric test for β-lactamase production (Young, 1977). All negative cultures were re-examined after incubation for 48 hours.

**Rapid carbohydrate utilization test (RCUT) and β-lactamase test**

Seven tubes (70 x 10 mm) were used for each isolate to be tested; 20 μl of 10 per cent aqueous (w/v) glucose, maltose, sucrose, lactose and fructose, and 20 μl of ampicillin (250 mg/ml in buffer-salt solution (BSS) (5.2 mm phosphosphate buffer containing 0.8 per cent (w/v) KCl and 0.01 per cent phenol red, pH 7.10 to 7.15) were added to six individual tubes, followed by 100 μl of BSS. After 24 hours if there was sufficient growth of the isolate to be tested a thick suspension was made with thorough mixing in 300 μl of BSS in the seventh tube; a 30 μl aliquot of this suspension was transferred to each sugar-containing tube and to the ampicillin tube. Tubes were shaken and incubated at 37°C in a water bath. A positive result usually developed within 30 to 60 min and a definite reading was possible at three hours. A yellow colour (occasionally yellow-orange) is positive; red is negative. Each day, a known strain of N. meningitidis and a penicillinase-producing strain of N. gonorrhoeae were set up as controls.

If, at 24 hours there was insufficient growth of the isolate to be tested, an overnight subculture was grown on MNYC medium and the RCUT and β-lactamase tests were done with this.

**Results**

The numbers and percentage of patients from whom N. meningitidis, N.
Identification of meningococci

Table I

<table>
<thead>
<tr>
<th>Neisseria spp isolated</th>
<th>Number (and percentage) of patients with positive throat cultures for Neisseria spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. meningitidis</td>
<td>119 (47-4)</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>7 (2-8)</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>6 (2-4)</td>
</tr>
<tr>
<td>Total</td>
<td>132 (52-6)</td>
</tr>
</tbody>
</table>

Lactamica or N. gonorrhoeae were isolated in the Table. Of the 132 Neisseria spp isolated, 90-2 per cent (119) were N. meningitidis, 5-3 per cent (7) were N. lactamica and 4-5 per cent (6) were N. gonorrhoeae.

Of the 119 meningococcal isolates, 70-6 per cent (84) were fully identified by 24 hours: six of the seven N. lactamica and three of the six N. gonorrhoeae isolates were identified within 24 hours. In two separate cases, a throat culture yielded a mixture of N. meningitidis and N. gonorrhoeae on the same culture plate and this necessitated subculture prior to full identification at 48 hours.

All of the neisseriae isolated gave a negative reaction for β-lactamase.

Discussion

The RCUT applied to primary isolation cultures provides a simple and rapid method for identifying pathogenic neisseriae. Since there is no need to subculture prior to the RCUT this makes a considerable saving in cost and technician time. In addition, the incorporation of a tube containing ampicillin in the normal identification scheme allows all isolates to be readily monitored for β-lactamase production. β-lactamase-producing N. gonorrhoeae (Laneet, 1976) and Branhamella (Neisseria) catarrhalis (Ninane, Joly, Piot and Kraytman, 1977) have already been described and it seems reasonable to look for β-lactamase-producing meningococci as a routine.

Although isolates of meningococci are normally subcultured prior to serotyping, it is possible to type strains by incorporating anti-serum in the primary isolation medium (Sivonen, Renkonen and Robbins, 1977). However, this procedure would be very expensive for routine screening.

MNYC medium was not compared directly with Thayer-Martin medium for the isolation of neisseriae in the present study, because our earlier work showed that Thayer-Martin medium was not a suitable source of growth for the RCUT (Young, Paterson and McDonald, 1976).

(Thanks are extended to Dr D. H. H. Robertson and colleagues of the Department of Venereology, Edinburgh Royal Infirmary for sending clinical specimens and to Professor J. G. Collee for helpful advice during the preparation of the paper.)
References


Individual susceptibility to neisserial infection?

BY

H. YOUNG, A. B. HARRIS, AND D. H. H. ROBERTSON

Reprinted from British Journal of Venereal Diseases, Vol. 55, No. 3, June, 1979
pp. 188-190

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Individual susceptibility to neisserial infection?

H. YOUNG, A. B. HARRIS, AND D. H. H. ROBERTSON
From the Department of Bacteriology, Edinburgh University Medical School, and the University Department of Venereology, Royal Infirmary, Edinburgh

SUMMARY Specimens from genital, anorectal, and pharyngeal sites from 1671 men and 1419 women were cultured for Neisseria gonorrhoeae. Pharyngeal specimens were also cultured for Neisseria meningitidis. N. gonorrhoeae was isolated from a genital site 2.7 times more often in men and 1.8 times more often in women who also carried meningococci in their pharynx than from those who did not; the meningococcus was isolated 3.4 times more often from men and 2.0 times more often from women with genital gonorrhoea than from those without. In both men and women the association of each organism with the other was statistically significant (p<0.001) and may be related to sexual behaviour rather than to individual susceptibility to neisserial infection.

Introduction

The gonococcus and the meningococcus, the two most important human pathogens in the genus Neisseria, are closely related genetically (Kingsbury, 1967; Kingsbury et al., 1969) and antigenically (Tramont et al., 1974). If there are specific host factors which increase the susceptibility of certain individuals to these organisms, their study could increase our knowledge of the pathogenicity of gonococcal and meningococcal infections.

In an investigation by Wilcox et al., (1977) of 150 cultures of throat exudates from patients (predominantly male) who were examined at a clinic for sexually transmitted diseases because they had a history of orogenital intercourse, the meningococcus was isolated nearly six times more often from those with genital gonorrhoea than from those without. In the same study the gonococcus was found 2.5 times more often in those who carried the meningococcus in the pharynx than in those who did not. These workers suggested (Wilcox et al., 1977) that confirmation of these findings would support the theory of individual susceptibility to the acquisition of neisseriae.

This paper reports our findings on all patients, male and female, from whom genital (and, in some cases, anorectal) and pharyngeal specimens were cultured during 1977.

Materials and methods

Specimens of genital and pharyngeal exudates obtained from 1671 men and 1419 women attending the Department of Venereology at the Royal Infirmary, Edinburgh, during 1977 were cultured in parallel for Neisseria gonorrhoeae. The pharyngeal specimens were also cultured for Neisseria meningitidis.

Urethral and pharyngeal specimens were taken from all male patients in the study while anorectal specimens were also taken for culture from homosexual men. Urethral, cervical, anorectal, and pharyngeal specimens were taken for culture from all female patients in the study. These patients comprised all known contacts of gonorrhoea. Patients were not routinely asked about recent oral contact, but if such a history was obtained they were included in the study.

Material was inoculated directly on to modified New York City medium (Young, 1978a) at the time of the patient's initial examination. After inoculation, plates were held at 36°C in a carbon dioxide-enriched (10%) atmosphere and transferred to the laboratory within four hours. Specimens were processed, and N. gonorrhoeae and N. meningitidis were identified by fluorescent antibody and rapid carbohydrate utilisation tests (Young, 1978b).

Statistical analysis was made by the $\chi^2$ method with Yates's correction.

Results

The results of cultures from the 1671 male and 1419
female patients are shown in Tables 1 and 2 respectively. The isolation of gonococci from genital sites is compared with that of meningococci in the pharynx of men and women in Tables 3 and 4 respectively; the isolation of meningococci from the pharynx compared with that of gonococci from genital sites is shown in Tables 5 and 6.

Table 1 Isolation of Neisseria gonorrhoeae from genital and anorectal sites and of Neisseria meningitidis from the pharynx of 1671 men

<table>
<thead>
<tr>
<th>Genital/anorectal sites</th>
<th>Pharyngeal culture results for N. meningitidis*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Culture results for N. gonorrhoeae</td>
<td>89</td>
<td>262</td>
</tr>
<tr>
<td>Positive</td>
<td>97</td>
<td>1223</td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>1485</td>
</tr>
</tbody>
</table>

*χ²: 89·8; p<0·001

Table 2 Isolation of Neisseria gonorrhoeae from genital and anorectal sites and of Neisseria meningitidis from the pharynx of 1419 women

<table>
<thead>
<tr>
<th>Genital/anorectal sites</th>
<th>Pharyngeal culture results for N. meningitidis*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Culture results for N. gonorrhoeae</td>
<td>96</td>
<td>256</td>
</tr>
<tr>
<td>Positive</td>
<td>148</td>
<td>919</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>1175</td>
</tr>
</tbody>
</table>

*χ²: 32·4; p<0·001

Table 3 Isolation of gonococci from genital and anorectal sites in relation to that of meningococci in the pharynx of 1671 men

<table>
<thead>
<tr>
<th>Meningococci cultures</th>
<th>No.</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningococci-positive</td>
<td>186</td>
<td>89</td>
<td>47·9</td>
</tr>
<tr>
<td>Meningococci-negative</td>
<td>1485</td>
<td>262</td>
<td>17·6</td>
</tr>
<tr>
<td>Total</td>
<td>1671</td>
<td>351</td>
<td>21·0</td>
</tr>
</tbody>
</table>

Table 4 Isolation of gonococci from genital and anorectal sites in relation to that of meningococci in the pharynx of 1419 women

<table>
<thead>
<tr>
<th>Meningococci cultures</th>
<th>No.</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningococci-positive</td>
<td>244</td>
<td>96</td>
<td>39·3</td>
</tr>
<tr>
<td>Meningococci-negative</td>
<td>1175</td>
<td>256</td>
<td>21·8</td>
</tr>
<tr>
<td>Total</td>
<td>1419</td>
<td>352</td>
<td>24·8</td>
</tr>
</tbody>
</table>

Table 5 Isolation of meningococci from the pharynx in relation to that of gonococci from genital and anorectal sites in 1671 men

<table>
<thead>
<tr>
<th>Genital/anorectal cultures</th>
<th>No.</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonococci-positive</td>
<td>351</td>
<td>89</td>
<td>25·4</td>
</tr>
<tr>
<td>Gonococci-negative</td>
<td>1320</td>
<td>97</td>
<td>7·4</td>
</tr>
<tr>
<td>Total</td>
<td>1671</td>
<td>186</td>
<td>11·1</td>
</tr>
</tbody>
</table>

Table 6 Isolation of meningococci from the pharynx in relation to that of gonococci from genital and anorectal sites in 1419 women

<table>
<thead>
<tr>
<th>Genital/anorectal cultures</th>
<th>No.</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonococci-positive</td>
<td>352</td>
<td>96</td>
<td>27·3</td>
</tr>
<tr>
<td>Gonococci-negative</td>
<td>1067</td>
<td>148</td>
<td>13·9</td>
</tr>
<tr>
<td>Total</td>
<td>1419</td>
<td>244</td>
<td>17·2</td>
</tr>
</tbody>
</table>

The gonococcus was isolated 2·7 times more often from men and 1·8 times more often from women who were pharyngeal carriers of meningococci than from those who were not (Tables 3 and 4). Conversely, meningococci were isolated from the pharynx 3·4 times more often from men and 2·0 times more often from women with gonorrhoea than from those without (Tables 5 and 6). The association of each organism with the other is highly significant, in both men (χ²: 89·08; p<0·001) and women (χ²: 32·4; p<0·001).

The incidence of pharyngeal gonorrhoea in our study was 5·4% (19/352) in women and 3·1% (11/351) in men with genital or anorectal gonorrhoea or both; this represents an incidence of 1·3% (19/1419) and 0·7% (11/1671) of all female and male patients respectively.

Discussion

Our results confirm the earlier finding (Willcox et al., 1977) that individuals who carry the meningococcus in the pharynx and who have been exposed to the risk of gonorrhoea are more likely to yield positive results to genital culture for N. gonorrhoeae and vice versa. Although in the earlier study (Willcox et al., 1977) the meningococcus was isolated approximately six times more often from patients with genital gonorrhoea than from those without, the corresponding factors in our study are lower, 3·4 for men and 2·0 for women. The former six-fold difference (Willcox et al., 1977) reflects a lower meningococcal carriage rate in the group without gonorrhoea (5·3%) and a higher rate in the group...
with genital gonorrhoea (31.3%) than the corresponding carriage rates in our study—7.4% and 13.9% in men and women without gonorrhoea and 25.4% and 27.3% in those with gonorrhoea respectively. Our finding that the gonococcus was isolated 2.7 times more often from men and 1.8 times more often from women who were pharyngeal carriers of the meningococcus than from those who were not, is in good agreement with the 2.5-fold difference quoted previously (Willcox et al., 1977).

Thus, in our study each organism was isolated two to three times more frequently in the presence of the other. The factors responsible for this association are unknown. It has been reported (Foster and Labrum, 1976) that there is an increased risk of gonococcal infection in blood group B subjects, and other workers (Miler et al., 1977) confirmed this for white patients but not for West Indians. It was concluded (Miler et al., 1977) that such data must be carefully collected on well-defined ethnic groups over a range of samples before a definite conclusion on intrinsic susceptibility to gonococcal infection based on blood group differences can be made. Analysis of the results by blood group or ethnic group was not attempted in the present study.

Another possibility is that our results, and those of Willcox et al. (1977), do not indicate that specific host factors are involved in individual susceptibility but rather reflect that the more frequent acquisition of both organisms is related to sexual behaviour. It has been suggested (World Health Organisation, 1978) that more frequent changes of sexual partner may have contributed to the increase in gonorrhoea found in many countries in recent years. Assuming that an intimate activity, such as kissing, is a common prelude to sexual intercourse, more frequent changes of partner might increase the chance of acquiring meningococci in the pharynx.

If the population examined in this study includes a group whose carriage of neisseriae merely reflects the association of intimate behaviour with the exchange of flora, then it might be worthwhile to investigate the carriage of other ‘marker’ organisms, for example, Streptococcus pyogenes and Haemophilus influenzae. If oropharyngeal carriage of these organisms indicates a similar correlation with genital gonorrhoea then the postulated host susceptibility factor for neisseriae is unlikely.

We thank Professor J. G. Collee for constructive criticism and helpful advice in the preparation of this paper.

References


SCREENING BY CULTURE FOR THE DETECTION OF GONORRHOEA IN WOMEN

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Department of Bacteriology, University Medical School and University Department of Venereology, Royal Infirmary, Edinburgh

Summary. The number of gonococcal infections detected by each of three sets of diagnostic cultures from the urethra, cervix, rectum and occasionally the throat, were calculated for 1976 when Thayer Martin (TM) medium was used and for 1977 when Modified New York City (MNYC) medium was used.

In 1977, 98.7 per cent (451/457) of the total infections diagnosed were confirmed by culture compared with 88.3 per cent (324/367) of infections diagnosed in 1976 (P<0.001). The first set of diagnostic tests detected 97.6 per cent (440/451) of culture-positive infections in 1977 compared with only 88.9 per cent (228/254) in 1976 (P<0.001). The efficiency of screening with a single endocervical culture was also calculated. This procedure would have detected 90.2 per cent (407/451) of culture-positive infections in 1977 when MNYC medium was used compared with only 78.1 per cent (253/324) in 1976 when TM medium was used (P<0.001). It is suggested that the statistically significant improvement in the culture results for 1977 resulted from the introduction of MNYC medium since all other diagnostic procedures were identical to those in 1976. Other advantages associated with the use of MNYC medium are discussed.

The high incidence of gonorrhoea in the United Kingdom (UK) (Nicol, 1976) and to a greater extent in the United States of America (USA) (Wiesner et al., 1976), imposes a considerable burden on both clinician and bacteriologist involved in the diagnosis of sexually-transmissible diseases. This situation, aggravated in many areas by shortages of staff and resources, makes a critical review of diagnostic procedures and screening policies a necessity.

Examination of Gram-stained smears of patients' secretions is a valuable aid in the diagnosis and control of gonorrhoea since it allows the patient to receive immediate and effective treatment. However, in the case of female patients, Gram-staining is not a very sensitive procedure and only 55 to 65 per cent of cases with positive cultures are detected by this means (Barlow et al., 1976; Chipperfield & Catteral, 1976; Evans, 1976). Cultural diagnosis, therefore, is of prime importance and in the UK most clinics consider that repeated testing of multiple sites is necessary to detect the maximum number of cases of gonorrhoea in women (Adler, 1978).

In the gonorrhoea control programme in the USA, screening for gonococcal infection by endocervical culture alone has been widely used in an attempt to detect the large reservoir of infected women without symptoms (Norins 1974).

Obviously the efficiency of screening for gonococcal infection depends on the quality of the microbiological service. Most studies which have evaluated the number of diagnostic tests required have been made in different centres and have involved relatively small numbers of patients (Barlow et al., 1976; Chipperfield & Catteral, 1976; Evans, 1976). From these studies it is difficult to assess the influence of factors such as the quality of the gonococcal culture medium.

In a small prospective study using both Thayer Martin (TM) and Modified New York City (MNYC) media the latter medium was shown to give a higher yield of positive cultures (Young, 1978a). As a result of this
study TM medium was replaced by MNYC medium for the cultural diagnosis of gonorrhoea. This paper compares culture results for the years 1976 and 1977, when TM and MNYC media respectively were used, in order to see if a change in the efficiency of cultural diagnosis in routine clinical practice coincided with the introduction of the new medium.

**Materials and methods**

This study included all women from whom microbiological tests for gonorrhoea were made at the Department of Venereology, Edinburgh Royal Infirmary, during 1976 and 1977.

Gram-stained smears of material from the urethra and cervix were examined microscopically whilst the patient was at the clinic. Cultures were made routinely from the urethra, cervix and rectum. Urethral material was obtained by inserting a cotton-wool applicator into the urethra after gentle downward milking of the urethra by means of the index finger inserted into the vagina; the cervical material was obtained by inserting a similar applicator into the endocervix after gently mopping vaginal material from the vaginal surface of the cervix and external os; the rectal sample was obtained from the anorectum by inserting an applicator past the anorectal junction and then withdrawing it (in occasional cases where this was difficult a child’s proctoscope was inserted and then the applicator was pushed past its orifice and withdrawn with the proctoscope). In addition, if the patient was a gonorrhoea-contact or admitted recent oro-genital contact throat cultures were also made. If the first set of cultures for Neisseria gonorrhoeae were negative these were repeated twice at one or two-week intervals. Test-of-cure cultures were usually made, one week, two weeks, and four weeks following treatment.

**Media.** Cultures were plated on to TM medium during 1976 and on to MNYC medium during 1977. TM medium comprised lab m Columbia agar base (London Analytical and Bacteriology Media Ltd., 50 Mark Lane, London) supplemented with 10 per cent (by vol.) heated (56°C. for 60 min.) human blood and the antibiotics vancomycin (4 μg./ml.), colistin (6 μg./ml.) and nystatin (10 μg./ml.). MNYC medium comprised Difco gonococcal base enriched with 10 per cent (by vol.) human blood lysed with 0.5 per cent (by vol.) saponin, 2.5 per cent (by vol.) yeast dialysate prepared as described by Faur et al. (1973), 0.1 per cent (by vol.) glucose, lincomycin (1.0 μg./ml.), colistin (6 μg./ml.), amphotericin B (1.0 μg./ml.), and trimethoprim lactate (6.5 μg./ml.). Both media were prepared in the Bacteriology Department and used within four days of preparation.

All cultures were inoculated directly at the clinic. After inoculation plates were held at 36°C. in a carbon dioxide (10%) enriched atmosphere and transferred to the laboratory within four hours. Cultures were incubated and N. gonorrhoeae identified by carbohydrate utilisation and fluorescent antibody tests as described earlier (Young, 1978b).

Statistical analysis of the results was made by the chi square method with Yates’ correction.

**Results**

Of 2,486 patients investigated in 1976, 367 episodes of gonococcal infection were detected by three sets of diagnostic tests: the same number of diagnostic tests detected 457 episodes of infection among 2,736 patients in 1977. Results of culture and microscopical examination of these patients are shown in Table I.

From Table I it can be calculated that 88.3 per cent (324/367) of infections in 1976 were confirmed by culture compared with 98.7 per cent (451/457) of infections in 1977. This difference is statistically significant ($\chi^2 = 37.5; p<0.001$).

The number of patients detected, and the sites positive, by each of three diagnostic tests during 1976 and 1977 are given in Tables II and III respectively.

From Tables II and III it is seen that 88.9 per cent of infections were diagnosed at the first set of diagnostic cultures in 1976 compared with 97.6 per cent of infections in 1977. This difference is also statistically significant ($\chi^2 = 23.4; p<0.001$). After two diagnostic tests, 98.7 per cent and 98.5 per cent of infections were diagnosed during 1977 and 1976, respectively.
Table I. Results of microscopic examination and culture for 367 and 457 episodes of gonorrhoea detected by diagnostic tests during 1976 and 1977 respectively.

<table>
<thead>
<tr>
<th>Pattern of results:</th>
<th>Episodes of gonorrhoea and pattern of results</th>
<th>On TM medium 1976</th>
<th>On MNYC medium 1977</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Culture + Smear +</td>
<td>229 62.4</td>
<td>277 60.6</td>
<td></td>
</tr>
<tr>
<td>Culture + Smear —</td>
<td>75 20.4</td>
<td>151 33.1</td>
<td></td>
</tr>
<tr>
<td>Culture + Smear 0</td>
<td>20 5.5</td>
<td>23 5.0</td>
<td></td>
</tr>
<tr>
<td>Culture — Smear +</td>
<td>43 11.7</td>
<td>6 1.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>367 100</td>
<td>457 100</td>
<td></td>
</tr>
</tbody>
</table>

+ = positive; — = negative; 0 = no smear

Table II. Number of infections detected, and the sites positive, by each of three diagnostic culture tests using TM medium (1976).

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Number (and percentage) of infections detected</th>
<th>Number (and percentage) of infections with positive cultures at following sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ur    (58.3)</td>
<td>Cx    (78.1)</td>
</tr>
<tr>
<td>1</td>
<td>288 (88.9)</td>
<td>189 (53.5)</td>
</tr>
<tr>
<td>2</td>
<td>31 (9.6)</td>
<td>17 (5.3)</td>
</tr>
<tr>
<td>3</td>
<td>5 (1.5)</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td>Total</td>
<td>324 (100)</td>
<td>209 (64.3)</td>
</tr>
</tbody>
</table>

Ur = urethra; Cx = cervix; Rect = rectum.

Table III. Number of infections detected, and the sites positive, by each of three diagnostic culture tests using MNYC medium (1977).

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Number (and percentage) of infections detected</th>
<th>Number (and percentage) of infections with positive cultures at following sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ur    (72.5)</td>
<td>Cx    (90.2)</td>
</tr>
<tr>
<td>1</td>
<td>440 (97.6)</td>
<td>327 (72.5)</td>
</tr>
<tr>
<td>2</td>
<td>5 (1.1)</td>
<td>4 (0.9)</td>
</tr>
<tr>
<td>3</td>
<td>6 (1.3)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Total</td>
<td>451 (100)</td>
<td>334 (74.1)</td>
</tr>
</tbody>
</table>

Ur = urethra; Cx = cervix; Rect = rectum.

Discussion

Between 1976 and 1977 the increase from 88.3 to 98.7 per cent in the proportion of infections confirmed by culture was statistically significant (p<0.001). In the same period there was also a statistically significant difference in the number or culture-positive infections detected by the first set of diagnostic tests (88.8% compared with 97.6%; p<0.001). These improvements in cultural diagnosis are reflected in the correlation between microscopy and culture for the two year period (Table I). The proportion of culture-positive smear-negative cases increased from 20.4 per cent in 1976 to 33.1 per cent in 1977. In the same period the proportion of smear-positive culture-negative cases decreased from 11.7 to 1.3 per cent: these cases were all gonorrhoea-contacts, themselves diagnosed by culture, suggesting that the smear results were reliable. Also, the proportion of patients with positive smears and cultures during 1976 and 1977 was similar (62.4% and 60.6% respectively) suggesting that there was no difference in the preparation and interpretation of Gram-stained smears during the two-year period.
The improvement in the cultural results in 1977 presumably resulted from the introduction of MNYC medium since all other diagnostic procedures were identical to those in 1976. This view, although impossible to prove in this study, is supported by the superior performance of MNYC medium when swabs were inoculated on to the two media in a randomized manner (Young, 1978a). MNYC and TM media differ in both their formulation and also the selective antibiotics involved. Either component, or a combination of both, could account for the observed difference in gonococcal isolation rate. Brorson et al. (1973) found that substitution of lincomycin for vancomycin resulted in a net gain of bacteriologically diagnosed cases of gonorrhoea of about 10 per cent. Strains of gonococci isolated on medium containing lincomycin (4 μg./ml.) but not on the parallel medium containing vancomycin (3 μg./ml.) did not grow in the presence of 1.25 μg. per ml. of vancomycin. In our earlier study (Young, 1978a) there was no direct support for vancomycin sensitivity as a cause of culture failure with TM medium since none of the strains isolated only on MNYC medium was sensitive to vancomycin at the concentration present in TM medium (4.0 μg./ml.). Nevertheless, the safety margin with lincomycin was much greater (minimum inhibitory concentration (MIC) usually 16 times the concentration present in the medium) than with vancomycin (MIC usually two to four times greater than the corresponding medium concentration). This safety margin combined with the better nutritional value of MNYC medium may be particularly important in the case of small inocula. While other factors such as a change in the presentation of the infection could explain the difference in the results this seems unlikely to occur in one region in such short a time.

The data presented in Tables II and III enable us to calculate the efficiency of screening by a single endocervical culture. The reported range of sensitivity for this procedure is 40 to 90 per cent and the most optimistic figures resulted from two studies with a total of only 162 infected women (Norins, 1974). Our results provide good evidence that a single endocervical culture can detect 90 per cent of infections in women: using MNYC medium, 90.2 per cent of the 451 culture-positive infections would have been detected by a single endocervical culture (Table III). However, our poorer results with TM medium (78.1% of culture-positive infections detected by a single cervical culture) suggest that a detection rate of 90 per cent might not be reached in areas where conventional TM medium is used.

In terms of a screening schedule more infections are detected by testing additional sites at the first test than by re-screening with cervical cultures. Using MNYC medium two additional endocervical cultures would have detected another 2.2 per cent of infections giving a total of 92.4 per cent for three endocervical cultures, whereas 97.6 per cent of infections would have been diagnosed by a single set of diagnostic tests (Table III).

Although the efficiency of examination of vaginal material was not determined in the present study, other workers (Schmale et al., 1969; Bhattacharyya et al., 1973) have drawn attention to the limitations of sampling this site.

Our results support the view of others (Barlow et al., 1976; Evans, 1976) that provided the microbiological service consistently reaches a high standard only two sets of investigations need to be performed to diagnose or exclude gonorrhoea in women. The few patients found to be positive by a third diagnostic test are likely to have acquired the infection after the first diagnostic test (Evans, 1976).

Apart from the great improvement in the number of infections detected by the first set of diagnostic tests, the use of MNYC medium has other advantages. Gonococcal growth is more rapid on MNYC medium which is also designed to allow biochemical identification directly from the primary isolation plate. This means that results are obtained with greater economy and in a shorter time, 87 per cent of infections being reported positive after only 24 hours (Young, 1978b), allowing patients with negative smears to be treated quickly. The patient is also less likely to default when the time interval between the initial visit and subsequent appointment is short.
REFERENCES


U.S.A. This programme includes DTP, polio, and measles, mumps, and rubella. The local authorities in Hong Kong include BCG—a controversial decision. There is clearly no indication for typhoid, cholera, or plague vaccinations.

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Hong Kong

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FIBRE AND POSTOPERATIVE ILEUS

Sir,—Dr Sculati and colleagues (June 7, p. 1252) found reduced postoperative ileus after a preoperative bran enriched diet. Since most of the people in our area traditionally eat a bran rich diet, mainly cassava leaves, we thought that it would be interesting to measure the delay of passage of gases after uncomplicated operations. 22 unselected cases for which we had accurate data were retrospectively analysed (7 caesarean sections, 5 abdominal hysterectomies, 5 adnexectomies, 1 cholecystectomy, 3 laparotomies, 1 bilateral cuneiform excision of ovaries).

<table>
<thead>
<tr>
<th>DELAY OF RETURN OF PASSAGE OF GAS AS INDICATOR OF DURATION OF POSTOPERATIVE ILEUS</th>
<th>Return by postoperative day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sculati's study</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
</tr>
<tr>
<td>Treated</td>
<td>19</td>
</tr>
<tr>
<td>Our cases</td>
<td>4</td>
</tr>
</tbody>
</table>

Although our results (see table) are not as striking as Sculati's they show that in populations where a fibre rich diet is widespread, reduced postoperative ileus is one of the numerous advantages conferred by high fibre consumption.

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KABAYA

HEMMOPHILUS IN SEXUALLY TRANSMITTED DISEASES

Sir,—Taylor et al.1 reported the presence, in the sera of non-gonococcal urethritis patients, of antigens to the contagious equine metritis agent HEMMOPHILUS equigentialis. On the basis of serological data and therapeutic results they suggested that this organism, or one related to it, might play a part in the aetiology of human non-gonococcal urethritis. From an epidemiological point of view the second possibility seems the more likely—and human hemophili may be the related bacteria. In 1978 we diagnosed some genital infections caused by H. influenzae and H. parainfluenzae in adults and subsequently specimens from patients with sexually transmitted diseases were routinely cultured for the detection of hemophili. Our studies (to be published elsewhere) showed that both species are important pathogens in sexually transmitted diseases.

The infections of the genital tract usually followed orogenital contact in both sexes, but genito-genital transmission also occurred, and in several cases both modes of infection may have been occurring simultaneously. We also noted mild and asymptomatic infections with persistent excretion of hemophili in the genital discharges or urine. Mixed infections with other STD agents (especially Trichomonas vaginalis) are not rare and some strains were isolated from post-gonorrheal urethritis.

Although non-gonococcal urethritis due to "influenza bacillus" was described by P. Colin 75 years ago, hemophilus infections of the genital tract in adults and the role of the sexual transmission seem to have been almost forgotten. Recently some severe neonatal infections, described by N. Khuri-Bulos and K. McIntosh (Am J Dis Child 1975; 129: 57) and G. Martin and E. Wald (Pediatrics 1976; 58: 863), focused attention upon the vaginal carriage of H. influenzae, but extremely low rates were found in pregnant women. Sporadic reports indicate, however, that the presence of H. influenzae in the vagina can lead to vaginitis, Bartholinitis, endometritis, tubo-ovarian abscess, and septic arthritis mimicking gonococcal arthritis.

During pregnancy septic abortions, amnionitis, and puerperal sepsis have been observed. It is surprising that sexual transmission (orogenital contact) has only occasionally been suspected as a mode of infection of the female genital tract, and H. parainfluenzae has never been recovered from the maternal genitalia.

Since sexually transmitted hemophilus infections seem to be common in patients with sexually transmitted diseases we suggest the introduction of routine examinations for the isolation of H. influenzae and H. parainfluenzae in departments seeing such patients and in urology and gynaecology clinics. For the selective cultivation of hemophilus heated blood agar plates containing vancomycin hydrochloride 25 μg/ml can be used. In small laboratories the use of heated blood agar plates with vancomycin 30–50 μg and lincomycin 10 μg paper discs on the surface of the media permits the simple isolation of both hemophilus and neisseria. Readings are made after incubation in 5% CO₂ in closed jars at 37°C for 2 days. Isolates should be tested for antibiotic resistance, because drug resistant strains (mainly H. parainfluenzae) do occur.

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M. Füzi

ISOLATION OF TRICHOMONAS VAGINALIS RESISTANT TO METRONIDAZOLE

Sir,—Although decreased sensitivity of Trichomonas vaginalis to metronidazole has not generally been considered to be a factor in the failure of metronidazole treatment, Thurmer and Meingassner1 reported decreased sensitivity by both in-vitro and in-vivo testing of stock isolated from such a therapeutic failure. Korner and Jensen2 found no evidence of resistance, from published reports, that treatment failures were becoming more common. Roe3 commented that there are very few treatment failures which cannot be attributed to reinfection, failure to take treatment prescribed, poor absorption of the drug, or its excessive destruction by vaginal flora.

on many occasions after the above course. In view of the denial of further sexual intercourse the reappearance of T. vaginalis was considered to be due to relapse rather than to reinfection. On Feb. 5, 1980, she had a yellow vaginal discharge containing numerous T. vaginalis. She denied having had intercourse for about 2½ years. She was admitted to the ward for treatment with metronidazole under supervision and for collection of blood samples to estimate plasma-metronidazole levels. Metronidazole was given orally in doses of 200 mg at 08-00, 12-00, and 16-00 hours on days 1 and 2; subsequently five 200 mg tablets were given orally at 08-00 and 16-00 hours from day 3 to day 12 inclusive, and treatment was supplemented by insertion of a metronidazole suppository daily into the vagina at noon on the last five days in doses of 500 mg, 1000 mg, 500 mg, 500 mg, and 500 mg respectively. The course of treatment, which was based on that given by Thurner and Meingassner,1 was started on Feb. 6 and completed by Feb. 17. The patient complained of an unpleasant taste in the mouth from the start of treatment until Feb. 29. Although she had a brownish vaginal discharge at that time, no T. vaginalis were seen then or on March 14, when she also had a slight vaginitis. On April 11, however, there was an unpleasant-smelling vaginal discharge, and numerous T. vaginalis were found. The trichomonad was isolated on April 15 in the medium of Lumiden, Roberson, and McNeillage,4 modified by the replacement of liver digest by neutralised liver digest (Oxoid) and by altering the pH of the buffer component to 6.8.

After serial passage by culture, the trichomonad from the patient was examined on May 25 for sensitivity to metronidazole. Stock of T. vaginalis isolated from another patient who had not had recurrent trichomonal infection was also assayed for metronidazole sensitivity as a control. The culture medium was prepared in 6 ml vials containing two-fold serial dilutions of metronidazole from 200 mg/l to 0.78 mg/l and also a control without metronidazole. Each bottle was inoculated with 0.2 ml from a 5-day-old culture containing actively motile organisms (about 10⁷). Cultures were examined up to day 4 for the presence of motile organisms. Subcultures were made from each into fresh medium without metronidazole and were examined for motility in the same way. For further study, stocks of the T. vaginalis in culture from the patient (D9559) and the control (E25) were stored separately by cryopreservation in liquid nitrogen.4 5 Samples from the frozen stocks from both patient and control were shown by culture to be viable after thawing.

Motility was lost in the control stock with the minimum concentration used (0.78 mg/l) but retained in the patient's stock until concentrations above 12.5 mg/l were attained (see table). Concentrations inhibiting motility also prevented subsequent multiplication on subculture in medium lacking metronidazole.

A specific and sensitive high-performance liquid-chromatographic (HPLC) method using 1-(2-hydroxyethyl)-2-ethyl-5- nitromidazole as internal standard was used for the determination of plasma-metronidazole concentrations.6 Venous blood samples were taken into heparin tubes at 10.00, 14.00, and 18.00 hours on days 1 and 2 and at noon on days 3, 6, 7, 8, 9, 10, and 12. After separation by centrifugation the plasma was stored at −20°C until the HPLC assay was carried out.

Plasma-metronidazole levels (see figure) ranged from 4.0 to 8.8 mg/l on the first two days with the dose of 200 mg orally thrice daily. Later, with the high oral dosage of 1.0 g twice daily, levels of 16 to 26 mg/l were reached. A peak corresponding with the metabolite, 1-(2-hydroxyethyl)-2-hydroxy- methyl-5-nitromidazole,4 appeared from each plasma. The low concentration of metronidazole on the 9th day, together with a high ratio of metabolite to metronidazole suggested a mistimed specimen or dose.

A report of polarographic studies of serum-metronidazole levels in two patients showed that on a dosage of 200 mg daily,

<table>
<thead>
<tr>
<th>Metronidazole (mg/l)</th>
<th>Patient</th>
<th>Control</th>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.78</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>3.77</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13-13</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.25</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25.00</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>50.00</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>100.00</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>200.00</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Subcultures made from medium containing corresponding metronidazole concentration.


Dose mg

<table>
<thead>
<tr>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

Concentrations of metronidazole in plasma.

Intravaginal doses are given in the text.
thrice daily by mouth serum concentrations of 2.2–5.9 mg/l were obtained in the morning sample before the first of the daily doses and that there was no evidence of accumulation of the drug in the serum. Levels attained in our patient (4.0–8.8 mg/l) did not suggest poor absorption on the usual dose of 200 mg three times daily. High levels were obtained on the high dose of 1-0 g given twice daily; the additional administration of vaginal metronidazole seemed to give little advantage.

Our findings of measurable in-vitro resistance of *T. vaginalis* to metronidazole in stock isolated from a therapeutic failure is added evidence to that given by Thurner and Meingassner that resistance can be a factor in failure of metronidazole in the therapy of *T. vaginalis* vaginitis. These findings are in contrast to earlier studies on metronidazole sensitivity. Squires and McFadzean found that none of 26 strains of *T. vaginalis* examined grew at a concentration greater than 1 mg/l metronidazole, while Korner and Jensen found that the mean trichomonacidal concentration of metronidazole for 44 strains of *T. vaginalis* was 2-79 mg/l (range 0-50 to 7-50 mg/l). Neither of these groups considered resistance of *T. vaginalis* to metronidazole as a causative factor in the failure to respond to the drug.

Serial culture done during the evaluation of susceptibility of the organism in vitro or in vivo may lead to loss of sensitivities to metronidazole, and cryopreservation may be of value where assays cannot be immediately carried out or when confirmation of findings is desirable: the stock of *T. vaginalis* isolated from the patient after relapse has now been stored frozen as a "stable" at -196°C so that its susceptibility to metronidazole can be studied further.

In trichomonal infections there is no certainly useful alternative to metronidazole, and because of the possibility of resistance it should be used only when *T. vaginalis* is shown by microscopy or culture to be present.

We thank our patients for their cooperation; May and Baker Ltd for the gift of metronidazole and HPLC internal standard; Dr F. R. Clark for his help; and Dr W. J. Herbert, Director of Animal Services, Dundee University Medical School, for cryopreservation facilities.

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PENICILLIN-INSENSITIVE PNEUMOCOCCI

Sir,—Dr Gratten and his colleagues (July 26, p. 192) reported that one-third of 57 strains of pneumococci isolated from patients in Port Moresby, Papua New Guinea, in 1978 were penicillin-insensitive with minimum inhibitory concentrations (MIC) of penicillin G ranging from 0.1 to 1.0 μg per ml. They noted that although this degree of resistance is not likely to cause therapeutic problems in the treatment of pneumonia, it may reduce the effectiveness of penicillin in the treatment of pneumococcal meningitis.

Pneumococci resistant to several antibiotics and with MIC of penicillin as high as 4 to 8 μg/ml have been reported from South Africa1 but have not yet emerged as a problem elsewhere. However, there have been reports of the isolation of pneumococci showing relative or intermediate resistance to penicillin similar to that reported by Dr Gratten and his colleagues. These have included isolations from patients in Britain,2–4 Canada,5 and U.S.A., where in two surveys in the winter of 1977–78 relative resistance (MIC 0-1 to 0-5 μg/ml) was found in 6-8% of 74 isolates in Cincinnati5 and 15-5% of 103 strains in Oklahoma City.6

Microbiology laboratories that provide a clinical diagnostic service should evidently have the means to screen pneumococci for intermediate levels of resistance to penicillin. For more than 10 years in this laboratory the 3-disc method1 has been used to test gonococci for variations in susceptibility to penicillin. This is a disc-diffusion test with discs of 6 mm diameter containing respectively 0-03 units (0-018 μg), 0-25 units (0-15 μg), and 1 unit (0-6 μg) of penicillin G (Mast Laboratories, Liverpool). Jacobs et al.7 in South Africa used a development of this method to test pneumococci and concluded that, with the 0-018 μg disc, strains of intermediate resistance (MIC 0-1–1-0 μg/ml) which gave much reduced zones of inhibition could be readily distinguished from those that were fully sensitive to penicillin (MIC 0-015–0-024 μg/ml).

We have used the 3-disc method to test pneumococci isolated from patients in Fazakerley Hospital, Liverpool. Between October, 1979, and July, 1980, isolates from 66 patients were tested—from sputum (59 specimens), blood cultures (2), pleural fluid (1), thoracotomy wound (1), eye swab (1), cerebrospinal fluid (1), and brain (1, necropsy). All behaved as fully-sensitive strains with zones of inhibition round the disc with the least penicillin (0-018 μg) ranging in diameter from 18 to 28 mm. Thus we did not find evidence of the emergence in Liverpool of pneumococci showing any degree of resistance to penicillin. However, in the light of reports from Port Moresby and elsewhere it is clear that routine sensitivity tests of pneumococci should be capable of detecting the development of intermediate resistance to penicillin by, for example, the use of the 0-018 μg or other low-potency disc.

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MARGARET A. Knowles

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WEIGHING BABIES IN HEALTH CLINICS

Sir,—In Britain it is usual for mothers to take their babies to a health clinic to be weighed so that progress, especially in regard to the adequacy of feeding, can be monitored. The method of documenting weight in clinics varies considerably, but the commonest it seems is for the baby's weight to be written, along with the date, on a card. Unfortunately, this method does not always convey a reliable picture of weight gain, as shown in two babies who were regularly taken to a health clinic. Their weights were recorded on a clinic card. When they were four months old there was some concern about their weight gain, and they were referred to hospital.

Detection of antigonococcal IgA in cervical secretions by indirect immunofluorescence: an evaluation as a diagnostic test

A McMILLAN,*† G MCNEILLAGE,+ H YOUNG,‡ AND S S R BAIN†

From the *Department of Sexually Transmitted Diseases, Black Street, Glasgow, and the †Departments of Venereology and ‡Bacteriology, University of Edinburgh, Edinburgh

SUMMARY Using an indirect immunofluorescent-antibody method, antigonococcal IgA was detected in the cervical secretions of 56 (72%) of 78 women with untreated gonorrhoea but in only 25 (5%) of 490 non-infected women. The low sensitivity—comparable to that of Gram-stain microscopy—the expense, and the laboriousness of the test militate against its use as a routine diagnostic procedure.

Introduction

Although gonococcal antibodies in the serum of patients with gonorrhoea may be demonstrated by various methods,1 the detection of these antibodies has not proved useful as a diagnostic test.2 A serum antibody response may not be apparent until infection has been established for several days, and thus early gonorrhoea may be missed.2 Serum antibodies, particularly of the IgG class, remain detectable for weeks or months after successful treatment;3 interpretation of tests based on the demonstration of IgG antibody is therefore difficult. Wilkinson,4 using a fluorescent-antibody test, demonstrated significantly high titres (>16) of IgM antibody in only 24 (43%) of 55 women with untreated gonorrhoea; Ison and Glynn5 observed that the detection of IgM antibodies by an enzyme-linked immunoassay was of little diagnostic help.

Gonorrhoea is essentially an infection of mucosal surfaces, and gonococcal antibodies may be demonstrated in the urethral and cervical secretions from infected men and women respectively.6,7 The local IgA antibody response antedates that of the serum, and in most cases antigonococcal IgA in the secretions cannot be detected two to three weeks after successful treatment.7

By microscopical examination of Gram-stained smears of material from the cervix and urethra, only about 55% of women with gonorrhoea will be identified.8 Culture on selective medium is the most sensitive procedure available for the diagnosis of gonorrhoea in women; however, results are not generally available until 48 hours after the collection of specimens. To control gonococcal infection within a community, a rapid and sensitive method of identifying infected, but asymptomatic, individuals is required. The purpose of this study was to determine if the detection of antibodies in cervical secretions could be used as a diagnostic test for gonorrhoea. Preliminary data are presented in this paper.

Patients and methods

STUDY POPULATION AND DIAGNOSIS

With the exception of pregnant women, all female patients who attended the Department of Sexually Transmitted Diseases, Black Street, Glasgow, and clinics held by one of us (SSRB) at the Department of Venereology, Royal Infirmary, Edinburgh, during a six-month period were studied.

The methods used in both centres for the diagnosis of gonorrhoea have previously been described.7 Patients were divided into three groups: (a) 78 women with untreated gonorrhoea; (b) 178 women who had previously been treated for gonorrhoea; and (c) 490 women who had no microbiological evidence of current gonococcal infection or a past history of gonorrhoea.

COLLECTION OF SPECIMENS

Under direct vision, a cottonwool-tipped applicator stick was inserted into the cervical canal to a distance
of about 1 cm and allowed to remain in contact with the secretions for about 15 seconds. Secretions were eluted from the swab by placing this in 0.5 ml of phosphate-buffered saline, pH 7.4, containing Tween 20 (0.05% v/v). Samples were frozen at -20°C until required. After thawing and centrifugation at 2000×g for 15 minutes, the supernate was used for estimating IgA and IgG concentrations and for the indirect immunofluorescent-antibody test.

**ESTIMATION OF IgA AND IgG CONCENTRATIONS**

Using commercially available, low-level immunodiffusion plates (Hoescht Pharmaceuticals, Hounslow, Middlesex) the concentrations of IgA and IgG in the supernate of diluted secretions from 18 patients selected at random were determined. Commercially available serum IgG (Travenol Laboratories, Norfolk) was used as reference standard; colostral IgA standard was used in the estimation of secretory IgA.

**INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST**

The procedure has been described in detail elsewhere. Strain 9 of *Neisseria gonorrhoeae*, as described by O'Reilly and co-workers, was used as antigen. The grading of fluorescence was as follows: 4+, brilliant fluorescence of all organisms; 3+, well defined fluorescence of all organisms in the field; 2+, low density fluorescence of at least 75% of organisms; and 1+, occasional fluorescing organisms. Only a 2+ fluorescence or higher reading was recorded as a positive result.

**STATISTICAL METHOD**

Statistical comparisons were made using the χ² test with Yates's correction.

**Results**

**IMMUNOGLOBULIN CONCENTRATIONS**

The mean IgA and IgG concentrations in the supernate of the diluted cervical secretions were 11·4×10⁻³ g/1 (standard deviation, SD 6·7×10⁻³) and 30·5×10⁻³ g/1 (SD 20·6×10⁻³) respectively.

**INDIRECT IMMUNOFLUORESCENT-ANTIBODY TEST**

The results obtained when cervical secretions from patients in each of the three groups were examined for IgA and IgG antibodies reactive with *N. gonorrhoeae* are given in Table I.

**IgA**

IgA antibody was detected in 56 (72%) of the 78 women with untreated gonorrhoea but in only 25 (5%) of 490 non-infected women; the mean duration of infection in the 22 women in whom antibody was not detected was 16·6 days (range 4-48 days).

Antigonnococcal IgA was found in cervical secretions from 24 (22%) of 110 women who had been treated more than 28 days previously (mean interval between treatment and sampling, 422 days).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>With previously treated gonorrhoea (days between treatment and sampling)</th>
<th>Non-infected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With untreated gonorrhoea</td>
<td>≤7</td>
<td>8-14</td>
</tr>
<tr>
<td>IgA only</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>IgG only</td>
<td>7</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>IgA and IgG</td>
<td>48</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>None</td>
<td>15</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>14</td>
<td>32</td>
</tr>
</tbody>
</table>

**TABLE II Results of immunofluorescent-antibody test to detect antigonnococcal IgA in cervical secretions from 78 women with untreated gonorrhoea compared with those of Gram-stain microscopy and culture**

<table>
<thead>
<tr>
<th>Fluorescent-antibody test</th>
<th>Gram-stain only</th>
<th>Culture only</th>
<th>Gram-stain and culture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigonnococcal IgA detected</td>
<td>1</td>
<td>16</td>
<td>39</td>
<td>56</td>
</tr>
<tr>
<td>Antigonnococcal IgA not detected</td>
<td>0</td>
<td>4</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>20</td>
<td>57</td>
<td>78</td>
</tr>
</tbody>
</table>
IgG antibody reactive with N. gonorrhoeae was demonstrated in the cervical secretions of 55 (71%) of 78 women with untreated gonorrhoea and in 89 (18%) of 490 non-infected patients. This antibody was found in the secretions of 19 (17%) of the 110 women who had been treated for gonorrhoea at least 29 days previously.

**Comparision with Gram-stain microscopy**
In relation to the results of culture, the sensitivity (72%) of the fluorescent-antibody test with antiserum against IgA was almost identical to that of Gram-stain microscopy (74%) (table II).

**Oral contraceptives and the fluorescent-antibody test**
Data on the method of contraception, if any, were available for 60 women with gonorrhoea. Table III shows the results of the fluorescent-antibody test with IgA antiserum in relation to the contraceptive method used. Antigonococcal IgA was detected in 22 (73%) of 30 women who used an oral contraceptive preparation but in only 12 (40%) of 30 patients who used some other form of contraception or none. This difference in antibody detection is statistically significant ($\chi^2 = 5.50; P<0.02$).

<table>
<thead>
<tr>
<th>Type of contraception</th>
<th>IgA present</th>
<th>IgA absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>22</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>IUD or none</td>
<td>12</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>26</td>
<td>60</td>
</tr>
</tbody>
</table>

**Discussion**

In a previous study, IgA reactive with N. gonorrhoeae was demonstrated in the cervical secretions (obtained by aspiration) of 71 (95%) of 75 women with untreated gonorrhoea. The rate of detection of antibody (72%) was significantly lower in the present series and probably reflects the different sampling method; the mean IgA concentration in the secretions eluted from the swabs was $11\cdot4\times10^{-3}$ g/l compared with $24\cdot0\times10^{-3}$ g/l in secretions obtained by aspiration. The collection of secretions by the latter method is however cumbersome and not suitable for routine use in a busy clinic.

The sensitivities of the immunofluorescent-antibody test with IgA antiserum and of Gram-stain microscopy were almost identical (72 and 74% respectively) but the former test was expensive and time-consuming, and results were not available for at least one hour after collection of specimens. Similar difficulties were encountered with direct fluorescent-antibody procedures.

Although antigonococcal IgA was detected in secretions from 25 (5%) of 490 women who had no microbiological evidence of gonococcal infection, it cannot be assumed that these patients had not recently been infected. In previous studies, IgA antibody was not detectable in the cervical secretions of women who had been treated more than 14 days previously. From the present study however, it is clear that this antibody can be detected in the secretions a month or even longer after satisfactory treatment. It is possible that some of these 25 women had been treated inadvertently before attending the clinic. As previously shown, a local antibody response may be detected in known contacts in whom repeated microbiological examination fails to show active gonococcal infection.

It was of interest that antigonococcal IgA was detected significantly more frequently in cervical fluid from women with gonorrhoea who used an oral contraceptive preparation. Chipperfield and Evans showed that the mean concentration of IgA in cervical mucus was significantly higher in women using a combined pill than in women with natural cycles.

The low sensitivity and specificity of the fluorescent-antibody test using IgG antiserum is clearly shown. IgG antibody is frequently detected in cervical secretions from patients with nongonococcal cervicitis and is probably the result of transudation of natural serum antibody through the inflamed mucus membrane.

As IgM antibody is detectable in the secretions of only about 40% of women with gonorrhoea, the fluorescent-antibody test with IgM antiserum was not performed.

We wish to thank the staff of the two clinics for their assistance in the collection of specimens.

This project was supported by a grant (reference number K/MRS/50/C182) from the Scottish Home and Health Department.

**References**


Detection of antibodies reactive with Neisseria gonorrhoeae in secretions on extra-genital surfaces

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Summary

Using an immunofluorescent-antibody test, antigonococcal antibody was found in the rectal secretions of six of 18 men with rectal gonorrhoea, being of the IgG class in all six and associated with IgA in three. This antibody was only detected in two of 14 women with rectal infection.

Antibody reactive with Neisseria gonorrhoeae was found in the saliva of each of 12 men, and in four of six women, with oropharyngeal gonorrhoea.

Salivary antibody was found in 10 of 18 homosexual, and in two of 25 heterosexual men with urethral or rectal gonorrhoea, but no apparent pharyngeal infection (P < 0.05).

Introduction

Gonorrhoea is an infection confined mainly to the mucosal surfaces of the body, and like other infections at these sites, likely to stimulate a secretory immune response. Antibodies reactive with Neisseria gonorrhoeae may be demonstrated in genital secretions by various techniques including precipitin reactions (Kelley, 1922), haemagglutination (Hirschberg, 1970) and immunofluorescent antibody tests (Kearns, O'Reilly, Lee and Welch, 1973; O'Reilly, Lee and Welch, 1976).

Most workers have investigated the secretory IgA response, with a few reports on the detection of the other immunoglobulin classes (Tapchaisri and Sirisinha, 1976).

Although principally an infection of the genital mucous membranes, the gonococcus not uncommonly infects the oropharynx; about five per cent of patients with genital infection may have concomitant pharyngeal gonorrhoea (Stolz and Schuller, 1974), most commonly as a result of fellatio. Ano-rectal infection in men results from anal intercourse, and is common in homosexual men attending sexually-transmitted diseases clinics (McMillan and Robertson, 1977). Rectal gonorrhoea in women is common, being found in up to 45 per cent of those with genital infection (Bhattacharyya and Jephcott, 1974).
There have been no reports on the production of antibodies against the gonococcus at these surfaces and it is the purpose of the present paper to investigate this aspect of the local immune response to gonococcal infection.

Patients and methods

Patients
Patients attending the Department of Venereology, Black Street Clinic, Glasgow, were investigated.

Material for microbiological examination was obtained from the urethra and oropharynx of all men in the study, and if there had been homosexual contact, from the ano-rectum.

In homosexual men, if tests taken at the patient’s initial visit failed to show evidence of gonococcal infection, cultures from the ano-rectum and oropharynx were repeated twice at weekly intervals. Only if these sets of investigations were negative was gonorrhoea at these sites excluded.

From women, specimens were taken from the urethra and cervix for microscopic and culture examination and for culture only from the oropharynx and ano-rectum. Gonococcal infection in women was only excluded if three sets of tests, taken at weekly intervals were negative.

Material for culture was sent to the laboratory in Stuart’s transport medium. The culture medium and methods of identification of Neisseriae have previously been described (McMillan and Young, 1978). A diagnosis of gonorrhoea was made only if N. gonorrhoeae was isolated on culture.

In order to obviate any difficulties in interpretation of results as a consequence of infection at multiple sites, homosexual men with gonorrhoea were selected for study only if the infection was confined to one site. Similarly, women with oropharyngeal gonorrhoea were included only if there was no evidence of concomitant ano-genital infection.

Patients who had received anti-microbial drugs within the preceding two months, or who had previously been infected with gonorrhoea or, who although showing no evidence of gonococcal infection, were known sexual contacts of persons with gonorrhoea, were not investigated.

Collection of secretions
Ano-rectal secretions were collected from each homosexual patient who attended; these secretions were not taken from heterosexual men. Material from the ano-rectum was taken from each woman who attended. In each case, a proctoscope, lightly lubricated with K-Y jelly (Johnson-Johnson, Slough, U.K.) was inserted into the anal canal, and secretions collected by rolling a cotton wool-tipped applicator stick over the mucosal surfaces of the rectum. Secretions were eluted by placing the swab immediately into 0.5 ml of phosphate buffered saline pH 7.4 (PBS) and leaving for one hour at room temperature. The diluted secretions were centrifuged at 1500 g for 20
minutes, a drop of sodium azide, 0.1 per cent in PBS, was added, and the secretions stored at -20°C until required. Saliva was obtained from each patient studied. The patient rinsed his/her mouth with 10 ml of PBS and ejected this into a universal container. After centrifuging at 1500 g for 20 minutes, the saliva was concentrated to a final volume of 1 ml using a positive pressure dialysis unit (Amicon Microfiltration System, Model 8MC, with a Diaflo Membrane, PM30, Amicon, Lexington, Massachusetts, U.S.A.). Sodium azide was added and the specimen stored as above.

Indirect immunofluorescent-antibody (IFA) test
Secretions were examined for antibody using an indirect immunofluorescent-antibody technique, as previously described for examination of serum (McMillan, McNeillage, Young and Bain, 1979). Strain 9 of N. gonorrhoeae as described by O'Reilly, Welch and Kellogg (1973) was used as antigen in the test, as it had been shown to contain antigenic characteristics common to a variety of gonococcal strains, but not shared by other Neisseria spp. Commercially-available (Wellcome Reagents Ltd., Beckenham, Kent) fluorescein-conjugated antisera against IgA (α-chain), IgG and IgM were used in the test.

Fluorescence was graded according to the system of Welch and O'Reilly (1973) - 4+ = brilliant fluorescence of all organisms in the field; 3+ = well-defined fluorescence of all organisms in the field; 2+ = low-intensity fluorescence of at least 75 per cent of organisms; 1+ = occasional fluorescing organisms. Only a 2+ fluorescence of higher reading was recorded as positive.

Statistics
The $\chi^2$ test with Yates' correction was used to compare data between the various groups of patients.

Results
Tables I and II show the sites infected in the patients studied.

Ano-rectal secretions
Antibody of the IgG class reactive with N. gonorrhoeae was demonstrated in the rectal secretions of six (33 per cent) of the 18 men with ano-rectal gonorrhoea, and of eight (28 per cent) of the 29 non-infected homosexual patients. Antigonococcal IgA was detected in three (17 per cent) of the infected, and in one (four per cent) of the non-infected men. Antibody of the IgM class against the gonococcus could not be demonstrated in any of the secretions.

Ano-rectal secretions from two (14 per cent) of 14 women with ano-rectal gonorrhoea were found to contain antibody reactive with the gonococcus,
Table I Sites of infection with N. gonorrhoeae in 114 men

<table>
<thead>
<tr>
<th>Site infected</th>
<th>No. of men</th>
<th>Heterosexual</th>
<th>Homosexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra only</td>
<td>25</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Ano-rectum only</td>
<td>0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Oropharynx only</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>30</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>59</td>
<td></td>
</tr>
</tbody>
</table>

Table II Sites of infection with N. gonorrhoeae in 66 women

<table>
<thead>
<tr>
<th>Site infected</th>
<th>No. of women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra and/or cervix only</td>
<td>11</td>
</tr>
<tr>
<td>Urethra and/or cervix, and ano-rectum</td>
<td>14</td>
</tr>
<tr>
<td>Oropharynx only</td>
<td>6</td>
</tr>
<tr>
<td>None</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
</tr>
</tbody>
</table>

being of the IgG class in one, and of both IgA and IgG classes in the other. Antigococcal IgM was not detected. No antibody reactive with N. gonorrhoeae was found in the rectal secretions of 35 non-infected women, or in those from 11 women with genital gonorrhoea only.

Saliva
Antibody of the IgA class reactive with N. gonorrhoeae was detected in each of the 12 men with oro-pharyngeal infection and was found in two (seven per cent) of 29 non-infected homosexual men and in two (seven per cent) of 30 non-infected heterosexual men. This class of antigococcal antibody was found in the saliva of 10 (71 per cent) of 18 homosexual men with ano-rectal gonorrhoea only and of two (eight per cent) of 25 heterosexual men with urethral infection only. The difference between the latter two groups of patients is significant (\( P < 0.005, \chi^2 = 9.4 \)).

Immunoglobulin G reactive with N. gonorrhoeae was found in the saliva of nine (75 per cent) of 12 men with oro-pharyngeal infection but in only four (14 per cent) of 29 non-infected homosexual, and three (10 per cent) of 30 non-infected heterosexual men. This antibody was demonstrated in the saliva of six (33 per cent) of 18 homosexual men with ano-rectal gonorrhoea, but no apparent pharyngeal infection, and in the saliva of one (four per cent) of 25 heterosexual men with only urethral gonorrhoea; this difference is significant (\( P < 0.05, \chi^2 = 4.3 \)).

Antibody of the IgM class reactive with N. gonorrhoeae was found in the saliva of three of the 12 men with oropharyngeal gonorrhoea, but in none of the other patients.

The saliva of four of six women with oropharyngeal infection contained
antibodies against the gonococcus, being of the IgA class in each, and associated with IgG in three. Immunoglobulin A antibody was found in one (three per cent) of the 35 non-infected women, and in two (eight per cent) of the 25 with ano-genital infection. Saliva from three of 25 (12 per cent) women with ano-genital gonorrhoea contained antigonococcal IgG as did the saliva of one (three per cent) of 35 non-infected women. Antigono-coccal IgM was not found in the saliva of any of the infected or non-infected women.

Discussion

Antibodies against the gonococcus may be demonstrated in up to 90 per cent of men and women with urethral and cervical gonorrhoea respectively. Both IgA and IgG classes of antibody are involved, IgM being found in only about half of the infected patients (McMillan, McNeillage and Young, 1979; McMillan, McNeillage, Young and Bain, in press). Although the number of patients available for investigation in the present study is small, an antibody response to infection in the oro-pharynx was found in most patients with infection of these surfaces. An interesting observation was the high proportion of homosexual patients with ano-rectal gonorrhoea, but no evidence of pharyngeal infection, who had antibody against the gonococcus in the saliva. Several factors may explain this situation. These men may have had pharyngeal gonorrhoea which was not detected. The diagnosis of pharyngeal infection is not always simple, multiple testing being required to exclude infection (McMillan and Young, 1978). Clearly it would have been undesirable to withhold treatment for ano-rectal infection in order to perform repetitive pharyngeal cultures. Another possibility may be that the patient had been infected, an immune response had been elicited, and the infection eradicated. Antigono-coccal antibodies may be demonstrated in the cervical fluid of women who, although named sexual contacts, are not found to have infection (McMillan, McNeillage, Young and Bain, 1979). A third hypothesis is that antigenic material may be absorbed from the ano-rectum and carried to other mucosal surfaces where a local response is elicited. This seems to be an unlikely explanation. Ogra and Karzon (1969) demonstrated that an immune response was produced in mucosal surfaces only to which antigen was applied. Analysis of lacrimal secretions from 30 patients with ano-rectal gonorrhoea, failed to reveal any antigono-coccal antibody (McMillan, unpublished data).

In only a small proportion of patients with ano-rectal infection could antigono-coccal antibody be detected. This is somewhat surprising in view of the demonstration of IgA- and to a lesser extent IgG- and IgM-containing plasma cells in the lamina propria of the rectal mucosa (Crabbe and Here-mans, 1966) and the proliferation of these cells in gonococcal infection (Harkness, 1948). A possible explanation may be the degradation of immunoglobulin by proteolytic enzymes in the rectal secretions. Secretary IgA is, however relatively resistant to proteolysis (Lindh, 1975) and in a
series of experiments (McMillan, unpublished data) in which secretory IgA was incubated in vitro with rectal secretions, no degradation was observed over a period of six hours.

The finding of antigonococcal IgG in the rectal secretions from over a quarter of homosexual men who showed no evidence of gonorrhoea may reflect previous infection which had been inadvertently treated. Significant levels of IgG antibody in genital secretions persists for months after successful treatment of gonorrhoea (McMillan, McNeillage, Young and Bain, 1979).

(This project was supported by a grant from the Biomedical Research Committee of the Scottish Home and Health Department (Research Grant No. K/MRS/36/C38) whose assistance is gratefully acknowledged.)

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Clinical Practice in Sexually Transmissible Diseases

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PITMAN MEDICAL
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*between pages 116 and 117*
Foreword

I entertain the hope that the Foreword may help to introduce this volume to a much broader readership than just those already committed to the specialty of Sexually Transmissible Diseases. The increased opportunities for the transmission of infection by sexual contact which contemporary patterns of living promote, and the widening spectrum of organisms of all genera involved, have enhanced the relevance of this volume’s subject matter to many areas of medical practice.

Sexual health problems, regrettably, are ignored or deliberately avoided by many doctors. I believe the reasons for their unease with these topics are linked with influences operating in the formative years of their medical careers. During their early professional training, doctors unconsciously acquire a necessary indifference to the nudity and intimate touching inseparable from clinical practice. Often their continuing comfort in the face of this physical closeness is only maintained if the patient’s sexuality is excluded from thought or discussion.

However, it is impossible to practice medicine competently wearing sexual blinkers, for many important problems of physical and mental health originate from sexual function or dysfunction. A new specialty—’Sexual Medicine’—has been adumbrated, but I wonder if it is the wisest course to attempt to segregate all sexual aspects of health in this way: they are too much an integral part of the general fabric of health care. I believe, therefore, that the information which Dr Robertson and his colleagues have brought together in this volume will be of value to many health professionals, but the book bears its own commendation in the blend of scientific erudition, humane concern and sound practical guidance which is so clearly evident throughout.

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Preface

This book has been written primarily for those who are actively engaged in the increasingly demanding clinical practice of venereology and who, by so doing in the United Kingdom at least, now tend to undertake so large a part of the primary care of adolescents and young adults. We have attempted to bring together information which is widely scattered in the literature and to present an appreciation of clinical and laboratory aspects of the various subjects in a way which we believe will be useful to our colleagues. It is our hope that the book will be of value, as a reference in teaching and also to the wider range of clinicians, for instance gynaecologists and urologists, who participate as we do in the practice of sexual and reproductive medicine and will therefore often require to consider the full range of sexually transmitted diseases. General practitioners and other clinicians, who are involved in clinics set up for counselling and for giving contraceptive and kindred advice, will find some answers to their questions, and physicians, who may not ordinarily look after adolescents and young adults, may find the inclusion of sexually transmissible infections in their differential diagnosis a rewarding exercise. It is clear that when precise information about the organisms involved is regarded as an essential discipline in the diagnosis of genito-urinary and pelvic inflammatory disease, the importance of transmission by sexual intercourse will become better appreciated and the application of isolation methods, more searching than conventional bacteriological investigations, will help in developing more rational care of patients.

Although this book is primarily for medical readers it is hoped that those involved in nursing or counselling patients, tracing contacts or in health education will be able to obtain some of the factual information which they require. Barriers between disciplines are tending to become inappropriate and those who share objectives in patient care will require to pool their knowledge to obtain the best results possible.

There is more to be achieved by those concerned than a technical understanding of one aspect of clinical medicine and microbiology because psychological and social barriers intrude and hamper at every level. Some aims of this text are primarily intellectual and are fundamental to practice in a subject which involves deep personal feelings to such an extent that patient, doctor and society may appear not
infrequently to be bewitched. The reader will be encouraged to adopt a logical attitude essential in clinical practice and an approach to the subject based on an acceptance of human diversity.
IDENTIFICATION OF PATHOGENIC NEISSERIAE BY GENETIC TRANSFORMATION

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Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh, EH8 9AG, Scotland

Developments in selective media and rapid identification procedures have considerably improved the cultural diagnosis of gonorrhoea (Jephcott and Rashid, 1978; Young, 1978a). When direct plating with immediate incubation is impracticable, several transport and growth systems such as Transgrow (Martin and Lester, 1971), Microcult GC (Willcox and John, 1976) and the biological environment chamber described by Martin and Jackson (1975) are now available, in addition to the conventional non-nutrient transport media such as Amies’s modification of Stuart’s medium (Amies, 1967).

Unfortunately, all of the above systems depend on maintaining the viability of Neisseria gonorrhoeae and in many localities transport-associated problems continue to impose limitations on their value. To overcome these problems it was recommended (World Health Organization, 1978) that methods for the detection of gonococcal components in the secretions of infected patients should be investigated.

Janik, Juni and Heym (1976) described a method for the detection of N. gonorrhoeae based on the ability of DNA samples from clinical isolates of gonococci to transform nutritional mutants of a particular strain of N. gonorrhoeae. At the time we wished to examine this technique in our own laboratory the proline auxotrophs used by Janik et al. (1976) could not be made available to us. Accordingly, we examined the feasibility of using a laboratory stock culture of N. gonorrhoeae strain F62, a strain known to require proline for growth in chemically defined medium (Catlin, 1973; Knapp and Holmes, 1975).

This paper reports our evaluation of the sensitivity and specificity of a genetic-transformation assay with this strain.

Materials and methods

Bacteria. N. gonorrhoeae strain F62 colony-type 2 was kindly supplied by Dr A. E. Jephcott, Public Health Laboratory, Myrtle Road, Bristol. The strain is autotrophic for proline and was used as recipient in the genetic transformation assay. N. gonorrhoeae strain 9 was kindly supplied by Dr D. S. Kellogg, Centre for Disease Control, Atlanta, Ga, USA. Stock cultures of the following strains of Neisseria were supplied by the National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London: N. elongata NCTC10660, N. cinerea NCTC10294, N. catarrhalis NCTC3622, N. cuniculi NCTC10297, N. canis NCTC10296, N. pharyngis var. flavus NCTC4590, N. pharyngis var. siccus NCTC4591, N. denitrificans
NCTC10295, and N. animalis NCTC10212. N. meningitidis strains of serogroups A, B, C, D, E, 29E, WI35, X and Z were originally obtained from the Neisseria Repository, Berkeley, California. The Oxford staphylococcus and a laboratory stock culture of Pseudomonas aeruginosa were obtained from the diagnostic section of this department. Subcultures of genital and oral isolates of neisseriae from patients attending the Department of Venereology, the Royal Infirmary, Edinburgh, were also obtained from the diagnostic laboratory; these included 169 strains of N. gonorrhoeae, 12 of N. meningitidis, three of N. lactamica, and one of N. perflava.

Gram staining and oxidase testing were done on all isolates. The clinical isolates were identified by the fluorescent-antibody test and the rapid carbohydrate-utilisation test as described by Young (1978a).

Culture media. Modified New York City (MNYC) medium (Young, 1978b) was used for subculture of isolates and for maintenance of N. gonorrhoeae strain 9. Stock strains of non-pathogenic neisseriae were maintained on MNYC medium lacking antibiotics.

Gonococcal genetic medium (GGM) (La Scola and Young, 1974) was used for growth and maintenance of N. gonorrhoeae strain F62 type 2 while it was auxotrophic for proline; the same medium lacking proline (GGM Pro−) was used to detect strain F62 when it became prototrophic for proline after transformation.

Preparation of transforming DNA. A sterile swab was charged with the growth from an overnight plate culture of the test organism. It was then placed in 0-5 ml of 0-025% (w/v) sodium dodecyl sulphate in standard saline citrate solution (0-15M sodium chloride, 0-015M sodium citrate) and agitated to remove bacteria. The resulting suspension was heated in a water bath at 65–68°C for 45–60 min. After heating, the crude DNA preparations were diluted with 0-3 ml of sterile distilled water to compensate for the increase in SDS concentration caused by evaporation.

Transformation assay. Three DNA samples, including DNA extracted from strain 9 as a control, were tested on a single GGM plate. The plate was divided into seven sections; two drops (approximately 0-04 ml) of a thick suspension (approximately 10^10 colony-forming-units (c.f.u.)/ml) of strain F62 colony-type 2 in saline citrate were placed on each of three sections of the plate and one drop on a fourth section. Two drops of each of the three crude DNA preparations were deposited over the recipient cells in each of the three areas that had received two drops of F62. A drop of each of the DNA samples was placed on the remaining three sections as a sterility control. After incubation of the plate for 3 h at 37°C in a CO2-enriched (10%) atmosphere, the contents of each section were streaked on to an identically marked plate of GGM Pro− medium which was then incubated for 48 h as described above. The GGM plate was also incubated to check the viability of F62 and to monitor for contamination.

After incubation, the GGM Pro− plates were examined with a Zeiss binocular stereoscopic microscope with substage lighting (Jephcott and Reyn, 1971). A positive result in the transformation assay was indicated by growth of several colonies (≥20 colonies) identical in size and appearance to the colonies of strain F62 growing on GGM. The results of the test specimens were compared with those obtained with N. gonorrhoeae strain 9. The section containing non-DNA-treated F62 streaked on to GGM Pro− was a control to detect spontaneous revertant prototrophic cells.

Relationship of colony-type of recipient to the efficiency of transformation. Viable counts were made on thick suspensions of N. gonorrhoeae strain F62 colony-types 2 and 3 by the method of Miles, Misra and Irwin (1938); the dilutions were made in saline citrate and the colonies counted on clear GC agar (Kellogg et al., 1963). A crude DNA preparation was made from N. gonorrhoeae strain 9. Hundredfold dilutions of the DNA preparation were made in saline citrate up to a dilution of 10^12 and assayed as already described with cells of colony-types 2 and 3 as recipients.

Assessment of sensitivity of the test procedure with N. gonorrhoeae strain 9 and N. meningitidis serogroup B. A crude DNA preparation was made from a thick suspension of N. gonorrhoeae strain 9: the number of bacteria present in the suspension was determined by the method of Miles et al., 1938. Hundredfold dilutions of the crude DNA were made up to a dilution of 10^12. Each dilution was assayed as previously described. The procedure was repeated with N. meningitidis serogroup B as test organism.
Assessment of proportion of clinical isolates auxotrophic for proline. Eighty-four of the clinical isolates were subcultured on to GGM and GGM Pro⁻ media to test their requirement for proline.

Duration of storage of test organisms on swabs. Nineteen MNYC plates were seeded with N. gonorrhoeae strain 9. After overnight incubation, the growth on each plate was harvested with a sterile serum-coated swab (Exogen Ltd, Clydebank Industrial Estate, Beardmore Street, Clydebank G81 4SA, Scotland). Nine of the swabs were stored at 4°C, nine at room temperature and the remaining swab was used immediately in a transformation assay. The test organisms on the swabs, stored as described above, were assayed at intervals up to 68 days.

RESULTS

Colony type of recipient in relation to the efficiency of transformation

Viable counts showed that suspensions of strain F62 types 2 and 3 used as recipient contained approximately 10¹⁰ c.f.u./ml. With a series of DNA dilutions up to 10¹² made from N. gonorrhoeae strain 9, strain F62 colony-type 2 gave a positive transformation result up to a dilution of 10⁶ DNA whereas colony-type 3 gave a positive transformation result up to a dilution of 10⁴.

Stock strains

Apart from N. gonorrhoeae strain 9 and N. meningitidis strains of serogroups A, B, C, D, E, 29E, W135, X, Z, all of the remaining stock cultures, including the various other members of the family Neisseriaceae failed to transform N. gonorrhoeae strain F62.

Clinical isolates

Of the 169 clinical isolates of N. gonorrhoeae, 150 (88·8%) gave a positive transformation assay. All 12 of the meningococcal isolates tested were positive as were the three strains of N. lactamica. N. perflava gave a negative transformation result.

Assessment of proportion of clinical isolates auxotrophic for proline

Of 84 clinical isolates of gonococci tested, 73 gave a positive transformation assay. Eighteen of the isolates grew on GGM and on GGM Pro⁻, 58 failed to grow on either medium, 8 grew only on GGM, and none grew only on GGM Pro⁻. Of the 8 strains growing only on GGM, 6 (75%) gave a negative result in the transformation assay. All of the 18 strains that grew on both media gave a positive transformation result.

Sensitivity of transformation assay

The thick suspension of N. gonorrhoeae strain 9 contained 1·7 × 10¹⁰ c.f.u./ml and gave a positive transformation result up to a dilution of 10⁶. Because 0·1 ml of this suspension was placed in 0·9 ml of the SDS solution, the
crude DNA preparation was obtained from cells at a concentration of \(1.7 \times 10^9\) c.f.u. Thus, 0.04 ml of the most concentrated sample of DNA tested in the transformation assay was derived from \(6.8 \times 10^7\) c.f.u. and 0.04 ml of the maximum dilution that gave a positive result corresponded to 68 c.f.u.

The thick suspension of \(N.\) meningitidis serogroup B contained \(4.7 \times 10^9\) c.f.u./ml and gave a positive transformation result up to a dilution of \(10^2\). Because 0.1 ml of this suspension was placed in 0.9 ml of the SDS solution, the crude DNA preparation was obtained from cells at a concentration of \(4.7 \times 10^8\) c.f.u. Thus, 0.04 ml of the most concentrated sample of DNA tested in the transformation assay corresponded to \(1.9 \times 10^7\) c.f.u. and the maximum dilution that gave a positive result corresponded to \(1.9 \times 10^5\) c.f.u.

Storage of test organisms on swabs

Swabs bearing test suspensions stored at 4°C and room temperature gave a positive result when tested at intervals up to 68 days. The effect of longer storage was not tested.

Discussion

Our results confirm the findings of Janik et al. (1976) that genetic transformation can be used as a tool for the identification of \(N.\) gonorrhoeae. Although we used a different strain as recipient, the overall sensitivity of the technique is comparable. Janik et al. (1976) found that approximately 50 c.f.u. of donor cells were required to give a positive result compared with approximately 70 c.f.u. in our studies with \(N.\) gonorrhoeae strain F62. As Janik et al. (1976) found, colony-type 2 was more efficient than colony-type 3 in the uptake of DNA.

With the exception of \(N.\) meningitidis and \(N.\) lactamica, the transformation assay is specific for \(N.\) gonorrhoeae. A positive result was obtained with all 12 clinical meningococcal isolates and the stock cultures of the different meningococcal serogroups. These results are also similar to those of Janik et al. (1976) who found that DNA preparations from only a few neisseriae other than the gonococcus were able to transform their proline auxotrophs; these preparations were unable to transform a uracil and arginine auxotroph in the standard transformation assay conditions. The lack of specificity with regard to \(N.\) meningitidis would not obviate the value of the assay in detecting ano-genital gonorrhoea because meningococci are relatively rare in the urogenital tract and anal canal (Givan, Thomas and Johnston, 1977; Blackwell, Young and Bain, 1978) and the number required to give a positive transformation assay is approximately 1000-fold higher than when gonococci are used. However, in view of the positive results given by meningococci the genetic-transformation assay described would not be suitable for diagnosing pharyngeal gonorrhoea.

Naturally-occurring proline auxotrophs do not appear to limit the value of the assay greatly because approximately 90% of the clinical isolates gave a
positive transformation assay. In another locality Bawdon, Juni and Britt (1977) reported that 97% of 71 clinical isolates of N. gonorrhoeae gave a positive transformation result in an assay that used a uracil and arginine auxotroph. Proline requirement is probably the main reason for negative results in our survey because 6 of 8 (75%) strains that failed to grow on GGM Pro- gave negative results whereas all of the 18 isolates that grew on both GGM and GGM Pro- gave positive results. However, our results with respect to the growth of isolates on GGM and GGM Pro- do not seem to be very reliable because 58 of 84 (69%) fresh clinical isolates failed to grow on both media; clinical isolates probably need to be adapted to growth in the laboratory before giving reliable growth on a minimal medium such as GGM.

After prolonged storage of gonococci on swabs, a positive transformation result could still be obtained. Therefore the ability of the assay to detect non-viable gonococci would make this test of value in overcoming problems associated with the transport of specimens before cultivation by conventional methods. A trial is now under way to assess the value of this procedure in clinical practice.

**Summary**

The detection of pathogenic neisseriae by genetic transformation of a naturally occurring proline auxotroph of Neisseria gonorrhoeae strain F62 is described. Of 169 clinical isolates of N. gonorrhoeae, approximately 90% gave a positive transformation assay. Twelve clinical isolates of N. meningitidis and stock cultures of the various meningococcal serogroups also gave a positive result. However, the sensitivity of the assay was found to be approximately 1000-fold lower with N. meningitidis as test organism. Eleven other members of the family Neisseriaceae failed to transform the recipient organism.

Although proline requirement did not appear to limit the value of the assay greatly, it probably was the main reason for negative results. The sensitivity of the assay and its ability to detect non-viable gonococci suggests that this method merits further investigation as a possible aid to diagnosis of gonococcal infection in special circumstances.

This work was supported in part by a grant from the Scottish Home and Health Department (research grant no. K/MRS/50/C22).

We thank Professor J. G. Collee for helpful advice in the preparation of this paper and Dr C. Caroline Blackwell for her interest and valuable comments made throughout the course of the work.

**REFERENCES**


Isolation of Neisseria lactamica from the female genital tract
A case report

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SUMMARY Neisseria lactamica was isolated from the genital tract of a young patient with a persistent vaginal discharge. Although infection with N lactamica occurs very rarely, the importance of complete biochemical identification of neisseriae is emphasised in view of the serious social and medicolegal consequences which could result from a misdiagnosis of gonorrhoea.

Introduction

Neisseria lactamica is similar to Neisseria meningitidis in many respects but differs in its relative lack of virulence and ability to utilise lactose.1 Although meningococcal colonisation of the genital tract is recognised with increasing frequency2,3 there has only been one previous report of the isolation of N lactamica from a genital site.4 This paper reports an additional isolation of N lactamica from the genital tract and emphasises its rarity.

Case report

CLINICAL HISTORY
An 18-year-old secretary, who was engaged to be married, presented to her general practitioner with depression and complaints of a yellow offensive vaginal discharge of some days’ duration. She was taking Ovranette (Wyeth Laboratories) contraceptive pills, and her menstrual cycle had been normal. A clinical diagnosis of Trichomonas vaginalis infection was made and a one-week course of treatment with metronidazole (600 mg daily) started. During the week of treatment, her symptoms improved but recurred within a few days of the end of the course. Two weeks after her initial visit she returned still depressed and complaining of a troublesome vaginal discharge, dysuria, and frequency of micturition. On examination she was apyrexial but had a foul-smelling yellowish vaginal discharge. The cervix was inflamed and the uterus, although of normal size, was tender on bimanual examination. The uterine appendages were normal. Cervical and high vaginal swabs were taken, placed in Stuart’s transport medium, and sent to the laboratory with a midstream specimen of urine (MSSU). Empirical treatment with clindamycin (600 mg daily) was started.

LABORATORY INVESTIGATIONS
Investigation of the MSSU showed many pus cells and Proteus mirabilis (≥10³/l). Direct microscopy of the vaginal and cervical swabs showed many pus cells and mixed organisms. No intracellular Gram-negative diplococci were seen. Routine culture yielded many Bacteroides fragilis and anaerobic streptococci; culture on modified New York City medium, in an atmosphere of 5% CO₂ in air, showed moderate numbers of Gram-negative diplococci, which were identified as Neisseria lactamica by a rapid carbohydrate utilisation test.6

TREATMENT AND FOLLOW UP
In view of the laboratory findings, the patient was given a 10-day course of cotrimoxazole (4 tablets daily) for her urinary tract infection. When she attended for follow up two weeks later, the urinary symptoms had resolved and her MSSU culture gave a negative result. The vaginal discharge remained troublesome and two high vaginal swabs (one taken at follow up and another a month later) showed large numbers of Bacteroides fragilis and anaerobic streptococci. Neisseriae were not seen in or cultured from any further specimens.
Discussion

In view of the persistence of the vaginal discharge after the disappearance of *N. lactamica* from the genital tract it is unlikely that this organism contributed to the patient's symptoms. The anaerobic streptococci and *Bacteroides fragilis* were probably more important in this respect, although their relationship to troublesome vaginal symptoms is not well understood.7

The rarity with which *N. lactamica* is isolated from the genital tract is emphasised by our failure to isolate it from over 20,000 patients attending a sexually transmitted diseases clinic over the last five years. Nevertheless, in view of the more frequent occurrence of meningococci in the genital tract,2 3 this report reinforces the view that full biochemical identification of oxidase-positive Gram-negative diplococci growing on selective medium is necessary to avoid the serious social and medicolegal consequences which could result from a misdiagnosis of gonorrhoea.

References

The diagnostic value of a gonococcal complement fixation test

H. YOUNG, C. HENRICHSEN AND A. McMILLAN*
The diagnostic value of a gonococcal complement fixation test

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(Received 20 August 1979)

The diagnostic value of a gonococcal complement fixation test with N. gonorrhoeae strain 9 as antigen was investigated.

To assess the test as a routine screening procedure sera from female patients attending a Sexually Transmitted Diseases clinic were investigated. The test was positive in 35% of women with culture-proven gonorrhoea: the performance of the test was noted to be directly related to the duration of infection, the positivity rate being 70% when the infection had been present for two weeks or more.

Of 297 patients attending the clinic but in whom there was no clinical, epidemiological, laboratory or presumptive evidence of gonococcal infection, past or present, 7% gave an unexplained positive result. The test was reactive in 1% of control patients attending the antenatal clinic: although there was no clinical or epidemiological evidence of gonorrhoea, these patients were not investigated fully.

The role of the GCFT in the diagnosis of gonorrhoea is discussed.

Key words: Gonorrhoea; GCFT.

Introduction

Gonorrhoea is becoming increasingly difficult to control, partly due to the large reservoir of infection provided by asymptomatic females, up to 90% of whom remain asymptomatic when infected with Neisseria gonorrhoeae. Males may also contribute to this problem since approximately 15% of infected men have few symptoms if any.

The need for serological tests in the detection and control of gonococcal infection has been emphasised. Although current research using radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) may eventually provide a suitable test the gonococcal complement fixation test (GCFT) is the only serological test for gonococcal infection that has been extensively used in clinical practice.

Magnusson & Kjellander found that the GCFT procedure used by them had a high specificity and was a worth-while complementary tool for the diagnosis of uncomplicated or complicated gonorrhoea. However, there were cross reactions in patients with meningococcal infection or chronic bronchitis. Watt, Ward & Glynn compared the GCFT of Magnusson & Kjellander, the GCFT of Reising, Schmale, Danielsson & Thayer and the bentonite flocculation test of Wallace, Diena, Yugi and Greenberg. They concluded that the Magnusson & Kjellander GCFT would be a useful adjunct in the diagnosis of gonorrhoea but the level of false positive results in all tests was too high to permit their use for routine screening for asymptomatic infection in the population.

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In contrast it has been stated that the GCFT has only a limited value\textsuperscript{12} and the results of the test are without diagnostic significance whatsoever.\textsuperscript{13}

In spite of these apparently conflicting reports the GCFT is still used in clinical practice. We therefore considered it worthwhile, particularly at a time when new serological tests are being evaluated, to report our evaluation of the GCFT using antigen prepared from a strain of \textit{N. gonorrhoeae} (S9) which O’Reilly, Welch & Kellogg\textsuperscript{14} considered to possess antigenic features that might be quantitatively or qualitatively specific to species of \textit{N. gonorrhoeae}.

Material and Methods

Patients and controls

The survey included 457 female and 75 male patients attending the Sexually Transmitted Diseases (STD) clinic at the Royal Infirmary of Edinburgh. A control group was provided by 419 women attending an antenatal (AN) clinic.

Patient investigation

In male patients attending the STD clinic, if a urethral discharge was present, or if there was presumptive evidence of gonorrhoeal contact, a Gram-stained smear of urethral discharge was examined microscopically and material inoculated directly on to selective medium and cultured for \textit{N. gonorrhoeae}.\textsuperscript{15} In the case of homosexuals, material from urethra, rectum and pharynx was cultured routinely and, if negative on the first occasion, repeated twice at weekly intervals.

In female patients attending the STD clinic, Gram-stained smears of material from urethra and cervix, and cultures from urethra, cervix, rectum and, if indicated, from pharynx, were taken. Generally, if the first cultures for \textit{N. gonorrhoeae} were negative these were repeated twice at weekly intervals.

In both male and female STD clinic patients note was made of any previous gonococcal infection and of any antibiotic therapy during the preceding month. Duration of infection was estimated, where possible, by careful reference to each case history and contact history.

The gonococcal complement fixation test (GCFT)

Sera examined. As a preliminary study, sera were obtained from 41 female and 75 male patients who were infected with \textit{N. gonorrhoeae}. The sera were stored at $-20^\circ$C and examined by the GCFT in two batches. Later, the sera from 416 female patients selected at random from those who attended the STD clinic over a three month period were screened by the GCFT as a routine. Sera from 419 women attending the AN clinic were also tested as controls.

Antigen preparation. The antigen was prepared from the strain of \textit{N. gonorrhoeae} coded No. 9 by O’Reilly, Welch & Kellogg.\textsuperscript{14} We propose to designate this test GCFT (S9) to distinguish it from GCFTs using other antigens.

A vial of lyophilized culture (predominantly colony type IV) was reconstituted, inoculated on to supplemented Difco GC base\textsuperscript{15} and incubated overnight at $37^\circ$C in a 10% CO\textsubscript{2} atmosphere. This culture was then used to inoculate a further ten plates of the same medium, which were incubated as above. Next day the growth was harvested into 10 ml 0.9% sterile saline, washed once by centrifugation, and resuspended in 10 ml 0.9% saline. The purity of this suspension was checked by Gram and immunofluorescence staining and by culture. The suspension was then heated in a water bath at $60^\circ$C for 30 min. with mixing every 5 min. After cooling to room temperature the suspension was stored at $4^\circ$C overnight before determining the optimal dilution of antigen for use in the GCFT, using immune rabbit serum against \textit{N. gonorrhoeae} strain 9. The antigen was then dispensed undiluted in 150$\mu$l aliquots and stored at $-20^\circ$C.

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GONOCOCAL COMPLEMENT FIXATION TEST

Test procedure. The tests were carried out by a standard technique using a unit volume of 25 μl in U-cup microtitre plates. Inactivated sera were screened at a dilution of 1 in 4 (final dilution 1 in 16) against the antigen at optimal titre (usually 1 in 40) with 1.5 minimum haemolytic doses (MHD) of complement as determined on the day of the test, and 2.5% sheep cells sensitized with rabbit haemolytic anti-sheep cell antibody at the pre-determined optimal sensitizing concentration. The fixation period was 1 h at 37°C and the incubation period after the addition of the sensitized cells was 30 min at 37°C. Any sera giving inhibition of lysis >25% were tested quantitatively at dilutions from 1 in 4 to 1 in 128. The titre was taken as the highest dilution giving 100% inhibition of lysis. Sera giving approximately 50% inhibition of lysis were scored as ‘inconclusive’, whilst those showing any degree of inhibition in the serum control at a 1 in 4 dilution were scored as anti-complementary.

Analysis of results. Statical analysis was made by the Chi Square method with Yates’s correction.

Results

Tests with sera from patients with gonorrhoea

In a preliminary study to determine the sensitivity of the modified GCFT, the test was applied to a battery of stored sera taken from patients who were each culture-positive for N. gonorrhoeae when the serum was taken. There were 75 males (Table 1) and 41 females (Table 2). The GCFT (S9) results are indicated in relation to the estimated duration of the gonococcal infection for each case when the serum was taken; this analysis was done retrospectively, so that the GCFT readings were made independently of the information.

The test was positive for 25 (33%) of 75 male patients and 23 (56%) of 41 female patients with untreated gonorrhoea. One serum of each of these groups was anti-complementary. The ability of the test to detect infected patients increased with the duration of infection: with

<table>
<thead>
<tr>
<th>Duration (days) of infection with N. gonorrhoeae when serum was taken</th>
<th>Number of sera giving the stated GCFT result</th>
<th>Total number of sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Anti-complementary</td>
</tr>
<tr>
<td>&lt; 7</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>8–14</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>&gt;14</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total number of sera</td>
<td>25</td>
<td>49</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration (days) of infection with N. gonorrhoeae when serum was taken</th>
<th>Number of sera giving the stated GCFT result</th>
<th>Total number of sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Anti-complementary</td>
</tr>
<tr>
<td>&lt; 7</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>8–14</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>&gt;14</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total number of sera</td>
<td>23</td>
<td>17</td>
</tr>
</tbody>
</table>
the group of 75 males it was positive in four of 18 (22%) when the infection had been present for seven days or less, and in 13 of 25 (52%) when the infection had been present for more than 14 days; with the 41 females studied it was positive in four of nine (44%) women infected for seven days or less, and in eight of 12 (67%) women infected for more than 14 days.

Tests with sera from female patients attending the STD clinic

The usefulness of the GCFT (S9) procedure as a routine screening test was now assessed in sera from 416 female patients attending the STD clinic, three of which were anti complementary. The results for the remaining 413 sera are given (Table 3) with subclassification of cases into groups in which gonococcal infection was: (i) proved but as yet untreated (49 patients); (ii) assumed on epidemiological evidence, and treated (7); (iii) proved by culture and treated (50); and (iv) not proved, but there was considerable clinical doubt or history of partial therapy or possible contact (10). In a fifth group (v) of 297 patients there was no clinical, epidemiological, laboratory or presumptive evidence of gonococcal infection.

In Group (i), 47 of the patients with untreated gonorrhoea gave a positive culture at the same time as the blood was tested by the GCFT (S9). One patient was culture positive one week later, whilst the remaining patient (treated at her first visit) was a smear-positive contact of a male with culturally proven gonorrhoea. In this group the test was positive for 17 (35%) of 49 patients, but the positivity rate increased with the duration of infection: the test was positive in one (6%) of 17 patients when the infection had been present for seven days or less, and in 12 (70%) of 17 patients with an infection of more than 14 days duration. The titre was 4 in 47% (8/17) of cases of untreated gonorrhoea. Only two patients (11.8%) of those with untreated gonorrhoea gave a titre $\geq 32$; the duration of infection was more than eight days in each case.

In Group (ii) the GCFT (S9) was positive in six out of seven of the patients treated for gonorrhoea on the basis of epidemiological evidence. All seven patients were contacts of males with culture proven gonorrhoea and each had had at least three sets of negative cultures.

In Group (iii), 16 (32%) of the 50 patients with a reliable history of gonorrhoea treated in the preceding 12 months gave a positive GCFT (S9) result. The positivity rates at one month, two to three months, and four or more months post-treatment were 61% (11/18), 17% (4/23) and 11% (1/9) respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>No. GCFT positive</th>
<th>No. of patients with a GCFT titre of:</th>
<th>No. GCFT negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Untreated gonorrhoea</td>
<td>49</td>
<td>17</td>
<td>8 7 2</td>
<td>32</td>
</tr>
<tr>
<td>Duration of infection (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7</td>
<td>16</td>
<td>1</td>
<td>1 0 0</td>
<td>15</td>
</tr>
<tr>
<td>8-14</td>
<td>16</td>
<td>4</td>
<td>1 2 1</td>
<td>12</td>
</tr>
<tr>
<td>&gt; 14</td>
<td>17</td>
<td>12</td>
<td>6 5 1</td>
<td>5</td>
</tr>
<tr>
<td>(ii) Gonorrhoea treated on epidemiological evidence</td>
<td>7</td>
<td>6</td>
<td>6 0 0</td>
<td>1</td>
</tr>
<tr>
<td>(iii) Treated gonorrhoea</td>
<td>50</td>
<td>16</td>
<td>9 6 1</td>
<td>34</td>
</tr>
<tr>
<td>Period since treatment (months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>18</td>
<td>11</td>
<td>8 2 1</td>
<td>7</td>
</tr>
<tr>
<td>2-3</td>
<td>23</td>
<td>4</td>
<td>0 4 0</td>
<td>19</td>
</tr>
<tr>
<td>&gt; 4</td>
<td>9</td>
<td>1</td>
<td>1 0 0</td>
<td>8</td>
</tr>
<tr>
<td>(iv) Clinically doubtful</td>
<td>10</td>
<td>10</td>
<td>6 4 0</td>
<td>0</td>
</tr>
<tr>
<td>(v) No evidence of gonorrhoea</td>
<td>297</td>
<td>20</td>
<td>14 6 0</td>
<td>277</td>
</tr>
<tr>
<td>Total number of patients</td>
<td>413</td>
<td>69</td>
<td>43 23 3</td>
<td>344</td>
</tr>
</tbody>
</table>

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Group (iv) in Table 3 comprises 10 patients in whom the clinical diagnosis was uncertain: four of these patients were contacts of culture proven cases of gonorrhoea but in each case there was no bacteriological evidence of infection on at least one occasion; an additional four patients, each of whom had had at least one casual contact in the preceding four months, had received antibiotic in the previous month; one patient who had had a casual contact five months earlier gave positive urethral and cervical smears but _N. gonorrhoeae_ was not isolated on culture and repeated cultures from her regular consort were negative; the husband of the final patient in this group had been treated for gonorrhoea on the basis of a positive smear result.

Of the 297 patients [Group (v)] in whom there was no clinical, epidemiological, laboratory or presumptive evidence of gonococcal infection, past or present, 20 (7%) gave an unexplained positive GCFT (S9) result; two of the 20 patients were pharyngeal carriers of _N. meningitidis_. However, the difference in the reactivity of the GCFT (S9) in this group, with no evidence of gonorrhoea, and the group with untreated gonorrhoea is highly significant (\(P < 0.001\)).

**Tests with sera (controls) from antenatal patients**

The GCFT (S9) was positive in four (1%) of 419 patients attending an antenatal clinic; the titres were, 4 (two patients), 8 and 16. The GCFT was inconclusive in another four patients. Although there was no clinical or epidemiological evidence of gonorrhoea these patients were not investigated fully.

**Discussion**

Although a highly sensitive and specific screening test would be of value in the detection and control of gonorrhoea definitive diagnosis must rely on culture and identification of _N. gonorrhoeae_. The results discussed below suggest that none of the existing serological tests are suitable for screening for gonococcal infection, although they may be a useful adjunct to culture in certain cases.

The GCFT (S9) was positive in 33% of all male patients with untreated gonorrhoea and in 52% of men with infections of more than 14 days duration. The reactivity of the test varied from 56% (Table 2) to 35% (Table 3) but increased to approximately 70% for infections of more than 14 days duration. A positive GCFT result was obtained in only 7% of patients attending the STD clinic but in whom there was no clinical or microbiological evidence of gonorrhoea. The corresponding figure for the 419 patients attending the AN clinic was 2% (i.e., assuming the four positive and four inconclusive results in this group were, in fact, false positives).

These results agree favourably with those of other workers who have examined the GCFT. Magnusson and Kjellander\(^7\) reported a positive GCFT result in 21% of infected men and 50% of infected women while only 1.3% of controls gave positive results. Rodas and Ronald,\(^1\) examining prenatal and gynaecology clinic patients, found that a positive GCFT result was obtained in 31% of those with positive cultures compared with 10.5% of those with negative cultures.

Our results also compare favourably with those obtained using more modern serological tests. Glynn and Ison\(^7\) reported that ELISA detected approximately 55% of infected women but was reactive in 11% of controls while RIA\(^6\) was positive in approximately 85% of culture-positive asymptomatic women and 13% of controls.

Although not proven in the present study it is likely that cross-reaction with _N. meningitidis_ was responsible for some of the unexplained positive reactions in the patients in whom there was no evidence of gonorrhoea: throat cultures were taken from only six of these patients and two were positive for _N. meningitidis_. Throat carriage of _N. meningitidis_ was shown to influence the levels of anti-gonococcal antibody detected by RIA\(^7\) as did a previous history of gonorrhoea.
From the comparisons made above there is little difference between the results obtained with the GCFT (S9) and those obtained with other serological tests. The present sensitivity and specificity of all of these tests, and the persistence of antibody due to past infection, limit their value in clinical practice.

Our conclusions agree with those of others17 who considered that serodiagnosis may be useful in conjunction with culture tests in suspected gonococcal pelvic inflammatory disease and disseminated gonococcal infection. It is important to emphasise that, at present, serological tests are not suitable for screening for gonococcal infection and should not be used in this way to diagnose or exclude gonorrhoea. Analysis of the information provided with routine GCFT requests suggests that the test is, in many instances, used with the aim of detecting or excluding uncomplicated gonococcal infection. This may be due in part to a reluctance of some clinicians to take appropriate genital specimens for culture tests or (in some areas at least) to difficulties associated with transport of specimens for culture.

We wish to thank Professor J. G. Collee, Department of Bacteriology, University of Edinburgh, and Dr D. H. H. Robertson, Consultant Venereologist, Royal Infirmary of Edinburgh, for their valuable help in the preparation of this paper.

References

Oropharyngeal flora and individual susceptibility to neisserial infection

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SUMMARY β-haemolytic streptococci were isolated from throat swabs from 49 (10·5%) of 466 patients undergoing cultural examination for gonorrhoea. Although β-haemolytic streptococci were isolated more frequently from patients with genital or anorectal gonorrhoea (15·9%) than from those without (9·2%), the difference was not statistically significant. When groupable (A, B, C, or G) and other (non-A, -B, -C, or -G) β-haemolytic streptococci were analysed separately, a statistically significant association between non-A, -B, -C, or -G streptococci and gonococci was observed but not between groupable β-haemolytic streptococci and gonococci.

Introduction
Since the preliminary observations of Willcox et al.,1 it has now been convincingly demonstrated that Neisseria meningitidis is isolated at least twice as often from the throats of patients with genital gonorrhoea than from patients without gonorrhoea and that Neisseria gonorrhoeae is isolated from a genital site approximately twice as often from patients with meningococci in the throat than from patients without.2 To date, only one study3 has found no such association between genital gonorrhoea and oropharyngeal carriage of meningococci.

Ødegaard and Gedde-Dahl4 observed that these results could indicate either that there is individual susceptibility to neisserial infection or that there is simply a difference in the behaviour patterns of patients who contract gonorrhoea, the conduct of the latter making them not only more likely to be infected with N gonorrhoeae but possibly more exposed to the acquisition of N meningitidis as well. We made a similar suggestion1 and also postulated that if these results on the carriage of neisseriae merely reflect the association of intimate behaviour with the exchange of flora then oropharyngeal carriage of other "marker" organisms should show a similar correlation with genital gonorrhoea.

This paper reports our findings with β-haemolytic streptococci as marker organisms.

Patients and methods
Unselected patients who attended the Department of Venereology at the Royal Infirmary, Edinburgh, for the first time during the months of September, October, November, and December 1979 were included in the survey. The sites sampled as part of the diagnostic routine, and the culture and identification methods for gonococci and meningococci, were as described previously.3

OROPHARYNGEAL CULTURE
An additional throat swab was taken from each patient and received at the laboratory within four hours. On receipt, each specimen was streaked on to blood agar medium (Gibco blood agar base + 5% human blood) and modified New York City (MNYC) medium.6 Both media were incubated at 37°C in an aerobic atmosphere enriched with 10% CO2. After incubation for 24 hours a representative colony from those plates with β-haemolytic colonies on the blood agar medium was inoculated into Todd-Hewitt broth and subcultured on to a blood agar plate. After overnight incubation the Todd-Hewitt broth culture was checked by Gram-staining and streptococci were grouped by a coagglutination method (Phadebact streptococcus test; Pharmacia Diagnostics, Uppsala, Sweden) with reagents specific for groups A, B, C, and G. The blood agar plate served as a purity check and confirmed the β-haemolytic nature of the isolate. MNYC plates were examined at 24 and 48 hours and any oxidase-positive Gram-negative diplococci were identified by standard methods.3

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Received for publication 1 February 1980
Oropharyngeal flora and individual susceptibility to neisserial infection

STATISTICAL METHOD

Statistical analysis of the results was made by the \( \chi^2 \) method with Yates’s correction.

Results

**N GONORRHOEAE**

Specimens from 466 patients (290 men and 176 women) were examined. \( N \) gonorrhoeae was isolated by culture from genital or anorectal sites of 88 (18·9%) patients (51 men and 37 women). \( N \) gonorrhoeae was also isolated from the throat of two of these patients and from the throat only of one additional patient.

**ß-HAEMOLYTIC STREPTOCOCCI**

ß-haemolytic streptococci (ß-HS) were isolated from throat swabs of 49 (10·5%) patients (36 men and 13 women). Of the 49 ß-HS isolates, two were group A, six group B, nine group C, three group G, and 29 were not A, B, C, or G.

β-HS (16 groupable and 19 non-A, -B, -C, or -G) were isolated from 35 (9·2%) of 378 patients with negative genital and ano-rectal culture results for \( N \) gonorrhoeae. ß-HS (four groupable and 10 non-A, -B, -C, or -G) were isolated from 14 (15·9%) of 88 patients with positive genital or ano-rectal culture results for gonococci. Gonococci were isolated from 74 (17·8%) of 417 patients with negative culture results for ß-HS and from 14 (28·6%) of 49 patients from whom ß-HS were cultured.

ASSOCIATION OF ORGANISMS

Although β-HS were isolated more frequently from patients with than from those without gonorrhoea, and gonococci were isolated more frequently from patients with than those without oropharyngeal ß-HS, the association of each organism with the other was not statistically significant (\( \chi^2 \); 2·7; 0·2 > P>0·1).

When the groupable and non-A, -B, -C, or -G ß-HS are analysed separately the following associations are obtained. Excluding the 29 patients with non-A, -B, -C, or -G ß-HS, groupable ß-HS were isolated from 16 (4·5%) of the 359 patients without gonorrhoea and from four (5·1%) of the 78 patients with gonorrhoea. Excluding the 20 patients with groupable ß-HS, non-A, -B, -C, or -G ß-HS were isolated from 19 (5·3%) of the 362 patients without gonorrhoea and from 10 (13·5%) of the 74 patients with gonorrhoea. The association of non-A, -B, -C, or -G ß-HS with gonococci was statistically significant (\( \chi^2 \); 3·9; 0·05>P>0·02) whereas there was no statistically significant association between groupable ß-HS and gonococci (\( \chi^2 \); 0·001; P>0·9).

**N MENINGITIDIS**

Meningococci were isolated from 17 (3·7%) of the 466 throat swabs plated in the laboratory: meningococci were isolated from seven (8%) of the 88 patients with gonorrhoea and from 20 (2·7%) of the 748 patients without gonorrhoea. The association of gonococci and meningococci was statistically significant (\( \chi^2 \); 4·3; 0·05>P>0·02).

Oropharyngeal specimens from 233 patients were examined for meningococci both by direct plating and by throat swabs sent to the laboratory: meningococci were isolated from 55 (23·6%) patients by direct plating compared with only 10 (4·3%) from swabs. The association of gonococci and meningococci in the 233 patients on whose throat specimens direct plating was done was significant (\( \chi^2 \); 6·9; P<0·01).

Discussion

Unfortunately, the question of whether or not the reported association of genital gonorrhoea and oropharyngeal carriage of meningococci is the result of individual susceptibility to neisserial infection or is attributable to behavioural factors cannot be answered from our results. This is partly due to the complication that arises since the association of the groupable ß-HS (A, B, C, and G) in patients with and without genital or anorectal gonorrhoea differs significantly from that of the non-A, -B, -C, or -G ß-haemolytic streptococci. Streptococci belonging to groups A, B, C, and G are the ones most often associated with human streptococcal infection. Therefore the division between groupable and non-groupable ß-HS made in this study is not without justification.

Our finding of an association between non-A, -B, -C, or -G ß-HS and gonorrhoea could possibly be explained as a result of quantitative differences in the oropharyngeal flora secondary to altered host resistance after infection with \( N \) gonorrhoeae. There are inherent difficulties in expressing what is essentially a quantitative situation by the qualitative parameters of growth or no growth on a culture plate. This is particularly true when it is borne in mind that over 90% of organisms collected on the swab will adhere so tightly that they will not be released on inoculation of the culture plate.

A suitable method of quantitative bacteriological sampling will probably be required before the factors governing the association of gonococci and meningococci can be elucidated by the aid of marker oropharyngeal organisms. It is also possible that both individual susceptibility and behavioural factors contribute to the observed association making the specific contribution of each difficult to assess.
The different behaviour of the non-A, -B, -C, or -G and groupable \( \beta \)-HS suggests that differences might also exist between certain sero-groups and non-groupable meningococci with reference to their association with gonococci. This possibility is under investigation at present. Whether or not different sub-populations of meningococci could explain the failure of Noble et al\(^5\) to demonstrate an association between gonococci and meningococci is open to speculation.

Thanks are extended to the staff of the Department of Venereology, Edinburgh Royal Infirmary, for sending clinical specimens and to Dr P W Ross and Professor J G Collee for their helpful advice during the preparation of the paper.

References

Chlamydia trachomatis and Ureaplasma urealyticum in men attending a sexually transmitted diseases clinic

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SUMMARY Urethral specimens from 480 heterosexual patients were examined for Chlamydia trachomatis. Chlamydia were isolated from 32-7% of men with non-specific urethritis (NSU), from 16-1% of men with gonorrhoea, and from 4-1% of men without urethritis. Chlamydial isolation was not related to duration of symptoms, presence of discharge, or past history of attendance at the clinic.

Urine from 176 heterosexual patients was examined for Ureaplasma urealyticum. Ureaplasmas were present in 53-8% of men with NSU, in 28% of men with gonorrhoea, and in 32-9% of men with no urethritis. Detection rates for ureaplasmas in patients with chlamydia-negative and chlamydia-positive NSU were similar, but ureaplasmas were present in significantly greater numbers in patients with chlamydia-negative NSU than in those with chlamydia-positive NSU.

Introduction

Non-specific urethritis (NSU) is now the commonest sexually transmitted disease in the United Kingdom,1,2 but the aetiology in many cases is still obscure. The association of Chlamydia trachomatis with nongonococcal urethritis was first described by Dunlop et al3 in 1965 and the causal nature of this relationship now seems established, with C trachomatis being isolated in 30-70% of cases.4-12

Ureaplasma urealyticum has also been implicated in the aetiology of nongonococcal urethritis and has been isolated from 40-60% of patients with NSU.13-16 This association has been said to be particularly strong in chlamydia-negative NSU,17 but despite evidence from studies with antibiotics which differentiate between chlamydial NSU and ureaplasmal NSU18-20 and from the self-inoculation study reported by Taylor-Robinson et al,21 the aetiological role of ureaplasmas in NSU is still in doubt.16 The results of quantitative studies have also been conflicting.13 16 17

The present study was performed to determine the prevalence of C trachomatis and U urealyticum infection in male patients attending a clinic for the treatment of sexually transmitted diseases. An attempt has also been made to evaluate the relationship between chlamydia and ureaplasmas in NSU.

Patients and methods

STUDY POPULATION

The study population was composed of 480 heterosexual male patients who attended the department of sexually transmitted diseases at the Edinburgh Royal Infirmary between October 1979 and January 1980. Those included were new patients and old patients returning with a fresh infection who presented between 9 am and 12 noon Monday to Friday and who had not received antibiotics during the six weeks preceding their initial visit.

DIAGNOSTIC CRITERIA

Gonorrhoea was diagnosed on the basis of positive urethral culture results for Neisseria gonorrhoeae with or without Gram-negative diplococci present on microscopy. Non-specific urethritis was diagnosed irrespective of symptoms in patients in whom microscopical examination of Gram-stained urethral material or threads from the first specimen of urine showed 10 or more leucocytes per high power field (×1000),8 when gonorrhoea was excluded as above.
**Chlamydia trachomatis** and Ureaplasma urealyticum in men attending an STD clinic

Asymptomatic patients were those who complained of neither discharge nor dysuria and in whom there was no microscopical evidence of urethritis.

Patients with symptoms but without signs of urethritis were excluded from both of the last two diagnostic groups.

### ISOLATION PROCEDURES

**Gonorrhoea**

Samples for microscopy and culture were taken with plastic disposable bacteriological loops (Nunc Products). Specimens for culture were plated directly on to modified New York City medium. After incubation at 36°C for 24 hours *N gonorrhoeae* was identified by rapid carbohydrate utilisation tests and delayed immunofluorescence or both.

**Chlamydia trachomatis**

Specimens from the anterior urethra were taken using a sterile ENT swab (Medical Wire and Equipment Company) and placed in 2SP medium. They were stored at −20°C for up to three hours and at −60°C in the laboratory for up to three days before being cultured. Isolation was carried out by the method of Thomas et al. with cycloheximide-treated cells. Incubation of the infected cells was continued for three days at 35°C and the coverslips stained with iodine to detect the glycogen inclusions.

**Ureaplasma urealyticum**

The first-voided sample of urine, usually 7-10 ml, was collected in a sterile universal container. The sample was stored at room temperature for up to three hours before being transported to the laboratory and processed as described by Young et al. Results were expressed as the total number of colour-changing units (ccu) present in the initial urine sample. In the absence of any evidence to suggest otherwise, it was assumed that the total number of organisms present in the initial urine specimen was an accurate reflection of the numbers present in the anterior urethra.

### STATISTICAL ANALYSIS

Statistical significance was determined by the χ² test with Yates's correction or Student's t test.

### RESULTS

**CHLAMYDIA TRACHOMATIS**

*C trachomatis* isolation was attempted in 480 heterosexual men; the organism was isolated from 48 (32.7%) of 147 men with NSU and from eight of 195 men with no evidence of urethritis (table I). This difference is highly significant (χ² = 48.8; P<0.001). Ten (16.1%) of the 62 men with gonorrhoea also harboured chlamydia.

<table>
<thead>
<tr>
<th>Primary diagnosis</th>
<th>No</th>
<th>% Of all diagnoses</th>
<th>Chlamydia-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonorrhoea</td>
<td>62</td>
<td>12.9</td>
<td>10</td>
</tr>
<tr>
<td>NSU</td>
<td>147</td>
<td>30.2</td>
<td>48</td>
</tr>
<tr>
<td>No symptoms</td>
<td>195</td>
<td>40.6</td>
<td>8</td>
</tr>
<tr>
<td>Other*</td>
<td>76</td>
<td>16.3</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
<td>100.0</td>
<td>77</td>
</tr>
</tbody>
</table>

*Patients with symptoms but without signs of urethritis at the time of initial examination.

The presence of a clinically detectable discharge and the duration of symptoms had no significant effect on the recovery of *C trachomatis*. Indeed among the 21 patients with NSU who had no symptoms the organism was isolated from 10 (47.6%) patients.

*C trachomatis* was isolated from 10 (35.7%) of 28 patients with ureaplasma-positive NSU and from 10 (41.7%) of 24 patients with ureaplasma-negative NSU. This difference is not significant.

### CONTACT TRACING

Although the study was not designed to include the contacts of the men examined, consort of 14 of the 77 men infected with chlamydia were seen. Chlamydia were isolated from the seven cases tested.

**UREAPLASMA UREALYTICUM**

*U urealyticum* was looked for in 176 heterosexual men; the organism was detected in 28 (53.8%) of 52 men with NSU but in only 32.9% of 73 asymptomatic men. This difference is significant (χ² = 4.67; 0.02<P<0.05). Ureaplasmas were present in 18 (56.3%) of 32 men with chlamydia-negative NSU and in 10 (50%) of 20 men with chlamydia-positive NSU (table II). This difference is not significant (χ² = 0.02; P>0.5).

Marked differences between chlamydia-negative and chlamydia-positive NSU occurred, however, when the number of ureaplasmas present was taken into account. Of patients with chlamydia-negative NSU 53.1% had large numbers (>10⁵ ccu) of ureaplasmas present in their urine compared with 15.0% of patients with chlamydia-positive NSU (table II). This difference is significant (χ² = 6.03; 0.01<P<0.02). The mean number of ureaplasmas present in men with chlamydia-negative NSU was 10⁻¹ compared to 10⁴.⁶ in men with chlamydia-positive NSU (=.2.13; P<0.05). The distribution of ureaplasmas in patients with chlamydia-positive and chlamydia-negative NSU is shown in the figure.

Although *U urealyticum* was found less often in men with gonococcal urethritis and in men without urethritis (table II), it was usually present in large
numbers. Ureaplasmas were present in numbers equal to or greater than $10^5$ ccu in five of the men with gonococcal urethritis who harboured ureaplasmas and in 16 of the men without urethritis.

**Discussion**

Any consideration of non-specific urethritis is complicated by the lack of universally accepted criteria for its diagnosis; even within England and Wales there are marked variations in the criteria applied. This may partly account for the differences in reported isolation rates for *C. trachomatis*, which range from 26% to 58.5% in recent surveys; there is a tendency for the highest isolation rates to be reported by those authors who restrict their diagnosis of urethritis to patients whose urethral exudates contain more than 20 leucocytes per high power field on microscopical examinations. The isolation rate of 33% for *C. trachomatis* in NSU in the study reported here is similar to that obtained by others using the same diagnostic criteria.

Unlike other authors we were unable to relate isolation of *C. trachomatis* to either severity or duration of symptoms, and we obtained particularly high isolation rates in patients with NSU who had no symptoms.

The significance of *U. urealyticum* in NSU is much less well established. The overall isolation rate of 53.8% reported here agrees with the 52.9% reported by Taylor-Robinson et al., but he found no significant difference between chlamydia-positive and chlamydia-negative NSU in terms of the numbers of organisms present. However, the results of quantitative studies have been conflicting, and Hare et al. detected "high numbers" of ureaplasmas in chlamydia-negative NSU more frequently than in chlamydia-positive NSU.

In the present study we found that *U. urealyticum* could be detected fairly frequently in sexually active men, and large numbers ($>10^5$ ccu) could be present without causing any evidence of urethritis. Nevertheless, the organism is isolated more often from men with NSU, and a causative role for *U. urealyticum* in some cases cannot be excluded. The factors which might influence the pathogenicity of the organism are not known, but the observation that ureaplasmas were found in low numbers in chlamydia-positive NSU might indicate some inhibitory action of *C. trachomatis*. It seems unlikely that detection of the organism is influenced by the presence of urethritis, as detection rates and numbers of the organisms present are similar for patients with gonococcal urethritis and those without urethritis.

This study provides further support for the aetiological role of *C. trachomatis* in NSU, but the case for such a role for *U. urealyticum* is still not proved.

We are grateful to our medical and non-medical colleagues in the University Department of Genitourinary Medicine, Edinburgh Royal Infirmary, for their help in the collection of specimens and data and in the preparation of this paper.

**References**

Chlamydia trachomatis and Ureaplasma urealyticum in men attending an STD clinic


Genitourinary infection with Ureaplasma urealyticum in women attending a sexually transmitted diseases clinic

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SUMMARY Ureaplasma urealyticum was detected in the cervix of 49.9% and in the urine of 47.7% of women attending a department of genitourinary medicine. Isolation of U urealyticum was not related to diagnosis, nor was there any association between the presence of the organism and any symptoms or signs of genital tract disease. Fewer organisms were detected in the cervix of women who had a concurrent infection with Chlamydia trachomatis than in women who had not. A pathological role for U urealyticum has not been established.

Introduction

Non-gonococcal urethritis (NGU) is now the commonest sexually transmitted disease in Britain.1,2 The substantial evidence for a causative role for Chlamydia trachomatis in this infection has been reviewed by Taylor-Robinson and Thomas.3 An aetiological role for Ureaplasma urealyticum, especially in non-chlamydial NGU, has been suggested,4-7 but despite evidence from self-inoculation8 and chemotherapeutic studies9-11 the pathological significance of the organism has yet to be confirmed.

U urealyticum has been detected frequently and in large numbers in sexually active women.12-13 Its transmission by sexual contact seems well established,14 and the colonisation of the female genital tract with U urealyticum has been related to sexual activity and the number of sexual partners.13-15 Although various reports have related U urealyticum infection to impaired fertility, abortion, and low birth weight,13-16 its pathogenicity in the female genital tract has not yet been established.

This study describes the prevalence of U urealyticum in women attending a clinic for the treatment of sexually transmitted disease and attempts to correlate ureaplasmal infection with signs and symptoms of genital tract disease.

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Accepted for publication 5 March 1981

Patients and methods

The patients studied were women who attended the department of genitourinary medicine at the Royal Infirmary, Edinburgh, for the first time between April and September 1980 and women who had attended the clinic previously but who presented during this period with a new complaint. All such women who presented between 9 am and 12 noon Monday to Friday were included unless they had received antibiotic therapy during the six weeks before their first visit.

DIAGNOSTIC PROCEDURES

Gonorrhoea

Specimens for microscopy were taken from the urethra and cervix. Specimens for culture were taken from the urethra, cervix, rectum, and throat (where indicated) and were plated directly on to Modified New York City medium.17 After incubation at 36°C for 24 hours Neisseria gonorrhoeae was identified by rapid carbohydrate utilisation tests or delayed immunofluorescence or both.18

Chlamydia trachomatis

Specimens were taken from the endocervix with sterile cotton-tipped swabs and placed in 2SP medium. They were stored at −20°C for up to three hours and at −60°C for up to three days before being cultured. Isolation was carried out by the method of Thomas et al.19 with cycloheximide-treated cells. Infected cells were incubated for three days at
Genitourinary infection with Ureaplasma urealyticum in women

35°C and the coverslips stained with iodine to detect the glycogen inclusions.

Ureaplasma urealyticum

Urine. The first 10-15 ml of voided urine were collected in a sterile universal container and stored for up to three hours at room temperature before being transported to the laboratory and processed as described by Young et al.12 Results were expressed as total colour-changing units (ccu) present in the initial sample.

Cervix. Endocervical swabs were placed in 1·8 ml of TALC colour change medium12 contained in bijou bottles for transport to the laboratory. On arrival, the swab was agitated in the medium and liquid expressed from the swab before it was removed from the bottle. A 200-μl volume was used to initiate a series of 10-fold dilutions up to 10^10 in 1·8-ml volumes of TALC medium. The medium from which the swab had been removed was considered to be a 1/10 dilution of cervical material.

Other STDs

Genital herpes simplex virus infection was confirmed by viral isolation and serum antibody titres. The presence of pox virus particles in molluscum contagiosum was established by electron microscopy. Vaginal secretions were examined microscopically for Trichomonas vaginalis and yeasts. Other STDs were diagnosed clinically.

Diagnostic Groups

Patients were assigned to one of four diagnostic groups:

(1) Women from whom N gonorrhoeae was isolated;
(2) Women who were identified as consorts of men with NGU;
(3) Women with any other STD; and
(4) Women with no evidence of STD; this group was subdivided into women who had conditions which required treatment and those which did not.

Allocation to any diagnostic group was made independently of the culture results for C trachomatis and U urealyticum.

Clinical Appearance of the Cervix

The difficulties associated with interpreting clinical signs in the cervix have been discussed in detail by Rees and her co-workers,20 who described a method for the standardised reporting of the clinical appearance of the cervix.

We used a similar method with the following classifications: (a) clinically healthy cervix; (b) simple cervical ectopy; and (c) cervicitis (congested oedematous cervix or cervical mucopus or pus or both).

Statistical Analysis

Statistical analysis was carried out using the χ² test with Yates's correction.

Results

Five hundred and twenty-eight women participated in the survey. Valid results for ureaplasmal isolation were obtained from the cervix in 413, from the urine in 375, and from both sites in 359 women. Several women from whom cervical specimens were obtained were unable to pass urine at their initial visit, and a batch of almost 100 specimens from both sites was contaminated accidentally.

Prevalence

U urealyticum was isolated from the cervix in 206/413 (49·9%) of women and from the urine in 179/375 (47·7%). Ureaplasmas were detected with equal frequency among all diagnostic groups (table 1). The isolation rate was not affected by age, U

Table 1: Prevalence of ureaplasmas in the cervix and urine in relation to diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total No of patients</th>
<th>Ureaplasma positive</th>
<th>With &lt;10⁵ ccu</th>
<th>With &gt;10⁵ ccu</th>
<th>Cervix</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No %</td>
<td></td>
<td></td>
<td>No %</td>
<td>No %</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>75</td>
<td>40 53·3</td>
<td>25 33·3</td>
<td>15 20·0</td>
<td>67</td>
<td>32 47·8</td>
</tr>
<tr>
<td>NGU contact</td>
<td>58</td>
<td>29 50·0</td>
<td>15 25·9</td>
<td>14 24·1</td>
<td>56</td>
<td>31 55·4</td>
</tr>
<tr>
<td>Other STD</td>
<td>113</td>
<td>51 45·1</td>
<td>37 32·7</td>
<td>14 12·4</td>
<td>96</td>
<td>43 44·8</td>
</tr>
<tr>
<td>Other conditions: requiring treatment</td>
<td>84</td>
<td>44 52·4</td>
<td>32 38·1</td>
<td>12 14·3</td>
<td>78</td>
<td>38 48·7</td>
</tr>
<tr>
<td>not requiring treatment</td>
<td>83</td>
<td>42 50·6</td>
<td>28 33·7</td>
<td>14 16·9</td>
<td>78</td>
<td>35 44·9</td>
</tr>
<tr>
<td>Total</td>
<td>413</td>
<td>206 49·9</td>
<td>137 33·2</td>
<td>69 16·7</td>
<td>375</td>
<td>179 47·7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ccu = colour-changing units</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

ccu = colour-changing units
Ureaplasma urealyticum was found was found in the cervix of 10 of 30 women over 35 years of age compared with 49 of 91 aged 15-19 years ($\chi^2 = 3.02; 0.1 < p < 0.05$) and in 45-8% (11/24) of the youngest women (aged 15-17 years) compared with 56-7% (38/67) of those aged 18-19 years ($\chi^2 = 0.46; p = 0.5$). Large numbers ($>10^5$ ccu) of ureaplasmas were present in the cervix in 16-7% and in the urine in 20-5% of women. The number of ureaplasmas present in either site was not related to age or diagnosis.

There was a significant correlation between isolation of ureaplasmas from the cervix and from the urine ($\chi^2 = 204; p < 0.001$); 46-2% of women had positive results in both sites, 6-1% in the cervix alone and 5-8% in the urine alone; 41-8% had negative results in both sites. This association was also quantitative; 45-3% of women had ureaplasmas present in both urine and cervix in numbers $>10^5$ ccu and 30% had small numbers at both sites (table II). In only 4 (1-1%) women were large numbers of ureaplasmas detected at one site if the other showed no growth.

**TABLE II** Ureaplasma isolation in urine and cervical specimens

<table>
<thead>
<tr>
<th>Cervical culture</th>
<th>Urine culture</th>
<th>$&lt;10^5$ ccu</th>
<th>$&gt;10^5$ ccu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>166</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>150</td>
<td>96</td>
</tr>
<tr>
<td>$&lt;10^5$ ccu</td>
<td>18</td>
<td>77</td>
<td>68</td>
</tr>
<tr>
<td>$&gt;10^5$ ccu</td>
<td>3</td>
<td>73</td>
<td>28</td>
</tr>
</tbody>
</table>

ccu = colour-changing units
+ = ureaplasm-positive; - = ureaplasm-negative

**RELATION OF UREAPLASMAS TO SYMPTOMS AND SIGNS**

In 179 women in whom other STDs were excluded the presence of *U urealyticum* was analysed in relation to symptoms and signs of genital tract disease. No significant association was found, either quantitatively or qualitatively, between the presence of ureaplasmas in the cervix or urine and any symptom or sign (tables III and IV). In particular, large numbers of ureaplasmas were as common in those with a healthy cervix as in those with cervicitis.

Of 34 women in whose urine *U urealyticum* was present in numbers $>10^5$ ccu, three (8-8%) complained of dysuria compared with 11/89 (12-4%) women whose urine was ureaplasm-negative.

**CONCURRENT CHLAMYDIAL INFECTIONS**

Ureaplasmas were detected in the cervix of 43/81 (53-1%) of chlamydia-positive women and in 157/319 (49-2%) of chlamydia-negative women (table V). Numbers $>10^5$ ccu were detected in nine (11-1%) chlamydia-positive women and in 17-9% (57/319) of chlamydia-negative women. This difference just fails to reach statistical significance at the 0-5% level ($\chi^2 = 2.9; 0.1 < p < 0.05$). If, however, only the groups with the highest incidence of *C trachomatis* (contacts of NGU and women with gonorrhoea) are considered, the difference in numbers of ureaplasmas present in chlamydia-positive and chlamydia-negative women is significant (table V; $\chi^2 = 4.5; 0.05 > p > 0.02$). No such difference was noted in women with other conditions, sexually transmitted or otherwise.

The presence of *C trachomatis* in the cervix was not correlated with the isolation of ureaplasmas from the urine, either quantitatively or qualitatively, in any diagnostic group.

**Discussion**

Braun et al$^{21}$ reported a strong association between the presence of *U urealyticum* in the cervix and in the urine. Our results confirm this association and demonstrate its quantitative nature. Braun, however, isolated ureaplasmas from the cervix alone in 18% of patients, whereas in this study ureaplasmas were detected in the cervix alone in only 6-1%. A similar number (5-8%) had positive results in the urine alone; the results suggest that if screening women for the presence of ureaplasmas is a useful investigation, samples from either the urine or the cervix would give equally valid results. Numbers of ureaplasmas $>10^5$ ccu were detected significantly more often in the urine than in the cervix, but this may simply reflect

**TABLE III** Frequency of ureaplasmal isolation from the cervix in relation to its clinical appearance

<table>
<thead>
<tr>
<th>Clinical appearance</th>
<th>Total No of specimens</th>
<th>Ureaplasma-positive</th>
<th>With $&lt;10^5$ ccu</th>
<th>With $&gt;10^5$ ccu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Healthy</td>
<td>51</td>
<td>27</td>
<td>52.9</td>
<td>17</td>
</tr>
<tr>
<td>Simple cervical ectopy only</td>
<td>47</td>
<td>24</td>
<td>51.1</td>
<td>17</td>
</tr>
<tr>
<td>Vaginitis only</td>
<td>22</td>
<td>8</td>
<td>34.8</td>
<td>5</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>58</td>
<td>31</td>
<td>53.4</td>
<td>17</td>
</tr>
</tbody>
</table>

ccu = colour-changing units
the difference in sampling procedures; an initial specimen of urine may contain a greater proportion of the organisms present than does an endocervical swab.

The overall prevalence of ureaplasmas found in this study (47.7% in the urine and 49.9% in the cervix) is slightly lower than that previously reported in similar populations. We were unable to detect any difference in isolation rates among the various diagnostic groups, and the number of ureaplasmas present was similar in every group. Young et al. reported that women whose consorts had NGU had a significantly higher incidence of ureaplasma infection, but they were also unable to detect any quantitative differences.

It might have been expected that the organism would occur less frequently among the youngest women, but no difference was found between those aged 15-17 years and the other age groups, presumably because sexual activity is already established in girls of this age attending the clinic. The only age group in which ureaplasmas were isolated less frequently was that over 35 years of age; these women comprised less than 10% of the study population and the difference was not statistically significant.

The lack of association, quantitative or otherwise, between the presence of ureaplasmas and any sign or symptom of genital tract disease makes a pathological role for the organism in women seem unlikely. In the same series of patients we noted an association between chlamydial infection and the clinical appearance of the cervix. These findings (unpublished observations) are similar to those reported by Rees and her co-workers.

Concurrent infection with C. trachomatis seems to affect adversely the number of ureaplasmas detected, although this effect is less clear-cut in women than it was in men with NGU. The presence of chlamydia may inhibit the multiplication of ureaplasmas, perhaps by preventing their association with cells; alternatively, ureaplasmas may be present in chlamydia-infected cells but are more difficult to detect. Masover et al. reported that the single identifying property of U urealyticum—the ability to hydrolyse urea—was lost or not expressed after association with mammalian cells in vitro. The mechanism of the association between ureaplasmas and cells in vivo is not known, but the possibility that it might be influenced in some way by the presence of C. trachomatis deserves further study.

This study confirms that U urealyticum is common in the urine and cervix of sexually active women, but it has not established a pathological role for the organism.

We thank our medical and non-medical colleagues in the Department of Genitourinary Medicine, Royal Infirmary, Edinburgh, for their help in the collection of specimens and data and in the preparation of this paper. Thanks are also due to Dr John Peutherer, Dr Isabel Smith, and Mr Angus MacAulay, Department

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**TABLE IV Ureaplasma isolation from urine in relation to symptoms**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Total No of specimens</th>
<th>Ureaplasma Positive</th>
<th>With &lt;10⁵ ccu</th>
<th>With ≥10⁵ ccu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>No symptoms</td>
<td>71</td>
<td>30.3</td>
<td>16.22.5</td>
<td>14.19.7</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>77</td>
<td>41.53.2</td>
<td>23.29.9</td>
<td>18.23.4</td>
</tr>
<tr>
<td>Genital irritation</td>
<td>60</td>
<td>34.56.6</td>
<td>20.33.3</td>
<td>14.22.3</td>
</tr>
<tr>
<td>Dysuria</td>
<td>19</td>
<td>8.42.1</td>
<td>3.26.3</td>
<td>3.15.8</td>
</tr>
</tbody>
</table>

ccu = colour-changing units

---

**TABLE V Ureaplasma and chlamydial isolation in relation to diagnostic groups**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total No of specimens</th>
<th>Ureaplasma Positive</th>
<th>With &lt;10⁵ ccu</th>
<th>With ≥10⁵ ccu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>All groups</td>
<td>81</td>
<td>43.53.1</td>
<td>34.42.0</td>
<td>9.11.1</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>319</td>
<td>157.49.2</td>
<td>100.31.3</td>
<td>57.17.9</td>
</tr>
<tr>
<td>NGU Contact</td>
<td>30</td>
<td>14.46.7</td>
<td>11.36.7</td>
<td>3.10.0</td>
</tr>
</tbody>
</table>

ccu = colour-changing units
+ = positive; - = negative
of Bacteriology, University of Edinburgh, who provided the facilities for the isolation of C trachomatis.

References
Preface

Since the last edition of this book there have been enormous changes in the field of sexually transmitted diseases. As a result there have been more relevant publications in the last five years than there were in the previous decade.

We have not attempted to cover the complete spectrum of venereology as it is now impossible to do this in one volume of Recent Advances. There are other topics which I would have liked to have included but I hope we can correct these omissions in the next edition.

I am grateful to the publishers who have allowed me to invite an international group of contributors and I am grateful to my contributors for making my editorial work so enjoyable. All those involved in this project hope that this book will be of benefit to everyone interested in this rapidly expanding branch of medicine.

London, 1981

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INTRODUCTION

Over the last few years, improvements in selective culture media combined with new transport and growth systems, and rapid identification methods, have increased the speed and efficiency of cultural diagnosis dramatically. Laboratories were quick to meet the challenge of screening for penicillinase-producing gonococci following their discovery in 1976 (Percival et al, 1976; Philips et al, 1976) and several suitable tests are now available. Unfortunately these advances have not been matched by improved rapid diagnosis at the clinic.

IMMEDIATE DIAGNOSIS

Immediate diagnosis is extremely important as it enables a presumptive diagnosis to be made in the clinic so that appropriate treatment can be given without delay.

Gram-staining

Gram-staining of genital secretions remains the only widely accepted routine procedure for making an 'on-the-spot' diagnosis of gonococcal infection.

An unequivocally positive or negative Gram-stained smear of urethral discharge from a man provides an immediate differential diagnosis between gonococcal and non-gonococcal urethritis in 85 per cent of male patients (Jacobs & Kraus, 1975).

For women, Gram-staining of smears of cervical and urethral secretions is less reliable, detecting only 55 to 65 per cent of patients who subsequently give positive cultures (Chipperfield & Catterall, 1976; Barlow et al, 1976; Evans, 1976). Recently, Barlow & Philips (1978) reported that Gram-staining of urethral samples made no significant contribution to the diagnosis of gonorrhoea in women. A similar conclusion was reached by Thin & Shaw (1979).

Gram-staining has no place in the diagnosis of pharyngeal infection in either sex and rectal smears are rarely carried out as a routine. In homosexual men, routine examination of Gram-stained smears of rectal material obtained by proctoscopy was found to be of limited diagnostic value (Deheragoda, 1977; McMillan & Young, 1979); the value of the technique may be increased if pus or mucopus can be collected for examination. Thin & Shaw (1979) reported a low yield from rectal smears in women. However, Barlow & Philips (1978) considered Gram-staining of material taken from the rectum under direct vision to be of some value in women who are known gonorrhoea contacts: of 607 episodes of gonorrhoea studied, a positive rectal smear was the only test positive in three patients.
Immediate immunofluorescence

Unfortunately, fluorescent antibody (FA) staining of secretions direct from the patient has not provided a more sensitive and reliable routine diagnostic technique than Gram-staining.

Results with the immediate FA test do not appear to be consistent when compared with Gram-staining and culture. Hare (1974) made a comparative assessment of microbiological methods for the diagnosis of gonorrhoea in women and examined results from several published trials. For standardisation the most sensitive method in each trial was expressed as 100 and other methods as percentages of this. In four of 11 studies the direct FA test was found to be the most sensitive method but in three it scored less than 60 per cent in comparison with other methods. Hare (1974) concluded that the FA test seems to be unsuitable for routine use but is useful in the investigation of special cases and in research.

Danielsson & Forsum (1975) in a detailed discussion of the methodology of immunofluorescence applied to the diagnosis of neisserial infections concluded that the immediate FA test requires highly skilled staff and is too laborious for routine use. Nevertheless, like Hare (1974), they considered that it could be useful on special occasions, for example when the patient had been given antibiotics prior to testing.

In support of these opinions, Tronca et al. (1974) found the direct FA test to be a valuable tool in the study of the pathogenesis of disseminated gonococcal infection (DGI) and in the rapid diagnosis of this disease. Direct FA staining of exudate from skin lesions of patients with DGI was found to be significantly more sensitive than either culture or Gram-staining. Nineteen (66 per cent) of 29 specimens of exudate from skin lesions examined simultaneously by all three methods were positive by at least one method: in 18 (62%) of the cases, gonococci were demonstrated by the FA test, in three (10%) by culture, and in four (14%) by Gram-staining. Great care must be taken in interpreting results obtained with exudates from skin lesions since specimens from two patients with staphylococcal pyoderma contained rounded organisms that stained non-specifically but did not resemble the bean-shaped diplococci typical of Neisseria gonorrhoeae.

FA testing of peripheral blood buffy coat smears also appeared to be a useful adjunct to routine blood cultures for detecting gonococcal bacteraemia, particularly in patients who had received recent antibiotics.

Although the FA technique as described above is of obvious value it might prove difficult to maintain an appropriate level of control in areas where the incidence of DGI is low.

CULTURE

Selective media

The importance of selective media is now established beyond doubt; over 70 per cent of laboratories in England and Wales now use some form of selective medium (Adler, 1978). The mechanism whereby a selective medium improves the cultural diagnosis of gonorrhoea is probably twofold: firstly it prevents the growth of other flora in the sample from overgrowing any gonococci present and masking the presence of any gonococci that do grow; secondly it prevents the growth of several
bacterial species that are capable of inhibiting the growth of gonococci. Shitobil (1976) noted that *N. meningitidis, Staphylococcus epidermis, Corynebacterium* species, *Staph. aureus*, group A streptococci and *N. subflava* could all inhibit the growth of gonococci. Inhibition of gonococci by components of the normal endocervical flora was also noted by Saigh et al (1978) who considered it possible that such antagonistic actions towards gonococci might prevent the establishment of infection. Certain strains of *Candida albicans* also produce a substance that is inhibitory for some strains of gonococci in vitro (Hipp et al, 1974; Hipp et al, 1975).

*Thayer Martin medium and modified Thayer Martin medium*

Although widely used the medium of Thayer & Martin (1966) which contains the antibiotics, vancomycin, colistin and nystatin has been criticised because: 3 per cent (Reyn, 1969) to 10 per cent (Brenson et al, 1973) of gonococcal strains are inhibited by vancomycin at the concentration used; it does not prevent spotting of cultures by overgrowth with *Proteus* species; nystatin at the concentrations used is not an effective inhibitor for *C. albicans*; and growth of gonococci is slow and colonies are small on Thayer Martin (TM) medium.

Superior culture results were obtained by Martin et al (1974) with a modified Thayer Martin (MTM) medium. MTM differed from TM in containing double the concentration of agar (2 per cent), additional glucose (0.25 per cent) and trimethoprim (5 μg/ml). When 328 cervical specimens were cultured in both media, 128 (37.5 per cent) were positive on MTM whereas only 110 (33.5 per cent) were positive on TM medium. Also, MTM completely eliminated spreading growth by *Proteus*, whereas 19 (5.8 per cent) of the cervical specimens were overgrown with spreading *Proteus* on TM medium.

*New York City medium and modified New York City medium*

In 1973, Faur and her colleagues (Faur et al, 1973a; Faur et al, 1973b) described a new selective medium designated NYC (New York City) medium, which provided luxuriant growth of pathogenic neisseriae after incubation for 24 hours. NYC medium essentially consists of a proteose peptone-corn starch agar buffered base to which is added a haemoglobin solution prepared from fresh horse erythrocytes, horse plasma, yeast dialysate, glucose, and vancomycin, colistin, amphotericin B and trimethoprim lactate.

As originally described by Faur et al (1973a, b), NYC medium is inconvenient for many service laboratories to prepare and the modified New York City (MNYC) medium described by Young (1978a) may be more suitable for routine use. The modified medium is much simpler to prepare than the original since it uses a commercially available gonococcal base. The other major simplification in MNYC medium is the use of completely lysed whole blood in place of plasma and haemoglobin solution made from fresh horse erythrocytes.

In MNYC medium, lincomycin is used in place of vancomycin since it is less inhibitory to gonococci. Amphotericin B is used in place of nystatin in both media because it is more inhibitory to yeasts: the importance of inhibiting *Candida* has already been discussed. Yeast dialysate is also used in both media since yeast extract may contain macromolecular substances that have some toxic effect on gonococci and these are eliminated by dialysis.
Although MNYC medium was not compared directly with the original NYC medium, both media resulted in similar improvements when they were compared with TM medium in separate trials. When specimens were inoculated as matched pairs on to TM and NYC media, Faur et al. (1973b) found that the yield of positive results in males was 56.9 per cent (198 of the 348 patients examined) with TM medium but 62 per cent (216 patients) with NYC medium. A significant increase in positive cultures was also found in women: 11.5 per cent (150 of 1302 patients examined) were positive with TM medium compared with 15.1 per cent (196 patients) with NYC medium. In a similar trial (Young, 1978a) the yield of positive results in males was 13.3 per cent (56 of the 422 patients examined) with TM medium but 17.3 per cent (73 patients) with MNYC medium: 11.3 per cent of women (29 of 256 patients examined) gave positive cultures with TM medium compared with 16.4 per cent (42 patients) with MNYC medium.

Rapidity of growth

Four et al. (1973b) found that after 24 hours of incubation 81.5 per cent of NYC cultures were positive compared with only 48.2 per cent of TM cultures read at the same time: the corresponding figures for MNYC medium compared with TM medium were 78.6 per cent and 47.3 per cent respectively (Young, 1978a). These findings are of great value in allowing rapid isolation and identification: for many of the identification techniques described later, young 18-24 hour colonies are preferable to 48 hour ones.

Other studies have also noted the importance of rapid growth of gonococci. Seth & Wilkinson (1976) found that the growth-promoting properties of brain heart infusion base for gonococci were impaired by autoclaving. Filtered medium gave consistently larger colonies than when it had been autoclaved. Although Seth & Wilkinson (1976) considered filter-sterilised medium preferable to medium sterilised by autoclaving it was appreciated that filtration was not practical for many laboratories. When gonococcal growth medium is sterilised by autoclaving careful control of the process must be maintained.

The use of Imferon, an iron-dextran complex, as a replacement for ferric nitrate in a defined supplement for GC agar (Imferon agar) significantly increased the average colony size of both gonococci and meningococci (Payne & Finkelstein, 1977). In comparison with TM medium Imferon agar increased the speed and yield of culture isolation of gonococci from clinical specimens: this was not a completely valid comparison since Imferon agar plates were read after 16 to 24 hours whereas TM plates were only examined after 40 to 48 hours.

Combination of a selective and non-selective medium

Recently, a significant proportion of clinical isolates of *N. gonorrhoeae* have been shown to contain mutations which result in a markedly increased susceptibility to a variety of antibiotics (Eisenstein & Sparling, 1978). These findings will lend support to the earlier suggestion (Reyn, 1969) that ideally a combination of a selective and a non-selective medium should be used. Such a procedure is too time consuming, technically demanding, and not cost-effective for routine use and in practice less than 10 per cent of clinics in England and Wales employ such a routine (Adler, 1978).
The recommendation of Jephcott & Rashid (1978) that a non-selective medium should be included in the subsequent examination of contacts of infected patients if their initial tests are negative is a more practical approach. The efficiency of selective media should also be carefully monitored. An indication of the percentage of gonococci failing to grow on selective medium, possibly due to antibiotic sensitivity, can be obtained by correlating the results of microscopy and culture. In Edinburgh where the selective medium contains vancomycin, colistin, amphotericin B and trimethoprim only 1 per cent of cases in women gave typical positive Gram-stained smears which were not confirmed by culture (Young et al., 1979). In Sheffield where a selective medium containing vancomycin, colistin, nystatin and trimethoprim (VCNT) is used for first sets of tests in women, only 1.8 per cent of cases diagnosed at their first visit were unconfirmed by culture (Jephcott & Rashid, 1978). Barlow et al. (1976) reporting from London found that 3 per cent of their female cases gave positive smears but negative cultures on their selective medium which also contained VCNT. Therefore, in several regions of the United Kingdom sensitivity of gonococci to antibiotics (including vancomycin) in selective media does not appear to be a significant problem at present.

TRANSPORT AND CULTURE SYSTEMS

When direct plating and immediate incubation is impracticable, several transport and culture systems are now available in addition to the conventional non-nutrient transport media such as Stuart's, or Amies' modification of Stuart's medium (Amies, 1967). These systems utilise a selective medium usually present in a small chamber containing CO₂ or a CO₂ generating system of the type described by Martin et al. (1974). The medium can be inoculated directly from the patient and transported to the laboratory either before or after incubation.

Transgrow

Transgrow (Martin & Lester, 1971) employs a selective medium containing vancomycin, colistin, and nystatin layered inside a CO₂-containing bottle. Condensation produced on incubation combined with the curvature of the glass frequently obscures the surface of the medium that accurate removal of suspect colonies for biochemical identification is impracticable and even identification by the delayed FA technique has to be applied blindly to samples from almost every culture (Jephcott et al., 1974).

Jembec/Neigon system

The problems associated with Transgrow are overcome in the Jembec/Neigon system which is based on the biological environment chamber described by Martin & Jackson (1975). It consists of a rectangular culture plate containing a selective (VCNT) gonococcal culture medium. The plate incorporates a small recess into which a CO₂-generating tablet is placed at the time of inoculation. The moisture of the plate ensures that CO₂ is generated due to the reaction between the citric acid and sodium bicarbonate in the tablet. The closed plate is placed inside a plastic bag which is sealed to make it gas-tight. This system offers easy direct access to the
surface of the culture medium so that suspect colonies can be tested by the oxidase reagent, and if positive also examined by Gram staining and the delayed FA test.

Jephcott et al (1976) found their conventional culture system (swabs transported in Amies' medium for inoculation at the laboratory) and the Jembec system were comparable in terms of yield of positive cultures but that the latter system allowed a much more rapid diagnosis. If the diagnostic laboratory is situated in or adjacent to the clinic then the Jembec system offers little advantage, apart from space saving, over conventional direct plating systems, but if transport is essential then its flexibility can be a great advantage.

Microcult GC
The Microcult GC kit consists of a small plastic container with a detachable lid, into which are set two wells each containing a 2 cm square cellulose matrix impregnated with dehydrated selective medium, containing lincomycin, colistin, amphotericin and trimethoprim. The kit is supplied dry and gamma-irradiated in a foil-lined sealed envelope. Immediately before use the medium is rehydrated with a few drops of 4 per cent (v/v) glycerol in water. After inoculating both squares of medium with the same specimen, the lid is replaced and the container placed along with a CO²-generating tablet into the foil envelope which is sealed prior to incubation at 35-37°C for 24 hours. After incubation, oxidase-positive growth is detected by pressing a paper strip impregnated with oxidase reagent (two strips supplied per kit) firmly against one culture area for 3-5 seconds. Any area showing a positive oxidase reaction should be scraped gently with a loop and a smear prepared for Gram-staining; a presumptive diagnosis of gonorrhoea is made on the basis of oxidase-positive Gram-negative diplococci. If the result is negative at 24 hours the culture is reincubated for a further 24 hours and the second area tested for cytochrome oxidase production as before.

Willcox & John (1976) found the Microcult system more efficient than culture on conventional TM medium for the diagnosis of urethral gonorrhoea in men; it was stated, however, that conventional cultures were giving unsatisfactory results at the time of the trial. Unsworth et al (1979) confirmed that the Microcult GC system was efficient for detecting gonorrhoea in men but they considered the method may be less sensitive for detecting infected women. The test may be difficult to interpret for rectal specimens and is not appropriate for throat cultures. Unsworth et al (1979) also reported a false positive oxidase reaction with 4.6 per cent of high vaginal swabs tested. Unfortunately, it was not tested whether Gram-staining would have refuted these positive results.

Willcox & John (1976) and Unsworth et al (1979) considered that the Microcult GC system might be useful in areas or countries which lack laboratory facilities. Exactly how useful it could be will depend on its sensitivity in detecting gonorrhoea in women.

Direct plating
Faur et al (1977) evaluated the recovery of N. gonorrhoeae with Tranagrow and with NYC medium contained in plates and in a biological environment chamber. When each system was incubated directly after inoculation with 178 clinical specimens the NYC-plates and NYC-chambers each yielded 36 (20.2 per cent) positive cultures
compared with 31 (17.4 per cent) for Transgrow. When 195 specimens inoculated on to NYC-chambers and Transgrow were left at room temperature for 48 hours before incubation, 52 (26.7 per cent) and 47 (24 per cent) positive cultures were obtained on NYC-chambers and Transgrow respectively, compared with 63 (32.3 per cent) positive results for NYC plates incubated without delay. After 72 hours at room temperature the yields from 162 specimens were, 20 (12.3 per cent) and 18 (11.1 per cent) with NYC-chambers and Transgrow respectively, compared with 39 (24 per cent) for immediate incubation. Faur et al (1977) concluded that the NYG-chamber system is an effective method for the handling, transport and culture of *N. gonorrhoeae* if the delay in transport and incubation does not exceed 24 hours. Unfortunately, in this study no mention was made of the sex of the patients, or of the sites from which the clinical specimens were obtained.

Thin & Shaw (1979) in a study of gonorrhoea in women found no significant differences between direct plating and Stuart's transport medium: the swabs were in transport for not longer than 19 hours. This finding applied to urethral, cervical and rectal specimens but only if rectal material was collected through a proctoscope. When material was collected by passing a swab straight up the anal canal ('anal canal specimens'), results with Stuart's transport medium were poorer than those obtained by direct plating.

Bhattacharya & Jephcott (1974) also using Stuart's transport medium found anal canal specimens poorer than rectal specimens whereas, using direct plating, Deheragoda (1977) in a study of male rectal gonorrhoea found similar results from anal swabs and rectal swabs passed through a proctoscope.

Danielson et al (1978) found that compared with direct plating there was a loss of approximately 10 per cent of positive specimens after transport in a modified Stuart's transport medium for less than one day. Prolonged transportation increased the loss of positive specimens, rising to as much as 55 per cent after two days. Although rectal specimens were included in this study it is not stated how they were taken and the results are not reported for individual sites.

The most suitable transport and culture system must be related to the constraints imposed in individual localities. In general, direct plating seems to be the system which most consistently provides the best results for most specimens. As discussed earlier, the Jembee system has the advantage over conventional transport media of providing a more rapid identification, and over the other transport and culture systems in allowing ready identification and antibiotic sensitivity testing of isolates. The Microcult system has the unique advantage of a very long shelf life. While future trials will clarify the clinical and microbiological value and limitations of such systems their cost may limit their widespread use.

**IDENTIFICATION**

A presumptive diagnosis of gonorrhoea made on the basis of oxidase-positive Gram-negative diplococci growing on selective medium is approximately 98 per cent accurate for specimens taken from a genito-urinary site. Further identification is necessary to provide a definitive diagnosis for infection at all sites but particularly in determining infection of the pharynx or rectum. However, in areas of the world with limited resources, culture and 'presumptive diagnosis' would be a significant
improvement over direct microscopy, particularly in the detection of gonorrhoea in women.

**Immunological methods**

*Delayed immunofluorescence*

In laboratories equipped with a suitable fluorescence microscope, the fluorescent antibody (FA) staining of a smear of suspect organisms obtained from the primary isolation plate (delayed FA test) can provide a simple, rapid and reliable confirmation of the gonococcus. The test is best carried out in conjunction with Gram-staining to prevent possible false positive fluorescence results due to the conjugate reacting with protein A-containing staphylococci. While cross-reaction with meningococci has been largely eliminated due to the conjugate being absorbed with strains of *N. meningitidis*, cross-reaction may occur with *N. lactamica*, an organism which grows readily on selective medium and is found in approximately 2 per cent of throat cultures. Due to the possible cross-reaction with other neisseriae delayed FA identification is not recommended for throat cultures (Lind, 1975).

*Coagglutination*

This is a rapid slide agglutination test which uses protein A producing staphylococci with rabbit anti-gonococcal antibodies, bound by their Fc portions to the protein. On mixing this reagent (available commercially as Phadebact gonococcus test) with gonococci a readily visible agglutination is produced. This reaction is compared with a control in which the staphylococci have not been coated with specific antibodies. For positive tests to be readable there must be an easily distinguishable difference between the test and control reaction. Experience with the test is necessary to distinguish between true agglutination and granular or auto-agglutination reactions (Barnham & Glynn, 1978). Inconclusive results can to a certain extent be converted to clearly positive or negative reactions by treating isolates with trypsin (Menck, 1976) or heat.

Barnham & Glynn (1978) found that 98 per cent of 140 strains of gonococci gave positive reactions. However the discrimination between *N. gonorrhoeae* and other neisseriae was poor. These findings differ from the results of Menck (1976) who found the test useful to distinguish between different Neisseria species. This technique, which is of potential value as a simple and rapid identification procedure, requires evaluation on a wider scale.

*Agglutination with anti-gonococcal lipopolysaccharide hen serum*

Wallace et al (1978) described a simple slide agglutination test for the identification of gonococci using antisera prepared in hens to ‘R’ type gonococcal lipopolysaccharide (LPS); hens appear to be excellent producers of antibodies to LPS. Anti-LPS hen serum proved highly efficient for confirming the identity of gonococci in both primary and secondary cultures and showed no agglutination with other neisseriae including *N. meningitidis*. Clearly this apparently sensitive specific and simple slide test requires further investigation.
Carbohydrate utilisation
Carbohydrate utilisation tests are necessary to establish the identity of neisseriae isolated from extra-genital sites. They are also necessary to identify isolates giving negative or equivocal results in other tests and provide a means of identifying gonococci antigenically distinct from those used in the production of antignonococcal antibodies for immunological tests. Carbohydrate utilisation tests are basically of two types, conventional and rapid.

Conventional tests
In conventional tests a solid or semi-solid medium containing the appropriate carbohydrate and a pH indicator is inoculated with the test organism. Although widely used, these tests are unsuitable since a positive reaction is dependent on adequate growth of the test organism which may require incubation for up to 72 hours (Pollock, 1976). One of the main problems with these tests is finding suitable media to support the growth of all strains of gonococci while at the same time giving reproducible and clear-cut indicator changes. Faur et al (1975) describing a plate system for carbohydrate utilisation based on NYC medium noted that the widely used semi-solid cystic-trypticase agar base (CTA) to which carbohydrates are added often gives equivocal results because of inadequate growth of fastidious strains of pathogenic Neisseria. Maniar (1977) reported good results with a guinea-pig serum agar medium which provided the adequate growth of fastidious gonococci essential to the validity of the test: guinea-pig serum was used since it lacks maltase. Hafiz et al (1979) described a liquid medium which they hoped would prove useful in determining carbohydrate utilisation reactions of fastidious neisseriae.

Rapid tests
In the rapid carbohydrate utilisation test (RCUT) system, pre-formed enzyme is measured by adding a suspension of the overnight growth of the suspect organism to a buffered non-nutrient solution containing the carbohydrate to be tested and a pH indicator. Since the test measures pre-formed enzyme it has the advantage of being independent of growth. It therefore presents no problems with fastidious strains since they can be grown on a suitable medium first. Unfortunately, as reported by Brown (1974) several laboratories were unable to obtain results comparable to those described by Kellogg & Turner (1973) who pioneered the method. A modified method (Young et al, 1976) gives easy to read and reproducible results. The test itself is simple to perform and very rapid; results usually being available within one hour. When MNYC medium is used the RCUT can be carried out directly from the primary isolation plate enabling the vast majority of isolates to be identified within 24 hours of seeing the patient (Young, 1978b).

Young & Prytula (1978) described a modified RCUT which utilised both preformed enzymes and enzymes formed by the bacteria as a result of growth in a super-enriched medium. Because of the combined action of two sources of enzymes this method is probably suitable for very small inocula. By the same principle however, it is unlikely that it could be used to characterise isolates directly from primary isolation cultures because of possible contamination by other bacteria.
ANTIBIOTIC SENSITIVITY TESTING

Antibiotic sensitivity tests have long been considered essentially retrospective since the majority of patients will already have been treated on the basis of Gram-stained smear results. Nevertheless they are important for epidemiological purposes (Jackson & Jephcott, 1976) and in planning rational therapy for use in the geographical area concerned. Although standardisation of sensitivity test procedures is desirable to allow accurate comparative assessment of sensitivity in different laboratories this is proving difficult to achieve, most laboratories carrying out disc or agar dilution procedures with media, inocula, and under conditions dictated by their individual needs and preferences. Rapid routine sensitivity tests, to penicillin at least, are now vital because of the recent discovery of strains of gonococci totally resistant to penicillin due to penicillinase (β-lactamase) production (British Medical Journal, 1976; Lancet, 1976).

Detection of penicillinase-producing gonococci

All strains of gonococci with decreased sensitivity to penicillin (minimum inhibitory concentration, \( \geq 0.125 \mu g/ml \)) must be tested for penicillinase production. A simple disc technique can be used to screen for such isolates which can then be tested for penicillinase production by one of the several tests now available (World Health Organisation, 1978).

One of these tests depends upon the hydrolysis, by penicillinase, of the lactam bond of a chromogenic cephalosporin substrate giving rise to a coloured product (O'Callaghan et al, 1972). The test can be applied either to colonies on a plate or to a bacterial suspension. The cephalosporin compound may be sensitising, so inhalation of dust, and contact with eyes and skin must be avoided (World Health Organisation, 1978). Other tests depend upon the bacterial breakdown of penicillin to penicilloic acid which is detected by the ability of penicilloic acid to dissociate a starch-iodine complex (Odugbemi et al, 1977) or by a pH indicator system (Phillips et al, 1976). The latter method, which uses very simple reagents, can be modified and carried out alongside the RCPt as part of routine identification, enabling all isolates to be tested, many directly from the primary isolation plate (Young, 1978b).

Hodge et al (1978) also described a simple, reliable and economical method based on the distortion that a penicillinase-producing organism causes to the zone of inhibition around a penicillin disc placed on a lawn of penicillin-sensitive organisms.

In screening for penicillinase-production, including preliminary disc sensitivity tests, it is important that the inoculum is derived from several colonies on the primary isolation plate since mixed infections containing both penicillinase-producing and penicillin-sensitive organisms can occur.

SEROLOGICAL DIAGNOSIS

Sensitive and specific tests for the reliable detection or exclusion of gonococcal infection are not available for routine use at present: serological diagnosis is however the subject of much active research (see Ch. 1).
GENERAL CONCLUSIONS

The improvements in culture media and identification procedures described in this review have increased the cost-effectiveness of screening for gonococcal infection since only two sets of investigations need now be performed to diagnose or exclude gonorrhoea in women: the proportion of infected women detected at their first attendance is now in the region of 95 to 98 per cent (Barlow et al., 1976; Thin & Shaw, 1979; Young et al., 1979). Interest in antibiotic sensitivity testing has been revitalised by the discovery of penicillinase-producing gonococci; this in turn may give added impetus to the campaign for standardisation of test methods. Although transport and culture systems have improved significantly there is still scope for developing cheaper and simpler methods. A simple serological test with the sensitivity and specificity required for routine use is still awaited. The development of new methods to detect gonococci or gonococcal antigen with reliability at the patient’s first visit to the clinic remains a practical goal in our research on procedures for the diagnosis of gonorrhoea.

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Serological diagnosis of gonorrhea: detection of antibodies to gonococcal pili by enzyme-linked immunosorbent assay

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Serological diagnosis of gonorrhoea: detection of antibodies to gonococcal pili by enzyme-linked immunosorbent assay

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(Received 6 May 1980)

An enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies to gonococcal pili in 498 women with gonorrhoea. The predictive value of a positive ELISA result was 35.6%, that of a negative result 94.4% and that of a positive result with respect to untreated or recently treated gonorrhoea 57.5%. Throat carriage of meningococci had no significant influence on ELISA reactivity. In its present form, the ELISA test for antibodies to gonococcal pili seems to be a specific test for gonococcal infection, but it lacks sensitivity.

Key words: gonorrhoea; ELISA.

Introduction

Although gonorrhoeal infections are usually confined to the mucosal surfaces of the body, a systemic antibody response is demonstrable by methods such as complement fixation,1 haemagglutination,2 indirect immunofluorescence,3 radioimmunoassay4,5 and enzyme-linked immunosorbent assay.6 Unfortunately, intensive efforts have not yet produced a satisfactory serological test for the diagnosis of gonorrhoea. Many of the existing tests are hampered by complicated methodology and/or lack of specificity, presumably attributable to cross-reactions with antibodies to other neisseriae.1,7 Although pili are considered to be much more specific than other gonococcal antigens,4 the radioimmunoassay method described for detecting antibodies to gonococcal pili has been submitted to a preliminary evaluation only.5

This paper reports our findings with an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to gonococcal pili in patients with gonorrhoea.

Materials and Methods

Patients

Sera were obtained from 498 women attending a Sexually Transmitted Diseases (STD) clinic at the Royal Infirmary of Edinburgh. These women were also examined for gonococcal infection by means of Gram-stained smears of material from the urethra and cervix and by taking cultures from the urethra, cervix, rectum and, if indicated, from the pharynx. If the first set of cultures for N. gonorrhoeae was negative, culture attempts were generally repeated twice at weekly intervals. The culture and identification procedure was as described previously.8
Control sera were obtained from 100 patients attending an ante-natal clinic. All of the sera used in this study had been sent for routine serological tests for syphilis.

Preparation of antigen (gonococcal pili)

Neisseria gonorrhoeae strain 82409 (kindly supplied by Dr K. Reimann, Neisseria Department, Statens Seruminstitut, Copenhagen) was selectively subcultured on clear GC medium to maintain colonies of the pilated type 2 morphology. Fifteen 16 h cultures of colony type 2 were used to inoculate a further 400 plates, which were then incubated for 16 h at 37°C in air with 10% carbon dioxide. Next day all plates were examined for colony type and tested by the oxidase reaction and Gram-staining. The growth was harvested into ice-cold 0.01 mol/L Tris/HCl buffer (pH 8.0) and pili isolated and purified by controlled homogenization, precipitation with polyethylene glycol, and caesium chloride density gradient centrifugation. Prior to precipitation with polyethylene glycol the material from a total of 1200 plates was pooled.

The protein concentration of the final preparation was estimated by reference to extinction values measured at 280 nm and 260 nm. Before being used as antigen, pili were shown to be present and to be free from material resembling lipopolysaccharide, by electron microscopy. The yield of purified pili was 0.8 mg per 10 g wet weight of cells.

Assay procedure

Two hundred and fifty microlitres of antigen (450 ng/ml) in carbonate–bicarbonate buffer (pH 9.6) was added to individual polystyrene tubes (10 x 55 mm), incubated at 37°C for 1 h and stored at 4°C overnight. Next day, antigen solution was removed with a vacuum suction pump, the tubes washed twice with PBS-Tween (phosphate buffered saline, pH 7.4 + 0.05% Tween 20) and 250 µl of each patient’s serum diluted 1 in 20 in PBS Tween added to individual tubes. After incubation at 37°C for 1 h the tubes were washed as before and 250 µl of alkaline phosphate conjugated antihuman IgG13 diluted to its working strength (Usually 1 in 1500) was added and the tubes incubated as above. The tubes were then washed as above and 250 µl of substrate (1 mg/ml p-nitrophenyl phosphate (Sigma) in PBS) added. After incubation at 37°C for 1 h the reaction was stopped by adding 250 µl of 1 mol/l sodium hydroxide. The colour reaction was read at 400 nm.

An extinction (E) value of 0.25 or greater was considered positive: this value was chosen as providing good discrimination between sera from patients with untreated gonorrhoea and those with no evidence or history of infection (Table 1). Positive and negative control sera were included with each batch of tests. The positive control was chosen to give an E value of 0.3 and if this fell below 0.25 the results were discarded.

Analysis of results

Statistical analysis of the results was made by the χ² method with Yates’s correction. The sensitivity of the test was defined as the percentage of culture-positive patients who were seropositive while specificity was defined as the percentage of culture-negative patients who were seronegative. The predictive value of a negative test was defined as the percentage of seronegative patients who were culture negative, while the predictive value of a positive test was defined as the percentage of seropositive patients who were culture positive.

Results

At the time the corresponding blood specimens were taken for serological tests 54 (10.8%) of the 498 STD clinic patients had untreated gonorrhoea; 37 had been treated for gonorrhoea in the preceding three months; 23 were known, and seven probable, contacts of cases of culture-proven gonorrhoea; one had received treatment for gonorrhoea on epidemiological grounds from her General Practitioner; and 376 patients had no clinical or epidemiological evidence of gonorrhoea, past or present.
Table 1 shows the range of extinction (E) values given by patients with untreated gonorrhoea, those with no clinical or epidemiological evidence of gonorrhoea past or present, and control sera from antenatal patients.

**TABLE 1**

*ELISA results grouped according to patient category and extinction value range*

<table>
<thead>
<tr>
<th>Extinction value range</th>
<th>Untreated gonorrhoea</th>
<th>No history or evidence of gonorrhoea</th>
<th>Antenatal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.124</td>
<td>7 (13.9)</td>
<td>173 (46.0)</td>
<td>79 (79)</td>
</tr>
<tr>
<td>0.125–0.249</td>
<td>16 (29.6)</td>
<td>181 (48.1)</td>
<td>15 (15)</td>
</tr>
<tr>
<td>0.250–0.374</td>
<td>20 (37.0)</td>
<td>20 (5.5)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>0.375–0.424</td>
<td>5 (9.3)</td>
<td>2 (0.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt; 0.425</td>
<td>6 (11.1)</td>
<td>0 (0)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>54 (100)</td>
<td>376 (100)</td>
<td>100 (100)</td>
</tr>
</tbody>
</table>

When E 0.25 is taken as the 'cut-off' point, 57.4% (31/54) of the infected women gave a positive result compared with only approximately 6% of STD clinic patients in whom there was no evidence of infection, and 6% of control antenatal sera. If the 'cut-off' point is lowered to E 0.2 then an additional two infected women, making a total of 61%, would be scored as positive. However, the reactivity would increase to 15% in the group without gonorrhoea and 10% in antenatal patients.

Taking E 0.25 or greater as positive the correlation between a positive ELISA result and a positive culture result (Table 2) is statistically highly significant ($\chi^2 = 60.34; P < 0.001$).

**TABLE 2**

*Results of ELISA and culture for 498 female patients attending the STD clinic*

<table>
<thead>
<tr>
<th>Pattern of results</th>
<th>Number (and percentage) of patients giving each pattern of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA + Culture +</td>
<td>31 (6.2)</td>
</tr>
<tr>
<td>ELISA + Culture −</td>
<td>56 (11.3)</td>
</tr>
<tr>
<td>ELISA − Culture +</td>
<td>23 (4.6)</td>
</tr>
<tr>
<td>ELISA − Culture −</td>
<td>388 (77.9)</td>
</tr>
<tr>
<td>Total</td>
<td>498 (100.0)</td>
</tr>
</tbody>
</table>

+ = positive, − = negative

Correlation between a positive ELISA result and a positive culture is highly significant ($\chi^2 = 60.34; P < 0.001$).

Of the 54 patients with untreated gonorrhoea at the time the corresponding blood specimens were taken for serological tests, 30 were in the ELISA + Culture + group, 23 were ELISA − Culture +, and one was ELISA + Culture − (this patient yielded a positive culture four days later). The remaining 56 patients in the ELISA + Culture − group comprised: 19 women who had been treated for gonorrhoea in the preceding three months; seven known and seven probable contacts of gonorrhoea; one woman who had received treatment for gonorrhoea on epidemiological grounds from her General Practitioner; and 22 women with no clinical, epidemiological or laboratory evidence of gonococcal infection within the preceding three months. Of the 388 patients in the ELISA − Culture − group, 16 were known contacts of culture-proven cases of gonorrhoea and 18 had been treated for gonorrhoea in the preceding three months.

The ELISA results in relation to carriage of *N. meningitidis* in the presence or absence of genital and/or ano-rectal gonorrhoea are shown in Table 3.
ELISA results in relation to throat carriage of meningococci in 495 women with or without genital and/or ano-genital gonorrhoea

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA +</td>
</tr>
<tr>
<td>Gonococcal culture positive with throat meningococci</td>
<td>11</td>
</tr>
<tr>
<td>Gonococcal culture positive without throat meningococci</td>
<td>21</td>
</tr>
<tr>
<td>Gonococcal culture negative with throat meningococci</td>
<td>5</td>
</tr>
<tr>
<td>Gonococcal culture negative without throat meningococci</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
</tr>
</tbody>
</table>

+ = positive, — = negative

Carriage of meningococci makes no significant difference to ELISA reactivity in gonococcal culture positive patients ($\chi^2 = 1.94; 0.2 > P > 0.1$) or in gonococcal culture negative patients ($\chi^2 = 1.04; 0.5 > P > 0.3$).

A total of 35 patients (7%) gave positive throat cultures for meningococci: three patients with positive throat cultures for N. lactamica were not included in this analysis. The carriage rate of meningococci in patients with gonorrhoea was 25.9% (14/54) compared with a carriage rate of 4.8% (21/441) in patients without gonorrhoea. Although the numbers are small there is no statistical evidence that carriage of meningococci influenced the ELISA reactivity in gonococcal culture positive patients ($\chi^2 = 1.94; 0.2 > P > 0.1$) or in gonococcal culture negative patients ($\chi^2 = 1.04; 0.5 > P > 0.3$).

The influence of a previous history of gonorrhoea on ELISA results is shown in Table 4.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA +</td>
</tr>
<tr>
<td>Gonorrhoea in preceding three months</td>
<td>19</td>
</tr>
<tr>
<td>No history of gonorrhoea in preceding three months</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
</tr>
</tbody>
</table>

+ = Positive, — = negative

A recent history of gonorrhoea has a significant effect on ELISA reactivity ($\chi^2 = 51.06; P < 0.001$).

The occurrence of gonorrhoea within the preceding three months has a significant effect on ELISA reactivity ($\chi^2 = 51.06; P < 0.001$), irrespective of treatment during that period. There was no difference between the ELISA results in patients with untreated gonorrhoea and those with gonorrhoea treated within the preceding three months: the reactivity of the ELISA was 57.4% (31/54) and 51.4% (19/37) respectively in the two groups ($\chi^2 = 0.12; 0.9 > P > 0.8$).

ELISA results in patients with negative cultures are shown in relation to gonorrhoea contact status in Table 5: the 54 patients with untreated gonorrhoea, 37 with treated gonorrhoea, seven patients with a dubious contact status, and the patient treated on epidemiological grounds were excluded from this analysis.

As calculated from Table 5 a positive ELISA result occurs significantly more often in contacts of gonorrhoea with negative cultures than in patients without a history of exposure to the gonococcus ($\chi^2 = 15.9; P < 0.001$).

TABLE 5
ELISA results with respect to gonorrhoea contact status in 399 women with negative cultures for N. gonorrhoeae

<table>
<thead>
<tr>
<th>Category</th>
<th>ELISA +</th>
<th>ELISA -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with gonorrhoea</td>
<td>7</td>
<td>16</td>
<td>23</td>
</tr>
<tr>
<td>No history of contact with gonorrhoea</td>
<td>22</td>
<td>354</td>
<td>376</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>370</td>
<td>399</td>
</tr>
</tbody>
</table>

+ = positive, − = negative
The ELISA positivity rate is significantly higher in contacts with gonorrhoea. \( \chi^2 = 15.9; P < 0.001 \).

Sensitivity, specificity and predictive value

From the results presented the sensitivity of the test is 57.4% (31/54) while the specificity is 87.4% (388/444). The predictive value of a positive ELISA result is 35.6% (31/87) and that of a negative ELISA result 94.4% (388/411). If a history of gonorrhoea within the preceding three months is taken into account then the predictive value of a positive ELISA result increases to 57.5% (50/87) with respect to untreated or recently treated gonorrhoea.

Discussion

Our results with the ELISA test for detecting antibodies to gonococcal pili compare favourably with other recently published studies on gonococcal serology. Radioimmunoassay detected antibodies to gonococcal pili in 85% of infected women and 13% of controls.\(^5\) Detection of antibody to an outer membrane protein with ELISA gave a rate of approximately 58% positive in infected patients and 8–11% in controls.\(^6\) In our study ELISA was reactive in 57.4% of infected women, but in only 5.9% of women attending the STD clinic with no clinical or epidemiological evidence of gonorrhoea, past or present. Even assuming that all of the positive ELISA results in patients attending the antenatal clinic were not due to gonococcal infection then the reactivity in this control group is similar (6%).

Holmes et al.\(^14\) considered that the two major limitations of serological tests performed on single sera for detection of gonorrhoea were cross-reactive antibody to N. meningitidis and the persistence of antibody from past infection. Glynn & Ison\(^6\) considered that a proportion of the 'false' positive results in their test may have been caused by extreme variation in a normal population—perhaps the result of stimulation by cross-reacting antigens. Also, in an earlier study\(^15\) it was suggested that nasopharyngeal meningococcal carriage was associated with the production of serum antibody to formalin-treated whole gonococcal cells in an indirect immunofluorescence test.

The low reactivity of sera from non-infected patients in our study may be explained by the finding that throat colonization by meningococci made no statistically significant difference \( P > 0.1 \) to the ELISA results in patients with and without gonorrhoea (Table 3). This finding, which requires confirmation on a larger series of patients, is extremely important in correlating positive results with the presence of anti-gonococcal antibodies, particularly when it is borne in mind that the meningococcus is isolated two to six times more often from patients with gonorrhoea than from those without.\(^16,17\) Holmes et al.\(^14\) using a radioimmunoassay for antibody to gonococcal pili, found that throat colonization by meningococci significantly increased the reactivity of their test. Although no statistical data was presented Reimann & Lind\(^2\) reported that only three of 21 patients with meningococcal infection gave a positive reaction in an indirect haemagglutination test with gonococcal pili as antigen. Whether these differences are attributable to variations in methodology or to the antigenic heterogeneity of gonococcal pili\(^18,19\) remains to be determined.
Although gonorrhoea is generally regarded as being of high infectivity, not all female contacts of men with gonorrhoea develop infection; the proportion of female contacts found not to be infected has been estimated as 34%20 24%21 and 22%.22 Our finding that a positive ELISA result occurs significantly more often (P < 0.001) in gonorrhoeal contacts with negative cultures than in patients without a history of exposure to the gonococcus is therefore of interest. McMillan et al.23 using indirect immunofluorescence on cervical secretions detected anti-gonococcal antibody to the IgA and IgG class in all 12 gonorrhoeal contacts found not to be infected. These results suggest that humoral mechanisms may be involved in preventing infection in certain individuals and should give encouragement to those engaged in the development of gonococcal vaccines.

Although the specificity of our test is high its value would be increased considerably if the sensitivity could be improved. The failure to detect just over 40% of infections could be due to the antigenic heterogeneity of gonococcal pili18,19 or to the testing of patients during the very early stages of the infection. There is some support for the latter suggestion since four of ten patients in the ELISA — Culture + group gave a positive ELISA result when tested two to four weeks later. McMillan et al.24 with indirect immunofluorescence found IgG antibody reactive with the gonococcus in 69 of 70 (98.6%) infected women but in only four (5.7%) non-infected women. However, the mean log titre of anti-gonococcal IgG was significantly higher in patients who had been infected for more than seven days than in those whose infection was of shorter duration. Anti-gonococcal IgM occurred most often in sera from patients who had been infected for less than 14 days.21 It is possible, therefore, that the sensitivity of our test could be improved by using anti-human IgM conjugated alkaline phosphatase in addition to the anti-human IgG conjugate. A further improvement in sensitivity might also result by testing sera against pili prepared from more than one strain of N. gonorrhoeae.

As discussed in detail by Holmes et al.14 the predictive value of a test is a function of its sensitivity and specificity, and the prevalence of gonorrhoea in the population tested. Detection of antibody against gonococcal pili by radioimmunoassay resulted in predictive values of 8 and 22% for a positive result where the prevalence of gonorrhoea in the population was 1.5 and 9.1% respectively.14 Our predictive value score of 35.6% for a positive result with ELISA in a population where the prevalence of gonorrhoea is 10.8% compares extremely favourably with these values. By testing a second specimen of serum a few weeks later we could have detected another four infected patients and increased the sensitivity to 64.8% and the predictive value to approximately 40%.

Detection of antibody to gonococcal pili by ELISA requires further development, particularly with a view to increasing its sensitivity. Lack of cross-reaction with N. meningitidis makes the test highly specific, although further work is needed to discover the cause of the 5—6% unexplained positive reactions. The methodology of the test is simple and readily applicable to large-scale screening and it should now be evaluated in parallel with culture in a low-risk population. The test might be usefully developed for epidemiological surveys to assess the prevalence of gonorrhoea in communities where suitable culture facilities are lacking.

We thank Dr D. H. H. Robertson and staff of the University Department of Venereology, Edinburgh Royal Infirmary and Dr C. C. Blackwell and staff of the Research and Development Laboratory, Department of Bacteriology, Edinburgh University for their interest and encouragement in the course of this work. Thanks are also extended to Professor J. G. Collee for constructive criticism and helpful advice in the preparation of the manuscript. This project was supported by a grant from the Biomedical Research Committee of the Scottish Home and Health Department (Grant No. K/MRS/50/C22) whose financial assistance is gratefully acknowledged.

References


Reactivity of the limulus lysate assay with uterine cervical secretions

A preliminary evaluation

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SUMMARY A limulus lysate assay was performed on cervical secretions from 66 women. When secretions were tested at a 1/100 dilution the assay gave a positive result in 15 (62.5%) of 24 patients with gonorrhoea confirmed by Gram-stained smear or culture or both. When secretions from seven of the nine remaining patients who had gonorrhoea but negative results to the limulus lysate test were retested at a 1/50 dilution, two gave a positive result, increasing the positivity rate of the test to 17 (70.8%) of 24 infected patients. Material from one patient with a history of contact with gonorrhoea and from three (7.3%) of the other 41 patients without any history of gonorrhoea gave positive reactions.

Introduction

Despite improvements in the cultural diagnosis of gonorrhoea1,2 and the advent of several transport and growth systems,3,4 problems associated with maintaining the viability of the gonococcus still exist in certain areas. Gram-staining of material from the genitourinary tract is the only widely accepted non-cultural method for the diagnosis of gonorrhoea. The reliability of Gram-staining for male cases is high; an unequivocally positive or negative result for smears of urethral discharge provides an immediate differential diagnosis between gonococcal and nongonococcal urethritis in 85% of patients.5 However, in female cases Gram-staining of urethral and cervical material will detect only 55-65% of patients from whom Neisseria gonorrhoeae is subsequently isolated by culture.6,8

An assay for endotoxin resulted from the finding by Levin and Bang9 that a lysate of washed amoebocytes of the horseshoe crab (Limulus polyphemus) formed a gel in the presence of minute amounts of endotoxin elaborated by Gram-negative bacteria. Since the demonstration by Rice and Kasper10 of the sensitivity of the limulus endotoxin assay for components of N gonorrhoeae, the system has been shown to be of value in the rapid presumptive diagnosis of gonococcal urethritis in men.11,12 Because of the lack of sensitivity of Gram-staining in female patients, any new non-cultural diagnostic method is of greater potential value in the diagnosis of gonorrhoea in women than in men. For these reasons we considered it worthwhile to report our preliminary evaluation of the limulus lysate assay when applied to cervical secretions.

Patients and methods

STUDY POPULATION AND DIAGNOSIS

Sixty-six women consecutively attending the department of genitourinary medicine at the Black Street Clinic, Glasgow, were investigated. Specimens were taken from the urethra and cervix for microscopical and cultural examination and from the rectum for culture only. Cultural and identification methods for N gonorrhoeae were as described.13

COLLECTION OF SECRETIONS

The cervix was cleaned with a cotton-wool swab held in sponge-holding forceps under direct vision. Secretions were collected from the endocervical canal by gentle aspiration through a sterile polythene capillary tube (chromatography column tubing, internal diameter 1·0 mm, obtained from Pharmacia Fine Chemicals, Uppsala, Sweden), one end of which had been inserted through the os to about 1 cm; the other end of the tube was attached to a 5-ml syringe.
containing 1 ml pyrogen-free water. Secretions in the tubing were ejected into a pyrogen-free plastic container. The secretions were stored at -20°C until required.

**LIMULUS ASSAY**

Frozen specimens of cervical secretions were thawed, mixed thoroughly, and diluted in pyrogen-free water to a final dilution of 1/100; 0.1 ml of the diluted secretion was mixed in a pyrogen-free plastic container with 0.1 ml of reagent reconstituted from the 50 Test Pyrotest™ vial (Difco Laboratories, Detroit, Michigan), incubated at 37°C in a water bath for one hour and read. Results were interpreted in accordance with the manufacturer’s instructions; a firm opaque gel which remained adherent to the bottom of the vial when inverted through 180° was scored as positive; the absence of a firm gel was scored as negative. Control samples with known positive and negative results were tested with each batch of assays. The sensitivity of the limulus assay was demonstrated by the detection of 0.125 μg/1 Difco Pyrotol positive control Escherichia coli endotoxin.

The limulus assays were read without previous knowledge of the conventional microbiological results. After the correlation between the limulus assay results and a diagnosis of gonorrhoea had been made, certain specimens showing an apparently false-positive limulus result were retested at a dilution of 1/200 while specimens showing a false-negative result were retested at a dilution of 1/50.

**PROTEIN ESTIMATION**

To determine whether false-negative results could possibly be due to the secretions being very dilute, the protein concentration of several secretions (four specimens with false-negative and five with confirmed positive results chosen at random) was determined.14

**STATISTICAL ANALYSIS**

The χ² test with Yates's correction was used to test the correlation between limulus assay results and conventional microbiological findings. Student's t test was used to compare the mean protein concentrations in secretions giving false-negative and confirmed positive limulus results.

**Results**

**CULTURE AND MICROSCOPY**

The results of culture and microscopy for N gonorrhoeae are shown in relation to limulus lysate assay results in the table. The limulus assay gave a positive result in 19 (28.8%) of the 66 patients investigated. The result was reactive in 15 (62.5%) of the 24 patients with cervical gonorrhoea (positive smear or culture result or both) compared with only four (9.5%) of the 42 patients without any microbiological evidence of gonorrhoea. This difference is statistically highly significant (χ² 18.4; p<0.001). When secretions from seven of the nine patients with gonorrhoea but negative limulus assay results at a dilution of 1/100 were retested at 1/50 two gave a positive result and this increased the positivity rate of the test to 17 (70.8%) of 24 infected patients.

**PRESENTING DIAGNOSES**

The presenting diagnoses (multiple in a few cases) of the 42 patients in whom there was no microbiological evidence of gonorrhoea were as follows: Chlamydia trachomatis infection, eight; trichomoniasis, 10; candidosis, eight; warts, five; and no infection detected, nine. The remaining eight patients, all with negative limulus assay results, had been treated for gonorrhoea within the preceding three months (range one week to three months).

The limulus assay gave a positive result in one patient who was a contact of gonorrhoea, one patient from whom C trachomatis was isolated, one patient with warts, and one patient in whom no abnormality was detected. If the patient who was a contact of gonorrhoea is excluded the presumed false-positivity rate is reduced to 7.3%; on retesting secretions from two of the three remaining patients at a dilution of 1/200 both gave negative results, reducing the false-positivity rate to 2.4%.

**Table** Results of conventional microbiological investigations for Neisseria gonorrhoeae and of the limulus lysate assay for endotoxin in cervical secretions from 66 women

<table>
<thead>
<tr>
<th>Microbiological results</th>
<th>No of patients</th>
<th>Results of limulus lysate assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture +/smear +</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Culture +/smear -</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Culture -/smear +</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Culture -/smear -</td>
<td>42</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>19</td>
</tr>
</tbody>
</table>

+ Positive - negative
MEAN PROTEIN CONCENTRATION

The mean protein concentration in the secretions from four patients with gonorrhoea who gave negative limulus results was 0·42 g/l compared with a mean concentration of 1·50 g/l in the secretions from five patients with gonorrhoea who gave positive limulus results. The difference between the two means is statistically significant (t 10·12; p<0·001).

Discussion

Although the number of infected patients was low there was a highly statistically significant correlation (p<0·001) between limulus lysate assay results and conventional microbiological evidence of gonorrhoea. The limulus assay result was positive in only 62·5% of women with cervical gonorrhoea and was less sensitive than Gram-staining, which detected 75% of infected women. However, the positivity rate of 62·5% for the limulus test is within the usual range (55-65%) of sensitivity found by Gram-staining.6-8

In the study of Spagna et al11 the limulus assay applied to urethral exudates from men gave a positive result in all 73 culture-positive cases of gonorrhoea tested. Our finding that the positivity rate for the limulus test could be increased to 70·8% by retesting limulus-negative secretions from patients with gonorrhoea at a dilution of 1/50, combined with the lower mean protein concentration found in secretions from such patients, suggests that variation in sampling may be the main reason for the poorer results in women. This variation could possibly be overcome by standardising the test on the basis of a determined protein concentration, for example, rather than by testing all specimens at a fixed dilution. A microdilution technique has also been reported to improve the sensitivity of the limulus assay.12

Positive limulus test results were considered to be unrelated to gonococcal infection in three (7·4%) of 41 patients. It is possible that these patients may have had a recent gonococcal infection, of which we were unaware. However, the limulus assay gave a negative result in all eight patients treated for gonorrhoea within the preceding three months (less than three weeks in four patients) suggesting that gonococcal components reactive in the assay are eliminated from the cervix in a fairly short time.

Clearly, the specificity of the limulus assay requires further evaluation on a larger series of women. In particular, it would be of value to combine these studies with quantitative aspects of the "commensal" flora of the female genital tract to determine the extent of interference due to cervical colonisation with coliform organisms and other endotoxin-positive bacteria. In men the situation is quite different; the urethra is normally free from heavy colonisation with endotoxin-positive bacteria and, as shown by Spagna et al,11 the gonococcus is almost invariably the cause of a positive limulus assay result for endotoxin.

Recently these workers15 extended their studies on the limulus assay to detect gonococcal endotoxin in cervical exudates diluted 1/800. The assay result was reactive in 17 (94%) of 18 of infected women, all of whom had a purulent cervical discharge. The lack of such a discharge in most of our infected patients might explain the lower reactivity rate in our study. Spagna et al15 noted that four of eight patients with nongonococcal cervicitis, in whom other Gram-negative bacteria were present, gave false-positive limulus assay results when exudates were tested at a 1/200 dilution; all eight results were negative when exudates were tested at a 1/800 dilution. Our combined findings suggest that it may prove difficult to obtain reliable results when exudates from all patients, irrespective of the presence or absence of a purulent cervical discharge, are tested at the same dilution.

This work was supported in part by a grant from the Scottish Home and Health Department (research grant No K/MRS/50/C22). We thank Professor J G Collee and Dr D H H Robertson for their helpful advice in the preparation of this paper.

References

Reactivity of the limulus lysate assay with uterine cervical secretions: a preliminary evaluation


Incidence of *Ureaplasma urealyticum* infection in women attending a clinic for sexually transmitted disease

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Summary

*Ureaplasma urealyticum* was isolated from the first 15–30 ml of voided urine from approximately 78 per cent of women attending a clinic for sexually transmitted diseases. Ureaplasmas were isolated significantly more often from patients with gonorrhoea, non specific genital infection, trichomoniasis, warts, and other conditions requiring treatment in the centre than from patients with conditions not requiring treatment in the centre. There was no significant difference in the quantity of ureaplasmas isolated from patients in any of the above categories. Titres of ureaplasmas found in urine specimens from women were higher than those obtained in a parallel study with urine specimens from men. Oral contraception made no significant difference to the incidence or quantity of ureaplasmas. There was a statistically significant correlation ($P < 0.01$) in urine culture results between matched pairs of regular partners. Ureaplasmas were also isolated significantly more often ($P = 0.02$) from women who were contacts of men with NGU, whether or not a diagnosis of non specific genital infection had been made, than from women who were not NGU-contacts.

Introduction

Nongonococcal urethritis (NGU) is one of the greatest problems in the management of the sexually transmissible diseases and is by far the most common condition requiring treatment in British Clinics (Willcox, 1979).

In the United States of America, NGU accounts for more than half of the cases of urethritis seen in sexually active males attending venereal disease clinics (Volk and Kraus, 1974; Holmes, Handsfield, Wang, Wentworth, Turck, Anderson and Alexander, 1975). There is now considerable evidence to implicate *Chlamydia trachomatis* as one of the causes of NGU (Bowie, Wang, Alexander and Holmes, 1977).

Evidence to implicate *Ureaplasma urealyticum* in the aetiology of NGU has resulted from human intraurethral inoculation of ureaplasmas (Taylor-Robinson, Csonka and Prentice, 1977) as well as from chemotherapeutic trials (Bowie, Alexander, Floyd, Holmes, Miller and Holmes, 1976; Coufalik, Taylor-Robinson and Csonka, 1979). Cultural studies by Bowie, Wang, Alexander, Floyd, Forsyth, Pollock, Lin, Buchanan and Holmes...
Ureaplasmas in women

(1977) showed that among men experiencing a first episode of urethritis \textit{U. urealyticum} was recovered more often and in greater numbers from chlamydia-negative cases of NGU than from chlamydia-positive cases or from men without urethritis. Wong, Hines, Brasher, Rogers, Smith and Schachter (1977) also found that ureaplasmas were particularly associated with non-chlamydial NGU although this was not found to be the case in the detailed study reported by Taylor-Robinson, Evans, Coufalik, Prentice, Munday, Csonka and Oates (1979).

Effective control of NGU must be dependent on the investigation and appropriate treatment of female contacts of infected men. Accordingly this paper records the incidence of ureaplasmas in women attending a clinic for sexually transmissible diseases.

Patients and methods

Patients
Two hundred and eighty seven new and return-new female patients attending the Sexually Transmitted Diseases Clinic at the Royal Infirmary, Edinburgh during the period November 1978 to October 1979 were routinely screened for the presence of \textit{Ureaplasma urealyticum} in an initial sample of urine. At the first clinic visit, after routine screening to exclude gonorrhoea, trichomoniasis, candidiasis and syphilis, each patient was asked to void urine and to collect the first 15-30 ml aliquot in a sterile universal container with a sterile funnel top (Bardic MSU kit); the funnel top was then removed, the container sealed with a sterile cap, and the specimen sent to the laboratory within three hours of collection.

Isolation of \textit{Ureaplasma urealyticum}

Culture medium
Trimethoprim, amphotericin, lincomycin, colistin (TALC) colour change medium was prepared by mixing the following ingredients: Difco PPLO broth (70 ml); yeast extract (10 ml of a 10 per cent w/v solution); Wellcome No. 3 Horse serum (20 ml); urea (0.2 ml of a 50 per cent w/v solution); trimethoprim (0.1 ml of 7.5 \(\mu\)g/ml trimethoprim lactate); amphotericin (0.1 ml of 1.0 \(\mu\)g/ml); lincomycin (1.0 ml of 1.0 \(\mu\)g/ml); colistin (0.1 ml of 6.0 \(\mu\)g/ml); and phenol red (1 ml of a 0.2 per cent w/v solution). The PPLO broth was sterilised by autoclaving. The other ingredients were sterilised by filtration and added aseptically. The complete medium was adjusted to pH 6-5 with 1 M HCl and dispensed aseptically in 1.8 ml aliquots in sterile bijou bottles.

Culture procedure
On receipt in the laboratory the volume of the first voided urine specimen was noted prior to centrifugation at approximately 3000 g for 10 minutes in
an MSE bench centrifuge. The supernate was discarded and the deposit resuspended in 1 ml of the above colour change medium minus antibiotics. A 200 μl volume of resuspended deposit was used to initiate a series of ten-fold dilutions in 1·8 ml volumes of TALC colour change medium: the final dilution was 10^10. Cultures were incubated at 37°C and examined after 24 and 48 hours. The presence of ureaplasmas was indicated by a colour change in the medium from yellow to pink. One colour-changing unit (c.c.u.) of activity was defined as the highest dilution of the ureaplasma suspension that produced a colour change (Taylor-Robinson, Martin-Bourgon, Watanabe and Addey, 1971). After allowing for the appropriate dilutions, the results were expressed as c.c.u./ml of initial urine.

Statistical analysis was made by the Chi-square method with Yates’s correction.

Results

Incidence of *Ureaplasma urealyticum*

Cultures for *U. urealyticum* prepared from first voided urine specimens from 287 women were contaminated in ten cases (3·5 per cent). Repeat cultures were positive in three patients and negative in one patient. No repeat cultures were available for six patients. Of the 281 valid cultures, ureaplasmas were present in 218 (77·6 per cent). A total of 300 diagnoses had been made in the 281 patients without reference to the ureaplasma culture results: Table I shows these culture results in relation to the various conditions diagnosed.

Table I  *Isolation of Ureaplasma urealyticum in relation to various conditions diagnosed in 281* women

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total number of patients with each diagnosis</th>
<th>Number (and percentage) of patients with positive cultures for <em>U. urealyticum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonorrhoea</td>
<td>15</td>
<td>13 (86·7)</td>
</tr>
<tr>
<td>NSGI</td>
<td>42</td>
<td>36 (85·7)</td>
</tr>
<tr>
<td>NSGI with arthritis</td>
<td>2</td>
<td>2‡</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>15</td>
<td>14 (93·3)</td>
</tr>
<tr>
<td>Genital Candidiasis</td>
<td>25</td>
<td>19 (76·0)</td>
</tr>
<tr>
<td>Genital Scabies</td>
<td>1</td>
<td>1‡</td>
</tr>
<tr>
<td>Pubic lice</td>
<td>1</td>
<td>1‡</td>
</tr>
<tr>
<td>Genital Herpes Simplex</td>
<td>3</td>
<td>2‡</td>
</tr>
<tr>
<td>Warts</td>
<td>17</td>
<td>15 (88·2)</td>
</tr>
<tr>
<td>Other conditions requiring treatment in the centre</td>
<td>117</td>
<td>98 (83·8)</td>
</tr>
<tr>
<td>Other conditions not requiring treatment in the centre</td>
<td>62</td>
<td>34 (54·8)</td>
</tr>
<tr>
<td>Total</td>
<td>300*</td>
<td>235 (78·3)</td>
</tr>
</tbody>
</table>

* More than one condition was diagnosed in several patients.

NSGI = non-specific genital infection (This diagnosis was made only in those patients in whom *Chlamydia trachomatis* was isolated from the cervix: these patients were usually but not always contacts of men with NGU).

‡ Percentages not given if total is less than 10.
The incidence of ureaplasmas was significantly higher in those with (a) gonorrhoea, 86.7 per cent ($\chi^2 = 4.0; 0.05 > P > 0.02$); (b) NSGI (including patients with arthritis), 86.4 per cent ($\chi^2 = 10.3; P < 0.01$); (c) trichomoniasis, 93.3 per cent ($\chi^2 = 6.3; 0.02 > P > 0.01$); (d) warts 88.2 per cent ($\chi^2 = 5.1; 0.05 > P > 0.02$); (e) other conditions requiring treatment in the centre, 83.8 per cent ($\chi^2 = 16.0; P < 0.001$) than in those with other conditions not requiring treatment in the centre, 54.8 per cent. Despite the difference in percentage isolation of ureaplasmas between the latter group and patients with candidiasis (76.0 per cent) the difference in proportions is not significant at the 5 per cent level ($\chi^2 = 2.5; 0.2 > P > 0.1$). There is no significant difference in ureaplasma isolation rate between the patients with candidiasis and those with gonorrhoea, NSGI, trichomoniasis, warts and other conditions requiring treatment in the centre ($\chi^2 = 2.7; 0.8 > P > 0.7$).

Apart from the 44 patients with NSGI (all chlamydia-positive whether or not known contacts of men with NGU) an additional 106 women were contacts of men with NGU. Ureaplasmas were isolated from 125 (83.3 per cent) of these 150 patients and from 93 (71.0 per cent) of the remaining 131 patients who were not diagnosed as suffering from NSGI and were not contacts of men with NGU. This difference is statistically significant ($\chi^2 = 5.43; P = 0.02$).

Quantitative results

The quantitative distribution of ureaplasmas in patients with various conditions is shown in Table II (Because of the small numbers, patients with pubic

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients with each diagnosis giving corresponding ureaplasma titre: c.c.u./ml of initial urine ($\log_{10}$)</th>
<th>Percentage $\geq 10^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>$\leq 5$</td>
<td>6</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>NSGI (including two patients with arthritis)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Warts</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Other conditions requiring treatment in the centre</td>
<td>38</td>
<td>16</td>
</tr>
<tr>
<td>Other conditions not requiring treatment in the centre</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>85</td>
<td>24</td>
</tr>
</tbody>
</table>

NSGI = non-specific genital infection.
lice, scabies and genital herpes simplex are excluded from this Table: patients in whom more than one condition was diagnosed are also excluded.

There is no significant difference between the various conditions with respect to isolation of ureaplasmas at high titre ($\geq 10^8$ c.c.u./ml) ($\chi^2 = 5.7; P = 0.5$).

**Effect of oral contraception**

Ureaplasmas were isolated from 117 (80.7 per cent) of 145 women using oral contraceptives compared with 78 (75.7 per cent) of women using other forms of contraception: this difference is not significant ($\chi^2 = 0.61; 0.5 > P > 0.3$). High titres of ureaplasmas ($\geq 10^8$ c.c.u./ml) were found in 64 (54.7 per cent) of the 117 pill-users with positive ureaplasma cultures compared with 54 (69.2 per cent) of the 78 non-pill users with positive cultures: this difference is not significant at the 5 per cent level.

**Results in matched pairs**

In 71 women a corresponding first voided urine specimen from their regular partner was cultured for ureaplasmas. Identical culture results were obtained in 47 matched pairs (30 positive and 17 negative); 22 male partners of women with positive ureaplasma cultures gave negative results (it is likely that a proportion of these men had received treatment prior to testing for ureaplasmas); and two men gave positive results although their female partners were negative. The correlation of culture results between patients is statistically significant ($\chi^2 = 10.67; P < 0.01$).

**Chemotherapy**

Repeat ureaplasma cultures were carried out on 64 patients seven days after commencing treatment with oxytetracycline 250 mg q.i.d.: the total oxytetracycline given was 5 g in seven patients and 7 g in 57 patients. Culture results are shown in Table III. Although patients were instructed to refrain from intercourse for 14 days some may have become re-infected.

A significantly higher proportion of patients (75.4 per cent) gave negative

<table>
<thead>
<tr>
<th>Oxytetracycline dosage</th>
<th>Positive</th>
<th>Significant decrease* (at least $10^4$ c.c.u./ml)</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 g</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>7 g</td>
<td>14</td>
<td>3</td>
<td>40</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4</td>
<td>40</td>
<td>64</td>
</tr>
</tbody>
</table>

* If post-treatment specimens were not negative the quantity of ureaplasmas present was usually the same as, but occasionally reduced ten-fold in comparison with, the pre-treatment specimen; these were scored positive. In four cases there was a decrease of at least ten thousand-fold ($\geq 10^4$ c.c.u./ml) reflecting a 99.99 per cent decrease in the number of ureaplasmas present in the post-treatment specimens.
results, or a decrease in titre of $\geq 10^4$ c.c.u./ml, after treatment with 7 g oxytetracycline than after treatment with 5 g (14.3 per cent): ($\chi^2 = 8.2; P < 0.01$).

A total of 12 patients were treated with doxycycline: 200 mg as an initial dose then 100 mg daily; treatment lasted for eight days or less in seven patients and for more than eight days in five patients (20 days in four patients and 11 days in one patient). Of the 12 patients treated, 83.3 per cent were either negative (nine patients) or showed a significant decrease (one patient). The two positive patients and the patient showing the significant decrease were in the group treated for less than eight days.

**Discussion**

The overall incidence of ureaplasmas (77.6 per cent) in women attending a clinic for sexually transmissible diseases is comparable to those of previously published reports for similar populations. Kundsin (1976) isolated ureaplasmas from urine specimens from 80 per cent of women with symptoms of genitourinary infection attending a genito-infectious disease clinic, from 51 per cent of women with a history of reproductive failure and from only 2 per cent of nuns. In another study (McCormack, Almeida, Bailey, Grady and Lee, 1972) of student and graduate nurses it was found that women with no history of sexual contact were usually free of ureaplasmas whereas 37.5 per cent of those with a history of intercourse with a single partner and 75 per cent of those who had intercourse with three or more partners were colonised by ureaplasmas. Prevalence of urethral ureaplasma infection in men is also correlated with total number of previous sex partners (McCormack, Lee and Zinner, 1973). A greater number of sex partners for patients in the various groups requiring treatment than for those patients who did not require treatment seems a likely explanation for the significantly lower ureaplasmal isolation rate in the latter group.

Taylor-Robinson (1977) considered it reasonable to expect ureaplasmas to be present in larger numbers if they were contributing to pathogenicity than if they had a commensal role only. In view of the lack of quantitative studies of ureaplasmas in women our finding that ureaplasmas at high titre ($\geq 10^6$ c.c.u./ml) were not isolated significantly more often from the various groups of patients requiring treatment than from patients who did not require treatment is of interest. Therefore, if ureaplasmas were responsible for genital symptoms in any of the patients it would appear that their actual numbers were of little significance.

The quantity of ureaplasmas found in first voided urine specimens from women is significantly greater than from men: high titres of ureaplasmas ($\geq 10^6$ c.c.u./ml) were isolated in 132 (60.8 per cent) of 217 women in this study whereas Hunter, Smith, Peutherer, MacAuley, Tuach and Young (1981) found that the total ureaplasma counts in first voided urine specimens from men was equal or greater than $10^6$ in only 41 (27.3 per cent) of 150
patients examined ($\chi^2 = 38.6; P < 0.001$). Since both studies were carried out in parallel with the same batches of media, this comparison appears to indicate a real difference in ureaplasma colonisation between the sexes. It may be that conditions in the female genito-urinary tract are much more favourable for the proliferation of ureaplasmas than are conditions in the male urethra. Oral contraception has no significant influence in this respect. Another possibility is that the high titres found in women result from colonisation of the periurethral mucosa.

McCormack, Rankin and Lee (1972) in a study of 132 women attending a prenatal and gynaecology clinic found that the greatest number of isolations (65 per cent) were obtained from vaginal cultures; cervical cultures were positive in 47 per cent of patients while culture of the urethra and posterior fornix showed intermediate results. Since bladder urine is normally sterile, these workers considered that the organisms were presumably picked up in transit from the periurethral mucosa. If this is the case, then the degree of colonisation must be very high indeed since titres of $10^8$ and $10^9$ were found in 72 (33.2 per cent) and seven (3.3 per cent) of 217 women respectively (Table II). A comparative study of ureaplasma titres in serial specimens of urine from individual patients is obviously required in order to determine whether or not ureaplasmas infect bladder urine.

We consider that culture of a centrifuged deposit of first voided urine is a simple and effective method of screening for ureaplasma infection. A strong association ($P < 0.001$) between the finding of ‘T’ strain mycoplasmas in the cervix and in the urine has been reported previously (Braun, Klein, Lee and Kass, 1970). Examination of urine also overcomes the recently reported problem of the inhibitory effect of wooden applicator swab sticks on growth of ureaplasmas (Poulin, Kundsin and Horne, 1979).

The results presented suggest that ureaplasma infection is very common in women attending a clinic for sexually transmitted diseases but do not allow us to draw conclusions regarding their ability to cause disease. In the present study no attempt was made to correlate ureaplasma isolation with genital tract symptoms or to study specifically the effect of chemotherapy. However, routine treatment which has been given without reference to ureaplasma culture results showed that a seven day course of oxytetracycline was better than a five day course at eradicating ureaplasmas ($P < 0.01$): this observation should be confirmed on a larger series of patients as re-infection, which would be more likely to occur in patients receiving the short course of treatment, may have exaggerated the difference between the two groups. Ureaplasma isolation with respect to symptomatology, and the effect of chemotherapy, merits a further prospective study. This is important not only in relation to disease in women but is also very relevant to the management and control of NGU in men.

(We thank Professor J. G. Collee for constructive criticism in the preparation of this paper and Dr D. H. H. Robertson and colleagues in the Department of Venereology for their help in the collection of specimens and data.)
References


Effect of *Neisseria meningitidis* group A polysaccharide vaccine on nasopharyngeal carrier rates

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Summary

The effect of *Neisseria meningitidis* group A polysaccharide vaccine on nasopharyngeal carriage was studied in the Finnish Armed Forces during an epidemic of meningitis caused by *N. meningitidis* group A.

In 1974, half of all new recruits received the vaccine (16,458 vaccinated) when entering service. Nasopharyngeal swabs were cultured for *N. meningitidis* at this time and again four weeks later in a follow-up cohort of about 1200 men. Another set of 1101 samples were collected and cultured seven to 16 weeks after entering service from recruits in all garrisons that had had cases of purulent meningitis. In most garrisons the carrier rates of group A *N. meningitidis* were low (average 1-2 per cent) or moderate (7 to 9 per cent) throughout the study without difference between the vaccinated and unvaccinated men. However, in three garrisons the carrier rates of group A strains were high (average 24 per cent) among those not vaccinated, and significantly lower (average 9 per cent) among group A vaccinees. Carriage of other, non-group A *N. meningitidis* strains was high, 75 per cent in all groups. Since group A strains were rare in men entering service (average 2.2 per cent in 1974), the group-specific inhibition of carriage by the vaccine was concluded to be due to its ability to prevent acquisition of group A strains in the nasopharynx.

Introduction

The efficacy of *Neisseria meningitidis* group A and C capsular polysaccharide vaccines (Gotschlich, Liu and Artenstein, 1969) in eliciting a serum antibody response and conferring group-specific disease protection is established (WHO Study Group, 1976). However, we have little information of their effect on nasopharyngeal carriage of *N. meningitidis*. This is difficult to study because of the low carrier rates of group A and C organisms, yet, this relation is important because of the probable role of nasopharyngeal carriers in the spread of infection. *Neisseria meningitidis* carrier rates generally rise in new military recruits, and this population may be at higher risk of disease (Aycock and Mueller, 1950; Fraser, Bailey, Abbot, Gill and Walker, 1973).

In some studies in army recruits, vaccination with group C polysaccharide vaccine was shown to reduce nasopharyngeal acquisition of group C *N. meningitidis*, especially in situations with higher carrier rates and rapid
Isolation of Ureaplasma Urealyticum from Seminal Plasma in Relation to Sperm Antibody Levels and Sperm Motility

T.B. HARGREAVE, M. TORRANCE, H. YOUNG and A.B. HARRIS

Introduction

Sperm antibodies are thought to account for some cases of infertility. Evidence for this is (1) these antibodies are found more frequently in the infertile population when compared with a fertile one (Hargreave et al. - 1980), (2) long-term follow up of couples with sperm antibodies has shown that when antibodies are detected above a certain level the chance of fertility is very low (Rumke et al. - 1974), (3) steroid treatment in some cases appears to result in fertility (Hendry et al. - 1979).

The etiology of these antibodies remains a mystery. It is possible there could be a breach in the blood testis barrier but in most antibody positive cases there is no obvious evidence of testicular damage when testicular biopsies are examined (Rumke and Hellinga - 1959) although detailed immunohistological techniques have not been widely used. A second possibility is that damage secondary to prostatitis or seminal vesiculitis may allow immunization through sperm resorption. There have been reports suggesting that chronic prostatitis secondary to seminal plasma T-mycoplasma (ureaplasma) growth may alter seminal parameters and account for some cases of infertility (Fowlkes et al. - 1975), Quesada et al. (1968) and Fjällbrant and Obrant (1968), found an association between agglutinating antibodies and prostatitis and in view of previous reports linking ureaplasmas with infertility it seemed worthwhile investigating whether ureaplasma infection bore any relationship to the genesis of antisperm antibodies. In this paper, we report the results of a study to determine whether seminal plasma ureaplasma cultures correlate with the presence of agglutinating and immobilising sperm antibodies.

Patients and Methods

115 men attending for fertility assessment had semen samples sent for ureaplasma culture as well as for routine semen analysis. In addition all men had blood samples and seminal plasma samples assayed for immobilising and agglutinating sperm antibodies. The samples were collected during a six month period and during this time the clinic appointments were weighted to give an increase in the number of men attending who were known on previous testing to have sperm antibodies. As well as these tests, all men and their wives gave a full history and were examined and in addition buccal smears were examined from all the men.

Key words: Spermatozoa, antibody — ureaplasma, infertility — mycoplasma, infertility — seminal plasma, ureaplasma
Table 1: Incidence of Ureaplasma Growth Tabulated in Relation to the Presence of Sperm Antibodies as Detected by Either the Trayslide Agglutination Test or the Micro Immobilisation Test

<table>
<thead>
<tr>
<th></th>
<th>Ureaplasma Culture Results: Log^{10} CCU*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0</td>
</tr>
<tr>
<td>negative antibodies</td>
<td>89</td>
</tr>
<tr>
<td>positive antibodies</td>
<td>26</td>
</tr>
</tbody>
</table>

* One colour-changing unit (CCU) of activity was defined as the highest dilution of the Ureaplasma suspension that produced a colour change.

Table 2: Ureaplasma Culture Results Tabulated in Relation to Tray Slide Agglutination Test Antibody Titres

<table>
<thead>
<tr>
<th>Serum Antibody Only</th>
<th>Ureaplasma Culture Results: Log^{10} CCU</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/64</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 1/64</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seminal plasma antibody only</th>
<th>Ureaplasma Culture Results: Log^{10} CCU</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/16</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 1/16</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibodies in both serum and seminal plasma</th>
<th>Ureaplasma Culture Results: Log^{10} CCU</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/64</td>
<td>7</td>
</tr>
<tr>
<td>&gt; 1/64</td>
<td>7</td>
</tr>
</tbody>
</table>

_Ureaplasma urealyticum culture_

Trimethoprim, amphotericin, lincomycin, colistin (TALC) colour change medium (Young et al. - 1981) was prepared by mixing the following ingredients: Difco PPLO broth (70 ml); yeast extract (10 ml of a 10 per cent w/v solution); Wellcome No. 3. Horse serum (20 ml); urea (0.2 ml of a 50 per cent w/v solution); trimethoprim (0.1 ml of 7.5 mg/ml trimethoprim lactate); amphotericin (0.1 ml of 1.0 mg/ml); lincomycin (1.0 ml of 1.0 mg/ml); colistin (0.1 ml of 6.0 mg/ml); and phenol red (1 ml of a 0.2 per cent w/v solution). The PPLO broth was sterilised by autoclaving. The other ingredients were sterilised by filtration and added aseptically. The complete medium was adjusted to pH 6.5 with 1 M HCl and dispensed aseptically in 1.8 ml aliquots in sterile bijou bottles.

Seminal plasma (0.2 ml) was added to 1.8 ml of TALC colour change medium and transported to the laboratory within four hours of collection. On receipt in the laboratory a series of tenfold dilutions was made in 1.8 ml volumes of TALC medium: the final dilution was 10^{10}. Cultures were incubated at 37° and examined after 24 and 48 hours. The presence of ureaplasmas was indicated by a colour change in the medium from yellow to pink. One colour-changing unit (CCU) of activity was defined as the highest dilution of the ureaplasma suspension that produced a colour change (Taylor-Robinson et al. - 1971).
Table 3: Relationship between Ureaplasma and Sperm Motility

<table>
<thead>
<tr>
<th>% Motility</th>
<th>Ureaplasma Culture Result: Log $^{10}$ CCU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>&lt; 20%</td>
<td>17</td>
</tr>
<tr>
<td>20–60%</td>
<td>19</td>
</tr>
<tr>
<td>&gt; 60%</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 4: Incidence of Positive Ureaplasma Culture in all Cases Compared to Those Couples with Unexplained Infertility

<table>
<thead>
<tr>
<th></th>
<th>Ureaplasma Culture Results: Log $^{10}$ CCU</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>All cases</td>
<td>115</td>
</tr>
<tr>
<td>Unexplained infertility*</td>
<td>17</td>
</tr>
</tbody>
</table>

* Unexplained Infertility mean: Wife = normal fallopian tubal patency and anatomy and normal ovulation. 

Husband = semen density greater than 20 millions/ml, spermatozoal motility greater than 60%, no history of testicular undescend, no evidence of varicocele and no evidence of sperm antibodies.

Antisperm Antibody Detection

Seminal plasma and serum samples were tested for agglutinating antibodies by the Tray Slide Agglutination Test (TAT) (Friberg - 1974) and for immobilizing antibodies by the micro immobilization test (MIT) (Husted and Hjort - 1976). In both test 5μl of patients serum/semen plasma were incubated under paraffin for 1 hour with 1 μl of good quality sperm from a donor. For the TAT the serum/semen plasma was tested at an initial dilution of 1/16 and for the MIT at an initial dilution of 1/8. For the MIT 1μl of complement was added after the incubation and a positive result is when the base line motility is reduced by 50%. In both tests positive and negative controls were used. Any positive specimens were then diluted out and the test repeated to find the titre of antibody.

Semen Analysis

Semen analysis was carried out on at least three occasions on all the men and the results reported in the tables are based on the best figures obtained in each case.

Results

In Table 1 the relationship between the numbers of ureaplasma isolated is tabulated in relation to whether antibodies were detected by either the tray slide agglutination test or by the micro immobilization test. In Table 2 the culture results are related to the tray slide agglutination titres. These two tables show there is no correlation between culture findings and antibody findings. The micro immobilization test was only positive in 4 cases and in
these cases the tray slide agglutination test was also positive; again there was no relationship between findings on culture results and from the micro immobilization test. Two further analyses were carried out to examine whether ureaplasma presence might be influencing infertility in other ways. In Table 3, the relationship between quantitative ureaplasma culture and sperm motility is shown. The motility recorded for the purpose of the analysis was the best of the three analyses. There is no correlation between the presence of ureaplasmas and poor motility: ureaplasmas were isolated from 11 (39.2%) of 28 patients with < 20% motility compared with 24 (46.2%) of 52 patients with > 60% motility. In Table 4 the results of quantitative ureaplasma cultures are analysed according to the fertility status of the couple in an attempt to see if ureaplasma infection may account for some cases of unexplained infertility. Seventeen couples were found in whom all other tests were normal and 9 had ureaplasma growth (53%). 51 out of 115 men (44%) originally tested were positive for ureaplasma; the difference between these two percentages is not significant.

Discussion

Following reports that ureaplasma infection may account for some cases of infertility (Fowlkes et al. - 1975) there have been other reports which have shown no difference between the presence of ureaplasma infection in a fertile population compared with an infertile population (de Louvois et al. - 1974). The isolation of ureaplasma from 51 (44.3%) of 115 men in our study is similar to the incidence (32.6%) reported previously in a similar population (Desai et al. - 1980). These isolation rates are also similar to those obtained in other populations: when urine specimens from men with non-gonococcal urethritis and from men with no urethritis were cultured, ureaplasmas were found in 53% and 33% of patients respectively (Hunter et al. - 1981). These findings lend support to the suggestion of Desai et al. (1980) that the presence of ureaplasmas in the semen is merely the result of contamination at the time of ejaculation. As might be expected in view of the ureaplasma culture findings in fertile infertile populations we have not found any relationship between ureaplasmas isolated from seminal plasma and the occurrence of sperm antibodies. Clearly localisation studies are required to define as precisely as possible the distribution of ureaplasmas within the genitourinary tract since our study does not preclude a correlation between ureaplasma infection of the prostate, for example and sperm antibody production.

It is also possible that subclinical prostatitis secondary to ureaplasma infection may have triggered sperm antibody production in the past but at the time we cultured the samples the ureaplasmas had spontaneously disappeared. This in fact is unlikely as some strains have been shown to persist for at least a year in the absence of sexual contact (Holmes et al. - 1974). It is not known whether serological studies to detect previous ureaplasma infection would be of value in this context.

The question of whether other organisms may cause subclinical genital tract infection which triggers sperm antibody production remains to be answered.

Summary

115 infertile men were studied to find out if there is any association between seminal plasma ureaplasma culture and agglutinating sperm antibodies. This was done to try to throw more light on the aetiology of sperm antibodies. No association was found.
Die Isolierung von Ureaplasma Urealytikum aus Seminalplasma in Relation zum Spermatozoen-Antikörperspiegel und zur Spermatozoenmotilität

Zusammenfassung


References


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DETECTION OF GONOCOCCAL ANTIGENS BY AN INDIRECT SANDWICH ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary. The detection of gonococcal antigens by an indirect sandwich ELISA system is described. The feasibility of using rabbit antiserum raised against whole cells of Neisseria gonorrhoeae strain 9 to detect gonococcal lipopolysaccharide, whole cells, and outer membrane (OM) protein was investigated. OM protein was found to be the main antigen detectable with the antiserum in a direct ELISA system. A positive result in the indirect assay could be obtained with a minimum of 46 to 92 ng of gonococcal OM protein or with $6.6 \times 10^3$ cfu of N. gonorrhoeae. The sensitivity of the assay was found to be approximately eightfold lower with OM complex from a strain of N. meningitidis serogroup B for which the minimum amount of OM protein detected was 375 ng. Negative results were obtained with OM complex from Streptococcus agalactiae, Bacteroides bivius and Escherichia coli. The assay seems to be highly specific for gonococcal antigens. The sensitivity of the assay and its specificity commend it for further evaluation in the detection of gonococcal antigens in clinical specimens.

Introduction

Recent attempts to provide a non-cultural diagnosis for gonorrhoea have included the detection of antibodies against gonococci (Buchanan et al., 1973; O'Reilly, Welch and Kellogg, 1973; Oates et al., 1977) as well as the detection of gonococcal components such as deoxyribonucleic acid (Janik, Juni and Heym, 1976; Sarafian and Young, 1980), endotoxin (Spagna, Prior and Perkins, 1979; Young, Sarafian and McMillan, 1981), and surface antigens (Thornley et al., 1979). Although these studies have met with only limited success, a reliable non-cultural method remains a worthwhile goal in gonococcal research.

An indirect sandwich enzyme-linked immunosorbent assay (ELISA) system described by Drow, Maki and Manning (1979) for the detection of Haemophilus influenzae type B capsular antigen in clinical specimens was found to be highly sensitive and specific. The aim of the present study was to assess the feasibility of detecting various gonococcal antigens by an ELISA system.

Received 12 Mar. 1982; accepted 30 Apr. 1982.

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Materials and methods

Reference bacteria. Neisseria gonorrhoeae strain 9 was kindly supplied by Dr D. S. Kellogg, Center for Disease Control, Atlanta, GA. N. gonorrhoeae strain 82409 was kindly supplied by Dr K. Reimann, Neisseria Department, Statens Serum Institut, Copenhagen. N. pharyngis var. flavus NCTC 4590 was supplied by the National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. A strain of N. meningitidis serogroup B was originally obtained from the Neisseria Repository, Berkeley, California. The following strains were obtained from the laboratory stock cultures of the Department of Bacteriology, University of Edinburgh Medical School: Streptococcus agalactiae NCTC 11078, Bacteroides bivius VP16822 and Escherichia coli NCTC 10418.

Clinical isolates were from patients attending the Department of Genito-Urinary Medicine, The Royal Infirmary, Edinburgh, and included 10 strains of N. gonorrhoeae, one of N. lactamica and one of N. perflava.

Culture media. Modified New York City (MNYC) medium (Young, 1978) was used for subculture of isolates and for maintenance of N. gonorrhoeae strain 9.

Before preparing antigen, neisseriae were inoculated on to supplemented Difco GC Base (Young, Paterson and McDonald, 1976) and incubated overnight at 37°C in a CO₂-enriched humidified atmosphere. Other bacteria were inoculated on to blood agar (Oxoid), and incubated aerobically, except for B. bivius which was incubated at 37°C by the standard anaerobic procedure of Collee et al. (1972).

Preparation of antigens for detection by enzyme-linked immunosorbent assay (ELISA). Lipopolysaccharide (LPS) was extracted from N. gonorrhoeae strain 9 colony-type 3 by the aqueous phenol procedure of Perry et al. (1975). Whole-cell gonococcal complement-fixation test (GCFT) antigen was prepared from N. gonorrhoeae strain 9 colony-type 3 as described by Young, Henrichsen and McMillan (1980). Outer membrane (OM) complex was prepared from all bacteria including stock cultures and clinical isolates as described by Poxton (1979). The bacteria were harvested and washed twice in phosphate-buffered saline (PBS, 0·15M NaCl containing 50 mM phosphate buffer pH 7·4) by centrifugation at 10 000 g for 30 min at 4°C. The pellets were resuspended in 10–30 ml of PBS pH 7·4 containing 10 mM ethylenediaminetetraacetic acid (EDTA), incubated at 45°C for 30 min, and treated in an ultrasonic bath (Model 6441A, Dawe Instruments Ltd, Western Avenue, London W3 OSJ) for 60 s. The treated cells were removed by centrifugation at 10 000 g for 30 min, leaving the OM complex in the supernate.

Quantitative analytical procedures. Protein was determined by the method of Lowry et al. (1951), with bovine-serum albumin as standard. Total carbohydrate was determined by the procedure of Dubois et al. (1956), with glucose as standard.

Preparation of antisera. Antisera against whole, untreated N. gonorrhoeae strains 9 and 82409 (both colony-type 2) were raised in New Zealand white rabbits. A pre-immunisation blood sample (5 ml) was taken from each rabbit.

The bacteria were grown overnight on GC agar, harvested and washed once with PBS pH 7·2 before resuspension in the same buffer to a concentration of 10⁹ cfu/ml. The rabbits received intravenous injections according to the following schedule: week 1, daily 0·5 ml for 4 days; week 2, daily 1·0 ml for 5 days; week 3, no injections; week 4, daily 1·0 ml for 5 days; week 5, test bleed (5 ml); 2 days later the rabbits were exsanguinated by cardiac puncture.

Antiserum against whole, untreated N. gonorrhoeae strain 9 colony-type 2 was also raised in CFI (albino) randomly bred mice. The mice received 0·2 ml intravenous injections, containing 10⁸ cfu/ml, according to the following schedule: week 1, daily for 3 days; week 2, daily for 3 days; week 3, no injections; week 4, daily for 3 days; week 5, daily for 3 days; week 6, test bleed (2 ml); 2 days later the mice were exsanguinated.

Antibody titres were determined by the GCFT as described by Young et al. (1980); the titres of the rabbit sera anti-strain 9 and anti-strain 82409 were 1024 and 256 respectively and that of the mouse serum anti-strain 9 was 256.

Determination of antibody titre of rabbit serum by ELISA with various gonococcal antigens was essentially as described by Voller, Bidwell and Bartlett (1976) and Poxton (1979), except that 4-h incubation periods at room temperature were reduced to 1 h at 37°C.

Microtitration plates were coated with graded amounts of the three antigen preparations as
ELISA TEST FOR GONOCOCCAL ANTIGENS

indicated: LPS (25 µg, 12.5 µg, 6.25 µg and 3.12 µg); whole-cell GCFT antigen (from a dilution of 10-1250 in a series of fivefold dilutions); OM complex (5-0.4 µg protein in twofold dilutions). Rabbit antiserum was serially diluted twofold to determine the antibody titre with LPS, and serially diluted fivefold for testing against whole-cell GCFT antigen and OM complex.

Anti-rabbit IgG conjugated to alkaline phosphatase (Miles laboratories Ltd, Stoke Poges, Slough, Bucks) was used at a dilution of 1 in 400, and the substrate p-nitrophenyl phosphate (Sigma) was used at a concentration of 1 mg/ml. Antigen was diluted in 50 mM carbonate buffer pH 9-6 containing sodium azide 0-02% (w/v). Rabbit antiserum and alkaline phosphatase conjugate were diluted in PBS pH 7-4 containing Tween 20 0-05% (v/v) and sodium azide 0-02% (w/v). Controls were set up lacking one or more of the following: antigen, rabbit antiserum, conjugate or substrate. Extinction values were read at 405 nm on a TitertekR Multiskan (Organon Teknika, Teknika House, Cromwell Road, St Neots, Huntingdon, Cambridgeshire PE19 2EU).

Effect of OM complex protein concentration on extinction value in direct ELISA. Serial doubling dilutions of the OM complex preparation from N. gonorrhoeae strain 9 (protein concentration of 1 mg/ml) were used to coat the wells over a range of dilutions from 2 to 32 768, corresponding to 25-0-003 µg of protein. Homologous rabbit serum and conjugate were used at dilutions of 1 in 5000 and 1 in 400 respectively.

Indirect sandwich ELISA for the detection of N. gonorrhoeae was essentially as described by Drow et al. (1979). Polystyrene balls (0-25 in., c. 0-6 cm) with frosted finish, RIA grade (Euro-Matic Ltd, Maycrete House, Boston Manor Road, Brentford, Middlesex) were armed with mouse antiserum, raised against whole untreated cells of N. gonorrhoeae strain 9, by immersion in antiserum diluted in 50 mM carbonate buffer pH 9-6, containing sodium azide 0-02% (w/v). Incubation was for 4 h in a shaking incubator at 37°C and thereafter static at 4°C overnight. Supernatant fluid was removed by suction with a pasteur pipette and the balls were washed three times with 0.15 M NaCl containing Tween 20 0-05% (v/v). The washed balls were then immersed in 10% (v/v) fetal-calf serum (FCS) in PBS pH 7-4 with sodium azide 0-2% (w/v) and thereafter incubated at room temperature for 1 h. The FCS was then removed by suction, and the balls placed on gauze in petri dishes for rapid-drying. The armed balls were stored at 4°C.

On the day of the assay, armed balls were placed in glass tubes (72 x 12 mm; one ball per tube). For antigen capture, 300 µl of antigen in PBS pH 7-4 containing Tween 20 0-05% (v/v) and sodium azide 0-02% (w/v) was added to each tube. After incubation in a 4°C water bath for 1 h, the balls were washed three times. Captured antigen was recognised by the addition of 300 µl rabbit anti-strain 9 serum diluted in PBS pH 7-4, containing Tween 20 0-05% (v/v) and sodium azide 0-02% (w/v). Incubation was at 40°C for 1 h. After washing as before, 300 µl of anti-rabbit IgG conjugated to alkaline phosphatase, diluted in the same buffer as that of the rabbit antiserum, were added to each tube. After incubation at 40°C for 1 h, the balls were washed as before and transferred to clean glass tubes; 300 µl of the enzyme substrate (1 mg/ml solution of p-nitrophenyl phosphate in 50 mM carbonate buffer pH 9-8, containing 1 mM MgCl₂) were added to each tube. After incubation at 40°C for 1 h, the solutions were quickly transferred to a microtitration plate, and the extinction values read at 405 nm on a TitertekR Multiskan.

Negative controls were set up in duplicate with every experiment; they comprised antigen diluent instead of antigen and were otherwise treated in the usual manner. The assay is represented schematically in fig. 1.

Determination of suitable levels of antibody for 'arming' and 'recognition'. Three sets of polystyrene balls (24 per set) were armed with mouse anti-strain 9 serum at dilutions of 1 in 100, 200 and 300. Rabbit anti-strain 9 serum at dilutions of 1 in 2500 and 5000 and conjugate at a dilution of 1 in 400 were used in the assay.

N. gonorrhoeae strain 9 OM complex (protein concentration 1 mg/ml) was serially diluted in fivefold steps to determine the minimum amount of protein detectable with the various dilutions of the 'arming' and 'recognition' antisera.

Specificity and sensitivity of the assay with OM complex preparations. The polystyrene balls were armed with mouse anti-strain 9 serum at a dilution of 1 in 300. Rabbit antiserum and conjugate were used at dilutions of 1 in 2500 and 1 in 400 respectively.
The specificity of the assay and the minimum amount of OM-complex protein detectable with this system were determined in tests with doubling dilutions of OM-complex preparations (protein concentration of 1 mg/ml) derived from N. gonorrhoeae strain 9, ten clinical isolates of N. gonorrhoeae, N. meningitidis serogroup B, S. agalactiae, B. hivus and E. coli.

Negative controls consisted of armed balls treated in the same manner except for the use of antigen diluent instead of antigen.

**Table I**

*Highest titres of rabbit antisera determined against antigen preparations by direct ELISA*

<table>
<thead>
<tr>
<th>Gonococcal antigen</th>
<th>Titre of rabbit antiserum raised against N. gonorrhoeae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 9 LPS</td>
<td>625</td>
</tr>
<tr>
<td>Strain 9 whole-cell GCFT antigen</td>
<td>625</td>
</tr>
<tr>
<td>Strain 9 OM complex</td>
<td>15625</td>
</tr>
<tr>
<td>Strain 82409 OM complex</td>
<td>15625</td>
</tr>
</tbody>
</table>
Detection of whole-cell antigen in a simulated clinical specimen. The overnight growth from a plate culture of *N. gonorrhoeae* strain 9 on GC medium was suspended in 1 ml of physiological saline. A viable count on GC medium was immediately done on 100 µl of the suspension. After the suspension had been held at room temperature for 2 h, 100 µl of 500 mM phosphate buffer pH 7.4 containing NaCl 0.85% (w/v), Tween 20 0.5% (v/v) and sodium azide 0.2% (w/v), were added to the 0.9 ml of suspension; the 10× concentrated buffer (antigen diluent) was therefore diluted tenfold, bringing it to its usual concentration. To the 1 ml of suspension an equal volume of Sputolysin (Calbiochem—Behring Corp., La Jolla, CA 92037), was added and the mixture serially diluted tenfold; 300 µl of each dilution were added to an appropriately labelled tube containing one armed ball, and the assay was done as described. Negative controls consisted of balls treated in the same manner except for the use of antigen diluent instead of antigen.

RESULTS

Direct ELISA: activity of rabbit antisera against LPS, whole-cell GCFT antigen, and OM complex

The LPS extracted from *N. gonorrhoeae* strain 9 consisted predominantly of carbohydrate with a low content of protein. The ratio of carbohydrate to protein was approximately 15 to 1. The OM-complex preparations consisted predominantly of protein with a low content of carbohydrate. The ratios of protein to carbohydrate were within the range of 8–12 to 1.

The ELISA extinction values obtained with negative controls varied within the range of 0.1–0.45. To obtain a distinct cut-off point, a value of 0.9 was chosen as the endpoint. Antibody titres are expressed as the highest serum dilution at which this endpoint was reached.

The highest titres of the rabbit antisera obtained with each series of antigen concentrations are shown in table I. The high antibody titres obtained with the antiserum raised against *N. gonorrhoeae* strain 9, with either homologous or heterologous OM-complex antigen, prompted its use in subsequent experiments.

The effect of strain 9 OM-complex protein concentration on extinction value in the direct ELISA system with homologous rabbit antiserum is shown in fig. 2. There was no further increase in extinction value beyond an antigen level of 0.78 µg of protein. Consequently, a tenfold dilution of the OM complex which corresponded to 5 µg protein per well was chosen for subsequent experiments; this allowed economic use of the antigen at a level well within the plateau.

Antibody titres of rabbit anti-strain 9 serum against homologous and heterologous OM complex preparations are shown in table II. If 100% activity corresponds to the antibody titre obtained with homologous OM complex, the relative activities obtained with the heterologous OM complexes were as follows: 100% (four gonococcal isolates); 50% (five gonococcal isolates); 25% (one gonococcal isolate and *N. meningitidis* serogroup B); 12.5% (*N. lactamica* and *N. perflava*), and 6.25% (*N. pharyngis*).

Indirect sandwich ELISA for the detection of *N. gonorrhoeae* OM complex antigen

The extinction values obtained with the negative controls varied within the range 0.35–0.47. To obtain a distinct “cut off” point, a value of 1.0 was chosen as the endpoint.

Various dilutions of mouse and rabbit anti-strain 9 sera were used to determine the
Fig. 2.—Effect of OM complex protein concentration on extinction value in direct ELISA with rabbit anti-whole cell serum.

most suitable levels of antibody for “arming” and “recognition”. The minimum amounts of homologous OM complex detectable in these conditions are shown in table III. Mouse and rabbit antisera at dilutions of 1 in 300 and 1 in 2500, respectively, were chosen for subsequent experiments; this allowed economic use of the mouse antiserum.

Table IV shows the minimum amounts of OM complex protein from 10 clinical

<table>
<thead>
<tr>
<th>Source of OM complex</th>
<th>Antibody titre of anti-strain 9 serum</th>
<th>Percentage activity of antiserum relative to antibody titre with strain 9 antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>12800</td>
<td>100</td>
</tr>
<tr>
<td>strain 9</td>
<td>12800</td>
<td>100</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>3200</td>
<td>25</td>
</tr>
<tr>
<td>serogroup B</td>
<td>1600</td>
<td>12-5</td>
</tr>
<tr>
<td><em>N. lactamica</em></td>
<td>1600</td>
<td>12-5</td>
</tr>
<tr>
<td><em>N. perflava</em></td>
<td>800</td>
<td>6-25</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>0*</td>
<td>0</td>
</tr>
<tr>
<td><em>B. hibius</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

0* = An extinction value below the defined cut-off point of 0·9 with undiluted anti-strain 9 serum (see Methods).
isolates of *N. gonorrhoeae* and organisms other than gonococci that could be detected by the indirect sandwich ELISA. OM complex from eight of the 10 clinical isolates was detectable at the same level as the homologous OM complex (46 ng protein); the endpoint with the two remaining clinical isolates was 92 ng OM-complex protein. The sensitivity of the assay decreased approximately eightfold with OM complex from a strain of *N. meningitidis* serogroup B; the minimum amount of OM protein detected was 375 ng.

**Detection of whole-cell antigen in a simulated clinical specimen**

The suspension of *N. gonorrhoeae* strain 9 contained $4.9 \times 10^7$ cfu/ml. When 0.9 ml was made up to 2 ml with 100 µl of the 10× concentrated buffer and 1 ml of sputolysin, the suspension contained $2.2 \times 10^7$ cfu/ml. Because 300-µl volumes from serial tenfold dilutions of this suspension were used in the assay, and a positive result obtained up to

---

**Table III**

Determination of appropriate dilutions of 'arming' (mouse) and 'recognition' (rabbit) antisera for maximum sensitivity

<table>
<thead>
<tr>
<th>Dilution of recognition antibody (rabbit anti-strain 9 serum): 1 in</th>
<th>Minimum amount (ng) of homologous OM complex protein detectable with balls armed with dilutions of mouse antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 in 100</td>
</tr>
<tr>
<td>2500</td>
<td>48</td>
</tr>
<tr>
<td>5000</td>
<td>48</td>
</tr>
</tbody>
</table>

---

**Table IV**

Sensitivity of indirect sandwich ELISA with OM complex from various sources

<table>
<thead>
<tr>
<th>Source of OM complex</th>
<th>Minimum amount (ng) of OM-complex protein detectable</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em> strain 9</td>
<td>46</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em> clinical isolate no.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>92</td>
</tr>
<tr>
<td>7</td>
<td>46</td>
</tr>
<tr>
<td>8</td>
<td>46</td>
</tr>
<tr>
<td>9</td>
<td>92</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>375</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>ND</td>
</tr>
<tr>
<td><em>B. hivus</em></td>
<td>ND</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not detectable at $3 \times 10^5$ ng.
a dilution of $10^3$, the minimum number of organisms detected corresponded to $6.6 \times 10^3$ cfu.

**Discussion**

Our direct ELISA results indicate that rabbit antiserum raised against whole untreated cells of *N. gonorrhoeae* strain 9 can be used to detect a variety of antigens from several gonococcal strains.

In spite of the location of LPS on the outermost aspect of *N. gonorrhoeae* (Johnston and Gotschlich, 1974; Wolf-Watz et al., 1975), the rabbit antiserum gave very low titres when ELISA plates were coated with this antigen. These results are in agreement with the findings of Perry, Diena and Ashton (1977) who have shown that LPS is a poor immunogen in many animals, including the rabbit, and gives rise to a significant antibody production only in hens. Furthermore, poor binding of LPS to polystyrene has been demonstrated by Ito et al. (1980) in ELISA experiments with $^{51}$Cr radiolabelled LPS as an antigen; satisfactory adsorption was achieved when Mg$^{++}$ was added to LPS. Consequently, the low titres obtained in the present study with LPS as antigen presumably reflect the antigenic heterogeneity of O side-chains, poor binding to polystyrene and relatively poor immunogenicity in the rabbit.

Poor binding is also the most likely explanation for the low titres obtained when whole-cell (GCFT) antigen was used in the direct assay; the antibody titres remained lower than those obtained in the GCFT. Most antigens adhere to polystyrene surfaces by physical adsorption, and antigens used for ELISA are soluble (World Health Organization, 1976). The GCFT antigen consisting primarily of whole cells was insoluble and may have been unable to adhere to the polystyrene surface during the washing stages. Recently, it has been shown by Ison, Hadfield and Glynn (1981) that whole cells can be attached to polystyrene by poly-L-lysine.

The most satisfactory direct ELISA results were obtained when OM complex rich in protein was used as antigen. These results confirm the evidence presented by other investigators for adequate binding of protein to polystyrene (Brodeur, Ashton and Diena, 1978; Glynn and Ison, 1978; Poxton, 1979; Young and Low, 1981). With homologous OM complex, the antibody titres of the anti-strain 9 and anti-strain 82409 rabbit serum were 15:3 and 12:2 times higher than when determined by GCFT. Because protein was the major component of the OM complex preparations, the high antibody titres obtained also indicated that OM protein is a major antigen eliciting an antibody response in the rabbit.

When the antibody titres of the two rabbit antisera were determined against their respective heterologous OM-complex preparations, the titres remained unchanged; that of the rabbit anti-strain 9 was higher with both homologous and heterologous (strain 82409) OM complex. Presumably, the common gonococcal antigens are better exposed on the whole cells of *N. gonorrhoeae* strain 9, thus provoking a greater immune response in the immunised rabbit. This is in agreement with the view that *N. gonorrhoeae* strain 9 possesses antigenic features that might be quantitatively or qualitatively specific to gonococci (O'Reilly et al., 1973). Our results with the direct ELISA system and the indirect sandwich ELISA confirm these findings (tables I and IV).

When the indirect assay was performed with a simulated clinical specimen
containing *N. gonorrhoeae* strain 9, a minimum of $6.6 \times 10^3$ cfu (or $2.1 \times 10^4$ cfu/ml) could be detected. Quantitative data on gonococci in genital secretions is limited to the work of Lowe and Kraus (1976) who found that the number of gonococci in cervical-vaginal aspirates from 52 women with gonorrhoea ranged from $4.0 \times 10^2$ to $1.8 \times 10^7$ cfu (mean = $1.45 \times 10^5$ cfu) present in the original 10-ml aspirates. Because our ELISA system appears to be able to detect gonococci within this range, further evaluation of the system with vaginal and cervical aspirates from infected patients is merited.

The cross reactivity observed with OM complex from *N. meningitidis* would not significantly limit the value of the assay in detecting ano-genital gonorrhoea because meningococci are relatively rare in the urogenital tract and anal canal (Givan, Thomas and Johnston, 1977; Blackwell, Young and Bain, 1978). However, in view of the occurrence of meningococci in the throat of all patients and their not infrequent occurrence in the rectum of homosexual men (Chapel, Gatewood and Keane, 1977), such an assay would be unsuitable for examining material from these sites.

This work was supported in part by a grant from the Scottish Home and Health Department (research grant No. K/MRS/50/C22) whose financial assistance is gratefully acknowledged. We also thank Professor J. G. Collee for his helpful advice in the preparation of this paper.

REFERENCES


Rapidity and reliability of gonococcal identification by coagglutination after culture on modified New York City medium

H YOUNG* AND A MCMILLAN†

From the *Department of Bacteriology, Edinburgh University Medical School and Royal Infirmary; and the †University Department of Genitourinary Medicine, Royal Infirmary, Edinburgh

SUMMARY The combination of culture on modified New York City (MNYC) medium and identification of neisserial isolates by the Phadebact® gonococcus test was evaluated in routine laboratory practice. The sensitivity of coagglutination was 96·7% (318/329 isolates) and that of immunofluorescence (IF) 97·9% (322/329 isolates); the specificity of both methods was 96·8% (120/124 isolates). Of the 329 gonococcal isolates, 286 (86·9%) could be tested by coagglutination and 309 (93·9%) by IF after only 24 hours’ incubation. Identification by coagglutination from primary cultures on MNYC medium is considered to be very rapid, simple, and efficient for the cultural diagnosis of anogenital gonorrhoea in women and urethral gonorrhoea in men. Because of the high prevalence of meningococci in the pharynx and their infrequent occurrence in the anorectum of homosexual men the identity of isolates from these sites is best confirmed by sugar utilisation tests.

Introduction

The Phadebact® gonococcus test (Pharmacia Diagnostics AB, Uppsala, Sweden) is a rapid slide test for the immunological identification of Neisseria gonorrhoeae. The test depends on the principle of coagglutination.1 An early report from the United Kingdom2 of gonococcal identification by coagglutination was critical of the specificity of the method. More recent reports from the United States,3,4 Australia,5 and Switzerland7 suggest that coagglutination can provide rapid and accurate immunological confirmation of N gonorrhoeae.

Rapid cultural diagnosis of gonorrhoea, particularly in women, is of prime importance in the control of infection within the community. Modified New York City (MNYC) medium promotes rapid gonococcal growth and enables biochemical characterisation to be carried out directly from the primary isolation plate, providing a rapid and economical system of identification.8 An improve-

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Accepted for publication 26 October 1981
COAGGLUTINATION TEST

Lyophilised reagents

Commercially available test kits (Phadebact® gonococcus test) were provided by Pharmacia Diagnostics AB, Sweden. A heavy suspension of each culture to be tested was made by removing the growth from the culture plate with a cotton-tipped swab and emulsifying it in 0.2 ml distilled water in a tube. The tube was covered and the suspension heated in a boiling water-bath for five minutes. After cooling to room temperature 40-μl aliquots of suspension were mixed with an equal volume of test reagent (rabbit antiguonococcal antiserum bound to dead staphylococci) and control reagent (non-immune rabbit serum bound to dead staphylococci) on a clear glass slide: three tests were carried out on a single slide (10.7 cm × 5.0 cm) with six clearly defined areas. The slide was rocked gently for two to three minutes by tilting to an angle of 45° every two to three seconds and the results read against a dark background using indirect light.

A markedly stronger reaction with the gonococcal reagent compared with the control reagent constituted a positive result. No reaction with the gonococcal reagent irrespective of any reaction in the control reagent was considered a negative result. A reaction of equal strength in both the gonococcal and the control reagents was classed as non-interpretable.

Isolates giving false-positive and false-negative results by sugar utilisation reactions were retested the next day as a 24-hour subculture. A suspension of growth from each subculture was also made in skimmed milk (10%) and stored frozen at -40°C.

Liquid reagents coloured with methylene blue

On completion of the trial isolates were reconstituted from the skimmed milk and tested with the new liquid reagents and with the conventional lyophilised reagents.

ANALYSIS OF RESULTS

Coagglutination and IF test results were correlated with sugar utilisation reactions. As the aim was to make an 'in use' evaluation, only gonococcal isolates giving clear-cut positive results with IF or coagglutination or both were scored positive; borderline reactions were scored negative. Conversely borderline reactions with non-gonococcal neisseriae were scored positive.

The specificity of coagglutination and IF methods was defined as the percentage of gonococcal isolates confirmed by carbohydrate utilisation tests that were IF-positive or coagglutination-positive, while specificity was defined as the percentage of non-gonococcal isolates confirmed by carbohydrate utilisation testing that was coagglutination-negative or IF-negative.

The significance of differences in the results with coagglutination and IF methods was determined by dividing the difference between the two totals to be compared by the standard error of the difference: a value equal to or greater than 1.96 indicates a significant difference at the 5% level.

Results

SPECIFICITY AND SENSITIVITY

There were 140 men (mean age 29.7 years) and 118 women (mean age 22.8 years) from whom oxidase-positive Gram-negative diplococci (GNDC) were isolated; 189 isolates of GNDC were obtained from 156 routine examinations of men and 264 isolates of GNDC from 131 routine examinations of women. The coagglutination, immunofluorescence (IF), and sugar utilisation results for the 453 isolates are shown in table 1.

The sensitivity of the coagglutination test was 96.7% (318/329 isolates) and that of the IF test 97.9% (322/329 isolates). This difference is not

<table>
<thead>
<tr>
<th>Site</th>
<th>No of cultures yielding oxidase-positive GNDC</th>
<th>Identification by sugar utilisation</th>
<th>Immunofluorescence</th>
<th>Coagglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N gonorrhoeae</td>
<td>N meningitidis</td>
<td>N lactamica</td>
<td>Positive</td>
</tr>
<tr>
<td>Urethra</td>
<td>170</td>
<td>170</td>
<td></td>
<td>166</td>
</tr>
<tr>
<td>Cervix</td>
<td>89</td>
<td>88</td>
<td>1</td>
<td>86</td>
</tr>
<tr>
<td>Rectum</td>
<td>49</td>
<td>48</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>Bartholin's gland</td>
<td>8</td>
<td>8</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Throat</td>
<td>137</td>
<td>15</td>
<td>115</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>453</td>
<td>329</td>
<td>117</td>
<td>326</td>
</tr>
</tbody>
</table>

GNDC = Gram-negative diplococci
*Includes one non-interpretable result
Rapidity and reliability of gonococcal identification

significant at the 5% level. The specificity of both coagglutination and IF was 96·8% (120/124 isolates). Four isolates of β-lactamase-producing gonococci gave positive results by both methods.

RAPIDITY OF DIAGNOSIS

Table II shows the rapidity of gonococcal identification by coagglutination and IF methods. The difference in the total number of isolates from all anatomical sites tested at 24 hours by each method was not significant at the 5% level.

Of the 115 meningococci isolated from throat cultures in men and women 96 (83·5%) were tested at 24 hours by coagglutination and 113 (98·3%) by IF.

FALSE-POSITIVE AND FALSE-NEGATIVE COAGGLUTINATION RESULTS

Seven isolates of gonococci (four from men and three from women) gave false-negative results when first tested. Only six of these isolates were available for retesting at the end of the trial and all gave a positive result. With the conventional freeze-dried reagents at least two minutes were required to produce coagglutination whereas this was clearly observable within 30 seconds with the liquid reagents containing methylene blue.

All four isolates of N meningitidis (two serogroup B and two non-groupable) that gave positive reactions initially gave negative results on retesting at the end of the survey.

Discussion

Our results indicate clearly that the overall sensitivity and specificity of coagglutination and IF are comparable. Similar results were obtained by Rufli et al. and by Johnston, who prepared her own coagglutination reagents. We found the coagglutination method was simple to perform, easy to interpret, and does not require expensive immunofluorescence equipment.

These advantages are of less benefit in laboratories with facilities for fluorescence microscopy and adequately staffed with skilled technologists. The cost of performing a coagglutination test with commercial reagents is approximately £0·50 when a 40-μl volume is used compared with a cost of £0·10 for an IF test with a 10-μl unit volume. If coagglutination can be performed equally well with a 10-μl volume, however, as reported by Futrovsky et al, this would reduce the cost per test to approximately £0·125.

Both coagglutination and IF methods have the same disadvantages: the failure to detect a small proportion of gonococcal isolates (2-3%) and the failure to obtain a clear-cut negative reaction with a small proportion of non-gonococcal neisseriae. These problems can be minimised by using a carefully absorbed antigonococcal serum known to be reactive in the coagglutination test with local gonococcal strains. Despite these limitations immunological identification is a very useful adjunct in the diagnostic laboratory. Confirmation of the identity of gonococci is obtained rapidly and with very small amounts of bacterial growth.

Provided that unequivocal results are obtained the specificity and sensitivity of immunological methods are such that further identification of urethral isolates from men and anogenital isolates from women is unnecessary provided that colonial morphology is typical and correlates with immunological findings. Whenever medico-legal proceedings may be involved full identification including sugar utilisation tests must be performed. Because meningococci are isolated from the throat much more often than gonococci, the small number of false-positive reactions that may occur decreases the utility of immunological identification of pharyngeal isolates.

Although there are several reports of the isolation of N meningitidis from anogenital sites, when the

<table>
<thead>
<tr>
<th>Site</th>
<th>No of isolates</th>
<th>No (%) of isolates tested at 24 hours by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Immunofluorescence</td>
</tr>
<tr>
<td>Male urethra</td>
<td>106</td>
<td>104(98·1)</td>
</tr>
<tr>
<td>Female urethra</td>
<td>64</td>
<td>56(87·5)</td>
</tr>
<tr>
<td>Cervix</td>
<td>88</td>
<td>85(96·6)</td>
</tr>
<tr>
<td>Male rectum</td>
<td>44</td>
<td>40(90·9)</td>
</tr>
<tr>
<td>Female rectum</td>
<td>8</td>
<td>8*</td>
</tr>
<tr>
<td>Bartholin's gland</td>
<td>7</td>
<td>5*</td>
</tr>
<tr>
<td>Male throat</td>
<td>8</td>
<td>7*</td>
</tr>
<tr>
<td>Female throat</td>
<td>8</td>
<td>7*</td>
</tr>
<tr>
<td>Total</td>
<td>329</td>
<td>309(93·9)</td>
</tr>
</tbody>
</table>

* Percentages not given if less than 10 observations.
incidence of such occurrences in relation to laboratory practice are considered the data must be interpreted carefully with reference to the particular patient groups concerned. In this study neisseriae were isolated from an anogenital site in 95 women (urethra, cervix, and rectum were tested routinely), but only one isolate (1%) proved to be a meningococcus. Since 3·2% of meningococcal isolates gave a false-positive reaction on initial testing a false-positive coagglutination result with a meningococcus from an anogenital site in a female patient is unlikely to occur in more than 1 in 3000 patients from whom anogenital neisseriae are isolated; this could be less than 1 in 30000 patients investigated. We consider identification by immunological methods acceptable at the above levels particularly when it is borne in mind that colonial morphology may alert a trained observer to a misdiagnosis.

In homosexual men non-gonococcal neisseriae appear to be isolated from the anorectum more frequently. In this trial one of five neisserial isolates from the anorectum of men was a meningococcus. In an earlier study we isolated N meningitidis from the anorectum of four (5-7%) of 70 homosexual men. We maintain our earlier view that it is important to confirm, preferably by a rapid carbohydrate utilisation test, the identity of neisseriae isolated from the anorectum of homosexual men. Tapsall and Cheng in a recent comparative study also noted that a rapid test using preformed enzymes was superior both in terms of accuracy and rapidity to two other systems, both of which required growth of the organism to produce a positive result.

The Phadebact® gonococcus test in conjunction with culture on MNYC medium provides a very rapid and simple method of gonococcal identification for isolates from anogenital sites in women and from the urethra in men. Because technical manipulations are kept to a minimum the scheme lends itself well to large-scale screening for gonococcal infection among high-risk groups and quality control is easy for laboratories that isolate few gonococci.

We are grateful to our medical and non-medical colleagues in the departments of genitourinary medicine and bacteriology for their help with the collection and processing of specimens.

References
Detection of penicillinase-producing Neisseria gonorrhoeae

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Detection of penicillinase-producing Neisseria gonorrhoeae

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(Received 16 November 1981)

Key words: gonorrhoeae; neisseria; penicillinase; Beta-lactamase.

Introduction

The estimated annual global number of gonococcal infections in the 1960s was 60 million, and during the 1970s this rose to approximately 200 million.1 Since its introduction in 1944 penicillin has been widely and successfully used in the treatment of gonorrhoea, although strains of Neisseria gonorrhoeae with decreased susceptibility to penicillin have been recognised since 1958.2 The effective therapeutic dosage of penicillin has been increased steadily to cope with chromosome-mediated increasing resistance of the gonococcus. Higher doses of penicillin, given together with probenecid to enhance blood levels, has maintained the balance in favour of penicillin. However, in 1976 penicillinase-producing N. gonorrhoeae (PPNG) strains emerged.3-4 Epidemiological data, including plasmid analysis, suggest that there are two separate endemic zones of infection, one in South-east Asia and the other in West Africa,5 while in recent years the prevalence of PPNG has increased markedly in certain parts of Europe.6-7 Although the majority of infections with PPNG reported in the United Kingdom are acquired in South-east Asia and West Africa, in 1979 41 patients (39% of the total infections with PPNG strains) contracted their infections in the United Kingdom.7 As the prevalence of PPNG strains increases, routine testing of all gonococcal isolates for penicillinase-production becomes more important.8 With low prevalence such tests have been restricted to those isolates with reduced sensitivity to penicillin, or isolates from cases of penicillin treatment failure. Of the tests used by laboratories to detect PPNG isolation a choice has to be made.

Comparative sensitivity of Test Methods

Certain tests have been compared qualitatively,9,10 and we have carried out a quantitative assessment of the sensitivity of the modified acidometric,8 Beta-lactamase test strip,11 iodometric starch paper,12 and chromogenic cephalosporin13 methods.

Our evaluation was carried out on 36 lyophilised cultures of PPNG, and the sensitivity of each of the various test methods is shown in Table 1.

The chromogenic cephalosporin method appears to be the most sensitive, followed by the Beta-lactamase strip, the acidometric method and lastly the iodometric method.
Penicillinase detection methods in routine use

Of 36 clinical isolates of *N. gonorrhoeae*, all gave a negative result for penicillinase-production when tested by each method. Each isolate gave a positive fluorescent antibody test with fluorescein-labelled anti-gonococcal conjugate and produced acid from glucose only in the rapid carbohydrate utilisation test when tested directly from the primary isolation culture after 24 h of incubation. Therefore negative results were not due to inadequate growth.

**PPNG isolated in Edinburgh**

Since January 1977 in our routine screening for penicillinase production by the acidometric method we have detected nine PPNG strains. Seven infections in males were acquired abroad: three in West Africa, three in South-east Asia and one in France. One woman acquired her infection in the UK from her husband on his return from West Africa, and the source of infection in one female patient was unknown.

Penicillinase testing directly from a 24 h primary isolation culture gave a positive result in six instances. In two cases there was insufficient growth for characterisation directly from the primary cultures, but in each case a positive result for penicillinase production was obtained with a 24 h subculture. One of these patients presented as a 'treatment-failure' – he had been treated with ampicillin plus probenecid one week earlier when a penicillin-sensitive gonococcus had been isolated. Penicillinase-testing of the primary culture from the woman who had acquired her PPNG infection from her husband gave a negative result. Ten separate colonies from the primary isolation culture were subcultured and all were tested for penicillinase-production the next day. Only one of the subcultures was identified as a PPNG strain.

**Discussion**

Our results demonstrate that PPNG strains can be identified by a variety of laboratory methods provided that the inoculum is adequate. By defining the minimum inoculum required to give a positive test result with each method we were able to show that the chromogenic cephalosporin method is approximately 20 times more sensitive than the Beta-lactamase test strip, 75 times more sensitive than the tube acidometric method, and 112 times more sensitive than the iodometric starch paper technique.

Sng et al. found that the chromogenic cephalosporin, rapid (tube) iodometric, and penicillin disc diffusion methods gave complete agreement, detecting 99 PPNG strains from 202 cultures tested; the filter paper iodometric method detected 98 PPNG strains. Likewise Shannon & Phillips have reported that chromogenic cephalosporin, an acidometric method, and the commercially available Intralactam method were equally effective in detecting eight PPNG strains among 18 gonococcal isolates tested.

Our data suggest that the chromogenic cephalosporin test, because of its greater sensitivity, should be the method of choice. The reagent is not readily available to all laboratories, however, and from a safety aspect it may be sensitising so inhalation of dust and contact with skin and eyes must be carefully avoided. Shannon & Phillips considered that the Intralactam method was as good as, and slightly more easily performed than, the chromogenic cephalosporin method. In our experience the Beta-lactamase test strip was also very easy to perform. Recently a filter paper acidometric method using bromocresol purple as pH indicator, similar to the commercially available reagents described above, has been evaluated by Sng et al., who considered the test very cheap, simple and effective in detecting PPNG strains.

Although the tube acidometric method is almost four times less effective than the Beta-lactamase test strip it is the most convenient test to perform if isolates are identified by the rapid carbohydrate utilization test. Whichever method is chosen for testing primary isolates we consider it important to use material from several colonies, and in addition to carry out a disc diffusion test with a 10 unit penicillin disc. With this test a zone of inhibition of less than 20 mm was obtained with 99 PPNG strains (16 mm in one instance, and 0 mm in 98) whereas all of 102 non-penicillinase producing strains gave zones greater than 20 mm.

The importance of testing material from several colonies was well illustrated in our study. Two of the infections with PPNG strains detected were most likely mixed infections in which penicillin-sensitive gonococci were also present. In one case a positive test for penicillinase-production was obtained with only one of ten gonococcal colonies subcultured. In the other case treatment with ampicillin most likely led to a selective increase in the proportion of penicillinase-producing organisms present.

Incidence of PPNG Infections

The risk of acquiring an infection due to a PPNG strain has increased markedly in certain geographical areas in recent years. For example the number of infections with PPNG strains in Singapore has risen from three in 1976 to 1792 (19.2%) of all gonococcal infections in 1977. In spite of measures such as intensive bacteriological surveillance, effective treatment, and determined contact-tracing, PPNG strains are not being contained in Singapore. When it is borne in mind that over two million visitors from all parts of Europe, Asia, Australasia and North America disembarked in Singapore during 1979 the real danger of global dissemination from such a busy port and tourist centre may be appreciated.

The increase in the proportion of infections with PPNG strains contracted in the United Kingdom is also ominous, and makes it mandatory for all laboratories in the UK to carry out routinely reliable surveillance for such strains. However, in many areas of the country the yield from such testing will probably be low. In Edinburgh during the years 1978–80 a total of 3163 episodes of gonococcal infection were confirmed by culture, and of these only four (0.13%) were due to PPNG strains. Therefore, in terms of laboratory practice routine surveillance may seem a tiresome and often unrewarding exercise. Under such conditions the highest standards of laboratory practice and quality control are imperative. Close liaison with clinical staff is also very important and should enable the laboratory to be altered whenever an infection may have been acquired in a high risk area such as South-east Asia or West Africa. In an attempt to minimise the risk of spread of PPNG strains it is recommended that gonococcal infections in patients returning from these areas should be treated with spectinomycin or cefuroxime.

*During the first two months of 1982, of 82 episodes of gonococcal infection in men three were due to PPNG strains: two of these were acquired in the U.K.
We wish to thank our medical and non-medical colleagues in the departments of genito-urinary medicine and bacteriology for their help with the collection and processing of clinical specimens. We are particularly grateful to Prof. J. G. Collee and Dr D. H. H. Robertson for their helpful advice during the preparation of this paper.

References

NON-CULTURAL DETECTION OF NEISSERIA GONORRHOEAE IN CERVICAL AND VAGINAL WASHINGS

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Summary. Genetic transformation, an indirect sandwich enzyme-linked immunosorbent assay (ELISA) and the Limulus amoebocyte assay were used to indicate the presence of products of Neisseria gonorrhoeae in vaginal and uterine cervical aspirates from 37 women attending a Department of Genito-Urinary Medicine. In parallel with these tests, qualitative and quantitative assessments of the microbial content of aspirates were made. There was wide variation in the numbers of gonococci cultured. The mean viable count for cervical aspirates was $1 \times 10^6$ cfu/ml and the range was $(5 \times 10^3)-(8 \times 10^6)$ cfu/ml; the mean count for vaginal aspirates was $8.4 \times 10^4$ cfu/ml and the range $(1 \times 10^5)-(1 \times 10^6)$ cfu/ml. Viable counts of organisms other than gonococci in vaginal aspirates were two to tenfold greater than the corresponding counts for cervical aspirates. Of 20 patients with gonorrhoea confirmed by conventional diagnostic cultures, aspirates from 15 (75%) gave a positive transformation result, and 12 (60%) a positive ELISA result; 16 (84.2%) out of 19 of these aspirates tested by the Limulus lysate assay were positive at a dilution of 1 in 100.

Introduction

Most methods for the laboratory diagnosis of gonorrhoea rely on culture and therefore depend upon maintaining the viability of Neisseria gonorrhoeae (Young, 1981). Recently, alternative approaches to the diagnosis of gonorrhoea, based upon the detection of gonococcal components in clinical specimens have been developed. These include detection of antigen in urine by solid-phase radioimmunoassay (Thornley et al., 1979), detection of gonococcal DNA by genetic transformation (Bawdon, Juni and Britt, 1977), the Limulus amoebocyte lysate assay for endotoxin (Spagna, Prior and Perkins, 1979 and 1980), gas liquid chromatography (Sud and Feingold, 1979), and the detection of the enzyme 1,2-propanediol oxidoreductase (Takeguchi et al., 1980).

Unfortunately, qualitative and quantitative assessments of the microbial content of cervical and vaginal aspirates have not been performed in parallel with the
development of the various tests. Lowe and Kraus (1976) found that the numbers of gonococci in cervico-vaginal aspirates from 52 women with gonorrhoea ranged from $40-1-8 \times 10^6$ cfu/ml (mean $1-45 \times 10^4$ cfu/ml). Recently we reported that an indirect sandwich enzyme-linked immunosorbent assay (ELISA) could detect gonococci within this range with clinical isolates and in a simulated clinical specimen (Sarafian and Young, 1982). Earlier studies (Sarafian and Young, 1980) had also shown that genetic transformation was capable of detecting numbers of gonococci within this range. The aim of the present study was to examine the value of these methods, and the Limulus lysate assay, in parallel with a quantitative microbiological investigation of cervical and vaginal aspirates.

**Materials and methods**

*Study population.* Thirty seven women attending the Department of Genito-Urinary Medicine, The Royal Infirmary, Edinburgh, were investigated; 25 of them were known contacts of men with urethral gonorrhoea and 12 were not known to be contacts.

*Specimens.* These were taken from the urethra and cervix for microscopic examination and culture, and from the rectum for culture only. Wet films of vaginal secretions were examined microscopically for the detection of *Trichomonas vaginalis*, and gram-stained smears for *Candida albicans*. Cervical secretions were collected from the endo-cervical canal as previously described (Young, Sarafian and McMillan, 1981).

To collect vaginal secretions, pyrogen-free saline (5 ml) was instilled into the posterior fornix of the vagina through a sterile polythene capillary tube (chromatography column tubing, internal diameter 1-0 mm; Pharmacia Fine Chemicals, Uppsala, Sweden) attached to a 5 ml syringe. After 30 s for equilibration, the washings were aspirated and expelled into a sterile, pyrogen-free, plastic Universal container (Sterlin).

Portions (200 μl) of vaginal and cervical secretions were dispensed in small plastic containers; material was left in the pyrogen-free vials for the Limulus lysate assay. The secretions were stored at $-20^\circ$C until required.

*Bacteriological investigation of vaginal and cervical secretions.* Immediately after collection, cervical and vaginal secretions were mixed thoroughly by a vortex mixer.

Viable counts were performed by serially diluting secretions in tenfold steps in GC broth (Kellogg et al., 1963) up to a dilution of $10^5$ and spreading 0-1 ml of each dilution on the following media: modified New York City medium (Young, 1978b), MacConkey agar (Oxoid), and blood agar (Oxoid). The counts on blood agar were performed in duplicate for aerobic and anaerobic incubation; the anaerobic methods were those of Collee et al. (1972). Plates were examined after incubation for 24 and 48 h.

*Statistical analysis.* The Student's $t$ test was used to assess the difference between means.

*Criteria for identification of isolates.* N. gonorrhoeae was identified by the fluorescent-antibody test and the rapid carbohydrate-utilisation test (Young, 1978a). *Chlamydia trachomatis* was isolated by the method of Thomas et al. (1977) with cycloheximide-treated cells. For the isolation and identification of *Ureaplasma urealyticum*, 100 μl of each specimen was transferred aseptically to 0-9 ml of modified U9 medium (Young, Tuach and Bain, 1981). Serial tenfold dilutions were made up to a dilution of $10^{10}$. The vials were incubated under standard conditions and examined at 24 and 48 h. The presence of *U. urealyticum* was indicated by a change of colour of the medium from yellow to pink. *Candida albicans* was identified on the basis of typical macroscopic and microscopic yeast-like morphology and the production of germ tubes (Taschdjian, Burchall and Kozinn, 1960).

The following organisms were identified according to the criteria outlined by Cowan (1974): *Staphylococcus* spp., haemolytic streptococci, enterococci (*Streptococcus faecalis*), lactobacilli, diphtheroids (diphtheroid-like organisms that were gram-variable, catalase negative and haemolytic) were presumptively identified as *Gardnerella vaginalis*, *Proteus* spp., *Escherichia coli*, anaerobic streptococci, *Bacteroides* spp., *Clostridium* spp.
Detection of gonococcal components

One portion from each of the 37 cervical and vaginal aspirates was thawed. All the specimens were processed in one batch by each of the following assays.

(i) Transformation assay. To each 200 µl of aspirate, 200 µl of saline citrate (pH 7-4) containing sodium dodecylsulphate 0-05% (w/v) was added and mixed thoroughly by a vortex mixer operated at full speed. The mixtures were incubated at 65°C for 1 h and processed as described by Sarafian and Young (1980). A proline auxotroph of N. gonorrhoeae strain F62 was used as recipient and a positive result was taken as the growth, on medium lacking proline, of ≥ 20 colonies identical in size and appearance to the colonies of strain F62 growing on the same medium containing proline.

(ii) Indirect sandwich ELISA. The method was based on that of Sarafian and Young (1982). To 180 µl of aspirate (preincubated at 56°C for 20 min to inactivate any intrinsic alkaline phosphatase), 20 µl of PBS (pH 7-4) was added; the molarity of the phosphate buffer was increased from 0-05M to 0-5M to allow for the tenfold dilution in the aspirate. NaCl was kept at 0-15M because the secretions were aspirated with physiological saline. The concentrations of Tween 20 and sodium azide were increased to 0-5% (v/v) and 0-2% (w/v) respectively. After the addition of 200 µl of sputolysin, the specimens were mixed thoroughly by vortex mixing and processed as described previously. Positive and negative controls were set up in duplicate; negative controls comprised antigen diluent instead of antigen and positive controls comprised a suspension of N. gonorrhoeae strain 9 in saline which was treated in the same manner as clinical specimens. An extinction value of ≥ 1-0 at 405 nm was considered to be a positive result.

(iii) Limulus lysate assay. The assay was performed on the cervical secretions in pyrogen-free containers. The specimens were mixed thoroughly by vortex mixing, diluted in pyrogen-free water to a final dilution of 1 in 100, and processed as described by Young et al. (1981). A firm opaque gel that remained adherent to the bottom of the vial when inverted through 180° was scored as positive.

After the correlation between the Limulus assay results and a diagnosis of gonorrhoea had been made, specimens that gave a positive result were retested at a dilution of 1 in 200.

RESULTS

Quantitative microbiology of cervical and vaginal aspirates

Gonococci were detected in cervical or vaginal aspirates, or both, from 19 of the 25 known contacts of men with gonorrhoea. Viable counts for gonococci cultured from aspirates are given in table I. Routine cultures taken at the first examination were also positive for gonococci in these 19 patients. An additional patient gave a positive culture at the second examination. None of the specimens from the 12 women who were not known contacts yielded gonococci on culture.

The number of N. gonorrhoeae isolated from cervical aspirates varied within a range of (5 x 10³)– (8 x 10⁶) cfu/ml (mean 1 x 10⁶ cfu/ml); the corresponding values for vaginal aspirates were (1 x 10⁴)– (1 x 10⁹) cfu/ml (mean 8-4 x 10⁴ cfu/ml). The difference between the two means is not statistically significant (t 1-8, 0-1 > p > 0-05).

Table II shows the frequency of isolation of each organism isolated from cervical and vaginal aspirates of 18 patients with and 15 patients without gonorrhoea. Quantitative data were not available for four of the patients in the study. When an organism was isolated from eight or more patients, the mean number of organisms present was calculated (table III).

Detection of gonococcal components in cervical and vaginal aspirates

The results obtained with specimens from the 20 patients with gonorrhoea are shown in table IV.
TABLE I
Viable counts of N. gonorrhoeae in cervical and vaginal aspirates from 19 women with gonorrhoea

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cervix</th>
<th>Vagina</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>66.0</td>
<td>28.0</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
<td>0.2</td>
</tr>
<tr>
<td>11</td>
<td>1.4</td>
<td>0.06</td>
</tr>
<tr>
<td>13</td>
<td>2.8</td>
<td>0.3</td>
</tr>
<tr>
<td>14</td>
<td>15.0</td>
<td>1.7</td>
</tr>
<tr>
<td>15</td>
<td>230.0</td>
<td>104.0</td>
</tr>
<tr>
<td>17</td>
<td>0.9</td>
<td>0.04</td>
</tr>
<tr>
<td>18</td>
<td>18.0</td>
<td>0.3</td>
</tr>
<tr>
<td>19</td>
<td>28.0</td>
<td>0.4</td>
</tr>
<tr>
<td>22</td>
<td>800.0</td>
<td>4.0</td>
</tr>
<tr>
<td>24</td>
<td>0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>29</td>
<td>580.0</td>
<td>1.7</td>
</tr>
<tr>
<td>30</td>
<td>8.0</td>
<td>0.6</td>
</tr>
<tr>
<td>31</td>
<td>37.0</td>
<td>0.1</td>
</tr>
<tr>
<td>32</td>
<td>140.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>33</td>
<td>0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>34</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>36</td>
<td>0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean</td>
<td>102</td>
<td>8.4</td>
</tr>
</tbody>
</table>

TABLE II
Qualitative assessment of microflora from cervical and vaginal aspirates of patients with and without gonorrhoea

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number (percentage) of patients with and without gonorrhoea from whom each organism was isolated from cervical (C) or vaginal (V) aspirates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With gonorrhoea (n=18)</td>
</tr>
<tr>
<td></td>
<td>Without gonorrhoea (n=15)</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>17* (94)</td>
</tr>
<tr>
<td>S. albus</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Haemolytic streptococci</td>
<td>0</td>
</tr>
<tr>
<td>Enterococci</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Anaerobic streptococci</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>10 (56)</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>10 (56)</td>
</tr>
<tr>
<td>G. vaginalis</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>0</td>
</tr>
<tr>
<td>Bacteroides bivius</td>
<td>1 (6)</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
</tr>
<tr>
<td>C. albicans</td>
<td>0</td>
</tr>
<tr>
<td>T. vaginalis</td>
<td>ND</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>4 (22)</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>15 (83)</td>
</tr>
</tbody>
</table>

* One patient with negative gonococcal cultures at the time of the quantitative examination gave positive cultures when tested one week later.
ND = not done.
Table III
Mean number, and range, of organisms isolated from patients with and without gonorrhoea

<table>
<thead>
<tr>
<th>Organism</th>
<th>Site*</th>
<th>Mean cfu (range)/ml of aspirate from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Patients with gonorrhoea</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>C</td>
<td>$1.0 \times 10^6$ ($5.4 \times 10^5$-$8.0 \times 10^5$)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>$8.4 \times 10^4$ ($1.0 \times 10^5$-$1.0 \times 10^5$)</td>
</tr>
<tr>
<td>S. albus</td>
<td>C</td>
<td>$2.3 \times 10^3$ ($1.0 \times 10^2$-$2.0 \times 10^2$)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>$1.9 \times 10^4$ ($3.0 \times 10^4$-$1.0 \times 10^5$)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>C</td>
<td>$3.1 \times 10^4$ ($2.0 \times 10^5$-$1.0 \times 10^8$)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>$5.1 \times 10^4$ ($4.8 \times 10^8$-$1.0 \times 10^9$)</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>C</td>
<td>$1.9 \times 10^5$ ($4.0 \times 10^5$-$1.0 \times 10^8$)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>$4.6 \times 10^6$ ($2.4 \times 10^7$-$1.0 \times 10^8$)</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>C</td>
<td>$1.9 \times 10^6$ ($1.0 \times 10^6$-$1.0 \times 10^9$)</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>$3.2 \times 10^6$ ($1.0 \times 10^6$-$1.0 \times 10^9$)</td>
</tr>
</tbody>
</table>

* C = cervix; V = vagina.
† For U. urealyticum, counts are expressed as colour changing units (ccu).

**Gram's stain.** Intracellular gram-negative diplococci were observed in gram-stained smears of cervical exudate from 11 (55%) of 20 patients with culture-positive gonorrhoea. None of the 17 patients who did not have gonorrhoea had a positive gram-stained smear.

**Transformation assay.** Cervical or vaginal aspirates from 15 (75%) of the 20 patients with gonorrhoea gave a positive transformation result; cervical aspirates were positive in 13 (65%) and vaginal aspirates in seven (13%) patients.

Of the 17 patients with no microbiological evidence of gonorrhoea, two (11.7%) gave a positive transformation result. In one of these, a contact of gonorrhoea, both the cervical and vaginal aspirates were positive; only the cervical aspirate was positive in the second patient. The condition diagnosed in both patients was trichomoniasis.

**Indirect sandwich ELISA.** Specimens from 12 (60%) of the 20 patients with gonorrhoea, gave a positive ELISA result; cervical aspirates were positive in 11 (55%) and vaginal aspirates in one (5%).

Cervical aspirates from two (11.7%) of the 17 patients with no microbiological evidence of gonorrhoea gave a positive ELISA result. The conditions diagnosed were urinary tract infection in one patient and non-specific genital infection, warts, and pubic lice in the second. Neither woman was a contact of a patient with gonorrhoea.

**Limulus lysate assay.** The test was positive in 16 (84.2%) of 19 patients with cervical gonorrhoea and in three patients (17.6%) with no microbiological evidence of gonorrhoea. The presenting diagnoses of two of these three patients were trichomoniasis and candidiasis respectively. No abnormality was detected in the third patient who was a contact of a man with gonorrhoea. On re-testing secretions from the three patients at a dilution of 1 in 200, all were negative, thus reducing the false positive rate to zero.

When the secretions from the 16 patients that gave a positive *Limulus* assay result at a dilution of 1 in 100 were re-tested at 1 in 200, the *Limulus*-positive-reactor rate was reduced to 13 (68.4%) of 19 infected patients.
Table IV
Results obtained with four methods of detecting gonococci in cervical and vaginal aspirates from 20 women with gonorrhoea

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Site</th>
<th>Smear</th>
<th>Transformation assay</th>
<th>Indirect sandwich ELISA</th>
<th>Limulus assay</th>
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<td>26*</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

C = cervix; V = vagina; + = positive result; - = negative result; ... = not done.
* A positive routine culture was obtained one week after the aspirates had been examined.

Discussion
The numbers of gonococci in clinical specimens are important when considering the development of a method of detecting gonococcal components. Our data (table I) suggest that any such method should be sensitive enough to detect components derived from as few as $1 \times 10^2$ cfu (vaginal aspirates) or $5 \times 10^3$ cfu (cervical aspirates) of N.
gonorrhoeae per ml of aspirate. These are minimum estimates as non-viable gonococci would also contribute to the total content of DNA, antigen or LPS in aspirates. Assuming that the viable counts at least provide an index of the total amount of gonococcal components present, the wide range of the numbers of gonococci isolated by irrigation of the cervix and vagina indicate that there may be a wide variation in the amounts of products available for detection.

*N. gonorrhoeae* was isolated from the vaginal aspirates of 17 (85%) of the 20 patients with gonorrhoea in this study. Although gonococci have been shown to adhere to vaginal epithelial cells in vitro (Forslin and Danielsson, 1980; Forslin, Danielsson and Falk, 1980), gonococci aspirated from the vaginal pool are probably derived from the cervix, the site of multiplication of the organisms (Ward, Watt and Robertson, 1974). The mean viable count of gonococci in vaginal aspirates (8.4 x 10^4 cfu/ml) was considerably lower than that in cervical aspirates (1 x 10^6 cfu/ml) although the difference did not attain statistical significance (p > 0.05).

Qualitatively, the microflora was similar in cervix and vagina. Quantitatively, however, there were differences. With the exception of the gonococcus, viable counts in aspirates from the vagina were approximately two to tenfold greater than the corresponding counts from the cervix. Staphylococci were isolated more frequently from the aspirates from patients with gonorrhoea. Organisms isolated only from patients with no microbiological evidence of gonorrhoea were haemolytic streptococci, *E. coli*, *C. albicans* and *T. vaginalis*. Unfortunately, an agar overlay assay of the type described by Saigh, Sanders and Sanders (1978) was not performed to determine whether the isolates in the present study were inhibitory to gonococci.

A positive transformation result was obtained with material aspirated from the cervix or vagina of only 75% of the patients with gonorrhoea. When crude DNA preparations from 169 clinical isolates from the same locality were tested in an earlier study (Sarafian and Young, 1980) c. 90% gave a positive transformation result. Therefore, negative transformation results with aspirates from patients with gonorrhoea were unlikely to be due to proline auxotrophy of donor gonococci. Insufficient numbers of gonococci also seems unlikely to be the cause of the five false-negative transformation results because aspirates from each of these patients yielded more than 1.7 x 10^3 cfu/ml, the minimum amount detectable by the transformation assay (Sarafian and Young, 1980). Enzymic degradation of DNA present in the aspirates may account for the false-negative results. We disagree with Janik, Juni and Heym (1976) who considered that genetic transformation was a simple, rapid and sensitive method of detecting gonococci in clinical specimens.

The indirect sandwich ELISA detected gonococcal antigens in aspirates from only 60% of infected women. In our earlier work (Sarafian and Young, 1982) the minimum number of gonococci detectable was 6.6 x 10^3 cfu which corresponds to 3.7 x 10^4 cfu/ml when a 180-µl portion is tested. Although 58% and 16% of cervical and vaginal aspirates respectively had numbers of gonococci above the minimum level, the detection of antigen did not correlate well with the numbers of viable gonococci isolated. Six of nine cervical aspirates with numbers of gonococci judged insufficient to be detectable by the assay gave a positive ELISA result whereas only five of eleven aspirates with sufficient numbers of gonococci gave a positive result. This lack of correlation could be explained if the ELISA depended largely on soluble antigen.
The *Limulus* lysate assay detected 84.2% of infected women and was clearly the most sensitive non-cultural method of diagnosing gonococcal infection. The *Limulus* assay, in detecting endotoxin, cannot be held to be specific; its significance in relation to the diagnosis of gonorrhoea depends upon the premise that other endotoxin-positive organisms are unlikely to produce sufficient amounts of endotoxin at the sites sampled. Moreover, at dilutions of secretions at which gonococci gave a positive result in the *Limulus* lysate assay, the presence of other endotoxin-positive bacteria did not seem to interfere with the results. Aspirates from three patients with no microbiological evidence of gonorrhoea were shown to contain endotoxin-positive bacteria but a negative *Limulus* result was obtained in each case. However, an unexplained positive result was obtained with secretions from three other patients without gonorrhoea. Retesting positive specimens at a dilution of 1 in 200 reduced the unexplained positive rate to zero but also reduced the true positive rate from 84.2% to 68.4%. It is suggested that further standardisation of specimens before testing could improve the sensitivity and the specificity of the *Limulus* lysate assay. Recently a test device has been developed to simplify and standardise the collection, dilution and testing of urethral exudates from men (Prior and Spagna, 1981). A similar approach to the problem of sampling cervical exudates merits study.

A major disadvantage of all of the above methods is that gonococci are not available for antibiotic sensitivity testing. This has become increasingly important with the discovery and increasing incidence of beta-lactamase-producing strains of *N. gonorrhoeae*. Perhaps some rapid method of detecting gonococcal beta-lactamase in secretions could also be developed and this would be complementary to the present approach. Another limitation of these methods in comparison with culture is related to diagnosis of infection in the rectum and pharynx. In spite of these limitations, we believe that the development of methods to detect gonococcal components is a worthwhile objective and could make a significant contribution to the rapid diagnosis and control of gonococcal infection. Of the methods examined, the *Limulus* lysate assay and an ELISA system are particularly worthy of further investigation. Because of the large numbers of gonococci and lower numbers of other organisms in cervical aspirates these are preferred to vaginal aspirates for the future development of such tests.

This work was supported in part by a grant from the Scottish Home and Health Department (research grant no. K/MRS/50/C22) whose financial assistance is gratefully acknowledged. We also thank Professor J.G. Collee for his helpful advice in the preparation of this paper.

**REFERENCES**


Neisserial colonisation of the pharynx

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From the *STD Diagnostic Laboratory, Department of Bacteriology, Edinburgh University Medical School, Edinburgh; and the †Department of Genitourinary Medicine, Royal Infirmary, Edinburgh

SUMMARY The spectrum of neisserial colonisation of the pharynx was determined from 3557 throat exudates cultured on modified New York City (MNYC) medium. Oxidase positive Gram-negative diplococci (GNDC) were isolated from 1204 (33·8%) of the throat cultures. Neisseria gonorrhoeae, N meningitidis, and N lactamica accounted for 20·3%, 74·2%, and 3·7% of the oxidase positive GNDC respectively. The observed coexistence of gonococci and meningococci in the pharynx (0·39%) was significantly different (p<0·001) from the theoretical expected value (1·7%). The prevalence of pharyngeal infection in patients with gonorrhoea was 4·3% for all men, 11% for homosexual men, and 7·9% for women.

Despite the risks of disseminated infection and spread to sexual partners, the detection of pharyngeal gonorrhoea is less important than that of endocervical infection in women, urethral infection in heterosexual men, and anorectal infection in homosexual men.

Introduction

The lack of correlation between pharyngeal colonisation with gonococci and symptoms of pharyngitis, combined with the reluctance of many patients to admit to orogenital contact, makes it difficult for the clinician to use these criteria in selecting patients from whom to take throat cultures. Because of the possible presence of other neisseriae in the pharynx, the microbiological examination of throat cultures is much more time consuming and technically demanding than the examination of material from genital sites. The use of a selective medium aids the examination of cultures from all anatomical sites but is particularly helpful in the diagnosis of rectal and pharyngeal infection. Since its introduction in 1977 modified New York City (MNYC) medium has improved the efficiency and the rapidity of cultural diagnosis in Edinburgh. Workers in other countries have also noted an improvement in culture results with MNYC medium and have adopted this medium for routine use.

Pharyngeal colonisation with penicillinase producing Neisseria gonorrhoeae (PPNG) may be of particular importance because of the potential for transfer of the TEM β-lactamase plasmid from N gonorrhoeae to N meningitidis if both organisms inhabit the same pharynx. The incidence of meningococcal colonisation of the pharynx is known to be higher (about 26%) in patients with genital gonorrhoea than in those without (about 11%).

Pharyngeal colonisation with gonococci ranges from 3·2% to 7·0% of men with gonorrhoea and from 2·9% to 11·3% of women with gonorrhoea. From these data simultaneous pharyngeal colonisation with gonococci and meningococci would be expected in I·2-5% of patients with gonorrhoea.

Patients and methods

The study population included all men and women from whom samples were taken for microbiological diagnosis of gonorrhoea at the department of genitourinary medicine, Edinburgh Royal Infirmary, during 1978 to 1981.

Gram-stained smears of material from the urethra in men and the urethra and cervix in women were examined microscopically while the patient was at the clinic. Cultures were made from urethral specimens in all men, whereas anorectal specimens were also taken from homosexual men. Urethral, cervical, and anorectal specimens were taken routinely from women. Pharyngeal specimens were taken from all women with gonorrhoea in a genital site, those who were contacts of men with gonorrhoea, and those who had a history of orogenital contact; pharyngeal specimens were also taken from patients considered to be at high risk of acquiring infection because of multiple sexual partners. Likewise, pharyngeal
Neisserial colonisation of the pharynx

specimens were taken from all male patients who were contacts of women with gonorrhoea, those with positive Gram-stained smears of urethral discharge, and those who gave a history of orogenital contact; pharyngeal cultures were taken routinely from all homosexual patients. If the first set of cultures from women were negative for N gonorrhoeae the tests were normally repeated twice at one or two weekly intervals. Test-of-cure cultures were normally carried out at one, two, and four weeks after treatment.

All specimens were inoculated directly on to MNYC medium\(^2\) at the clinic. After inoculation plates were held at 36°C in a carbon dioxide (10%) enriched atmosphere and transferred to the laboratory within four hours. Cultures were incubated and examined for oxidase positive Gram-negative diplococci (GNDC) which were tested for penicillinase production and further identified by carbohydrate utilisation and fluorescent antibody tests as described.\(^10\)

Details of patients with pharyngeal infection were obtained retrospectively from the case notes. Statistical analysis was by the \(\chi^2\) method with Yates’s correction.

Results

Table 1 shows the total number of cases of gonorrhoea diagnosed over the four year period 1978-81. Although the annual number of cases of gonorrhoea decreased during the four year period, the proportion of patients from whom throat cultures were taken remained fairly constant. There was a slight increase in the proportion of patients with pharyngeal gonorrhoea. The increase between 1978 and 1981, however, although more noticeable in women, was not statistically significant (\(p>0.1\)).

**INCIENCE**

**Men**

The 93 separate episodes of pharyngeal gonorrhoea (table I) occurred in 88 patients and yielded 107 isolates of gonococci. The average age of the patients was 24±8 years. Sixty nine (78-4%) of the patients were single, 11 (12-5%) married, and eight (9-1%) separated or divorced. Most patients (69-3%) had one or more casual sexual partners.

Rectal cultures were taken from 272 homosexuals, of whom 30 (11%) had pharyngeal gonorrhoea compared with 4-3% of all men.

Of 92 episodes of pharyngeal infection for which data were available, 80 (87-0%) were diagnosed at the first diagnostic test and seven (7-6%) at the second. In five (5-4%) patients pharyngeal gonorrhoea was diagnosed after treatment for anogenital infection.

Urethral infection was also present in 63 (68-5%) and rectal infection in 11 (12-0%) patients. The throat was the only site giving positive results in 21 (22-8%) patients; this corresponds to 1-0% of all patients with gonorrhoea in whom throat cultures were taken. The throat was the only site to give positive results in 13 (4-8%) of the 272 homosexuals.

**Women**

The 106 separate episodes of pharyngeal infection (table I) occurred in 105 patients and yielded 137 isolates of gonococci. The average age of the patients was 22±4 years. Sixty nine (65-7%) women were single, 17 (16-2%) married, 18 (17-1%) separated or divorced, and one (1-0%) widowed: 31-4% had one or more casual partners and 81% of the women were known contacts of men with gonorrhoea.

Of the 106 episodes of pharyngeal infection, 101 (95-3%) were diagnosed at the first diagnostic test and three (2-8%) at the second. Pharyngeal infection was diagnosed in two patients (1-9%) after treatment for anogenital infection. The throat was the only site giving positive results in 11 (10-4%) patients: this corresponds to 0-8% of all patients with gonorrhoea in whom throat cultures were taken.

**SIGNS AND SYMPTOMS**

Five (5-4%) of the 93 episodes of pharyngeal infection in men were associated with signs or symptoms referable to the throat compared with 14 (13-2%) of the 106 episodes of pharyngeal infection in women: this difference was not statistically significant \(\chi^2 = 2-7; p=0.1\). None of the patients had any symptoms relating to disseminated gonococcal infection.

**TABLE I Number of patients with gonorrhoea and pharyngeal gonorrhoea detected over the four year period 1978-81**

<table>
<thead>
<tr>
<th>Year</th>
<th>No of patients with gonorrhoea</th>
<th>No (% of patients with throat cultures taken</th>
<th>No (% of patients with pharyngeal gonorrhoea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>1978</td>
<td>702</td>
<td>453</td>
<td>639 (91±0)</td>
</tr>
<tr>
<td>1979</td>
<td>598</td>
<td>424</td>
<td>557 (93±1)</td>
</tr>
<tr>
<td>1980</td>
<td>583</td>
<td>403</td>
<td>517 (88±7)</td>
</tr>
<tr>
<td>1981</td>
<td>549</td>
<td>331</td>
<td>484 (86±2)</td>
</tr>
<tr>
<td>Total</td>
<td>2432</td>
<td>1611</td>
<td>2197 (90±3)</td>
</tr>
</tbody>
</table>

*Expressed as a percentage of patients in whom throat cultures were taken.*
RESULTS OF TREATMENT
The effectiveness of different dosages of ampicillin in treating pharyngeal infection in men and women is shown in table II: patients who were treated with other antibiotics and those cases without test-of-cure cultures were excluded.

In men the cure rate with a single dose of ampicillin was not significantly different from that after a course of treatment (p>0·5). In women, however, there was a highly significant difference between the cure rates obtained with a single dose and with a course of treatment ($\chi^2 = 19.5 ; p<0.001$).

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Results of treatment of pharyngeal gonorrhoea in 47 men and 72 women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>No (%) of patients</td>
</tr>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Ampicillin 2 g (single dose)*</td>
<td>7</td>
</tr>
<tr>
<td>Ampicillin 2 g + oxytetracycline 250 mg four times daily (5 days)</td>
<td>23</td>
</tr>
<tr>
<td>Ampicillin 2 g + ampicillin 250 mg four times daily (3 or 7 days)</td>
<td>13</td>
</tr>
<tr>
<td>Ampicillin 3 g + ampicillin 250 mg four times daily (5 or 7 days)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
</tr>
</tbody>
</table>

*Probenecid 1 g orally was given with ampicillin in each case.

NEISSERIAL IDENTIFICATION
As indicated in table I, 3557 pharyngeal specimens were taken for culture from 4043 patients with gonorrhoea. Oxidase positive GNDC were cultured from 1204 (33·8%) of the 3557 pharyngeal cultures: table III shows the identity of these organisms.

Of the 244 isolates of gonococci from the pharynx, 107 were from men and 137 from women. Gonococci and meningococci were isolated together at only 14 (0·39%) of the 3557 examinations. The theoretical proportion of patients in whom we would expect both organisms to be isolated together is 0·017, calculated on the basis that meningococci were isolated from 25·1% of patients and gonococci from 6·9% of patients (table III). The calculation of $\chi^2$ from a comparison between the actual and theoretical distribution showed a highly significant difference ($\chi^2 = 37·8 ; p<0.001$).

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>Identity of 1204 oxidase positive Gram-negative diplococci (GNDC) isolated from 3557 throat cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism</td>
<td>No of isolates</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>244</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>104</td>
</tr>
<tr>
<td>Neisseria lactamica</td>
<td>44</td>
</tr>
<tr>
<td>Neisseria flavic</td>
<td>1</td>
</tr>
<tr>
<td>Neisseria perflava</td>
<td>6</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>12</td>
</tr>
<tr>
<td>Unidentified</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>1204</td>
</tr>
</tbody>
</table>

Discussion
The importance of diagnosing pharyngeal infection should be evaluated in relation to: the spread of infection from the throat to genital or other sites in a sexual partner; the risk to the individual of developing a disseminated gonococcal infection (DGI); the potential for transfer of the TEM $\beta$-lactamase plasmid from $N$ gonorrhoeae to $N$ meningitidis if both organisms inhabit the same pharynx; and the resources available.

The prevalence of pharyngeal gonorrhoea in Edinburgh (4·3% of men and 7·9% of women with gonorrhoea) is comparable with that found elsewhere. The much higher incidence of pharyngeal infection (11%) in homosexual men, in 4·8% of whom the throat was the only site giving positive results, has also been noted by others.11

Thompson2 considered that transmission from the throat to any other body site of the sexual partner was undocumented. Others considered that mouth-to-mouth transfer and mouth-to-genital transfer may occasionally have taken place.11,13 Mouth-to-genital transfer probably occurs more frequently in certain geographical areas.6,14

The possibility of transfer of the TEM$\beta$-lactamase plasmid from the gonococcus to the meningococcus obviously depends on the prevalence of PPNG and the incidence of pharyngeal colonisation with gonococci and meningococci. The observed association of gonococci and meningococci in this study was significantly different (p<0·001) from the predicted value. This difference is particularly interesting in view of the strong positive association between genital gonorrhoea and meningococcal colonisation of the pharynx.7,8 Our finding requires corroboration since it implies that the opportunities for transfer of genetic material between gonococci and meningococci in the pharynx are much fewer than might be expected.

The above difference between the expected and observed values could be explained by technical
difficulties in detecting gonococci and meningococci in the same specimen; for example prolific growth of one organism might mask the presence of the other. Alternatively, meningococcal colonisation of the pharynx may have an inhibitory effect on gonococcal colonisation. The possible protective effect of meningococcal urethritis against gonococcal infection by bacteriocin production has been reported.15

Although effective treatment may eliminate the risks associated with pharyngeal gonorrhoea, infection at this site is often resistant to treatment.11,16 Single-dose treatment, which was effective in about 95% of genital infections, failed in over 50% of women with pharyngeal infection. The same treatment was effective in 85% of men with pharyngeal gonorrhoea: all but one of the men treated with a single dose had acquired their pharyngeal infection from heterosexual contacts. As most heterosexual men attended the clinic because of symptomatic urethritis, the outcome of single-dose treatment may be influenced by the duration of pharyngeal colonisation.

Pharyngeal gonorrhoea can only be diagnosed by identifying N gonorrhoeae in cultures of pharyngeal exudate. It is good practice also to identify, whenever possible, all non-gonococcal oxidase positive GNDC. The spectrum of oxidase positive GNDC encountered after culture on MNYC medium (table III) indicates the microbiological workload involved in this task. N meningitidis and Branhamella catarrhalis were the two organisms most often confused with N gonorrhoeae when suspected gonococcal isolates were submitted to a reference diagnostic laboratory for confirmation.17 In this study, despite the large numbers of isolates examined, we did not encounter either of these problems with the rapid carbohydrate utilisation system.10

Identification of problem cultures by both biochemical and immunological methods has been highly recommended.17 In our experience the sensitivity and specificity of immunofluorescence and coagglutination are similar, although the latter has several advantages in performance.18 N lactamica which occurred in 1.3% of all throat cultures may produce a cross-reaction in the coagglutination test.19

To prevent mis-identification an O-nitrophenyl-β-D-galactopyranoside (ONPG) test should be performed on all non-genital isolates which are positive by coagglutination. Other non-pathogenic neisseriae were rarely isolated, suggesting that MNYC medium is effective in inhibiting these organisms.

In this geographical area there seems to be little risk associated with pharyngeal gonorrhoea, either to the individual or to their sexual partner(s). In general, the detection of endocervical infection in women, urethral infection in heterosexual men, and anorectal infection in homosexual men is much more important than the detection of pharyngeal gonorrhoea in either sex and should have priority of resources. Careful monitoring of PPNG and pharyngeal gonorrhoea should, however, be undertaken by certain centres to provide valuable epidemiological data.

We thank our medical and non-medical colleagues in the departments of genitourinary medicine and bacteriology for their help in the preparation of this paper and in the collection and processing of specimens.

References

Ano-genital gonorrhoea and pharyngeal colonisation with meningococci: a serogroup analysis

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* Department of Genito-Urinary Medicine, The Royal Infirmary, Edinburgh
† Meningococcus Reference Laboratory {Scotland), Department of Laboratory Medicine, Ruchill Hospital, Glasgow

Summary

Among patients attending a clinic for sexually transmitted diseases, women without gonorrhoea were significantly less likely to be colonised with meningococci than were women with gonorrhoea, men with gonorrhoea and men without gonorrhoea: the respective carriage rates (per cent) for groupable plus non-groupable meningococci were 16, 26, 23 and 31. Considering groupable and non-groupable meningococci separately it was found that women without gonorrhoea were also significantly less likely to be colonised with groupable meningococci but there were no significant differences in the carriage rates of non-groupable meningococci. The association between ano-genital gonorrhoea and meningococcal colonisation of the pharynx observed previously with certain groups of patients most likely results from increased mouth-to-mouth contact in ‘high-risk’ patients rather than individual susceptibility to neisserial infection. The possibility that there is a difference in the predominant means of spread of groupable and non-groupable meningococci is discussed.

Introduction

Following the preliminary report by Willcox and colleagues,1 the association between ano-genital gonorrhoea and pharyngeal colonisation with Neisseria meningitidis has been confirmed by many workers.2-5 To what extent this association reflects an individual susceptibility to neisserial infection as suggested by Willcox or the degree of mouth-to-mouth contact in any given population2-5 requires clarification. As discussed in detail by William and co-workers5 the approximately two- to three-fold higher risk of gonorrhoea for pharyngeal carriers of N. meningitidis may reflect the greater rate of recent mouth-to-mouth contact in the more sexually active and hence higher risk patient: saliva is considered to be the vehicle of transmission in this form of direct person-to-person spread resulting from kissing. In contrast, the usual mode of transmission of N. meningitidis is thought to be by droplet spray.

Because of the absence of serogroup analysis in previous reports, and the possibility that meningococcal colonisation may result from two different mechanisms of transmission, the aims of the present study were: (i) to determine the serogroup and sulphadiazine sensitivity of meningococci carried by men and women attending a clinic for sexually transmissible diseases and...
(ii) to determine the carriage rate of groupable and non-groupable meningococci and examine their association with ano-genital gonorrhoea.

**Patients and methods**

Specimens of genital/ano-genital and pharyngeal exudates obtained from 1177 men and 1071 women attending the Department of Genito-Urinary Medicine, Edinburgh Royal Infirmary, between 21 January and 31 October 1980 were cultured for pathogenic Neisseriae. These patients were selected on the basis of criteria applied within the Department of Genito-Urinary Medicine for taking pharyngeal cultures. Pharyngeal cultures were taken from all women with gonorrhoea in a genital site, those who were contacts of men with gonorrhoea and those who had a history of oro-genital contact; pharyngeal cultures were also taken from patients considered to be at high risk of acquiring infection because of multiple sexual partners. Likewise, pharyngeal cultures were taken from all male patients who were contacts of women with gonorrhoea, those with 'positive' Gram-stained smears of urethral discharge and those who gave a history of oro-genital contact; pharyngeal cultures were taken routinely if there was a possibility of homosexually acquired infection.

Material was inoculated directly on to modified New York City medium at the time of the patient’s initial examination and cultures transported to the laboratory within four hours. Specimens were processed and *Neisseria gonorrhoeae* and *N. meningitidis* identified by fluorescent antibody and rapid carbohydrate utilisation tests. Meningococci were subcultured, harvested into Amies’s transport medium and sent to the Meningococcus Reference Laboratory (Scotland) for serogrouping and sulphadiazine sensitivity testing.

Statistical analysis was made by the $\chi^2$ method with Yates’s correction.

**Results**

Between 21 January and 31 October 1980 *N. meningitidis* was isolated from the pharynx of 296 men and 204 women. Serogrouping was performed on 247 (83.4 per cent) of the isolates from men and 168 (82.4 per cent) of those from women. The distribution of the various serogroups is shown in Table I.

Serogroup B accounted for 40 per cent of isolates while all other serogroups comprised only 23.5 per cent. A large proportion (36.5 per cent) of isolates were non-groupable.

The sensitivity to sulphadiazine of isolates within the various serogroups is shown in Table II.

Only 19 of 415 isolates (4.6 per cent) were resistant to sulphadiazine. Partial resistance was demonstrated by 203 isolates (48.9 per cent) while 193 isolates (46.5 per cent) were fully sensitive.

Meningococci were isolated from 28.8 per cent (120 of 417) of men with gonorrhoea compared to 23.2 per cent (176 of 760) of men without gonorrhoea ($P < 0.05$). Likewise, meningococci were isolated from 29.7 per cent (81 of 273) of women with gonorrhoea compared to 15.4 per cent (123 of 798) of women without ($P < 0.001$).

For a more detailed analysis of the relationship between throat carriage of
Meningococcal carriage and gonorrhoea

Table I  Number (and percentage) of isolates of Neisseria meningitidis within each serogroup

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>4 (2.5)</td>
<td>6 (1.5)</td>
</tr>
<tr>
<td>B</td>
<td>110 (44.5)</td>
<td>56 (33)</td>
<td>166 (40)</td>
</tr>
<tr>
<td>C</td>
<td>16 (6.5)</td>
<td>5 (3)</td>
<td>21 (5)</td>
</tr>
<tr>
<td>W-135</td>
<td>13 (5)</td>
<td>12 (7)</td>
<td>25 (6)</td>
</tr>
<tr>
<td>X</td>
<td>0</td>
<td>3 (2)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Y</td>
<td>9 (3.5)</td>
<td>7 (4)</td>
<td>16 (4)</td>
</tr>
<tr>
<td>Z</td>
<td>13 (5)</td>
<td>12 (7)</td>
<td>25 (6)</td>
</tr>
<tr>
<td>Z1</td>
<td>1 (0.5)</td>
<td>0</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Non-groupable</td>
<td>83 (33.5)</td>
<td>69 (41)</td>
<td>152 (36.5)</td>
</tr>
<tr>
<td>Total</td>
<td>247 (100)</td>
<td>168 (100)</td>
<td>415 (100)</td>
</tr>
</tbody>
</table>

Table II  Sulphadiazine sensitivity in relation to serogroup of 415 meningococcal isolates

<table>
<thead>
<tr>
<th>Sulphadiazine sensitivity</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>W-135</th>
<th>X</th>
<th>Y</th>
<th>Z1</th>
<th>Z</th>
<th>Non-groupable</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive: grow at 0.1 not 1 mg/l</td>
<td>2</td>
<td>73</td>
<td>11</td>
<td>14</td>
<td>2</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>72</td>
<td>193</td>
</tr>
<tr>
<td>Partially resistant: grow at 1.0 not 10.0 mg/l</td>
<td>0</td>
<td>85</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>16</td>
<td>1</td>
<td>75</td>
<td>203</td>
</tr>
<tr>
<td>Resistant: grow at 10.0 mg/l</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>166</td>
<td>21</td>
<td>25</td>
<td>3</td>
<td>16</td>
<td>25</td>
<td>1</td>
<td>152</td>
<td>415</td>
</tr>
</tbody>
</table>

meningococci and genital (or ano-genital) gonorrhoea only new patients were considered: the criteria for taking throat cultures could be applied most uniformly to new patients thus minimising individual clinician variation in selection of patients in whom throat cultures were taken. The incidence of groupable and non-groupable meningococci in new male and female patients with and without gonorrhoea is shown in Table III.

As calculated from Table III, the incidence of all meningococci (groupable and non-groupable) is significantly lower in new female patients without gonorrhoea than in other patient groups. When non-groupable meningococci are excluded the incidence of groupable meningococci remains significantly lower in new female patients without gonorrhoea than in other patient groups. However, when groupable meningococci are excluded there are no significant differences between any of the patient groups with respect to non-groupable meningococci.
Table III  Incidence of groupable and non-groupable meningococci in new male and female patients with and without gonorrhoea

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Number (and percentage) of patients from whom meningococci were isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groupable</td>
</tr>
<tr>
<td>(a) New male patients with gonorrhoea $n = 271$</td>
<td>41 (15)</td>
</tr>
<tr>
<td>(b) New male patients without gonorrhoea $n = 234$</td>
<td>50 (21·5)</td>
</tr>
<tr>
<td>(c) New female patients with gonorrhoea $n = 221$</td>
<td>37 (17)</td>
</tr>
<tr>
<td>(d) New female patients without gonorrhoea $n = 418$</td>
<td>39 (9)</td>
</tr>
</tbody>
</table>

There are significant differences at the 5 per cent level between (a) and (d) $P < 0·05$; (b) and (d) $P < 0·001$; and (c) and (d) $P < 0·01$ both for total meningococci and groupable meningococci. There is no significant difference at the 5 per cent level for non-groupable meningococci.

Table IV  Serogroup of meningococcal strains carried in Scotland compared with those isolated in Edinburgh

<table>
<thead>
<tr>
<th>Source</th>
<th>Year</th>
<th>Number of isolates tested</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Z'</th>
<th>W-135</th>
<th>Non-groupable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotland</td>
<td>1976</td>
<td>185</td>
<td>2·5</td>
<td>41</td>
<td>7</td>
<td>1·5</td>
<td>6·5</td>
<td>0</td>
<td>5·5</td>
<td>13·5</td>
<td>22</td>
</tr>
<tr>
<td>Scotland</td>
<td>1977</td>
<td>51</td>
<td>6</td>
<td>29</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>49</td>
</tr>
<tr>
<td>Scotland</td>
<td>1978</td>
<td>75</td>
<td>5·5</td>
<td>30·5</td>
<td>5·5</td>
<td>2·5</td>
<td>4</td>
<td>0</td>
<td>6·5</td>
<td>13·5</td>
<td>32</td>
</tr>
<tr>
<td>Scotland</td>
<td>1979</td>
<td>79</td>
<td>6·5</td>
<td>31·5</td>
<td>6·5</td>
<td>1·5</td>
<td>7·5</td>
<td>0</td>
<td>7·5</td>
<td>9</td>
<td>30·5</td>
</tr>
<tr>
<td>Edinburgh</td>
<td>1980</td>
<td>415</td>
<td>1·5</td>
<td>40</td>
<td>5</td>
<td>0·5</td>
<td>4</td>
<td>0·2</td>
<td>6</td>
<td>6</td>
<td>36·5</td>
</tr>
</tbody>
</table>

Discussion

The serogroups of meningococci isolated from carriage sites are fairly constant year by year in Scotland. As shown in Table IV the serogroups isolated from patients attending the sexually transmissible diseases clinic are similar to the pattern found for Scotland as a whole. However, proportionally more strains were fully sensitive to sulphadiazine and fewer strains partially resistant than was seen in the hospital and contact population surveyed in previous years (Table V). Sulphadiazine resistance was not associated with aberrant sugar degradation results. Kingsbury$^9$ isolated several maltose-negative strains of meningococci in which the inability to ferment maltose appeared to be
Table V  Sulphadiazine sensitivity of meningococcal strains carried in Scotland compared with those isolated in Edinburgh

<table>
<thead>
<tr>
<th>Source</th>
<th>Year</th>
<th>Sensitive</th>
<th>Partially resistant</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scotland</td>
<td>1976</td>
<td>39</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>Scotland</td>
<td>1977</td>
<td>28</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>Scotland</td>
<td>1978</td>
<td>39</td>
<td>57</td>
<td>4</td>
</tr>
<tr>
<td>Scotland</td>
<td>1979</td>
<td>28</td>
<td>66</td>
<td>6</td>
</tr>
<tr>
<td>Edinburgh</td>
<td>1980</td>
<td>47</td>
<td>48</td>
<td>5</td>
</tr>
</tbody>
</table>

Sensitive: grow at 0.1 not 1 mg/l.
Partially resistant: grow at 1 not 10.0 mg/l.
Resistant: grow at 10 mg/l.

genetically linked to sulphadiazine resistance (≥ 10 mg/l). Although 19 (5 per cent) of the 415 meningococcal isolates in our study were resistant to greater than 10 mg/l sulphadiazine all utilised maltose in carbohydrate degradation tests.

When the association between ano-genital gonorrhoea and meningococcal colonisation of the pharynx was analysed in relation to new patients the only significant difference in the prevalence of meningococci was the lower carriage rate in new female patients without gonorrhoea (Table III). This difference was apparent in relation to groupable but not to non-groupable meningococci. These results suggest that the female patients without gonorrhoea sampled in this study comprise a different behavioural group from those women with gonorrhoea and from the male patients.

It is very difficult to identify 'high risk' groups with any degree of accuracy. To a large extent this is easier in men: the vast majority of male patients from whom throat cultures were taken attended the clinic with symptomatic urethritis whereas many women attended as asymptomatic contacts. The finding that 271 (54 per cent) of 505 men from whom throat cultures were taken had gonorrhoea compared with only 221 (35 per cent) of 639 women from whom throat cultures were taken lends numerical support to this view. Therefore, the high incidence of meningococcal colonisation of the pharynx appears to be associated with the greater rate of recent mouth-to-mouth contact in the more sexually active and hence 'high risk' patient rather than directly correlated with gonococcal infection. Noble and colleagues in a study of 21,000 patients (40 per cent women) attending a venereal disease clinic in Kentucky, U.S.A. failed to demonstrate an association between ano-genital gonorrhoea and meningococcal colonisation of the pharynx. This result could be explained by all patients in their study being 'high risk' and therefore in a similar behavioural group. Because of the different health care system in the U.S.A., patients attending sexually transmitted disease clinics tend to belong to the lower socio-economic groups and a greater proportion are symptomatic.
Irrespective of whether the observed association between ano-genital gonorrhoea and meningococcal colonisation of the pharynx is due to behavioural factors, as suggested here, or to individual susceptibility, the different results with non-groupable and groupable meningococci are extremely interesting. These differences could result from a difference in the predominant means of spread of the two categories of meningococci. If colonisation with non-groupable meningococci results primarily from spread via droplet spray then most individuals will be exposed to a similar extent. In contrast, if colonisation with groupable meningococci results predominantly from direct salivary spread then they would be expected to be of higher incidence in our ‘high risk’ patients by virtue of their greater degree of mouth-to-mouth contact.

There are obvious differences between groupable and non-groupable meningococci demonstrable in the laboratory and in the ability of the former to cause disease. However, we do not know what determines the ability of a meningococcus to invade as opposed to colonise. Non-groupable strains may be of two types, non-groupable but smooth and the majority being non-groupable because they are auto-agglutinable in saline: detailed characterisation of non-groupable meningococci would be of value. Our results suggest that the factors governing the colonisation with groupable and non-groupable meningococci are fundamentally different and worthy of further study.

(We wish to thank W. Brown, B.A., FIMLS of the Meningococcus Reference Laboratory (Scotland) for serogrouping and sulphadiazine sensitivity testing of the meningococcal isolates. Thanks are also extended to medical and non-medical colleagues in the Departments of Genito-Urinary Medicine and Bacteriology for their help with the collection and processing of clinical specimens. We are grateful to Professor J. G. Collee for his helpful advice during the preparation of this paper.)

References
Serogrouping *Neisseria gonorrhoeae*: Correlation of coagglutination serogroup WII with homosexually acquired infection

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From the STD Diagnostic Laboratory, Department of Bacteriology, University Medical School, Edinburgh

SUMMARY With coagglutination reagents prepared against W antigens, 205 clinical isolates of *Neisseria gonorrhoeae* were classified into three serogroups WI, WII, and WIII. Of 195 strains isolated from patients who acquired their infection in the Edinburgh area, 85 (44%) belonged to serogroup WI and 110 (56%) to serogroup WII. Serogroup WII accounted for 90% of all isolates from homosexual men and for 34% and 47% of isolates from heterosexual men and women respectively (p<0.001). In homosexual men serogroup WII predominated regardless of the anatomical site from which the strain was isolated, and accounted for 94% of rectal, 90% of urethral, and 81% of pharyngeal isolates.

Introduction

The serological classification of *Neisseria gonorrhoeae* by coagglutination was first developed by Sandström and Danielsson. Although these workers described three different antigen classes, W, J, and M, only the W antigens proved suitable for serogrouping *N gonorrhoeae*. Reactions with W reagents were not altered by changes in colonial morphology and were shown to be stable, reproducible, and resistant to periodate. With reagents prepared against W antigens, gonococcal strains could be classified into three serogroups WI, WII, and WIII.

Recent work has shown that these coagglutination W serogroups correlate with the serotyping system of Buchanan and Hildebrandt based on antigenic differences in purified outer membrane protein I. Serogroups WI and WII/WIII correspond to two mutually exclusive forms of outer membrane protein I, protein IA and protein IB. It appears that serogroup WII represents a minor but prominent antigenic variant of protein IB rather than a third specific moiety.

Bydgeman et al showed that the distribution of W serogroups differed appreciably in two areas of central Sweden. Stockholm had a higher proportion of WII strains whereas Örebro had a higher proportion of WI strains. Antibiotic susceptibility of *N gonorrhoeae* also varies in relation to serogroup; non-penicillinase-producing *N gonorrhoeae* of serogroup WI were most susceptible and those of serogroup WII were the most resistant.

A very strong relation between serogroup WII and rectal isolates from men has been shown, but no correlation has been made between W serogroups and other anatomical sites of isolation. Moreover the distribution of W serogroups in the United Kingdom has not been reported, although workers in Bristol have shown that the W serogroup of gonococcal strains was the same from a limited number of contact pairs.

The aim of this study was twofold: to examine the distribution of W serogroups in the Edinburgh area, and to examine the serogroup of gonococcal strains from male homosexuals in relation to the anatomical site of isolation.

Materials and methods

CLINICAL ISOLATES

We obtained 205 gonococcal isolates from patients attending the department of genitourinary medicine at the Royal Infirmary, Edinburgh; 163 were from infections contracted in the Edinburgh area between November 1982 and June 1983, 32 were from infections contracted in Edinburgh during 1981 and 1982 and were preserved as lyophilised cultures before serogrouping, and 10 were from infections contracted abroad between November 1982 and June.
Serogrouping N gonorrhoeae: serogroup WII and homosexually acquired infection

All isolates were identified as N gonorrhoeae by the rapid carbohydrate utilisation test.\textsuperscript{10}

**STOCK CULTURES**

N gonorrhoeae major outer membrane protein (MOMP) reference strains A-1, B-2, C-3, D-4, E-5, F-6, N-10, R-11, S-12, U-14, and V-15\textsuperscript{11} were kindly supplied by Dr D. Danielsson, department of clinical bacteriology and immunology, Central County Hospital, Örebro, Sweden. Staphylococcus aureus NCTC8530 was obtained from the National Collection of Type Cultures, Colindale, London. All gonococcal strains were maintained on modified New York City (MNYC) medium.\textsuperscript{12} Staph aureus was maintained on blood agar.

**PREPARATION OF ANTISERUM**

Antiserum to each reference strain was prepared as described by Sandström and Danielsson.\textsuperscript{13} Table I shows the strains used for immunisation. Antiserum to S-12 and F-6 was kindly supplied by Dr E Sandström, department of dermatology, Karolinska Institutet, Stockholm, Sweden.

**TABLE I Immunisation and absorption scheme for producing antisera for coagglutination reagents**

<table>
<thead>
<tr>
<th>Immunising strain</th>
<th>Absorption strain</th>
<th>Serogroup defined by corresponding coagglutination reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOMP E-5</td>
<td>MOMP N-10</td>
<td>W1</td>
</tr>
<tr>
<td>MOMP D-4</td>
<td>MOMP C-3</td>
<td>W1</td>
</tr>
<tr>
<td>MOMP V-15</td>
<td>MOMP C-3</td>
<td>W1</td>
</tr>
<tr>
<td>MOMP N-10</td>
<td>MOMP D-4</td>
<td>WII</td>
</tr>
<tr>
<td>MOMP S-12</td>
<td>MOMP A-1</td>
<td>WII</td>
</tr>
<tr>
<td>MOMP U-14</td>
<td>MOMP R-11</td>
<td>WII</td>
</tr>
<tr>
<td>MOMP F-6</td>
<td>MOMP B-2 &amp; U-14</td>
<td>WIII</td>
</tr>
</tbody>
</table>

MOMP = major outer membrane protein.

**ABSORPTION OF ANTISERUM**

MOMP reference strains used for absorption of the antisera (table I) were grown on gonococcal (GC) agar (Difco) supplemented with L-glutamine, thiamine pyrophosphate, ferric nitrate, and glucose\textsuperscript{14} for 18 hours, harvested and washed once in 0.01 mol/l phosphate buffered saline (PBS) pH 7.2. Ten plates gave about 0.3-0.5 g wet weight of gonococcal cells. The cells were resuspended in 5 ml PBS, boiled for 30 minutes, and centrifuged at 4000 x g for 20 minutes. The pellet was used for absorption. Equal volumes of antiserum and pellet were incubated at 37°C for four hours and then held overnight at 4°C. The absorbed antiserum was collected by centrifugation, and coagglutination reagents were prepared to test the specificity of the antiserum. Absorption was repeated if cross reactions persisted.

**PREPARATION OF COAGGLUTINATION REAGENTS**

This was essentially as described by Jephcott.\textsuperscript{15} Staph aureus NCTC8530 was grown on GC agar for 18 hours, harvested, washed thoroughly in 0.01 mol/l PBS pH 7.2, resuspended in PBS containing 0.5% (v/v) formalin, and incubated at room temperature for three hours. The cells were then washed twice, resuspended in PBS, and heated at 80°C for five minutes. The stabilised cells were then washed twice, resuspended to 10% (w/v) in PBS, and stored at -20°C in 1 ml aliquots.

**PREPARATION OF SENSITISED TEST SUSPENSION**

Absorbed antigonococcal serum (0.1 ml) was mixed with 1 ml of washed stabilised staphylococcal cell suspension and held at room temperature for 15 minutes. The cells were then harvested by centrifugation at 2500 \times g for 15 minutes and the deposit washed twice in PBS. The sensitised cells were finally resuspended in 10 ml sterile PBS. The control reagent was prepared in exactly the same way, with normal rabbit serum substituted for antigonococcal serum.

**TEST PROCEDURE**

Test suspensions of N gonorrhoeae were prepared by harvesting organisms from half a culture plate into 400 µl distilled water and boiling for 10 minutes. Tests were performed with 20 µl test gonococcal suspension and 20 µl coagglutination reagent thoroughly mixed on a glass slide and rocked for two minutes. Slides were examined under oblique light against a dark background, and the amount of agglutination with the test reagent compared with that obtained in a similar test with the control reagent. The reactions were graded negative (-) and weakly positive (+), moderately positive (2+), and strongly or very strongly positive (3+).

**SEROGROUING PROTOCOL**

In a pilot study 58 strains were tested with each coagglutination reagent. The coagglutination reagents anti E-5 and N-10, defining serogroups W1 and WII respectively, reacted with most of the gonococcal strains within these serogroups. Each test gonococcal strain was therefore thereafter screened against the three reagents anti E-5, N-10, and F-6, defining serogroups W1, WII and WIII. When the serogroup of the strain was known, the reaction pattern of the strain with the remaining reagents within its serogroup was assessed. On the few occasions when a cross reaction occurred with anti N-10 and F-6 reagents, the strain was cloned before repeating the serogrouping.
Results

SEROGROUPS OF ISOLATES FROM INFECTIONS ACQUIRED IN THE EDINBURGH AREA

All strains of *N. gonorrhoeae* tested could be serogrouped. Table II shows the reaction patterns obtained in coagglutination tests with local gonococcal isolates: 85 (44%) of all isolates belonged to serogroup WI and 110 (56%) to serogroup WII. Most of the gonococcal strains, 66% of WI and 73% of WII, reacted with all three of the reagents defining each group. In addition, some minor reaction patterns were obtained.

None of the 195 gonococcal strains acquired in Edinburgh were asserogrouped as WII, although a few serogroup WII strains gave equivocal reactions with this reagent. Two strains acquired in West Africa and the Far East were classified as serogroup WIII, and one strain acquired in Thailand was grouped as WII/WIII.

TABLE II Coagglutination serogrouping of 195 gonococcal strains isolated from infections acquired in the Edinburgh area

<table>
<thead>
<tr>
<th>Coagglutination reagents</th>
<th>WI reagents</th>
<th>Pattern of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti E-5</td>
<td>+ + + +</td>
<td></td>
</tr>
<tr>
<td>Anti D-4</td>
<td>+ - + +</td>
<td></td>
</tr>
<tr>
<td>Anti V-15</td>
<td>+ + - +</td>
<td></td>
</tr>
<tr>
<td>No of gonococcal strains</td>
<td>56 16 7 5 1</td>
<td>(total 85)</td>
</tr>
<tr>
<td>WII reagents</td>
<td>+ - - -</td>
<td></td>
</tr>
<tr>
<td>Anti N-10</td>
<td>+ - + -</td>
<td></td>
</tr>
<tr>
<td>Anti S-12</td>
<td>+ - - -</td>
<td></td>
</tr>
<tr>
<td>Anti U-14</td>
<td>+ + - -</td>
<td></td>
</tr>
<tr>
<td>No of gonococcal strains</td>
<td>80 17 5 3 4</td>
<td>(total 110)</td>
</tr>
<tr>
<td>WII reagent</td>
<td>+ - - -</td>
<td></td>
</tr>
<tr>
<td>Anti F-6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>No of gonococcal strains</td>
<td>195</td>
<td></td>
</tr>
</tbody>
</table>

+ = agglutination; - = no agglutination

STRAINS OF *N. GONORRHOEAE* ISOLATED FROM HOMOSEXUAL MEN COMPARED WITH THOSE ISOLATED FROM HETEROSEXUAL MEN AND WOMEN

Table III shows the distribution of the W serogroups in the three groups of patients. Serogroup WII accounted for 90% of all isolates from homosexual men and for 34% and 47% of isolates from heterosexual men and women respectively. This difference in distribution of serogroup WII between gonococcal isolates from homosexual and heterosexual men was highly significant ($\chi^2 = 36.0; p<0.01$) as was the difference between strains isolated from homosexual men and heterosexual women ($\chi^2 = 25.1; p<0.01$).

TABLE III Distribution of serogroups WI and WII among homosexual men and heterosexual men and women

<table>
<thead>
<tr>
<th>Patient group</th>
<th>WI (%)</th>
<th>WII (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexual men</td>
<td>6 (10)</td>
<td>53 (90)</td>
<td>59</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>37 (66)</td>
<td>19 (34)</td>
<td>56</td>
</tr>
<tr>
<td>Women</td>
<td>42 (53)</td>
<td>38 (47)</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>85 (44)</td>
<td>110 (56)</td>
<td>195</td>
</tr>
</tbody>
</table>

SEROGROUPS OF ISOLATES FROM VARIOUS ANATOMICAL SITES

Table IV shows the serogroups of isolates from various sites. In homosexual men serogroup WII predominated regardless of the anatomical site from which the strain was isolated. Genital, mainly cervical, isolates from women were equally divided between serogroups WII and WI, whereas 69% of urethral isolates from heterosexual men were in serogroup WII ($\chi^2 = 2.6; 0.5>p>0.1$).

TABLE IV Serogroups of isolates from various anatomical sites

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Anatomical site</th>
<th>No (%) of strains</th>
<th>WI (%)</th>
<th>WII (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Throat</td>
<td>9 (50)</td>
<td>9 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>11 (58)</td>
<td>8 (42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genitalia</td>
<td>21 (50)</td>
<td>21 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>Throat</td>
<td>4 (50)</td>
<td>4 (50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urethra</td>
<td>33 (69)</td>
<td>15 (31)</td>
<td></td>
</tr>
<tr>
<td>Homosexual men</td>
<td>Throat</td>
<td>3 (19)</td>
<td>13 (81)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>2 (6)</td>
<td>31 (64)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urethra</td>
<td>1 (10)</td>
<td>9 (90)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>85 (44)</td>
<td>110 (56)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Right hip aspirate

Discussion

Strains of serogroup WI and WII accounted for 43·5% and 56·4% respectively of all the isolates from Edinburgh, or 58% and 42% respectively if isolates from homosexual men are excluded. The overall distribution is consistent with data reported from Stockholm which did not distinguish between heterosexual and homosexual men.

In a later study of homosexual male patients in Stockholm, Bygdeman found that 30 (76·9%) of 39 gonococcal strains were of serogroup WII, and this was shown to be an appreciable association. This considerable association of WII gonococcal strains with male homosexuals has also been noted in Seattle, United States of America. In our Edinburgh study 53 (90%) of 59 strains isolated from
homosexual men were of serogroup WII. Our finding of a strong association between serogroup WII and isolates from anatomical sites other than the rectum in homosexual men, suggests that the serogroup antigen is stable once acquired. As transmission of the organism among homosexual men is primarily rectal, it has been postulated that the rectal environment, which is rich in hydrophobic molecules such as fatty acids, selects gonococcal strains with mutations that reduce the permeability of the outer membrane to hydrophobic molecules. It seems probable that this same hostile environment selects the particular subgroup of the protein I molecule which is detected by the coagglutination WII reagents. Comparison of the relative distribution of WI and WII strains isolated from the genital regions of male and female heterosexual patients suggests that selective pressures may perhaps be exerted at sites of infection other than the hydrophobic rectal environment.

No WII strains were isolated from patients infected in Edinburgh, but two such strains were isolated from patients who had contracted their infections in Thailand and Nigeria. This is consistent with other studies, which have shown that WII strains are uncommon and are generally associated with infections acquired in the Far East.

A gonococcal strain has occasionally been classified as serogroup WII/WIII, which appears to be the result of shared antigenicity between the protein 1B molecules detected by both WII and WII coagglutination reagents. In this study an isolate from Thailand reacted very strongly with both WII and WII reagents and was subsequently typed WII/WIII.

Although the coagglutination W serogroups have been shown to correspond to different outer membrane protein I molecules, it must be remembered that other antigens, such as minor membrane proteins and lipopolysaccharide, probably contribute to the reaction patterns recorded and may be responsible for the equivocal cross reactions seen with some WII strains in tests with the WII reagent. Cross reactions could also result if a patient was colonised with more than one serogroup. Our data suggest this is unlikely as no cross reactions were observed with the reagents defining the frequently isolated serogroups WI and WII.

Although its precise value has yet to be established, the coagglutination W serogrouping system is easy and quick to perform and has achieved a more general acceptance than other forms of differentiation between strains.

We thank Professor JG Collee for helpful advice in the preparation of this paper. KGR also gratefully acknowledges the receipt of a Faculty of Medicine Research Scholarship.

References

Immunological identification of Neisseria gonorrhoeae with monoclonal and polyclonal antibody coagglutination reagents

H Young, Katherine G Reid
Immunological identification of *Neisseria gonorrhoeae* with monoclonal and polyclonal antibody coagglutination reagents

H YOUNG, KATHERINE G REID

*From the STD Diagnostic Laboratory, Department of Bacteriology, University of Edinburgh Medical School, Edinburgh EH8 9AG*

**Summary** The reliability of immunological identification of *Neisseria gonorrhoeae* using polyclonal and monoclonal antibody coagglutination reagents has been evaluated. When clinical isolates of neisseriae were tested in an “in use” trial the sensitivity and specificity of each reagent were similar and the overall agreement with carbohydrate utilisation was 97.9% (141/144) for the polyclonal antibody reagent and 97.2% (140/144) for the monoclonal reagent. When results of testing 13 stock cultures of *N lactamica* and five stock cultures of β-lactamase producing *Branhamella catarrhalis* were combined with the results for clinical isolates of non-gonococcal neisseriae the agreement with carbohydrate utilisation was 86.5% (64/74) for the polyclonal reagent and 97.3% (72/74) for the monoclonal reagent: this difference is statistically significant at the 5% level.

Calculation of positive and negative predictive values showed differences in the reliability of the coagglutination reagents when testing Gram negative diplococci isolated from various anatomical sites. The value and limitations of the polyclonal and monoclonal reagents were similar with respect to anogenital isolates: *N gonorrhoeae* was confirmed by a positive result but not excluded by a negative result. The monoclonal reagent was superior for testing throat isolates; although a negative result with either reagent confirmed Gram negative diplococci as non-gonococcal neisseriae, a positive result with the monoclonal reagent was more reliable (predictive value 93%) than a positive result with the polyclonal reagent (predictive value 86%).

Gonorrhoea remains a prevalent infection; 55 784 cases were identified and treated in clinics in England during the year ending 30 June 1980.1 Because of the heavy microbiological workload this imposes there is a considerable incentive to develop and implement simple, rapid, and reliable methods of differentiating between gonococcal and non-gonococcal neisseriae. The development of the coagglutination reaction2 increased the popularity of immunological identification of *Neisseria gonorrhoeae* and coagglutination has now superseded immunofluorescence in many laboratories.3-5 Some immunological cross reactions are found between common antigens on *N gonorrhoeae* and non-gonococcal neisseriae. Such reactions are not unexpected since the polyclonal antibodies used in the coagglutination reagent, although absorbed to remove non-reacting antibodies, are prepared by immunising rabbits with whole cells of several strains of gonococci. The cross reactions limit the usefulness of coagglutination in identifying isolates from sites such as the throat, where *N meningitidis* and to a lesser extent *N lactamica* are the most common isolates,6 and the anorectum of homosexual men, where meningococci are not uncommon.7

Monoclonal antibodies which recognise a single antigenic determinant should facilitate the production of more specific diagnostic reagents. We have evaluated the immunological identification of *N gonorrhoeae* with coagglutination reagents which employ monoclonal antibodies produced against the major outer membrane protein (protein I) of *N gonorrhoeae*.8

**Material and methods**

Clinical isolates of neisseriae were obtained from...
patients attending the department of genitourinary medicine of the Royal Infirmary. Anogenital and pharyngeal material was inoculated directly on to Modified New York City (MNYC) medium and transported to the laboratory within 4 h. After overnight incubation at 36°C in air enriched with carbon dioxide (5–10%) cultures were screened for oxidase positive Gram negative diplococci (GNDC). Suspected neisserial colonies (oxidase positive GNDC) were subcultured on to MNYC medium and incubated overnight before carrying out the rapid carbohydrate utilisation and coagglutination tests. If more than one anogenital site from a female patient yielded Gram negative diplococci, cultures from one site only, usually the cervix, were tested by coagglutination.

In the first part of the study all Gram negative diplococci were tested in parallel with polyclonal and monoclonal coagglutination reagents. Thirteen stock cultures of *N. lactamica* and five of β-lactamase producing *Branhamella catarrhalis* were also tested with both types of reagent. During the second half of the study only monoclonal reagents were used, and test performance was compared with bacterial suspensions prepared by boiling and by treatment with antigen releasing agent.

**COAGGLUTINATION**

Coagglutination with polyclonal antibody reagents (Phadebact Gonococcus test, Pharmacia Diagnostics AB, Sweden) was performed with a boiled suspension of organism as described elsewhere except that a 20 μl unit volume was used.

Coagglutination with monoclonal reagents (GONO GEN, New Horizons Diagnostic Company, Columbia, MD) was carried out with boiled suspensions of organisms and with suspensions treated with an antigen releasing agent available from the same source.

**BOILED SUSPENSION METHOD**

A heavy suspension of each culture to be tested was made by removing the growth from the culture plate with a cotton tipped swab and emulsifying it in 0.2 ml of distilled water in a tube. The tube was covered and the suspension heated in a boiling water bath for 5 min. After cooling to room temperature 20 μl aliquots of suspension were mixed with an equal volume of test reagent (murine monoclonal antiprotein I antibody bound to dead staphylococci) and control reagent (non-immune rabbit IgG bound to dead staphylococci) on a clear glass slide. Three tests were carried out on a single slide (7.6 cm × 5.0 cm) with six clearly defined areas. The slide was rocked gently in a rotary fashion for 1 min and the results read against a dark background using indirect light.

**ANTIGEN RELEASING AGENT**

Suspensions of each test organism were made on a glass slide by mixing several colonies with 20 μl volumes of antigen releasing agent (diluted 1/5 with distilled water). Test reagent (20 μl) and control reagent (20 μl) were mixed with the suspensions and the slides rocked and read as above.

Results were interpreted as follows:

- Positive—clumping or agglutination with test reagent significantly stronger than with control reagent.
- Equivocal—clumping or agglutination slightly stronger with test reagent than with control reagent.
- Non-specific—reaction of equal strength with test and control reagents.
- Negative—no reaction with the test reagent irrespective of any reaction with the control reagent.

Calculation of sensitivity and specificity were as follows:

**Sensitivity**—percentage of gonococcal isolates by carbohydrate utilisation that were coagglutination positive.

**Specificity**—the percentage of non-gonococcal isolates by carbohydrate utilisation that were coagglutination negative.

Predictive values were calculated according to the formula given by Veehio.

The predictive value of a positive test (PV+) is the probability that the Gram negative diplococci giving a positive coagglutination test are gonococci and is calculated according to the formula:

\[
PV^+ = \frac{pa}{pa + (1-p)(1-b)} \times 100
\]

The predictive value of a negative test (PV-) is the probability that the Gram negative diplococci giving a negative coagglutination test are non-gonococcal neisseria (or *Branhamella*) and the formula is:

\[
PV^- = \frac{(1-p)b}{(1-p)b + p(1-a)} \times 100
\]

where \( p = \) prevalence of gonococcal Gram negative diplococci within the total population of Gram negative diplococci isolated from a given site, \( a = \) test sensitivity, and \( b = \) test specificity.

The significance of differences in the results was determined by the \( \chi^2 \) test with Yates’ correction.

**Results**

Table 1 shows the results of polyclonal and mono-
clonal antibody coagglutination results with boiled suspensions of clinical isolates of neisseriae. The sensitivity and specificity of the monoclonal reagent were 97-7% (86/88) and 96-4% (54/56), respectively, compared with values of 100% (88/88) and 94-6% (53/56) for the polyclonal reagent. The overall agreement with carbohydrate utilisation was 97-9% (141/144) for the polyclonal antibody reagent and 97-2% (140/144) for the monoclonal reagent. These differences are not significant at the 5% level.

Whereas all 13 stock cultures of *N lactamica* were negative with the monoclonal reagent seven gave a positive result with the polyclonal reagent. All five isolates of β-lactamase producing *B catarrhalis* were negative with both reagents. When these results were combined with those for clinical isolates of non-gonococcal neisseriae the agreement with carbohydrate utilisation was 97-3% (72/74) for the monoclonal reagent and 86-5% (64/74) for the polyclonal reagent. This is a significant difference ($\chi^2 = 4-5; p < 0-05$).

Results of the monoclonal reagent with bacterial suspensions prepared by boiling and by treatment with antigen releasing agent are given in Table 2. Suspensions prepared with releasing agent tended to be stringy compared with the uniform suspensions obtained by boiling. On many occasions the stringy nature of the suspensions made it impossible to score results unequivocally positive or negative. The overall correlation with carbohydrate utilisation was 97-1% (166/171) for the test with boiled suspension and 80-7% (138/171) when antigen releasing agent was used. This is a highly significant difference ($\chi^2 = 21-6; p < 0-001$). The difference in test performance remains significant when gonococcal ($p < 0-01$) and non-gonococcal neisseriae ($p < 0-001$) are considered separately.

<table>
<thead>
<tr>
<th>Identity by carbohydrate utilisation</th>
<th>No of isolates</th>
<th>Polyclonal</th>
<th>Monoclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Non-specific</td>
<td>Negative</td>
</tr>
<tr>
<td><em>N gonorrhoeae</em></td>
<td>88</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>N meningitidis</em></td>
<td>52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>N lactamica</em></td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>N perflava</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>90</td>
<td>53</td>
</tr>
</tbody>
</table>

*Negative after two subcultures.

<table>
<thead>
<tr>
<th>Identity by carbohydrate utilisation</th>
<th>No of isolates</th>
<th>Results with suspensions prepared by boiling (and by treatment with releasing agent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Equivocal</td>
</tr>
<tr>
<td><em>N gonorrhoeae</em></td>
<td>117</td>
<td>112 (95)</td>
</tr>
<tr>
<td><em>N meningitidis</em></td>
<td>51</td>
<td>0 (1)</td>
</tr>
<tr>
<td><em>N lactamica</em></td>
<td>2</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>N perflava</em></td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>112 (69)</td>
</tr>
</tbody>
</table>

The differences are not significant at the 5% level.

Whereas all 13 stock cultures of *N lactamica* were negative with the monoclonal reagent seven gave a positive result with the polyclonal reagent. All five isolates of β-lactamase producing *B catarrhalis* were negative with both reagents. When these results were combined with those for clinical isolates of non-gonococcal neisseriae the agreement with carbohydrate utilisation was 97-3% (72/74) for the

### Table 1
Results of polyclonal and monoclonal antibody coagglutination reagents tested with 144 clinical isolates of neisseriae.

<table>
<thead>
<tr>
<th>Identity by carbohydrate utilisation</th>
<th>No of isolates</th>
<th>Polyclonal</th>
<th>Monoclonal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
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<td>0</td>
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<tr>
<td><em>N lactamica</em></td>
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<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>N perflava</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
<td>90</td>
<td>53</td>
</tr>
</tbody>
</table>

*Negative after two subcultures.

### Table 2
Monoclonal antibody coagglutination test applied to suspensions of 171 neisseriae isolates prepared by boiling and by treatment with antigen releasing agent.

<table>
<thead>
<tr>
<th>Identity by carbohydrate utilisation</th>
<th>No of isolates</th>
<th>Results with suspensions prepared by boiling (and by treatment with releasing agent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Equivocal</td>
</tr>
<tr>
<td><em>N gonorrhoeae</em></td>
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</tr>
<tr>
<td><em>N perflava</em></td>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>112 (69)</td>
</tr>
</tbody>
</table>

### Table 3
Predictive values of monoclonal and polyclonal antibody coagglutination reagents for Gram negative diplococci isolated from various sites.

<table>
<thead>
<tr>
<th>Source of Gram negative diplococci</th>
<th>Proportion of gonococcal Gram negative diplococci</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female anogenital and heterosexual male urethra</td>
<td>0.996</td>
<td>99.99</td>
<td>99.98</td>
</tr>
<tr>
<td>Homosexual male anogenital</td>
<td>0.953</td>
<td>99.91</td>
<td>99.80</td>
</tr>
<tr>
<td>Homosexual male rectum</td>
<td>0.920</td>
<td>99.84</td>
<td>99.65</td>
</tr>
<tr>
<td>Throat (all patient groups)</td>
<td>0.203</td>
<td>93.18</td>
<td>86.42</td>
</tr>
</tbody>
</table>
Immunological identification of Neisseria gonorrhoeae

Calculation of predictive values

The positive and negative predictive values of the polyclonal and monoclonal reagents applied to Gram negative diplococci isolated from various anatomical sites are given in Table 3. The values of a (sensitivity), b (specificity), and p (prevalence) used in calculating the predictive values given in Table 3 were derived as follows.

The sensitivity and specificity of the monoclonal reagent were 96.6% (198/205) and 98.2% (108/110) respectively; these figures represent all clinical isolates tested by the boiled suspension method. The sensitivity and specificity of the polyclonal reagent were 97.4% (406/417) and 96.1% (173/180), respectively. Because of the small number of isolates tested with the polyclonal reagent these are composite results of the present and previous study, which gave a sensitivity of 96.7% (318/329) and a specificity of 96.8% (120/124) with the same reagents.

The values of p (the proportion of gonococcal Gram negative diplococci within the total population of Gram negative diplococci from various anatomical sites) as given in Table 3 were taken from the finding that over a 4 year period N. gonorrhoeae accounted for 20.3% of 1204 throat isolates of Gram negative diplococci; and that of the Gram negative diplococci isolated over a 5 year period N. gonorrhoeae accounted for 99.6% of 1944 anogenital isolates from women, 99.7% of 2623 urethral isolates from heterosexual men, 95.3% of 384 anogenital isolates, and 92.0% of 225 rectal isolates from homosexual men.

Discussion

The advent of monoclonal antibodies raises the possibility of improved and more widely applied immunological methods of identifying microorganisms. In our "in use" evaluation, however, there was no significant difference in sensitivity and specificity of monoclonal and polyclonal antibody reagents for the routine identification of clinical isolates of N. gonorrhoeae.

Although factors such as sensitivity and specificity are important in evaluating new methods, the utility of a test result (the predictive value) depends on the prevalence of gonococcal Gram negative diplococci among the total population of Gram negative diplococci tested. As shown in Table 3, although the sensitivity and specificity of the test method remain the same, the predictive value varies greatly depending on the anatomical site of isolation. From these results we can make sound recommendations regarding the application of immunological methods for the laboratory identification of N. gonorrhoeae.

Clearly, coagglutination testing with either polyclonal or monoclonal reagents may be recommended for the identification of gonococci from an anogenital site, although reliability is less in the case of isolates from homosexual men, when 1 to 3 per 1000 positive tests will be false positives; the corresponding rate with isolates from heterosexual patients is 1 to 2 false positives per 10,000 positive tests. A positive coagglutination result on an isolate from the throat is less reliable than in the case of anogenital isolates; there will be 7 false positives per 100 positive tests with the monoclonal reagent and 14 false positives per 100 positive tests with the polyclonal reagent. Although this difference is not significant at the 5% level with 100 positive results, it becomes significant with 200 positive results.

It should be emphasised, however, that our criteria in assessing these reagents are extremely strict. Of 110 non-gonococcal neisseriae 108 gave unequivocally negative results, giving a specificity of 98.2%. One isolate of N. lactamica gave a non-specific reaction and one strain of N. meningitidis gave a positive reaction on first isolation but was negative when tested after two subcultures. In calculating the positive predictive value, these two results were included as "positive tests." It is of interest that these two results occurred in the first part of the survey, when we had least experience of the reagents; during the second part of the survey the specificity was 100% (54/54). In a preliminary evaluation of the same monoclonal reagents Philip et al. found all 27 isolates of non-gonococcal neisseriae tested gave a negative reaction. Although a larger study is required, we consider that monoclonal coagglutination reagents are likely to achieve a level of specificity such that the predictive value of a positive test result is a reliable indicator of pharyngeal gonorrhoea in patients attending a clinic for sexually transmitted diseases.

Because of the predominance of meningococci in the throat a negative coagglutination result is a reliable indicator of non-gonococcal neisseriae; fewer than one negative result in 100 will be a false negative. Because of the extremely rare occurrence of meningococci in anogenital specimens from heterosexual patients, however, a negative coagglutination result is not a reliable indicator of non-gonococcal neisseriae; about 9 of 10 negative results will be false negatives and gonococcal infection could pass undiagnosed. Although meningococci are isolated more frequently from anogenital sites in homosexual men, a negative coagglutination result with these isolates remains unreliable since 2 to 4 of 10 negative results will be false.

Because of its slightly greater sensitivity the negative predictive value of the polyclonal reagent is
Marginal better than that of the monoclonal reagent. A sensitivity of 99-99% would be required, however, to give a negative predictive value of 97.5% for an anosgenital isolate from a heterosexual patient; a sensitivity of 99-90% would give a negative predictive value of only 79.7%. It will obviously be difficult to achieve a sensitivity greater than 99-90% given that the sensitivity of the monoclonal reagent was 96.6% (198/205) in our study and is comparable to the figure of 96% (48/50) found by Philip et al.12

Monoclonal antibodies have been criticised as diagnostic reagents on the grounds that their very high specificity results in decreased sensitivity. To overcome this problem individual antibodies are normally combined to produce a broadly reactive reagent. Although the authors are unaware of the detailed characterisation of the monoclonal antibodies used in the GONO Gen reagent, other studies13 suggest that suitable combinations of monoclonal antibodies will result in broadly reactive reagents with high sensitivity. The 16 monoclonal antibodies described by Tam et al13 recognise sub-group antigens rather than strain specific antigens, which may be distinguished by serotyping.14 One antibody (4-GS) identified an epitope shared by all 14 gonococcal reference strains containing the protein IA molecule whereas another monoclonal antibody (2-H1) detected an epitope shared by all 20 reference strains containing the protein IB molecule; the other 14 monoclonal antibodies recognised subsets of reference strains within the protein IA or IB groups.

Gonococcal serogroups WI and WI/WIII as determined by coagglutination15 correlate with the serotyping system of Buchan and Hildebrandt14 based on antigenic differences in purified outer membrane protein I. Protein IA and IB are mutually exclusive forms of outer membrane protein I corresponding to serogroups WI and WI/WIII respectively.15 Serogroup WIII most probably represents a minor but prominent antigenic variant of protein IB rather than a specific moiety.*

Broadly reactive reagents containing combinations of monoclonal antibodies will require careful monitoring as the relative proportion of strains falling within a particular serogroup is likely to show considerable geographical and temporal variation. For example, of 195 gonococcal strains isolated from patients who acquired their infections in the Edinburgh area 44% belonged to serogroup WI and 56% to WII*: in contrast, of six strains isolated from infections acquired outside the Edinburgh area three (West Africa one and Far East two) were serogroup WIII.

The use of a boiled suspension of organisms may help in the detection of a wide range of serotypes with the minimum number of monoclonal antibodies. Monoclonal antibodies reacted more strongly and gave additional positive reactions with boiled organisms when compared with unheated control suspensions, which suggests an increased accessibility of antibody to certain epitopes after boiling.13 Although autolytic conditions such as an alkaline pH (pH 8.3) and the presence of divalent cation chelators have been reported to enhance the coagglutination reaction and give increased sensitivity,17 the use of the "antigen releasing agent" used in this study can not be recommended.

We conclude that a positive coagglutination test with either monoclonal or polyclonal reagents on Gram negative diplococci isolated from an anogentral site provides reliable identification of N gonorrhoeae and no further tests are required. Monoclonal reagents are recommended when testing Gram negative diplococci isolated from the throat, and provided that N gonorrhoeae has been isolated from an anogentral site a positive result is a reliable indicator of pharyngeal gonorrhoea and no further tests are required. Although negative coagglutination results with either polyclonal or monoclonal reagents need not be confirmed in the case of Gram negative diplococci isolated from the throat, it is mandatory to identify coagglutination negative isolates from anogentral sites by biochemical tests. Rapid carbohydrate utilisation tests18 would appear to be particularly useful for establishing the identity of such isolates. For laboratories not wishing to prepare the appropriate reagents, reagent impregnated neisseria identification discs are available commercially (Oxoid, Basingstoke).

Katherine G Reid gratefully acknowledges receipt of a Faculty of Medicine Research Scholarship.

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Immunological identification of Neisseria gonorrhoeae

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Differentiation of gonococcal and non-gonococcal neisseriae by the superoxol test

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SUMMARY We evaluated the superoxol (catalase) test as a means of differentiating gonococci from related species of organisms which were cultured from a variety of anatomical sites. An isolate was almost certainly not a gonococcus if it gave a negative superoxol test result, as all but one of 596 available gonococci gave positive test results. However, a proportion of the non-gonococcal neisseriae and isolates of Branhamella catarrhalis, also gave positive test results. The superoxol test is thus a useful addition to the techniques available for the characterisation of gonococci, but additional procedures are needed to confirm the identities of isolates giving positive results.

Introduction

The isolation of gonococcal and non-gonococcal neisseriae and other related organisms from genital and extragenital sites makes it necessary to distinguish the clinically important isolates. Carbohydrate degradation tests, serological techniques, or both may be used. Where laboratories perform only conventional carbohydrate utilisation tests or fluorescent antibody tests, however, identification of certain Neisseria gonorrhoeae cultures may pose problems. Arko et al found that N meningitidis and Branhamella catarrhalis were the two organisms most often confused with N gonorrhoeae.1 Coagglutination is useful in identifying gonococci from anogenital sites in women and from the urethra in men.2 The frequent occurrence of meningococci in the anorectum of homosexual men3 and in the pharynx limits the value of coagglutination in identifying isolates from these sites because of cross reactivity. Recently Saginur et al described a simple, rapid, low cost screening test for gonococci, the superoxol (catalase) test,4 which was more reliable than coagglutination.

The rationale of the superoxol test was provided by Norrod and Morse,5 who found increased levels of catalase in gonococci compared with other neisseriae. Gonococci could be differentiated from meningococci by using 20% or 30% hydrogen peroxide (superoxol test) but not by the conventional catalase test with 3% hydrogen peroxide.4 Although results were not analysed in relation to culture source, a positive superoxol test result on an isolate growing on Thayer-Martin medium was considered strongly indicative of N gonorrhoeae. As various non-gonococcal neisseriae also gave superoxol positive results, however, biochemical confirmation was necessary. Owing to the high sensitivity of the test, a negative superoxol test result was considered to mean that an isolate was not gonococcal. We evaluated the superoxol test in Edinburgh and Sydney with specific reference to the anatomical source of cultures.

Patients and methods

In Edinburgh, genital or anogenital and pharyngeal material from patients attending the department of genitourinary medicine of the Royal Infirmary was inoculated directly onto modified New York City (MNYC) medium,6 and transported to the laboratory within four hours. Cultures were incubated for 24 hours, and any suspected neisserial colonies were tested by touching the colony with a cotton bud soaked in oxidase reagent; oxidase positive bacteria turn the contact area of the bud purple within five to 15 seconds. If colonies were oxidase positive, identical ones were Gram stained and examined by the superoxol test. If both urethral and cervical cultures were oxidase positive only those from one site, usually the cervix, were examined by the superoxol test. Oxidase positive Gram negative diplococci (GND) were further identified by the rapid carbohydrate utilisation test,7 and also on occasions by coagglutination.2 Cultures giving negative reactions were re-examined after incubation for 48 hours.

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Accepted for publication 13 September 1983

Br J Vener Dis 1984; 60:87-9
STOCK CULTURES
The following stock cultures were also examined by the superoxol test: 16 maltose negative variants of *N. meningitidis* serogroup B (kindly supplied by Dr J A Saenz Nieto, servicio de bacteriologia, Centro Nacional de Microbiologia, Virologia, y Immunologia Sanitarias, Majadahonda, Madrid), and 13 strains of β-lactamase producing *B. catarhalis* isolated from sputum specimens submitted to the clinical bacteriology laboratory of Edinburgh University Medical School for microbiological investigation.

In Sydney, material obtained from patients attending the sexually transmitted diseases clinic at the Prince of Wales Hospital was inoculated onto MNYC medium. After incubation for 24 and 48 hours suspected neisserial colonies were Gram stained and an oxidase test was performed. As a check for purity oxidase positive GNDC were subcultured onto a horse blood agar medium to which was added a 30% saponin solution. The growing colonies were emulsified on the face of a glass slide, and enclosed in a 6% samples were tested for the rapid carbohydrate utilisation test by the patient. Table I shows the results of the superoxol test applied to GNDC isolated from various anatomical sites.

Of the 252 isolates confirmed as being *N. gonorrhoeae* by the rapid carbohydrate utilisation test, 251 (99.6%) gave positive results to the superoxol test. Eight (5.3%) of the 150 non-gonococcal neisseriae also gave positive superoxol test results. Of the 150 non-gonococcal neisseriae, 133 (88.7%) were identified as *N. meningitidis*, 13 (8.7%) as *N. lactamica*, two (1.3%) as *N. perflava* and two (1.3%) as *B. catarhalis*. Four (3.0%) of the meningococcal isolates and three (23.1%) of the *N. lactamica* isolates gave positive superoxol test results.

In Edinburgh 430 oxidase positive GNDC isolates were examined by the superoxol test applied to these strains. All but one of 344 isolates confirmed as *N. gonorrhoeae* by the rapid carbohydrate utilisation test gave positive superoxol test results.

In Sydney 387 oxidase positive GNDC isolates were examined from July 1982 to February 1983. Table II shows the results of the superoxol test applied to these strains. All but one of 344 isolates confirmed as *N. gonorrhoeae* by the rapid carbohydrate utilisation test gave positive superoxol test results.

### TABLE I

<table>
<thead>
<tr>
<th>Identity (by rapid carbohydrate utilisation test)</th>
<th>Source</th>
<th>No of Isolates</th>
<th>Superoxol test results:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td>Urethra</td>
<td>129</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Throat</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Urethra</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Throat</td>
<td>129</td>
<td>4</td>
</tr>
<tr>
<td><em>Neisseria lactamica</em></td>
<td>Throat</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td><em>Neisseria perflava</em></td>
<td>Throat</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Branhamella catarhalis</em></td>
<td>Throat</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>402</td>
<td>259</td>
</tr>
</tbody>
</table>

H Young, A B Harris, and J W Tapsall

Results

In Edinburgh during a four month period, 430 oxidase positive colonies growing on MNYC medium were examined by the superoxol test and were Gram stained; and 402 were shown to be GNDC. The other 28 were Gram negative bacilli, and were not investigated further (27 (96.4%) of them were from throat cultures.) Table I shows the results of the superoxol test applied to GNDC isolated from various anatomical sites.

None of the 16 maltose negative variants of meningococci gave positive superoxol test results, whereas six (46.5%) of the 13 β-lactamase producing strains of *B. catarhalis* isolated from sputum samples gave positive results. The 16 maltose negative meningococci gave a positive superoxol test reaction in the rapid carbohydrate test.

In Sydney 387 oxidase positive GNDC isolates were examined from July 1982 to February 1983. Table II shows the results of the superoxol test applied to these strains. All but one of 344 isolates confirmed as *N. gonorrhoeae* by the rapid carbohydrate utilisation test gave positive superoxol test results.
TABLE II Superoxol test applied to 387 oxidase positive Gram negative diplococci isolated from patients attending the sexually transmitted diseases clinic at the Prince of Wales Hospital, Sydney

<table>
<thead>
<tr>
<th>Identity (by rapid carbohydrate utilisation test)</th>
<th>Source</th>
<th>No of Isolates</th>
<th>Superoxol test results:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Urethra</td>
<td>261</td>
<td>Positive 260</td>
</tr>
<tr>
<td></td>
<td>Cervix</td>
<td>52</td>
<td>Positive 52</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>14</td>
<td>Positive 14</td>
</tr>
<tr>
<td></td>
<td>Throat</td>
<td>16</td>
<td>Positive 16</td>
</tr>
<tr>
<td></td>
<td>Eye</td>
<td>1</td>
<td>Negative 1</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>Throat</td>
<td>34</td>
<td>Negative 4</td>
</tr>
<tr>
<td>Neisseria lactamica</td>
<td>Throat</td>
<td>2</td>
<td>Negative 2</td>
</tr>
<tr>
<td>Neisseria perflava</td>
<td>Throat</td>
<td>1</td>
<td>Negative 1</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>Throat</td>
<td>6</td>
<td>Negative 4</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>387</td>
<td>Equivalent 32</td>
</tr>
</tbody>
</table>

Discussion

These results confirm the findings of Saginur et al.4 regarding the sensitivity of the superoxol test in identifying N gonorrhoeae, with only one of 596 gonococci tested giving a negative result. The test is therefore valuable in screening for gonococcal colonies on culture plates. It should be noted, however, that the superoxol test is not specific for N gonorrhoeae, so that while a negative test result virtually excludes the possibility of an isolate being a gonococcus, other means are required to identify all isolates giving positive results. The Gram stain and oxidase tests are necessary procedures in this process, and either coagglutination or carbohydrate utilisation tests would provide satisfactory confirmation for urethral isolates in men or for strains from genital sites in women.2

This study has shown, however, that the superoxol test is of less value for identifying isolates from the pharynx and rectum, as it gives positive results with a considerable number of meningococci, other neisseriae, and the related B catarrhalis. These organisms may be isolated on selective media9 and were grown on the media used in this study. It has been shown that the value of coagglutination in identifying strains from the pharynx and the rectum is reduced by cross reactivity between gonococci and other organisms.2 Rapid carbohydrate utilisation tests would, however, appear to be particularly useful for strains from these sites.18 This is supported by the finding that meningococci, which are maltose negative by conventional growth dependent degradation tests, are maltose positive by the rapid utilisation tests. Odugbemi and Arko found a negative superoxol test result valuable in differentiating Kingella denitrificans from N gonorrhoeae.10 K denitrificans has a colonial morphology very similar to that of the gonococcus, is coccoïd to rod shaped, and produces acid from glucose. The superoxol test therefore seems to be a useful procedure for identifying gonococci if it is used in conjunction with currently available techniques and its limitations are recognised.

References

The Pathogenic Neisseriae
Proceedings of the Fourth International Symposium, Asilomar, California, 21-25 October 1984

Editor: Gary K. Schoolnik
Coeditors: Geo. F. Brooks
          Stanley Falkow
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American Society for Microbiology
Washington, D.C. 1985
Serogrouping of *Neisseria gonorrhoeae*: Correlation with Site of Infection

KATHERINE G. REID,1 H. YOUNG,1 AND A. MCMILLAN2

Department of Bacteriology, University Medical School,1 and Department of Genitourinary Medicine, Royal Infirmary,2 Edinburgh, Scotland

A total of 339 clinical isolates of *Neisseria gonorrhoeae* from infections acquired in the Edinburgh area over two consecutive 6-month periods were studied. Isolates were serogrouped by using coagglutination reagents prepared with polyclonal antibodies defining the three coagglutination W serogroups, WI, WII, and WIII. The overall distribution of serogroups for the two periods was the same: 44% of strains belonged to serogroup WI and 56% belonged to serogroup WII. A significantly higher proportion of WII strains was found among isolates from homosexual men than among those from heterosexual men and women (P < 0.001). Serogroup WII strains predominated in isolates from homosexual men regardless of site of isolation. Over the two time periods, a significant change in the proportion of serogroups was seen in heterosexual men; in the first study 66% of male isolates belonged to serogroup WI whereas in the second period 41% belonged to serogroup WI (P < 0.01). This difference resulted from a change in the distribution of serogroups among urethral isolates. In a retrospective study no correlation was found between the serogroup of the infecting strain and the presence or absence of symptoms in women. A comparison of the serogroups of isolates from 20 women and their respective partners, from 1 woman and her 2 partners, and from 1 woman, her husband, and her child showed that those for each set of contacts matched.

The serological classification of *Neisseria gonorrhoeae* by coagglutination was first developed by Sandström and Danielsson (16). By using reagents prepared with antisera against selected major outer membrane protein (MOMP) reference strains (11), clinical isolates can be serogrouped into three groups, WI, WII, and WIII. It has recently been shown that the W serogroups are based upon antigenic differences in the protein I molecules of the outer membrane (17). Serogroups WI and WII correspond to two mutually exclusive forms of protein I, protein IA and protein IB, respectively (15). It appears that serogroup WIII represents a minor but prominent antigenic variant of protein IB rather than a third specific moiety (15). A number of studies using reagents prepared with selectively adsorbed antisera have been carried out to determine the epidemiological and clinical value of the coagglutination serogrouping system. Differences in the geographical distribution of serogroups have been described (5, 9). In Sweden WII strains have been shown to predominate in large towns whereas WI strains predominate in smaller towns (9); WIII strains are rarely isolated and are much more common in the Far East (7, 15). Recently, coagglutination reagents prepared with monoclonal antibodies have been used in similar studies (6). Serogroup WI has been associated with disseminated gonococcal infections and with the Arg− Hxy− Ura− auxotype (9). In addition, WI strains have been shown to have greater susceptibility to antibiotics than strains from serogroups WII and WIII (3). Strains of all three serogroups isolated in the Far East have been shown to be more resistant than similar strains isolated in Europe (3), and multiresistance has been genetically linked to determinants for serogroup WII (4). Serogroup WII has been strongly associated with homosexually acquired infection (2, 13). However, no correlation had been made between W serogroup and anatomical sites other than the rectum. Moreover, the distribution of W serogroups in the United Kingdom has not been reported. In addition to studying W serogroups in relation to patient group and site of isolation, we

66
wished to determine the correlation of serogroup of infecting strains with the presence or absence of symptoms in women.

MATERIALS AND METHODS

A total of 339 clinical isolates of *N. gonorrhoeae* were obtained from the Department of Genitourinary Medicine, Royal Infirmary, Edinburgh, over two consecutive 6-month periods. All isolates were identified as *N. gonorrhoeae* by the rapid carbohydrate utilization test (19). The strains were serogrouped by using reagents prepared with selectively adsorbed polyclonal antibodies as previously described (14, 16). Antisera prepared against MOMPs E-5, D-4, and V-15 represented serogroup WI; antisera against MOMPs N-10, S-12, and U-14 represented serogroup WII; and antisera against MOMP F-6 represented serogroup WIII. Coagglutination serogrouping was carried out by mixing 20 µl of boiled suspensions of clinical isolates with 20 µl of reagent as described previously (14).

A retrospective study of the case notes of 52 women patients in whom gonorrhea was diagnosed and other genital infections were excluded was carried out to correlate the coagglutination serogroup of the infecting strain with presence or absence of symptoms. Only one isolate per patient, generally a cervical isolate, was used for analysis of clinical data.

All statistical analysis was carried out by the χ² test with Yates's correction.

RESULTS

All strains of *N. gonorrhoeae* tested in the two study periods, November 1982-June 1983 and July-December 1983, were serogrouped.

Table 1 shows the distribution of coagglutination W serogroups within patient groups for the first study period. Overall, 44% of strains belonged to serogroup WI and 56% belonged to serogroup WII. Serogroup WII accounted for 90% of all isolates from homosexual men and for 34% and 47% of isolates from heterosexual men and women, respectively. This difference in distribution of serogroup WII among gonococcal isolates from homosexual men and the heterosexual patients is highly significant (χ² = 36.5; *P* < 0.001). A higher proportion of isolates from male homosexuals belonged to serogroup WI (66%) when compared with isolates from women (53%). However, this difference is not significant (χ² = 1.96; *P* > 0.05). In the second study period (Table 2), the overall distribution of coagglutination serogroups was unchanged: 44% of strains belonged to serogroup WI and 56% belonged to serogroup WII. Two isolates, one from a homosexual man and one from a heterosexual man, reacted with both the WI and WII reagents. No isolates belonged to serogroup WIII. In the second study period all isolates from homosexual men belonged to serogroup WII compared with 59% of those from heterosexual men and 49% of those from women. Thus, the distribution of coagglutination serogroups among homosexual men remains significantly different from that in the heterosexual population. However, the major difference in the second period of study was the increase in the number of WII strains isolated from heterosexual men. In the first study 34% of isolates belonged to serogroup WII compared with 58% in the second study period. This is a significant difference (χ² = 7.58; 0.01 > *P* > 0.001).

However, there is no significant difference between the distribution of coagglutination serogroups when isolates from heterosexual men are compared with those from women for the second period. The overall distribution of serogroups

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. (%) of strains within serogroup:</th>
<th>Total strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WI</td>
<td>WII</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>0 (0)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>38 (41)</td>
<td>53 (58)</td>
</tr>
<tr>
<td>Women</td>
<td>51 (51)</td>
<td>49 (49)</td>
</tr>
<tr>
<td>Total</td>
<td>89 (44)</td>
<td>113 (55)</td>
</tr>
</tbody>
</table>

TABLE 1. Distribution of coagglutination serogroups among homosexual men and heterosexual men and women for the first study period, November 1982-June 1983

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. (%) of strains within serogroup:</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WI</td>
<td>WII</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>0 (0)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>38 (41)</td>
<td>53 (58)</td>
</tr>
<tr>
<td>Women</td>
<td>51 (51)</td>
<td>49 (49)</td>
</tr>
<tr>
<td>Total</td>
<td>89 (44)</td>
<td>113 (55)</td>
</tr>
</tbody>
</table>

TABLE 2. Distribution of coagglutination serogroups among homosexual men and heterosexual men and women for the second study period, July-December 1983
among isolates from women in the second period is very similar to that found in the first period.

Distribution of serogroups in relation to site of isolation. The distribution of coagglutination serogroups in relation to site of isolation for the first period is shown in Table 3. In homosexual men, serogroup WII predominated regardless of the anatomical site from which the strain was isolated. This held true for the second study period also (Table 4). In the first study period (Table 3) female genital isolates (mostly cervical isolates) were evenly distributed between serogroups WI and WII. The same distribution was also seen in the second study period (Table 4).

However, the main difference was found in urethral isolates from heterosexual men. In the first study period (Table 3), 69% of urethral isolates belonged to serogroup WII, whereas in the second period (Table 4), 43% belonged to serogroup WII (χ² = 7.27; P < 0.01).

Correlation of symptoms with serogroup of infecting strain. Table 5 shows the correlation of symptoms with the coagglutination serogroup of the infecting strain for 52 women. Overall 30 of 52 women (58%) had asymptomatic infections. In those presenting with symptoms, 15 had a vaginal discharge not associated with other vaginal pathogens such as Candida or Trichomonas. 3 had dysuria, 3 had pelvic inflammatory disease, and 1 had a disseminated gonococcal infection. Of the 22 women with symptoms, 55% had infections with gonococcal strains of serogroup WI compared with 47% of the 30 women with asymptomatic infection. Although women with symptomatic infections had a slightly higher proportion of WI strain than women with asymptomatic infections, this difference is not significant (χ² = 0.078; P > 0.5).

Study of isolates from 22 women and their respective partners. The serogroup of gonococcal isolates from 20 women and their respective partners, 1 woman and her 2 partners, and 1 woman, her husband, and her child were compared. The serogroups of isolates from each respective set of contacts were found to match.

**DISCUSSION**

This study confirms the previously reported association between gonococcal strains of coagglutination serogroup WII and homosexually acquired infection (2, 13). In addition, we were able to show that this association holds true regardless of the

---

### TABLE 3. Serogroup in relation to anatomical site of isolation for the first study period, November 1982-June 1983

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Anatomical site</th>
<th>No. (%) of strains within serogroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WI</td>
</tr>
<tr>
<td>Women</td>
<td>Throat</td>
<td>9 (50)</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>11 (58)</td>
</tr>
<tr>
<td></td>
<td>Genital</td>
<td>21 (50)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>1*</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>Throat</td>
<td>4 (50)</td>
</tr>
<tr>
<td>men</td>
<td>Urethra</td>
<td>33 (69)</td>
</tr>
<tr>
<td>Homosexual</td>
<td>Throat</td>
<td>3 (19)</td>
</tr>
<tr>
<td>men</td>
<td>Rectum</td>
<td>2 (6)</td>
</tr>
<tr>
<td></td>
<td>Urethra</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>85 (44)</td>
</tr>
</tbody>
</table>

* Right hip aspirate.

### TABLE 4. Serogroup in relation to anatomical site of isolation for the second study period, July-December 1983

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Anatomical site</th>
<th>No. (%) of strains within serogroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WI</td>
</tr>
<tr>
<td>Women</td>
<td>Throat</td>
<td>9 (56)</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>9 (47)</td>
</tr>
<tr>
<td></td>
<td>Genital</td>
<td>33 (51)</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>Throat</td>
<td>4 (33)</td>
</tr>
<tr>
<td>men</td>
<td>Urethra</td>
<td>34 (43)</td>
</tr>
<tr>
<td>Homosexual</td>
<td>Throat</td>
<td>0</td>
</tr>
<tr>
<td>men</td>
<td>Rectum</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urethra</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>89 (44)</td>
</tr>
</tbody>
</table>

### TABLE 5. Correlation of symptoms with serogroup of infecting strain

<table>
<thead>
<tr>
<th>Symptoms present</th>
<th>No. (%) of strains within serogroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WI</td>
</tr>
<tr>
<td>Yes</td>
<td>12 (55)</td>
</tr>
<tr>
<td>No</td>
<td>14 (47)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (50)</td>
</tr>
</tbody>
</table>
anatomical site from which the strains are isolated. A much higher proportion of strains isolated from homosexual men belonged to serogroup WII (approximately 90% in the first period and 100% in the second period) than in previously reported studies. Bydgosman in Stockholm, Sweden, and Morse et al. in Seattle, Wash., reported respectively that 30 of 39 (76.9%) and 42 of 57 (74.0%) gonococcal strains isolated from homosexual men belonged to serogroup WII (2, 13). Morse et al. suggested that in homosexual men the rectal environment, which is rich in hydrophobic molecules such as fecal lipids, selects gonococcal strains with mutations (mtr) that reduce the permeability of the outer membrane to hydrophobic molecules (13). It was proposed that the same environment may select strains with the particular protein I molecule detected by serogroup WII reagents. Although these authors suggested that antibiotic pressure may be involved in this selection, it was not considered the main driving force (13). Our results may suggest that once the characteristic of serogroup WII is acquired, it remains stable regardless of anatomical site of isolation. Alternatively, it is possible that some other force acting at all anatomical sites exerts a selection pressure in favor of serogroup WII.

Although the overall distribution of coagglutination serogroups remained unchanged over the two study periods, there was a significant ($\chi^2 = 3.9; P < 0.05$) change in the distribution of serogroups among strains isolated from heterosexual patients. In the first study period 79 of 136 (58%) isolates from heterosexual patients (men and women) belonged to serogroup WII compared with 89 of 192 (46%) in the second study period. This change in the distribution of coagglutination serogroups within the heterosexual population was caused by a significantly higher ($P < 0.01$) isolation rate of WII strains from heterosexual men in the second study period: 58% compared with 34% in the first period. There was no significant change in the distribution of coagglutination serogroups for gonococcal strains isolated from women over the two time periods.

Among the possible reasons for this change in distribution of serogroups among isolates from heterosexual men are seasonal influx of strains from elsewhere, buildup of immunity to serogroup WI strains, failure to detect WI infections, and inclusion of an undeclared group of homosexual men in the heterosexual population.

The second study period included the summer months when there is a large influx of tourists into the Edinburgh area, particularly during the Edinburgh International Festival. A proportion of heterosexual men attending the Department of Genitourinary Medicine during this period may therefore have acquired their infections from sources outside the Edinburgh area. This influx may lead to a seasonal variation in the distribution of serogroups among heterosexual men such as the one observed here. Alternatively, the influx may lead to a continuing long-term change in the distribution of coagglutination serogroups. Such a change has been reported in Sweden (6). Over a 3-year period, the proportion of WII strains in Stockholm gradually increased to approximately 70% overall (6), whereas in small Swedish towns WI strains predominated, with an approximate isolation rate of 80% (5, 9).

An immunological model has been proposed to explain differences in the distribution of coagglutination serogroups between men and women, and also between major cities and small towns in Sweden (6). It has been shown by using monoclonal serogrouping reagents that among WI strains isolated from major Scandinavian cities there is usually one dominating WI serovar with a range of only 1 to 5 WI serovars (6), whereas with WI strains the range is greater, 6 to 17, with no particular serovar dominating. With the assumption that protective antibodies are produced, it has been proposed that the likelihood of being reinfected with a WI strain of a different serovar is very small but is much greater with WI strains. The effect of this would be to reduce the overall number of WI strains in the population. Although it has been reported that in cases of complicated gonococcal infection such as salpingitis, antibodies may protect against reinfection with gonococcal strains of the same protein I serotype (1), it is unclear whether protective antibodies play an active role in localized gonococcal infections. The number of “repeat” infections in the total population would also have a significant effect on the dynamics of this immunological model. Hence, at present we can only speculate as to the effect of immunity on the distribution of coagglutination serogroups among the sexually active population.

It has also been suggested that the lower number of WI strains in men may be a result of undetected asymptomatic infections. Available data suggest that only 10 to 15% of heterosexual men have asymptomatic infections (18), although this figure may be higher in male contacts of women with pelvic inflammatory disease (10). Asymptomatic urethral infections in men have been reported to be caused predominantly by strains of the Arg\textsuperscript{+} Hx\textsuperscript{+} Ura\textsuperscript{+} auxotype (8), an auxotype common among WI strains (9). In contrast, in homosexual men where there is a very high rate (70 to 75%) of asymptomatic pharyngeal and rectal infections (2, 12), serogroup WII predominates at these sites. Although the highest occurrence (approximately 60%) of asymptomatic urethral infections occurs in women, serogroup correlation studies are lacking. In a retrospective study of 52 women, 58% of whom were asymptomatic, we found no significant association between serogroup W1 and asymptomatic infections in Edinburgh.
During the second study period, there was a large reduction in the number of men attending the Department of Genitourinary Medicine who declared that their infections were homosexually acquired. This period coincided with a great deal of publicity on the acquired immune deficiency syndrome which probably caused some homosexual men to alter their patterns of behavior. This may have led to an unknown number of homosexual men being counted among the heterosexual men studied, thus contributing toward the swing to serogroup W11 observed in the second study.

When determining causes for the differences observed in the distribution of coagglutination serogroups between men and women and between different geographical areas encompassing large cities, it is crucial to prevent a bias toward serogroup W11. Therefore, we strongly recommend that where there is a known homosexual component in the population studied the serogrouping data for this group be considered separately.

ACKNOWLEDGMENT

K.G.R. gratefully acknowledges receipt of a Faculty of Medicine Scholarship.

LITERATURE CITED


CORRELATION OF CELL-ENVELOPE PHENOTYPES OF NEISSERIA GONORRHOEAE WITH SITE OF INFECTION AND SEROGROUP

KATHERINE G. REID, JANET WARBRICK AND H. YOUNG

Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG

SUMMARY. The envelope phenotypes and coagglutination (CoA) W serogroups of 301 unselected clinical isolates of Neisseria gonorrhoeae were studied. Of the 287 isolates from infections acquired in the Edinburgh area, 252 (88%) were of the wild-type phenotype, 17 (6%) were of Env phenotype with increased permeability of the cell envelope, and 18 (6%) were of Mtr phenotype with reduced permeability of the cell envelope. Mtr strains were isolated significantly more often from homosexual men than from heterosexual men and women (p<0.001). Of the isolates from homosexual men there were considerably fewer Mtr phenotypes among rectal isolates than among urethral and throat isolates. All isolates from homosexual men, 57% from heterosexual men and 52% from women were of CoA serogroup WII (including WII/ WIII). Although the Mtr phenotype was strongly associated with serogroup-WII isolates from homosexual men, there was no such correlation between the Mtr phenotype and serogroup-WII isolates from heterosexual patients. It is suggested that hydrophobic compounds in the rectal environment are not the major factor in selecting serogroup-WII strains of Mtr phenotype associated with homosexually acquired infection. The hypothesis that the Mtr phenotype and serogroup WII are selected independently as a result of a more general selective pressure such as antibiotic usage, common to all infected sites, is discussed.

INTRODUCTION

Cell-envelope mutations of Neisseria gonorrhoeae alter the organism’s permeability and hence its sensitivity to various hydrophobic agents, including antibiotics (Maness and Sparling, 1973; Maier et al., 1974, 1975a and b).

Two separate mutations of this sort designated env (Maness and Sparling, 1973) and mtr (Maier et al., 1974) influence the organism’s sensitivity to these agents by altering both the amount of a minor protein (mol. wt 52 000) and peptidoglycan cross-
linkage in the outer membrane (Guyman et al., 1978). Thus an env mutant, which has increased membrane permeability, is markedly more sensitive (hypersensitive) and an mtr mutant, which has reduced permeability, is much more resistant than a representative wild-type strain. The env mutation suppresses the mtr locus if the two occur in the same strain (Sarubbi et al., 1975). Gonococci with mtr mutations have been associated with homosexually-acquired infections (Morse et al., 1982).

Gonococci can be divided into three serogroups—WI, WII and WIII—by coagglutination (CoA) serogrouping (Sandstrom and Danielsson, 1980). CoA depends on the principle that immunoglobulin G binds by its Fc portion to protein A on the surface of *Staphylococcus aureus* leaving the Fab site free to bind with the corresponding or related antigen to produce a coagglutination matrix easily visible to the naked eye. In gonococcal serogrouping, specific antibody raised against reference strains of gonococci (Johnston et al., 1976) are selectively absorbed to produce coagglutination reagents which define the three major serogroups, WI, WII and WIII.

Strains of CoA serogroup WII have been associated with homosexually-acquired infection (Bygdeman, 1981a; Reid and Young, 1984) and reduced sensitivity to antibiotics (Bygdeman, 1981b). Strains isolated from homosexual men often have both the mtr and CoA serogroup WII characteristic (Morse et al., 1982). Although both confer resistance to antibiotics they represent two separate loci (Morse et al., 1982).

It has been suggested that the mtr mutation and CoA serogroup WII are selected by the rectal environment, which is rich in hydrophobic molecules. Accordingly, the present study was undertaken to increase our understanding of the role of different anatomical sites within the host in the selection of gonococcal strains with particular outer-membrane properties.

**Materials and methods**

**Clinical Isolates.** During the period July 1983 to May 1984, 301 isolates of *N. gonorrhoeae* were obtained from patients attending the Department of Genito-Urinary Medicine, The Royal Infirmary, Edinburgh. Of these strains, 14 were from patients who had acquired their infections either elsewhere in Britain or abroad. All isolates were identified as *N. gonorrhoeae* by the rapid carbohydrate utilisation test (Young et al., 1976).

**Reference Strains.** *N. gonorrhoeae* strains with known genotypes were kindly supplied by Dr P. F. Sparling, University of North Carolina, Chapel Hill, USA. These were FA140 (pen A2 mtr-2 pen B2), FA102 (pen A2), FA19 (wild type), BR87 (env-2 str-7 pen A2 mtr-2 pen B2) and FA136 (pen A2 mtr-2). All strains were maintained on MNYC medium (Young, 1978) and envelope phenotypes were determined on the same medium lacking selective antibiotics (SMNYC medium).

**Determination of cell-envelope phenotype.** Each isolate was tested for sensitivity to the following hydrophobic compounds: erythromycin (Abbot Laboratories Ltd, Queenborough, Kent ME11 5EL), fusidic acid (Leo Laboratories Ltd, Princes Risborough, Bucks), Triton X-100 (Fisons Scientific Apparatus, Loughborough, Leics) and crystal violet (Koch-Light Laboratories, Colnbrook, Berks).

A 19-point hand-held replicator (Mast Laboratories Ltd, Mast House, Serby Road, Bootle, Merseyside) was used to seed separate plates of SMNYC medium containing: erythromycin (doubling concentrations from 0.015 to 2.0 mg/L); crystal violet (doubling concentrations from 0.5 to 4.0 mg/L); fusidic acid (0.03, 0.25 and 0.5 mg/L); and Triton X-100 (0.06, 1.0 and 2.0 g/L). The inoculum was c. 10^3 cfu of an overnight culture of each test strain.

Each batch of plates was set up in duplicate and included the reference strains FA140, BR87, FA19 and FA136. After incubation for 48 h at 37°C in air with 10% CO_2, plates were examined and the MIC determined as the lowest concentration of each agent that prevented visible growth.
Strains with the following MICs, which were given by the Mtr reference strains FA140 and FA136, were designated the Mtr phenotype: erythromycin $\geq 0.5$ mg/L, fusidic acid $\geq 0.5$ mg/L, crystal violet $\geq 2.0$ mg/L and Triton X-100 $\geq 2.0$ mg/L. Strains with the following MICs, which were given by the Env reference strain BR87, were designated the Env phenotype: erythromycin $\leq 0.06$ mg/L, fusidic acid $\leq 0.03$ mg/L, crystal violet $\leq 0.5$ mg/L and Triton X-100 $\leq 0.06$ mg/L.

The wild-type reference strain FA19 had the following MICs: erythromycin 0.12 mg/L, fusidic acid 0.25 mg/L, crystal violet 1.0 mg/L, and Triton X-100 1.0 mg/L. Test strains with erythromycin MICs of 0.12-0.25 mg/L were designated wild type.

Serogrouping by coagglutination. Coagglutination reagents defining CoA serogroups WI, WII, WIII were prepared and isolates serogrouped as described by Reid and Young (1984).

Statistical analysis. Significance was tested by the $\chi^2$ method with Yates's correction.

RESULTS

Table I shows the distribution of cell-envelope phenotypes among isolates from infections acquired in the Edinburgh area by homosexual contact. There is a highly significant difference in the proportion of wild-type to Env and Mtr phenotypes when gonococcal isolates from 20 homosexual men are compared with those from 116 heterosexual men ($\chi^2 = 23.96; p < 0.001$) and 151 women ($\chi^2 = 25.89; p < 0.001$). There is no such significant difference when isolates from heterosexual men and women are compared ($\chi^2 = 0.01; p = 0.9$). The envelope phenotypes of fourteen isolates from infections acquired outwith the Edinburgh area are given in table II. Seven (50%) of these 14 isolates were of the Mtr phenotype. Of the five isolates linked with Thailand, four were from heterosexual men whereas the two Mtr strains linked with London were from homosexual men.

Table III shows the distribution of envelope phenotype in relation to site of isolate and homosexual or heterosexual acquisition. The proportion of Mtr-phenotype strains among rectal isolates from homosexual men is not significantly different from the proportion of Mtr strains among rectal isolates from women ($\chi^2 = 0.04; p > 0.8$). However, a significantly higher proportion of urethral isolates from homosexual men had the Mtr phenotype when compared with genital isolates from heterosexual men and women ($\chi^2 = 21.3; p < 0.001$). Likewise, a significantly higher proportion of throat isolates from homosexual men had an Mtr phenotype when compared with throat isolates from heterosexual men and women ($\chi^2 = 10.8; p = 0.001$). With the isolates

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Env</th>
<th>Wild type</th>
<th>Mtr</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexual men</td>
<td>0(0)</td>
<td>12(60)</td>
<td>8(40)</td>
<td>20</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>9(8)</td>
<td>103(89)</td>
<td>4(3)</td>
<td>116</td>
</tr>
<tr>
<td>Women</td>
<td>8(5)</td>
<td>137(91)</td>
<td>6(4)</td>
<td>151</td>
</tr>
<tr>
<td>Total</td>
<td>17(5.9)</td>
<td>252(87.8)</td>
<td>18(6.3)</td>
<td>287</td>
</tr>
</tbody>
</table>
TABLE II
Envelope phenotype and serogroup of 14 isolates from male infections acquired outwith the Edinburgh area

<table>
<thead>
<tr>
<th>Country or area of infection acquired</th>
<th>Envelope phenotype</th>
<th>CoA serogroup</th>
<th>Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thailand</td>
<td>Mtr</td>
<td>WII</td>
<td>Homosexual</td>
</tr>
<tr>
<td>Thailand</td>
<td>Mtr</td>
<td>WII</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>Thailand</td>
<td>Mtr</td>
<td>WII</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>Thailand</td>
<td>Mtr</td>
<td>WII/III</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>Kenya</td>
<td>Wild type</td>
<td>WII</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>Spain</td>
<td>Wild type</td>
<td>WII</td>
<td>Homosexual</td>
</tr>
<tr>
<td>Germany</td>
<td>Wild type</td>
<td>WII</td>
<td>Homosexual</td>
</tr>
<tr>
<td>London</td>
<td>Mtr</td>
<td>WII</td>
<td>Homosexual</td>
</tr>
<tr>
<td>London</td>
<td>Mtr</td>
<td>WII</td>
<td>Homosexual</td>
</tr>
<tr>
<td>England</td>
<td>Wild type</td>
<td>WII</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>Northern Ireland</td>
<td>Wild type</td>
<td>WII</td>
<td>Heterosexual</td>
</tr>
<tr>
<td>Inverness</td>
<td>Wild type</td>
<td>WII</td>
<td>Heterosexual</td>
</tr>
</tbody>
</table>

TABLE III
Cell-envelope phenotype in relation to patient group and site of isolation

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Site of isolation</th>
<th>Number (and percentage) of isolates with the following envelope phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Env</td>
<td>Wild type</td>
</tr>
<tr>
<td>Homosexual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>U</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>0(0)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>0(0)</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>U</td>
<td>9(9)</td>
</tr>
<tr>
<td>Men</td>
<td>T</td>
<td>0(0)</td>
</tr>
<tr>
<td>Women</td>
<td>G</td>
<td>4(4)</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>2(7)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>2(11)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>17(5-9)</td>
</tr>
</tbody>
</table>

U = urethra; G = female genital; R = rectum; T = throat.

From homosexual men there were considerably more Mtr phenotypes among urethral and throat isolates than among rectal isolates ($\chi^2 = 3.71; 0.1 > p > 0.05$).

Table IV shows the distribution of envelope phenotype in relation to CoA serogroup and patient group. All isolates from homosexual men, 57% from heterosexual men and 52% from women were of serogroup WII (including WII/III). With regard to envelope phenotype, 13 (72-2%) of the 18 Mtr strains and 151 (56-1%) of the 269 non-Mtr strains were serogroup WII; this difference is not significant at the 5% level ($\chi^2 = 1.2; p > 0.2$). However, the Mtr phenotype occurred significantly more often among WII isolates from homosexual men than among WII isolates from heterosexual men ($\chi^2 = 14.3; p < 0.001$) and women ($\chi^2 = 20.4; < p 0.001$). In heterosexual men and women there was no significant difference in the distribution of Mtr
Table IV

Relationship of cell-envelope phenotype with patient group and CoA W serogroup

<table>
<thead>
<tr>
<th>Patient group</th>
<th>CoA serogroup</th>
<th>Number (and percentage) of isolates with the following envelope phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexual men</td>
<td>WI</td>
<td>0(0) 0(0) 0(0) 0</td>
</tr>
<tr>
<td></td>
<td>WII or WII/III</td>
<td>0(0) 12(60) 8(40) 20</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>WI</td>
<td>6(12) 43(86) 1(2) 50</td>
</tr>
<tr>
<td></td>
<td>WII or WII/III</td>
<td>3(4-5) 60(91) 3(4-5) 66</td>
</tr>
<tr>
<td>Women</td>
<td>WI</td>
<td>4(5-5) 65(89) 4(5-5) 73</td>
</tr>
<tr>
<td></td>
<td>WII or WII/III</td>
<td>4(5) 72(92) 2(3) 78</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>17(5-9) 252(87-8) 18(6-3) 287</td>
</tr>
</tbody>
</table>

*A total of 17 strains serogrouped as WII/III: 3 from homosexual men; 5 from heterosexual men and 9 from women.

Discussion

Of 287 unselected gonococcal strains isolated from infections acquired in the Edinburgh area 252 (87-8%) had a wild-type phenotype, 17 (5-9%) had an Env phenotype and 18 (6-3%) had Mtr phenotypes. Morse et al. (1982) found 128 (82-9%) wild type strains, 7 (4-6%) Env strains and 19 (12-5%) Mtr strains among 152 isolates collected during a 5-year period. Although the genotypes of our isolates were not confirmed by transformation experiments, the proportions of Env and wild-type phenotypes are similar in the two studies. In spite of a significantly lower proportion of Mtr strains in our series (p < 0-05) we confirm the findings of Morse et al. (1982) that Mtr strains are significantly more prevalent among isolates from homosexual men than among those from heterosexual men and women (p < 0-001). However, our findings cast doubt on the hypothesis (Morse et al., 1982) that the rectal environment, essential for the transmission of gonococci within the homosexual population, exerts a selective pressure favouring the emergence of the Mtr phenotype. We found Mtr strains considerably more often among urethral and throat isolates from homosexual men than among rectal isolates; and Mtr strains were no more common among male rectal isolates than female rectal isolates (p > 0-8).

The lack of association between the Mtr phenotype and male rectal isolates was surprising. Eisenstein and Sparling (1978) reported that the mtr mutation consistently resulted in reduced rates of exponential growth in enriched broth cultures, whereas introduction (by transformation) of an env mutation resulted in partial restoration to normal growth rates in vitro. Therefore, mtr mutants may be less able to establish rectal infection as their poorer growth characteristics could make them less capable of competing with the complex microbial flora of the rectum.
In homosexual men, serogroup WII predominates regardless of anatomical site of isolation and has been shown to account for 94%, 90% and 81% of rectal, urethral and throat isolates respectively (Reid and Young, 1984). Although the Mtr phenotype has been reported to be strongly correlated with CoA serogroup WII (Morse et al., 1982), our data (table IV) show that this applies only to WII strains isolated from homosexual men; strains isolated from heterosexuals show no correlation between Mtr and CoA serogroup WII. It would appear, therefore, that CoA serogroup WII and the Mtr phenotype are selected independently in the homosexual population rather than by a close genetic linkage.

Our results suggest that the rectal environment is not responsible for selection of Mtr phenotypes. The report of an Mtr-independent system (McFarland et al., 1983) for resistance to faecal lipids also casts doubt upon the role of hydrophobic compounds in the rectal environment in selecting gonococcal strains with reduced membrane permeability. We consider it likely that the rectal environment is not responsible for selecting serogroup WII but that a more general selective pressure such as exposure to antibiotics common to all infected sites is the driving force for CoA WII and Mtr selection in isolates from homosexual men. The following associations between serogroups and antibiotic sensitivity observed in other geographical areas support our hypothesis.

Gonococcal strains with decreased susceptibility or so-called multi-resistant strains belong to serogroups WII and WIII whereas those highly sensitive to β-lactam antibiotics, tetracyclines and other antibiotics belong to serogroup WI (Bygdeman, 1981b). Bygdeman et al. (1982) confirmed a genetic linkage between serogroup specificity and antibiotic multi-resistance. Although the resistance pattern of the WII transformants studied was very similar to that resulting from the mtr locus, there was no increase in the 52 000-mol. wt protein associated with the mtr mutation. Further transformation experiments are required to determine the precise nature of the genetic locus.

Bygdeman et al. (1983) considered that greater antibiotic pressure in larger towns than in smaller ones might account for their observed higher incidence of WII strains in larger towns. Isolates from infections acquired in the Far East, where antibiotic pressure is very high because of the lack of antibiotic control, exhibit reduced sensitivity to a wide variety of antibiotics and are predominantly of serogroups WII and WIII (Bygdeman, 1981b). Our serogrouping of a limited number of strains supports these findings and demonstrates a high incidence of the Mtr phenotype among heterosexual men (table II).

The similarity in the serogroup and envelope phenotype of isolates from homosexual men and those from the Far East suggests that the same selective pressure may be responsible. Homosexual men experience a wider spectrum and a higher rate of sexually transmitted diseases than heterosexual men (Fluker, 1976; Wilcox, 1981). Although difficult to prove, it is likely that overall antibiotic usage is greater among homosexual men than among heterosexuals. Evidence for antibiotic selection independent of serogroup WII was reported by Bygdeman (1981a) who found that serogroup-WI isolates from homosexual men were more resistant to penicillin than were unselected WI isolates.

It is not unexpected that such differences which most likely result from antibiotic pressure should also occur within serogroup WI because the different protein antigens
representing serogroups WI, WII and WIII act as “porins”, enabling selective passage of molecules across the cell wall (Douglas et al., 1981). However, the proteins IA (representing serogroup WI) and IB (representing serogroups WII and WIII) (Sandstrom et al., 1982) have different structures and orientations in the outer membrane (Barrera and Swanson, 1984) and differ significantly in apparent pore size (Blake, personal communication cited by Barrera and Swanson, 1984). These differences may account for the preferential selection, in response to antibiotic pressure, of protein-IB antigens among isolates from homosexual men.

We thank Professor J. G. Collee for his helpful advice in the preparation of this paper. K. G. R. gratefully acknowledges receipt of a Faculty of Medicine Scholarship.

REFERENCES


Leucocytes in the Ejaculate from Fertile and Infertile Men

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University Department of Surgery/Urology, Western General Hospital; Department of Bacteriology and Medical Computing and Statistics Unit, University Medical School, Edinburgh

Summary—The presence of leucocytes and their subpopulations was studied in the ejaculate from 69 men with an infertile marriage and 12 fertile men. Monoclonal antibodies specific for human leucocytes were used in an immunoperoxidase technique. In addition to the standard sperm analysis, each specimen was also tested for sperm antibodies, aerobic and anaerobic micro-organisms, Ureaplasma urealyticum and Chlamydia trachomatis. Leucocytes were found in large numbers in the fertile men compared with the patients. Lymphocytes were found in 20% of the patients. Micro-organisms were cultured from a similar proportion of both groups. We found no correlation between leucocyte counts, sperm density and motility, sperm antibodies and growth of micro-organisms. Our results cast doubt on the conventional criteria of subclinical genital tract infection, namely positive culture and excess leucocyte counts.

The role of subclinical genital tract infection in male infertility is controversial. Some studies have shown no difference in the results of culture for micro-organisms from semen samples from fertile and infertile populations and no correlation between sperm measurements (motility and density) and bacteriology. Comparing semen characteristics of fertile and infertile groups, McGowan et al. (1981) found that infection in the semen did not significantly affect the count, motility or volume of the specimen. Comhaire et al. (1980) and Naessens et al. (1986) also found no significant differences in the semen motility and density of infertile males with and without infection.

Others believe that there is a substantial proportion of infertile men with very minor symptoms but significant infection. Eliasson et al. (1967) showed that approximately 40% of infertile men in Sweden had cytological or bacteriological findings indicating infection or inflammation of the accessory genital glands. They suggested that asymptomatic genital infection may be a major cause of infertility (Eliasson and Johannisson, 1978). Queseda et al. (1968), Moberg et al. (1980) and Swenson et al. (1986) also reported that infection of the semen may reduce sperm motility and density and hence affect fertility. There are two important reasons for this controversy. The first is a lack of agreement about criteria to diagnose subclinical genital tract infection and the second is that in many studies there has been a failure to culture the complete range of pathogenic micro-organisms; in most cultures Chlamydia has been omitted.

Traditional diagnosis is made by finding an excess of leucocytes in the ejaculate. However, it is difficult to distinguish leucocytes from the immature sperm cell using current techniques, which have many limitations and are not specific. In view of these problems, this study was undertaken to try and improve the diagnosis of infection by the use of a new technique to stain leucocytes. We have also undertaken culture from the same ejaculate for aerobic and anaerobic organisms, Chlamydia and genital Mycoplasma.
Patients and Methods

The study was carried out on 81 men, 69 of whom were attending the infertility clinic because of an infertile marriage. The remainder were fertile men attending for vasectomy. The fertile men had fathered at least two children and at the time of testing had one child under 2 years of age. All were given clearly written instructions about the production and delivery of the semen specimens. In addition, a full medical history was obtained and a complete physical examination was performed.

Semen

Semen was collected by masturbation into sterile plastic containers after 3 days of sexual abstinence. Using sterile precautions, two aliquots were extracted from each specimen, one for standard semen analysis and the other for cultures for microorganisms. The remainder of the specimen was prepared for immunocytochemical staining for leucocytes. Semen analysis was performed as described in the World Health Organisation (WHO) Laboratory Manual (Belsey et al., 1980). Antisperm antibody measurement was done using the tray agglutination test (TAT) (Friberg, 1974) and the MAR test (Jager et al., 1978).

Microbiological screening

Bacterial cultures were done for aerobic and anaerobic organisms, Chlamydia trachomatis and Ureaplasma urealyticum. The methods have been described elsewhere (Hargrave et al., 1982; Young et al., 1983). All micro-organisms (aerobes, anaerobes, Ureaplasma urealyticum and Chlamydia trachomatis) were identified and reported separately (Taylor-Robinson et al., 1971; Duerden et al., 1980).

Seminal leucocyte staining

1. Preparation of a single cell suspension of the ejaculate. 1 ml of the freshly collected ejaculate was mixed with 9 ml Hanks Balanced Salt Solution (HBSS), calcium- and magnesium-free, in a 10 ml centrifuge tube and centrifuged at 200 x g for 10 min at room temperature. The supernatant was decanted and the cell pellet was resuspended in 10 ml HBSS and centrifuged again at 200 x g for 10 min. The cell pellet was then resuspended at a concentration of 5 to 10 million sperm/ml in HBSS.

   This procedure removed seminal plasma proteins and resulted in minimal cell loss and damage.

2. Preparation of cytocentrifuge smears of washed ejaculate. 100 µl of the cell suspension were put in each cytospin block and spun down on to alcohol-cleaned glass slides using a Shandon Cytocentrifuge (Shandon-Elliot, Southern Instruments Ltd). This was achieved by spinning at 800 rpm for 10 min. The smears were circled with a diamond marker, air dried for 1 h and then fixed in acetone for 10 min at room temperature. The smears were either stained immediately or stored, wrapped in aluminium foil and kept at −20°C until used.

3. Immunoperoxidase staining. The indirect immunoperoxidase technique was used throughout. The procedure has been fully described by El-Demiry et al. (1986). The essential steps were as follows. Smears were incubated with the different anti-human leucocyte monoclonal antibodies, followed by peroxidase conjugated rabbit anti-mouse antibodies. Full details of the antibodies used are given in Table 1. In the case of negative controls the primary antibodies were omitted. As positive controls the monoclonal antibodies were applied to cytocentrifuged smears of human peripheral blood leucocytes. The colour reaction was developed by incubating the smears with diaminobenzidine-hydrogen peroxide substrate. Smears were finally counter-stained with haematoxylin, dehydrated and mounted in DPX.

Smears were examined by light microscopy (magnification ×320). The positively labelled cells were identified by their dark brown cell membrane staining (Figs 1 and 2). Stained cells with any individual monoclonal antibody were counted in 10 different fields for each smear. The average was taken and multiplied by the dilution (the number of ml in which the pellet was resuspended).

The resulting numbers of leucocytes reported in the Tables have no independent meaning but they allow comparison between patients and distinction of those with high or low leucocyte numbers.

Table I *Monoclonal Antibodies Reacting with Human Leucocytes

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-human leucocytes (Hle-1)</td>
<td>All leucocytes</td>
</tr>
<tr>
<td>Anti-Leu 4</td>
<td>All T lymphocytes</td>
</tr>
<tr>
<td>Anti-Leu 3a</td>
<td>Helper/inducer T lymphocytes</td>
</tr>
<tr>
<td>Anti-Leu 2a</td>
<td>Suppressor/cytotoxic T lymphocytes</td>
</tr>
<tr>
<td>Anti-Leu 12</td>
<td>B lymphocytes</td>
</tr>
</tbody>
</table>

*These monoclonal antibodies were obtained from Becton-Dickenson, Lab Impex Limited, Twickenham.
between leucocyte or bacterial counts and subsequent fertility were tested by couple-months analysis (Hargreave and Elton, 1983).

**Results**

**Microbiological investigations**

Sixty-five patients and eight fertile men were tested for the following micro-organisms: aerobes, anaerobes, *Ureaplasma urealyticum* and *Chlamydia trachomatis*: 57 of the 65 patients and 7 of the 8 fertile men were found to have positive bacterial cultures for one or more of these micro-organisms (Table 2). Eight patients and one fertile man had completely negative results. There were no positive cultures for *Chlamydia*.

Aerobic organisms were isolated either alone or in association with anaerobes or *Ureaplasma* from 55 patients and seven fertile men. Anaerobes were isolated from 43 patients and from all of the fertile men with positive cultures. Nine patients and one fertile man had positive culture for *Ureaplasma urealyticum*.

There were no significant differences in bacterial counts among individual organisms or groups except for *Proteus*, which was significantly more common in the fertile men (2/8) compared with the patients (2/65). The most frequent organism in both patients and fertile men was *Staphylococcus albus* (Table 3) with a mean (range) count of colony forming units isolated of $1.1 \times 10^5$ (1.5×$10^2$–1.5×$10^6$). Correlating the bacterial count of every individual organism detected with sperm density and motility, the results were not significant except

![Image](image1.png)

**Fig. 1** Immunoperoxidase staining of cytocentrifuge smear of semen with antihuman leucocyte (Hle-1) monoclonal antibody. (×320).

![Image](image2.png)

**Fig. 2** Leu 2a+ cells (suppressor/cototoxic T lymphocytes) in semen stained with anti-Leu 2a monoclonal antibody. (Immunoperoxidase × 320).

**Statistical methods**

Between-group comparisons of leucocyte and bacterial species and counts were made using Wilcoxon rank sum tests, and the significance of relationships between these measurements was assessed by Kendal rank correlation co-efficients. Relationships

### Table 2

<table>
<thead>
<tr>
<th>No. of bacterial species isolated per patient</th>
<th>No. of patients with corresponding number of species (and total number of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infertile patients</td>
</tr>
<tr>
<td>0</td>
<td>8 (0)</td>
</tr>
<tr>
<td>1</td>
<td>12 (12)</td>
</tr>
<tr>
<td>2</td>
<td>11 (22)</td>
</tr>
<tr>
<td>3</td>
<td>17 (51)</td>
</tr>
<tr>
<td>4</td>
<td>12 (48)</td>
</tr>
<tr>
<td>5</td>
<td>3 (15)</td>
</tr>
<tr>
<td>6</td>
<td>2 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>65 (160)</td>
</tr>
</tbody>
</table>

*Excluding *Ureaplasma urealyticum*

**Note:** Mean number of organisms per control sample = 1.75 (14/8). Mean number of organisms per infertile sample = 2.46 (160/65). None of the controls had >3 bacterial species present per sample.
Table 3 Isolation Rate of Bacteria from 73 Semen Samples

<table>
<thead>
<tr>
<th>Species group</th>
<th>Infertile patients (n=65)</th>
<th>Controls (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus albus</td>
<td>48 (73.8)</td>
<td>5 (62.5)</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>27 (41.5)</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>haemolytic streptococi</td>
<td>10 (15.4)</td>
<td>0</td>
</tr>
<tr>
<td>haemolytic streptoccci</td>
<td>1 (1.5)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>1 (1.5)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>2 (3.1)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3 (4.6)</td>
<td>0</td>
</tr>
<tr>
<td>Bacteroides spp</td>
<td>31 (47.7)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>(a) Anaerobic cocci</td>
<td>31 (47.7)</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>(b) Ureaplasma urealyticum</td>
<td>9 (13.9)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>(c) Others</td>
<td>6 (9.2)</td>
<td>0</td>
</tr>
</tbody>
</table>

(a) Bacteroides and/or anaerobic cocci were isolated from 43 samples (66.2%) from infertile patients and from 3 samples (37.5%) from controls.
(b) Ureaplasma isolation rate based on 70 valid cultures.
(c) Includes Fusobacterium nucleatum 1; Eubacterium lentum 2; Propionibacterium acnes 1; Gardnerella vaginalis 1; and Lactobacillus spp 1.

for Bacteroides asaccharolyticus, which showed a negative correlation with density and motility (P<0.05).

Leucocytes in the semen

Comparison of patients with an infertile marriage and fertile men. All of the specimens from the 12 fertile men showed cells reacting with the pan-antileucocyte monoclonal antibody with a mean count of 44.9 ± 25.8 leucocytes per high power field (×320). Most of these cells appeared from their morphological appearances to be polymorphonuclear (PMN) leucocytes. No cells reacting with the pan-antileucocyte reagent were detected.

Leucocytes were detected in 90% (63/67) of the specimens from the patients, with a mean count of 14.6 ± 17.1 leucocyte per HPF (×320). T lymphocytes (Leu 4+) with predominance of the suppressor/cytotoxic phenotype (Leu 2a+) (Fig. 2) were also demonstrated in 13 patients with a mean count of 4.5 ± 3.3 T lymphocytes per HPF (×320). The number of PMN leucocytes was calculated by subtracting the pan-lymphocyte count from the pan-leucocyte count. Wilcoxon rank sum tests showed that the total leucocyte count and the polymorphonuclear leucocytes were significantly higher in fertile men (P<0.001 in both), but there were no significant differences in the counts of T lymphocytes or their subsets.

Relationship of leucocytes to bacterial counts. Each individual bacterial count, Ureaplasma and bacterial groups were correlated with the leucocyte and leucocyte subpopulation counts. The results were not statistically significant (Tables 4, 5 and 6) except that β-streptococci showed a significant positive correlation with T lymphocytes (P<0.05).

Table 4 Relationship between Number of Bacterial Species per Semen Sample and Leucocyte Count

<table>
<thead>
<tr>
<th>No. of bacterial species isolated per patient</th>
<th>No. of patients with leucocyte counts of</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>1-20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>38</td>
</tr>
</tbody>
</table>

Kendall rank correlation = t = 0.01 (not significant).

Table 5 Relationship between Presence of Anaerobes and Leucocyte Count

<table>
<thead>
<tr>
<th>Leucocyte count</th>
<th>Anaerobes present</th>
<th>Anaerobes absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>12</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>1-20</td>
<td>22</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>&gt;20</td>
<td>7</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>22</td>
<td>63</td>
</tr>
</tbody>
</table>

Wilcoxon rank sum test showed non-significant correlation (z = 1.07).

Table 6 Relationship between Ureaplasma Culture and Leucocyte Count in Infertile Men

<table>
<thead>
<tr>
<th>Ureaplasma culture</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of leucocytes per field</td>
<td>No. of patients (% of +ve)</td>
<td>No. of patients (% of -ve)</td>
</tr>
<tr>
<td>&lt;1</td>
<td>2 (22.2)</td>
<td>12 (23)</td>
</tr>
<tr>
<td>1-20</td>
<td>5 (53.5)</td>
<td>30 (37)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>2 (22.2)</td>
<td>11 (21)</td>
</tr>
</tbody>
</table>

Relationship of leucocytes to antisperm antibodies. Serum TAT results were available for 50 patients and MAR IgG for 19. Of the correlations with leucocyte counts, only that between MAR IgG and PMN leucocytes was significant (positive P<0.05).
However, there was no significant correlation with serum TAT.

Relationship of leucocytes to sperm density, motility and subsequent fertility. There was no correlation between the leucocyte count and the duration of involuntary infertility, sperm motility and sperm density (Kendal rank correlation of—0.03, 0.07 and 0.05 respectively, NS). Furthermore, using the couple-months analysis method, there was no significant relationship between leucocyte counts and subsequent fertility (Table 7).

Table 7a Relationship of Total Leucocyte Counts to Subsequent Fertility

<table>
<thead>
<tr>
<th>Pan leucocyte count</th>
<th>Observed pregnancies</th>
<th>Expected pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2</td>
<td>1.47</td>
</tr>
<tr>
<td>0&lt;10</td>
<td>2</td>
<td>3.94</td>
</tr>
<tr>
<td>10&lt;20</td>
<td>4</td>
<td>2.24</td>
</tr>
<tr>
<td>&gt;20</td>
<td>1</td>
<td>1.35</td>
</tr>
</tbody>
</table>

\[ x^2 = 2.63 \text{ df (NS)} \]

Table 7b Relationship of T lymphocytes to Subsequent Fertility

<table>
<thead>
<tr>
<th>T lymphocyte count</th>
<th>Observed pregnancies</th>
<th>Expected pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>7.11</td>
</tr>
<tr>
<td>&gt;0</td>
<td>4</td>
<td>1.89</td>
</tr>
</tbody>
</table>

\[ x^2 = 2.991 \text{ df (NS)} \]

Discussion

In this study we tried to find a possible relationship between subclinical genital tract infection and male infertility. We defined precisely the leucocytes and leucocyte subpopulations in the ejaculate using specific monoclonal antibodies in an immunoperoxidase technique. We also performed cultures for a range of micro-organisms.

Leucocytes were detected in all of the fertile men (12) and in 63 of the 65 patients (90%) with an infertile marriage. Polymorphonuclear leucocytes were the predominant cell type in both groups. The fertile men were found to have a significantly larger number of leucocytes (mainly PMN leucocytes) compared with the patients. T lymphocytes were detected in only 13 of the 63 patients. A high bacterial isolation rate was found: 87% of the semen cultures of both the fertile and infertile groups were positive for micro-organisms with no difference between the patients and fertile groups. However, there was no significant correlation between leucocyte counts and the counts of positive cultures for all micro-organisms. The significant correlation between β-streptococci and T lymphocytes \( P < 0.05 \) is difficult to explain but may be a spurious result from multiple statistical tests. Furthermore, no correlations were found between leucocyte counts and sperm density, motility and subsequent fertility.

In 1981 Fowler reviewed the literature and found little evidence to support infection as a frequent cause of infertility.

Our results are also in agreement with those of other studies. Naessens et al. (1986) reported that 97.7% of the semen cultures were positive for aerobes and anaerobes and they found that no single aerobic or anaerobic organism could be related to abnormal semen samples. Comhaire et al. (1980) found no significant difference in semen characteristics of infertile males with and without infection. McGowan et al. (1981) found that infection in the semen did not significantly affect the count, motility or volume of the specimen. In his experience with 290 patients who had semen cultured, Hendry (1983) found that pregnancies were produced with roughly equal frequency with or without positive semen cultures irrespective of whether the organisms were treated with antibiotics or not.

Our observation that none of the controls and the infertile men was positive for Chlamydia may be due to the fact that all men examined had no history of specific or non-specific genital tract infections and Chlamydia is not a commensal organism. However, Ulstein et al. (1976) were unable to culture Chlamydia from the urine, expressed prostatic secretion or semen of 21 asymptomatic men with unexplained infertility.

Our finding that the fertile men had large numbers of polymorphonuclear leucocytes compared with the patients with an infertile marriage raises many questions about the normal role of these cells in the ejaculate. These cells are important in host defence against invading micro-organisms but are also able to destroy tissue cells. It is possible that these polymorphonuclear leucocytes have a role in the phagocytosis of the residues of degenerating germ cells and of residual bodies in the ejaculate. Another possibility is that they play a role in the destruction and removal of the dead and/or immature sperm. In this connection it is interesting to note that leucocyte phagocytosis of
antibody coated sperm has been demonstrated by London et al. (1985).

In conclusion, the evidence from both the leucocyte and the bacterial studies suggests that subclinical genital tract infection in asymptomatic patients has no major role in male infertility. We found no correlation between sperm density, sperm motility, the presence of antisperm antibodies, positive bacterial cultures and the leucocyte response in the semen. Our results cast doubts on the currently accepted criteria of subclinical genital tract infection.

References


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Identification of pathogenic Neisseria by enzyme profiles determined with chromogenic substrates

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(Received 21 December 1984. Accepted 18 February 1985)

One hundred and forty-six clinical isolates of Neisseria species were tested in parallel by carbohydrate utilisation, coagglutination and Gonocheck II™, a new identification system for pathogenic neisseriae. Gonocheck II identifies N. gonorrhoeae, N. meningitidis, N. lactamica and Branhamella catarrhalis in a single 30 min test on the basis of their enzyme profiles determined with chromogenic substrates. The presence of the enzyme \( \gamma \)-glutamyl amino-peptidase in N. meningitidis differentiates it from N. gonorrhoeae. N. gonorrhoeae and N. lactamica are identified by the presence of proline iminopeptidase activity and \( \beta \)-galactosidase respectively. None of these enzymes are present in B. catarrhalis.

The overall correlation between Gonocheck and carbohydrate utilisation (97.5%) and the coagglutination test and carbohydrate utilisation (96.9%) was virtually identical. Of 55 clinical isolates of N. meningitidis two strains were misidentified as N. gonorrhoeae and one strain as B. catarrhalis; all three strains were identified correctly when tested from a fresh subculture. One of 55 clinical isolates of N. gonorrhoeae was repeatedly misidentified as B. catarrhalis. Gonocheck II is potentially a very useful identification system but further studies are required to establish the accuracy of the method.

Key words: Bacteriological technics. Chromogenic substrates. Neisseria.

Introduction

The current prevalence of gonorrhoea imposes a considerable burden on the microbiological laboratory. The high incidence of pharyngeal gonorrhoea combined with an increased awareness of anogenital colonisation with non-gonococcal neisseriae accentuates the need to differentiate accurately between Neisseria gonorrhoeae and non-gonococcal neisseriae such as N. meningitidis and N. lactamica. Consequently it is important to develop and assess new methods of neisserial identification.

Tests currently in use include the rapid carbohydrate utilisation test (RCUT), immunological methods such as the fluorescent antibody test and co-agglutination, and the superoxol test. The RCUT, which measures preformed enzyme, has proved to be an accurate method for identifying Neisseria species, its main disadvantage being the requirement for a fairly heavy inoculum. Some strains of N. gonorrhoeae have proved difficult to identify using fluorescent antibody tests and in many laboratories co-agglutination has become the more popular immunological technique. However, cross reactions with other strains of Neisseria—particularly N. lactamica—have been noted. The superoxol test is valuable for screening as a negative result virtually excludes the gonococcus; but other tests are required to identify isolates giving positive results.
Recently, enzyme profiles determined with chromogenic substrates have been used to identify Neisseria species. The presence of the enzyme gamma-glutamyl amino-peptidase (GGA) in N. meningitidis differentiates it from N. gonorrhoeae. N. gonorrhoeae and N. lactamica are identified by the presence of proline imino-peptidase (PA) activity and \( \beta \)-galactosidase respectively. None of these enzymes are present in B. catarrhalis. The Gonocheck II\(^9\) identification system utilises appropriate chromogenic substrates for these enzymatic reactions to identify N. gonorrhoeae, N. meningitidis, N. lactamica and Branhamella catarrhalis in a single 30 min test. The aim of this study was to evaluate the Gonocheck II system by comparing it with methods currently used in our laboratory. Accordingly, all suspect neisserial isolates from clinical specimens were tested in parallel by the Gonocheck II system, the RCUT and co-agglutination.

**Materials and methods**

Material from anogenital and pharyngeal sites of patients attending the Department of Genito-urinary Medicine at Edinburgh Royal Infirmary was inoculated directly on to modified New York city (MNYC) medium.\(^{10}\) Cultures were held at 37°C in an aerobic atmosphere enriched with 5\% CO\(_2\), and transported to the laboratory within 4 h. Cultures were then incubated under similar conditions for 24 h and examined for oxidase-positive Gram-negative diplococci (GNDC). The suspect neisserial organisms were further tested by the RCUT\(^3\) and co-agglutination (Phadebact Gonococcus test\(^{11}\)) as described previously.\(^{12}\) The Gonocheck II test\(^9\) was carried out as outlined below.

Whenever there was sufficient growth organisms were identified directly from the primary isolation plate; if there was insufficient material the tests were carried out the next day with a 24 h sub-culture.

**Clinical isolates and stock cultures**

Of routine clinical isolates, 146 were tested by all three methods. In addition, 10 maltose-negative variants of N. meningitidis serogroup B\(^{13}\) and three strains of N. gonorrhoeae with markedly reduced membrane permeability (Mtr phenotype)\(^{14}\) were also tested.

**Gonocheck II**

Each commercially-prepared Gonocheck II tube was reconstituted with 50 \( \mu l \) sterile distilled water and incubated with eight to 10 colonies picked from MNYC medium with a sterilised nichrome loop. The tests were incubated at 37°C for 30 min and examined for colour change. If there was no colour change 25 \( \mu l \) of EY-20 reagent (reconstituted with 0.5 ml sterile distilled water) were added and the tubes re-examined for a colour change.

Any specimen giving discordant results between any of the tests was re-tested by all three methods using a 24 h subculture. The Gonocheck II test was carried out as described above, but if there was no colour development after 30 min incubation, 25 \( \mu l \) of 0.2 mol/l Tris buffer pH 8.4 was added to ensure an optimum pH for GGA activity in N. meningitidis and PA activity in N. gonorrhoeae.

**Results**

Interpretation of results was as shown in Table 1. The identities of the 146 clinical isolates of Neisseria by the RCUT were N. gonorrhoeae (86), N. meningitidis (55) and N. lactamica (5). The results of Gonocheck and co-agglutination tests on these clinical isolates and the 13 stock cultures are given in Table 2.

The overall correlation between Gonocheck and the RCUT (97.5\%) and the coagglutination test and the RCUT (96.9\%) are almost identical. All of the stock cultures tested were identified correctly by both Gonocheck and co-agglutination. Reactions given by the four isolates wrongly identified by coagglutination are given in Tables 3 and 4.

The three strains of meningococci which were misidentified were tested from the primary isolation plate. On testing a 24 h sub-culture all three strains produced a yellow colour in the test and were correctly identified as N. meningitidis.

On several repeat tests carried out at pH 8.5 the isolate of N. gonorrhoeae failed to produce a red colour after the addition of EY-20 reagent and was consistently misidentified as B. catarrhalis.

One strain of N. lactamica gave a negative co-agglutination reaction after sub-culture and was identified as a non-gonococcal neisseria. The other four strains gave the same result on repeat testing and would have been mis-identified as N. gonorrhoeae.

Table 1. Interpretation of results

<table>
<thead>
<tr>
<th>Colour of tube</th>
<th>Identification</th>
<th>Chemical Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td><em>N. lactamica</em></td>
<td>Hydrolysis by β-D-galactosidase yields a blue colour from the colourless substrate</td>
</tr>
<tr>
<td>Yellow</td>
<td><em>N. meningitidis</em></td>
<td>Hydrolysis by γ-glutamyl aminopeptidase releases yellow p-nitroaniline from the colourless substrate</td>
</tr>
<tr>
<td>Red/pink (after adding) EY-20</td>
<td><em>N. gonorrhoeae</em></td>
<td>Hydrolysis of the β-napthyl amino acid derivative by proline iminopeptidase releases free β-naphthylamine derivative which complexes with the diazonium salt derivative (EY-20) to produce a red colour</td>
</tr>
<tr>
<td>No colour change (before or after adding EY-20)</td>
<td><em>B. catarrhalis</em></td>
<td>None of the above enzymes are present</td>
</tr>
</tbody>
</table>

Table 2. Results of Gonocheck II and co-agglutination tests applied to 159 isolates of *Neisseria*

<table>
<thead>
<tr>
<th>Identification by RCUT</th>
<th>No. of isolates</th>
<th>Gonocheck</th>
<th>Phadebact</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>89</td>
<td>88 (98.9)</td>
<td>89 (100)</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>65</td>
<td>62 (95.4)</td>
<td>63* (96.9)</td>
</tr>
<tr>
<td><em>N. lactamica</em></td>
<td>5</td>
<td>5 (100)</td>
<td>2* (40)</td>
</tr>
<tr>
<td>Total</td>
<td>159</td>
<td>155</td>
<td>154</td>
</tr>
</tbody>
</table>

* Identified as non-gonococcal *Neisseria*.

Table 3. Details of four neisserial isolates misidentified by Gonocheck II

<table>
<thead>
<tr>
<th>Identification by RCUT</th>
<th>No. of isolates</th>
<th>Identification by Gonocheck</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>1</td>
<td><em>B. catarrhalis</em></td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>3</td>
<td><em>N. gonorrhoeae</em> (2)</td>
</tr>
</tbody>
</table>

Table 4. Details of five neisserial isolates misidentified by coagglutination

<table>
<thead>
<tr>
<th>Identification by RCUT</th>
<th>No. of isolates</th>
<th>Identification by co-agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. meningitidis</em></td>
<td>2</td>
<td><em>N. gonorrhoeae</em></td>
</tr>
<tr>
<td><em>N. lactamica</em></td>
<td>3</td>
<td><em>N. gonorrhoeae</em></td>
</tr>
</tbody>
</table>

Discussion

The results show no significant differences between the Gonocheck II and co-agglutination tests for the identification of *N. gonorrhoeae*. An advantage of the Gonocheck II system is its potential ability to identify *Neisseria* species rather than simply divide isolates into gonococcal and non-gonococcal neisseriae. However, the enzyme profile used to identify *N. meningitidis* is not unique to this species, and its accurate identification relies upon the inhibition of non-pathogenic neisseriae by selective media. As *N. flava* and *N. perflava* account for approximately 0.6% of GNDC isolated from throat specimens cultured on selective medium a low level of...
misidentification is likely. The Gonocheck test requires a smaller inoculum than that required for the RCUT but the latter gives a more comprehensive and accurate identification at species level. Unfortunately many laboratories experience difficulties with carbohydrate utilisation tests.

The main disadvantage of the Gonocheck test was insufficient GGA activity in a few strains of meningococci: because the yellow p-nitroaniline was not released the tube was treated with the diazonium salt derivative (EY-20) which resulted in an identification of N. gonorrhoeae or B. catarrhalis (Table 3), depending on the level of PA activity in the particular isolate. This problem occurred with isolates tested from primary isolation cultures on MNYC medium, the correct enzyme profile was obtained with a fresh sub-culture on the same medium. Primary cultures were usually incubated longer than sub-cultures and, since MNYC medium promotes very luxuriant growth the organisms may have been beyond the growth phase corresponding to optimal enzyme activity. Gonocheck may prove particularly useful in identifying aberrant strains of meningococci which give false-negative maltose reactions in cystine trypticase agar (CTA) carbohydrate tests. A representative sample of such strains did utilise maltose when tested by the RCUT in both this and a previous study.

Unlike the mis-identification of meningococci which was corrected by re-testing a sub-culture, the strain of N. gonorrhoeae which was identified as B. catarrhalis consistently failed to demonstrate PA activity. Although cell envelope mutations could play a part in such a phenomenon this seems unlikely since three Mtr phenotype strains with markedly reduced membrane permeability were identified correctly.

We consider that Gonocheck II is potentially a very useful identification system but that a much larger study is required to determine (i) the accuracy of the test on MNYC medium primary isolation cultures and (ii) the incidence of PA-deficient gonococci.

References
11 Pharmacia (Great Britain) Ltd, Prince Regent Road, Hounslow, Middlesex TW3 1NE, England.
Characterisation of *Branhamella catarrhalis* and differentiation from *Neisseria* species in a diagnostic laboratory

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Characterisation of *Branhamella catarrhalis* and differentiation from *Neisseria* species in a diagnostic laboratory

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SUMMARY To distinguish *Branhamella catarrhalis* from *Neisseria* species a study of 140 strains was made on simple laboratory media, with particular reference to deoxyribonuclease (DNase) production, superoxol reaction, and growth characteristics. All 97 clinical isolates of *B catarrhalis* (58 of which were β-lactamase positive) and eight strains of *B catarrhalis* from the National Collection of Type Cultures were DNase positive and superoxol positive. None grew on modified New York City medium, modified Thayer Martin medium, MacConkey agar, crystal violet blood agar, nor under anaerobic conditions. Of the 16 different non-pathogenic *Neisseria* species tested, all were DNase negative, eight (50%) were superoxol reaction negative, and 13 (81%) grew on crystal violet blood agar.

Using simple laboratory media, DNase, and superoxol tests, it was possible to identify *B catarrhalis* and to distinguish it from pathogenic and non-pathogenic *Neisseria* species.

The unresolved problem of classification and differentiation of the family Neisseriaceae has a long history. In 1906 Von Lingelsheim¹ first described the identification system for Gram negative cocci using microscopic and macroscopic features and carbohydrate reactions. The same year Kutscher² and subsequently Arkwright in 1907³ extended the identification system of *Neisseria* spp and discussed their possible role in clinical disease. By 1909 Elser and Huntoon⁴ had overcome some of the difficulties in the classification of *Neisseria* spp by comparing their cultural and biochemical features. In 1921 Gordon⁵ reclassified the known *Neisseria* spp on the basis of carbohydrate fermentation reactions. By the late 1960's it was clear that *Neisseria catarrhalis* was quite different from the other *Neisseria* spp in its biochemical reactions and fatty acid and DNA base composition. In 1970 Catlin⁶ proposed a new genus, *Branhamella*, for *N catarrhalis* on the basis of biochemical and DNA base differences. Members of the genus *Neisseria* and the genus *Branhamella* are now classified in the family Neisseriaceae, along with the genera *Kingella*, *Moraxella*, and *Acinetobacter*⁷. The distinction between pathogenic and non-pathogenic strains of *Neisseria* spp and *B catarrhalis* has become important because of the changed perception of their pathogenicity. It is well known that pathogenic *Neisseria* spp are not restricted to their classical anatomical sites: pharyngeal infection with *N gonorrhoeae* and anogenital tract infection with *N meningitidis* are increasingly being recognised. Arko et al⁸ found that *B catarrhalis* and *N meningitidis* were the two organisms most often confused with *N gonorrhoeae*. Knapp et al⁹ discussed the difficulties in differentiating *B catarrhalis* and *N cinerea*. Conventional methods using carbohydrate utilisation tests require careful control of physical and biochemical conditions. The reports by Catlin in 1961¹⁰ and 1970¹¹ and subsequently by others¹¹ that *B catarrhalis* was unique among Neisseriaceae in producing a DNase, and more recently, work by Young et al¹² on the superoxol test to differentiate gonococcal from non-conococcal species, could offer useful means of differentiating *Neisseria* spp and *B catarrhalis*. We therefore examined a large number of *B catarrhalis* strains and various non-pathogenic *Neisseria* spp to compare and contrast DNase production, superoxol reactions, and growth characteristics on different media.

Accepted for publication 18 May 1987
Material and methods

A total of 140 strains of *B catarrhalis* and *Neisseria* spp were studied. Clinical isolates were used as test strains and known reference strains were used for control and comparison. The clinical isolates comprised 97 strains of *B catarrhalis* and 13 strains of *N perflava*, isolated from sputum specimens (bacteriology laboratory, City Hospital, Edinburgh) from patients with bronchopulmonary infection, and considered to be clinically important, and eight strains of *N lactamica* isolated from throat cultures (department of bacteriology, University of Edinburgh). The following bacteria used as controls were obtained from the National Collection of Type Cultures (NCTC), Colindale, London: eight strains of *B catarrhalis* (all are non-β-lactamase producers) (NCTC numbers 3622, 3623, 3625, 4103, 11015, 11016, 11017, and 11020), and the following 14 recognised strains of *Neisseria* spp: *N catavia* (10293), *N mucosa* var *heidelbergensis* (10777), *N* species (11049), *N* animalis (10212), *N* elongata subspecies glycolytica (11050), *N* canis (10296), *N* pharyngis (4590), *N* mucosa var *mucosa* (10774), *N* cuniculi (10297), *N* denitrificans (10295), *N* flavescens (8263), *N* ovis (11018), *N* elongata (10690), *N* cinerea (10294).

All the clinical isolates of *B catarrhalis*, *N perflava*, and *N lactamica* were primarily identified by using previously described laboratory criteria, comprising morphology, oxidase and catalase reactions, and rapid carbohydrate utilisation tests (RCUT).14

All the test strains and NCTC strains were examined for DNase and superoxol activity. A gonococcus coagglutination test (Phadebact) was performed to determine possible cross reactivity with the gonococcal antigen.

DNase test11

Oxoid DNase agar (Code CM321, Oxoid Ltd, Basingstoke, England) was used. Freshly prepared plates were divided into four to six sections. Each section of the plate was inoculated heavily with the strain to be tested and spread to form a circle of about 6 mm in diameter. The Oxford strain of *Staphylococcus aureus* (NCTC 6571) was used as a positive control. A known strain of *S epidermidis* served as a negative control. After 24 hours of incubation the plates were flooded with 0.1 M hydrochloric acid—the appearance of a clear zone around the inoculum in three to four minutes was taken as a “positive” test. A known strain of *B catarrhalis* was also used as an additional positive control because the test gives a much weaker reaction with *B catarrhalis* than it does with *S aureus*.

Superoxol test12

A few colonies of the culture to be tested were picked from the primary isolation plate with a plastic loop and emulsified in a drop of 30% H2O2 placed on a clean glass slide. A known strain of *N gonorrhoeae* and of *N perflava* were similarly tested as positive and negative controls, respectively. A positive superoxol test was defined as abundant production of bubbles within two to three seconds. Weak or delayed bubbling after three seconds indicated a negative reaction.

All isolates were tested by the Phadebact Gonococcus test (Phadebact Diagnostics, AB Sweden) according to the manufacturer’s instructions. Beta lactamase production was detected using chromogenic cephalosporin.15

CULTURE CHARACTERISTICS

To study the effect of various commonly used media on the growth characteristics of *B catarrhalis* and *Neisseria* spp an inoculum containing 10⁴–10⁵ colony forming units was applied to the surface of plates containing the following media:

1. Modified New York City medium (MNYC)16 containing the selective agents trimethoprim lactate 6.5 mg/l, amphotericin 1 mg/l, lincomycin 1 mg/l, and colistin 4 mg/l.

2. Modified Thayer Martin medium (MTM) containing vancomycin 3 mg/l, colistin 7.5 mg/l, trimethoprim 5 mg/l and nystatin 12500 U/l (Difco Laboratories).

3. MacConkey agar (Oxoid code No CM7).

4. Crystal violet blood agar, bi-layer plate: lower layer, columbia agar (Oxoid code CM331); upper layer, Columbia blood agar with crystal violet at final concentration 0.0002%.

5. Nutrient agar (Oxoid code CM3).


7. Neomycin sulphate blood agar—Columbia agar base + 7% defibrinated horse blood + 5 mg/l of menadione and 1 ml/l of 7000 mg/l neomycin sulphate.

Nutrient agar plates were incubated at 22°C and 37°C aerobically and 37°C anaerobically. A nutrient agar plate seeded with a known culture of *Pseudomonas aeruginosa* was included in each anaerobic jar as a control. All other plates were incubated at 37°C in air and 8% carbon dioxide. Cultures were read after overnight incubation and growth was recorded as +, ++, +++, representing light, moderate, or heavy growth.

Results

DNase activity was exhibited by all 97 clinical isolates of *B catarrhalis* and by the eight NCTC *B catarrhalis*
Neisseria \(^/-\)lactamase negative

Neisseria elongata (NCTC 10660)

Neisseria ovis

Neisseria cuniculi

Neisseria mucosa

Positive isolates

Neisseria pharyngeal (NCTC 4590)

Neisseria perflava

Neisseria elongata subspecies glycolytica (NCTC 10296)

Neisseria canis (NCTC 10261)

Neisseria flavescens (NCTC 8263)

Neisseria eloncata (NCTC 10660)

Neisseria cinerea (NCTC 10294)

Organisms

(No of isolates)

DNase

Superoxol

Phaeoact

MNYC and CMC media agar

MTM media agar

Crystal violet agar

Nutrient agar 22°C

Nutrient agar 37°C

Nutrient agar 37°C (anaerobic)

Branhamella catarrhalis

Clinical isolates

β-lactamase positive

β-lactamase negative

NCTC strains

Neisseria perflava

Neisseria lactamica

Neisseria caviae (NCTC 10293)

Neisseria mucosa var heidelbergensis (NCTC 10777)

Neisseria species (NCTC 10499)

Neisseria animalis (NCTC 10212)

Neisseria elongata subspecies glycolytica (NCTC 10599)

Neisseria canis (NCTC 10296)

Neisseria pharyngis (NCTC 4590)

Neisseria mucosa var mucosa (NCTC 10774)

Neisseria cuniculi (NCTC 10297)

Neisseria denitrificans (NCTC 10295)

Neisseria flavescens (NCTC 8263)

Neisseria eloncata (NCTC 10660)

Neisseria cinerea (NCTC 10294)

Growth characteristics

Table  Characterisation of \(B\) catarrhalis and Neisseria spp

<table>
<thead>
<tr>
<th>Organisms</th>
<th>(No of isolates)</th>
<th>DNase</th>
<th>Superoxol</th>
<th>Phaeoact</th>
<th>MNYC and CMC media agar</th>
<th>MTM media agar</th>
<th>Crystal violet agar</th>
<th>Nutrient agar 22°C</th>
<th>Nutrient agar 37°C</th>
<th>Nutrient agar 37°C (anaerobic)</th>
</tr>
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<tbody>
<tr>
<td>Branhamella catarrhalis</td>
<td>Clinical isolates</td>
<td>97</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>β-lactamase positive</td>
<td>58</td>
<td>+</td>
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<tr>
<td></td>
<td>β-lactamase negative</td>
<td>39</td>
<td>+</td>
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<td></td>
<td>NCTC strains</td>
<td>8</td>
<td>+</td>
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<tr>
<td>Neisseria pharyngeal</td>
<td>15</td>
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<tr>
<td>Neisseria perflava</td>
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<td>Neisseria lactamica</td>
<td>8</td>
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<td>Neisseria caviae (NCTC 10293)</td>
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<td>Neisseria mucosa var heidelbergensis (NCTC 10777)</td>
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<td>Neisseria species (NCTC 10499)</td>
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<tr>
<td>Neisseria animalis (NCTC 10212)</td>
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<td>Neisseria elongata subspecies glycolytica (NCTC 10599)</td>
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<td>Neisseria canis (NCTC 10296)</td>
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<td>Neisseria pharyngis (NCTC 4590)</td>
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<td>Neisseria cuniculi (NCTC 10297)</td>
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<td>Neisseria denitrificans (NCTC 10295)</td>
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<td>Neisseria flavescens (NCTC 8263)</td>
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<td>Neisseria eloncata (NCTC 10660)</td>
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<tr>
<td>Neisseria cinerea (NCTC 10294)</td>
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</table>

strains; it was not detected in the clinical isolates of \(N\) perflava nor \(N\) lactamica nor in the 14 NCTC strains of Neisseria spp. All 105 (100%) strains of \(B\) catarrhalis were positive in the superoxol test as were some uncommon non-pathogenic Neisseria spp—namely, \(N\) caviae, \(N\) animalis, \(N\) elongata subspecies glycolytica, \(N\) canis, \(N\) cuniculi, \(N\) denitrificans, \(N\) flavescens and \(N\) ovis A334/72. All clinical isolates of \(N\) perflava and of \(N\) lactamica and the following NCTC strains—\(N\) mucosa var heidelbergensis, \(N\) mucosa var mucosa, \(N\) elongata and \(N\) cinnerea were negative in the superoxol test.

\(N\) elongata subspecies glycolytica and two of the eight (25%) isolates of \(N\) lactamica were positive in the Phadebact Gonococcus test. Beta lactamase production was detected in 58 (60%) of the clinical isolates of \(B\) catarrhalis. None of the remaining strains of \(B\) catarrhalis produced β-lactamase.

All the strains of \(B\) catarrhalis and \(N\) perflava grew on nutrient agar at 22°C and 37°C. All strains of \(N\) lactamica failed to grow on nutrient agar at 22°C. The growth of the other Neisseria spp on nutrient agar at 22°C was variable (table). No difference was detected in any of the tests between β lactamase producing and non-β-lactamase producing strains of \(B\) catarrhalis. All the 140 strains tested grew on blood agar, chocolate agar, and nutrient agar media at 37°C.

None of the 140 strains studied grew on neomycin or bacitracin containing media. None of the \(B\) catarrhalis strains grew under anaerobic conditions, whereas all the strains of \(N\) perflava, \(N\) lactamica, and other non-pathogenic Neisseria spp, with the exception of \(N\) caviae, \(N\) elongata as subspecies and \(N\) cuniculi grew anaerobically.

Discussion

Increasing evidence that \(B\) catarrhalis is an important pathogen in diseases such as bronchopulmonary infections,\(^{13,17,18}\) otitis media,\(^{19}\) maxillary sinusitis,\(^{20}\) and conjunctivitis,\(^{21}\) warrants its proper identification and differentiation from non-pathogenic Neisseria spp. Morphological similarities and biochemical variations among different Neisseria spp and \(B\) catarrhalis may cause confusion and result in an error or delay in their recognition.

Results of our study indicate that the DNase test is reliable and simple to perform and because of its high specificity, it can be used as a confirmatory test in the
identification of *B. catarrhalis*. The superoxol test is a simple and economical test which can be performed on colonies from primary culture plates. It can differentiate *B. catarrhalis* from *N. perflava*, *N. pharyngis* and several other non-pathogenic *Neisseria* spp (table). In a previous study Young *et al.* found that 128 of 133 isolates of *N. meningitidis* were superoxol negative. The superoxol test cannot be used to differentiate between *N. gonorrhoeae* and *B. catarrhalis* as all but one of 596 gonococci tested were positive in the test. The Phadebact gonococcus test, however, is highly specific for *N. gonorrhoeae*. In our study all the isolates of *B. catarrhalis* were negative in the Phadebact gonococcus test. Although some non-gonococcal strains of *Neisseria* spp reacted in the Phadebact test, such cross reactions can be eliminated by use of the currently available Phadebact gonococcus test which uses monoclonal antibodies (H Young, unpublished data). Thus superoxol positive isolates may be differentiated using the Phadebact test into *B. catarrhalis* and *N. gonorrhoeae* on the same day. Together, the superoxol and DNase tests constitute reliable and cost effective means of differentiating *B. catarrhalis* from other *Neisseria* spp.

Differences in growth characteristics of *Neisseria* spp and *B. catarrhalis* on various media may also be used advantageously in presumptive identification. *B. catarrhalis* and *N. perflava* (the most common isolated *Neisseria* spp) do not grow on MNYC and MTM media as the minimum inhibitory concentrations of colistin for these strains is lower than the concentration used in these media. Additional characteristics that distinguish *B. catarrhalis* from *N. perflava* are inability of *B. catarrhalis* to grow on MacConkey crystal violet, and on nutrient agars under anaerobic conditions.

Growth at 22°C on basic media has been considered to be a property of non-pathogenic *Neisseria* spp. It has been suggested that β-lactamase producing strains of *B. catarrhalis* do not grow at 22°C. In this study, however, all the isolates of *B. catarrhalis* did grow on nutrient agar at 22°C (table). All the isolates of *B. catarrhalis* and *Neisseria* spp failed to grow on media containing bacitracin or neomycin. Therefore, a non-selective medium should be added where *B. catarrhalis* is likely to be a significant isolate. *N. catarrhalis* may colonise the oropharynx and less commonly the genital tract. Difficulties have been experienced in differentiating *N. catarrhalis* from *N. gonorrhoeae* and *B. catarrhalis* in several rapid systems used for the identification of pathogenic *Neisseria* spp. Knapp *et al.* used DNA hybridisation to identify *N. catarrhalis*, but this test is not readily available in most laboratories. We feel that DNase production, the superoxol test, and growth characteristics on various media are sufficient (table).

We conclude that simple laboratory tests like DNase production, superoxol reaction, anaerobic culture and growth characteristics on routinely used laboratory media such as MNYC, MTM, MacConkey and crystal violet blood agars are enough to distinguish *B. catarrhalis* from pathogenic and non-pathogenic *Neisseria* spp.

We are grateful to Mrs M Wideski and Miss E Dagg for secretarial help.

References
18 Johnson AM, Drew WL, Roberts M. *Branhamella* (*Neisseria*)
Characterisation of *B* catarrhalis and differentiation from *Neisseria* sp in a diagnostic laboratory

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Requests for reprints to: Dr Fareeduddin Ahmad, Department of Microbiology, Central Middlesex Hospital, Park Royal, Acton Lane, London NW10 7NS, England.
Serological classification of *Neisseria gonorrhoeae* with monoclonal antibody coagglutination reagents

D V COGHILL, H YOUNG
Serological classification of *Neisseria gonorrhoeae* with monoclonal antibody coagglutination reagents

D V COGHLI, H YOUNG

*From the STD Diagnostic Laboratory, Department of Bacteriology, University Medical School, Edinburgh*

**Summary** A total of 357 clinical isolates of *Neisseria gonorrhoeae* from 286 patients were classified serologically using two independently developed panels of monoclonal coagglutination reagents. The Pharmacia (Ph) Diagnostics panel comprised 14 reagents, five specific for serogroup WI strains and nine specific for serogroup WII/III strains, whereas the Genetic Systems (GS) panel comprised 14 reagents, seven specific for serogroup WI strains and seven specific for serogroup WII/III strains.

Serogroup WI represented 45% and WII/III represented 55% of the patients. Using the monoclonal antibody reagents, the serogroups could be further subdivided into so-called serovars. The Ph reagents identified four WI serovars and 21 WII/III serovars, whereas the GS reagents identified 10 WI serovars and 18 WII/III serovars. By combining the results obtained with each panel, 15 Ph/GS WI serovars and 33 Ph/GS WII/III serovars were recognised. In the WI isolates, one predominating serovar was recognised, whereas in the WII/III isolates, no single serovar predominated and a much greater variety of serovars was identified. The serovar patterns for men and women patients were very similar, except for one WII/III serovar that was 10 times more common in isolates from men than from women. Most isolates from different anatomical sites in the same patient were of the same serogroup and serovar. Two double infections were found. One patient had a genital infection with serogroup WII/III and a rectal infection with serogroup WI. Another patient with genital, rectal, and throat infections with serogroup WI was found to have gonococci of different GS serovars at each site.

It was concluded that the level of discrimination achieved with the monoclonal antibody reagents should prove to be valuable in studying the microepidemiology of gonococcal infection.

The serological classification of *Neisseria gonorrhoeae* using polyclonal antibodies directed against protein I epitopes has been in use since 1980. Using selectively absorbed polyclonal rabbit antisera, Sandstrom and Danielsson were able to divide strains into three antigen classes W, J, and M. Only the W antigens were found to be suitable for serological classification, and they enabled gonococci to be divided into three serogroups termed, WI, WII, and WIII. These serogroups correlate with the previously described microimmunofluorescence system and the subsequently developed protein I enzyme linked immunosorbent assay (ELISA) serotyping system. The co-agglutination W serogrouping system and protein I ELISA serotyping system both detect epitopes on the protein I molecule of the outer membrane of *N gonorrhoeae*. Two mutually exclusive protein I molecules are recognised; protein IA molecules that contain the epitopes recognised as serogroup WI, and protein IB molecules that contain the epitopes recognised as serogroups WI and WII. Serogroup WII appears to represent a minor but prominent antigenic variant of protein IB, rather than a third specific moiety.

The W serogrouping system has proved to have important epidemiological and clinical potential. Serogroup WI correlates with resistance to the bactericidal action of normal human serum, the arginine, hypoxanthine, and uracil requiring (AHU-) auxotype, disseminated gonococcal infection (DGI), and asymptomatic gonorrhoea. Serogroup WII/III correlates with resistance to several antimicrobial agents, the mtr mutation, and with homosexually ac-
quired infection.\textsuperscript{5,9–11} The geographical distribution of serogroups has also been described.\textsuperscript{8,10,12}

Unfortunately, reliable and reproducible subdivision of the serogroups is not possible using polyclonal antibodies. Using monoclonal antibodies against gonococcal outer membrane protein I, it is now possible to resolve the two major W serogroups (W1 and WII/III) into a large number of serovariants or serovars.\textsuperscript{13,14} These monoclonal antibodies have been incorporated into a coagglutination test. Studies to try to establish a panel of standardised coagglutination reagents for the serological classification and identification of \textit{N. gonorrhoeae} have been carried out.\textsuperscript{5,13–15} Serovar analysis using such a panel has potential in many aspects of gonococcal infection.\textsuperscript{16}

To apply serovar analysis to a given epidemiological situation, it is important to know the distribution of gonococcal strains in a community. To date, gonococcal serovar patterns have not been reported from any part of the United Kingdom. This study was undertaken to examine the diversity and distribution of gonococcal serovars in the Edinburgh area and also to compare the classification of isolates with the two panels of reagents.

Patients, materials, and methods

\textbf{Clinical isolates}

A total of 357 gonococcal isolates were obtained from 286 patients attending the department of genito-urinary medicine at the Edinburgh Royal Infirmary from November 1985 to April 1986 inclusive. Initial culture was on modified New York City (MNYC) medium,\textsuperscript{17} and all isolates were identified as \textit{N. gonorrhoeae} by the rapid carbohydrate utilisation test\textsuperscript{18} and the Phadebact monoclonal GC test (Blomqvist \textit{et al.}, unpublished observation presented at 6th International meeting for STD research, Brighton, 1985), which recognises serogroups W1 and WII/III separately. Once identified as \textit{N. gonorrhoeae}, isolates were subcultured on to MNYC and clear gonococal (GC) agar\textsuperscript{19} and incubated for 18–24 hours at 37\textdegree C in a moist 10\% carbon dioxide atmosphere for storage and assessment of serovars.

\textbf{Storage of isolates}

All isolates were stored in 5 ml trypticase soya broth containing 6\% lactose. A sterile cotton swab was used to remove as much growth as possible from an MNYC plate, and the swab was broken off into trypticase soya broth and stored at −20\textdegree C. Isolates with distinctive or rare serovars and isolates from the same patient but with different serovars were retained for testing again, whereas other isolates were discarded.

\textbf{Serovar identification}

\textbf{Monoclonal coagglutination reagents}

Serovars were identified using two different sets of monoclonal coagglutination reagents obtained from Genetic Systems (GS) and Pharmacia (Ph).\textsuperscript{15} Both sets of reagents were kindly supplied by Dr S Bygdeman, Huddinge University Hospital, Stockholm, Sweden. The GS reagents consisted of seven W1 reagents (A1, A2, A3, A4, A5, A6, and A7) and seven WII/III reagents (B1, B2, B3, B4, B5, B6, and B7), and the Ph reagents consisted of five W1 reagents (Ar, Ao, As, At, and Av) and nine WII/III reagents (Br, Bo, By, Bu, Bv, Bs, Bt, and Bx).

\textbf{Antigen preparation}

Isolates were incubated for 18–24 hours on GC medium. Using a cotton swab, they were harvested into 1 ml phosphate buffered saline pH 7.2 to give a smooth milky suspension. This suspension was boiled for 10 minutes and allowed to cool before testing. Prepared antigen was stored at 4\textdegree C for up to two weeks if not tested immediately.

\textbf{Test procedure}

Monoclonal coagglutination reagents and antigen preparations were each mixed well before testing. The tests were carried out by adding 20 ml of prepared antigen suspension to 20 ml of each coagglutination reagent on defined areas of surface treated plastic plates (Bioplate Type II, Biotest Folex Ltd, Moseley, Birmingham, England). Plates were rocked gently for two minutes, and coagglutination reactions were read using an oblique light against a dark background. Included with each set of reagents was a control containing antigen only, to check for autoagglutination.

\textbf{Scoring results}

Reactions were scored according to the strength of the coagglutination observed. They were graded negative (−) if they showed a smooth milky background; borderline (+) if they showed a slightly granular milky background; weakly reactive (1+) if they showed a granular background with some clearing; or moderately reactive (2+), strongly reactive (3+), or very strongly reactive (4+) if they showed increasing degrees of clumping and background clearing.

\textbf{Designation of serovars}

A serovar is defined as the pattern of reactivity of a test strain with a given set of monoclonal antibodies specific for either the W1 or WII/III serogroup.\textsuperscript{20} Each serovar is therefore depicted by an upper case letter A if it belongs to serogroup W1 and a B for WII/III isolates; these letters are then followed by lower case letters representing positive reactions with the corresponding coagglutination reagents.\textsuperscript{13} Reac-
tions of 1+ or more were scored as positive in designating a strain to a serovar.

Retesting isolates
Isolates that gave borderline reactions and those that autoagglutinated were grown again from storage in trypticase soya broth and retested. Isolates from multiple anatomical sites in the same patient that were shown to belong to different serovars were grown again from storage in trypticase soya broth, and 10 colonies from each site were tested to investigate the possibility of a mixed infection.

Results
A total of 357 gonococci was isolated from 264 genital (urethral, cervical, or both), 61 rectal, and 32 pharyngeal cultures. Gonococci of serogroup WI were isolated from 62 (37%) and WII/III from 107 (63%) of 169 infected men. Serogroup WI gonococci were isolated from 67 (57%) and WII/III from 51 (43%) of 117 infected women (one with a WI and a WII/III strain). Gonococci were isolated from both genital and extragenital (rectal, pharyngeal, or both) sites in 49 (42%) women and in 14 (8%) men. Except in the woman infected with both serogroups (WI rectally and WII/III genitally), gonococci isolated from different anatomical sites in the same patient were of the same serogroup.

Table 1 Distribution of Ph serovars in WI isolates from 62 men and 67 women

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Men (%)</th>
<th>Women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arost</td>
<td>46 (74)</td>
<td>48 (72)</td>
</tr>
<tr>
<td>Aros</td>
<td>2 (3)</td>
<td>6 (9)</td>
</tr>
<tr>
<td>Av</td>
<td>5 (8)</td>
<td>3 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>67</td>
</tr>
</tbody>
</table>

Table 2 Distribution of GS serovars in WI isolates from 62 men and 67 women

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Men (%)</th>
<th>Women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedgkih</td>
<td>46 (74)</td>
<td>46 (69)</td>
</tr>
<tr>
<td>Aedgli</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Aedgk</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Aedg</td>
<td>4 (6)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Aegk</td>
<td>2 (3)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Aedgih</td>
<td>8 (13)</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Aedih</td>
<td>5 (8)</td>
<td>5 (8)</td>
</tr>
<tr>
<td>Ac</td>
<td>2 (3)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>69*</td>
</tr>
</tbody>
</table>

*Multiple isolates from one patient gave slightly different serovars: Aedg from cervix; Aedg from rectum; and Aedgk from fauces.

WI isolates
The distribution of Ph and GS serovars in the 129 patients infected with WI isolates is given in tables 1 and 2. One predominating serovar (Ph-Arost and GS-Aedgkih) was seen with each set of reagents. The isolates could be divided into four Ph serovars and 10 GS serovars. The four most commonly occurring GS serovars, however, comprised 98% (61) of isolates from men and 88% (59) of isolates from women. Other GS serovars were usually represented by only one or two strains.

Table 3 gives the correlation between Ph and GS serovars in the WI isolates. The predominating Ph serovar correlated well with the predominating GS serovar, this combination accounting for 68% (89) of the 129 patients infected with WI isolates. Fifteen different combinations were recognised. Six of the 10 GS serovars each corresponded to only one Ph serovar, but only one of the four Ph serovars corresponded to one GS serovar (Av/Ae). In one woman with a WI infection, isolates from multiple anatomical sites each had slightly different GS serovars. The serovars remained different when tested again. In all other patients infected at multiple sites with WI isolates, the Ph and GS serovars of isolates from each site were identical.

Table 3 Correlation between Ph and GS gonococcal serovars in WI isolates from 129 patients

<table>
<thead>
<tr>
<th>Ph serovars</th>
<th>GS serovars</th>
<th>Aedgkih</th>
<th>Aedgli</th>
<th>Aedgk</th>
<th>Aedg</th>
<th>Aegk</th>
<th>Adg</th>
<th>Agk</th>
<th>Aedgih</th>
<th>Aedih</th>
<th>Ac</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arost</td>
<td>89</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>94</td>
</tr>
<tr>
<td>Arst</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Aros</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Av</td>
<td>Total</td>
<td>91</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>131*</td>
</tr>
</tbody>
</table>

*Multiple isolates from one woman patient gave slightly different GS serovars.
WII/III ISOLATES
The distribution of Ph and GS serovars in the 158 WII/III isolates is given in tables 4 and 5. There was a greater variety of WII/III Ph and GS serovars than WI serovars. The WII/III isolates consisted of 21 Ph serovars and 18 GS serovars. The serovars (Ph-Bropt and GS-Bajk) occurring most commonly with each panel of reagents did not predominate to the same extent as the WI predominating serovars.

The prevalence of Ph and GS serovars was comparable in men and women, except that Ph-Bropt and GS-Bajk were 10 times more prevalent in men than women. Table 6 shows the correlation between Ph and GS serovars in the 158 WII/III isolates. The most common combination, which accounted for 32% (50) of the WII/III isolates, corresponded to the most common Ph and GS serovars.

Thirty-three WII/III combinations were recognised, over twice the number recognised in WI isolates. Sixteen of the 21 Ph serovars each corresponded to only one GS serovar, the most notable of these being the Av/Bx/Behj combination. Nine of the 18 GS serovars each corresponded to one Ph serovar.

In all patients infected at multiple sites with WII/III isolates, the Ph and GS serovars of isolates from each site were identical.

Discussion
Using monoclonal coagglutination reagents, the distribution of serogroup WI was found to be 45% and of WII/III was 55%. This distribution was consistent with that found in a previous study employing polyclonal antibody reagents. These results are also similar to findings in Scandinavia, where WI strains were shown to predominate in larger towns and WI strains predominated in the smaller towns. We compared serogroup patterns between strains isolated from men and women. Of isolates obtained from 169 men, 37% belonged to serogroup WI and 63% to serogroup WII/III, and of isolates obtained from 117 women, 57% belonged to serogroup WI and 43% to serogroup WII/III. These results are consistent with previously reported Scandinavian data in which WI strains were more common in strains isolated from women than men.

Strains belonging to serogroup WI could be resolved into four Ph serovars and 10 GS serovars, with a total of 15 Ph/GS serovar combinations. Serogroup WII/III isolates could be resolved into 21 Ph serovars and 18 GS serovars, with a total of 33 Ph/GS serovar combinations. When one serovar in one system corresponded to two or more serovars in the other system, the latter were closely related, reaction patterns differing in only one or two reagents.

The Ph and GS reagents used in this study and four additional GS reagents (two specific for WI and two for WII/III) have been used to identify strains, mostly obtained from northern Europe and the United States of America, in a worldwide epidemiological study. Isolates belonging to serogroup WI could be resolved into 12 Ph serovars and 21 GS serovars. Isolates belonging to serogroup WII/III could be resolved into 38 Ph serovars and 62 GS serovars. A total of 27 WI Ph/GS serovar combinations and 93 WII/III Ph/GS serovar combinations were recognised. The larger
numbers of serovars recognised in such a worldwide study compared with a more localised study suggest that a large variety of different strains is circulating in different geographical locations.

A similar study carried out on strains isolated from Australia, New Zealand, Bangkok, Singapore, and Korea resolved 165 WI strains into seven Ph serovars, seven GS serovars, and eight Ph/GS serovar combinations; and 395 WI/III strains resolved into 25 Ph and 19 GS serovars with 41 Ph/GS serovar combinations. Several additional studies using selected panels of these Ph and GS reagents have been reported. These include studies that compared the discriminatory power of each panel and showed varying numbers of different serovars in the gonococcal populations studied.

Serovar patterns in the two major serogroups (WI and WI/III) differed noticeably. Strains belonging to serogroup WI/III could be subdivided into more serovars than strains belonging to serogroup WI. This has been noted in previous studies. One serovar combination (Arost/Aedgkiih) was found to predominate in the WI isolates. This was consistent with data reported in a worldwide study. The important foci of this serovar combination are thought to be Europe, North America, and some parts of Australia, but not South East Asia. Bygdemman reported that the GS serovars Aedgkiih, Aedih, and Ae were the three most common serovars in 533 isolates from different geographical areas, and accounted for 92% of the isolates. These serovars were also the most common serovars in WI isolates from Edinburgh, accounting for 85% of the isolates. In a survey of serovars of gonococcal isolates from different parts of the world, 13 WI serovars were recognised, 59% of which were accounted for by the GS serovars Aedgkiih, Aedih, and Ae. The geographical origin of these isolates, however, was not reported.

No predominating serovar was recognised in strains belonging to serogroup WI/III. This observation has been made in previous studies. In the same survey as that described for WI strains, Bygdemman reported that 1333 WI/III strains could be resolved into 36 GS serovars. The 10 most common serovars (Bajk, Bacejk, Bak, Bacek, Bacejk, Begik, Back, Begrhik, Bcgk, and Bcgk) accounted for 98% of the WI/III isolates. In the present study WI/III strains from 158 patients could be resolved into 21 GS serovars. The 10 most common serovars (Bajk, Bacejk, Bacejk, Bak, Bacek, Bacejk, Begik, Back, Begrhik, Bcgk, and Bcgk) accounted for 92% of the WI/III isolates. Five of these serovars are included amongst the 10 most common WI/III serovars reported by Bygdemman. These findings support the observation made by Bygdemman that there are great differences in WI/III serovar patterns between different regions, and that the serovar Bajk is common to all geographical regions. One WI/III serovar combination (Brov/Bak) is notable by the observation that strains of this serovar are 10 times more often isolated from men than women. As serogroup WI/III has been correlated with homosexually acquired infection, this serovar is probably related to homosexuality.
The small variety of serovars noted in isolates belonging to serogroup WI could be a result of the poor discriminatory power of the WI monoclonal antibodies or, alternatively, could reflect the small number of different epitopes of protein IA. The latter possibility would suggest the need for an alternative target antigen to protein I for use in the serological classification of WI strains. Sandstrom et al found it more difficult to obtain different antibodies against WI epitopes than WII/III epitopes, and studies on the antigenic drift of gonococcal protein I suggest that the WI serogroup is more antigenically stable than the WII/III serogroup. Auxotype analysis has been combined with serovar analysis to try to improve the resolution of the serovar system. Although several correlations between auxotype and serovar have been reported, these studies show that the combination of serological classification with auxotyping achieves greater discrimination than either system is used alone. Auxotyping, however, is not as easy to perform as serogrouping by coagglutination, and its use is therefore more restricted.

Despite the limitations, the degree of discrimination achieved by serological classification alone has proved valuable in many aspects of gonococcal epidemiology and pathogenesis. Serovar analysis has proved useful in contact tracing. In a study by Backman et al, gonococcal strains from 95% of known contact pairs belonged to the same serovar in each couple. Non-matching serovars from presumed sexual partners indicate that additional partners may have to be traced, or that there are double infections. The occurrence of double infections can be assessed by serovar analysis. Double infections were rare in this locality. One woman (less than 1%) out of 117 was infected simultaneously with gonococcal strains of different serogroups at each site. Another woman was infected with gonococci belonging to slightly different GS serovars at each site. Backman et al reported that two (1%) of 218 women, two (7%) of 28 heterosexual men, and three (25%) of 12 homosexual men had two or more gonococcal isolates simultaneously. These workers drew attention to the problem of recognising a double infection at one site with strains belonging to the same serogroup but different serovars.

Serovar analysis has great potential for the control of microepidemics of gonococcal infection, particularly with a strain with a distinctive serovar or one that has low prevalence in the population. In this study, a distinctive strain that cross reacted with Ph Av and Ph Bx reagents was detected in 14 (5%) of infected patients. All these strains belonged to GS serovar Behj and, furthermore, this serovar was found uniquely in such strains. A worldwide study reported that no isolates reacted with the Ph Bx reagent. More recently, however, the same Av/Bx cross reactive strains have been reported. These strains were also found to cross react with the GS Al reagent, one not included in the panel used in the present study. It has been proposed that these strains are the missing link between serogroups WI and WII/III. We are currently monitoring all Av/Bx isolates and carrying out intensive contact tracing to prevent this serovar becoming more widely established in the community.

Serovar analysis has potential value in medicolegal cases, particularly when the serovar of tested strains is related to the prevalence of such strains in the geographical area concerned. Serovar analysis has also been found to facilitate the classification of isolates taken before and after treatment as representing treatment failure or reinfection.

Correlations between serovars and various aspects of gonococcal infection extend the potential value of serovar analysis. These correlations include an association between serovar and susceptibility to antibiotics, serovar and sexual preference, and serovar and auxotype. Auxotype AHU +, which was previously correlated with disseminated gonococcal infection, has been correlated with particular serovars, indicating that serovar analysis could prove useful in identifying strains capable of causing serious or complicated infections. Serovar and auxotype analysis have also been correlated with plasmid profile, and serovar analysis has potential for monitoring outbreaks of infection with penicillinase producing strains of Neisseria gonorrhoeae (PPNG), thus helping to control such infections in the community. A knowledge of PPNG serovars allows outbreaks to be recognised early and contact tracing efforts to be concentrated on people infected with the epidemic strain. The loss of the β-lactamase encoding plasmid from PPNG strains has been reported. Serovar analysis could therefore have an advantage over detection methods that rely on production of the β-lactamase enzyme to identify PPNG strains, in that the spread of infection could continue to be monitored, even when one or more of the contacts in the chain of infection are infected with gonococci that have lost their β-lactamase plasmid.

The diversity of antigenic types of gonococci circulating in the Edinburgh area were illustrated in this study. To date, there are no published data on serovar patterns in the United Kingdom. A knowledge of currently existing serovars in a particular geographical area is necessary to interpret results obtained from serovar identification. Monitoring strains by serovar identification should also prove useful in developing antigen detection systems and in evaluating the use of monoclonal antibodies for serological classification.
Serological classification of Neisseria gonorrhoeae with monoclonal antibody coagglutination reagents

Continued monitoring of serovar patterns will be necessary, as temporal changes may occur because of genetic alteration of existing strains and the importation of strains from different geographical regions.

Serovar analysis by the coagglutination test is an easy and reproducible method for classifying gonococci. Although resolution could be improved by incorporating additional reagents to the panel, a compromise must be reached between improving resolution and making serovar analysis too complex and time consuming. The temporal monitoring of strains in particular geographical regions should make it possible to design panels suitable for the different regions, as the range of gonococci in any one area is limited. A standardised panel, however, will be preferable to compare results from around the world. Local monitoring of serovar patterns from geographical regions around the world should allow the origin of outbreaks to be identified and the worldwide spread of gonorrhoea to be studied.

DVC gratefully acknowledges receipt of a Faculty of Medicine Scholarship. The work was supported by a Scottish Home and Health Department research grant K/MRS/50/C875. We are indebted to Dr Solgun Bygdeman for her helpful advice and infectious enthusiasm.

References
25 Knapp JS, Sandstrom EG, Holmes KK. Overview of epidemi-


Detection of urogenital gonorrhoea in men by dot-blot immunoassay

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A simplified enzyme-linked immunosorbent assay (ELISA) system involving “dot-blotting” of antigen on to nitrocellulose has been developed. Antigen was detected with a mixture of antisera raised against whole cells of gonococci belonging to serogroup WI (strain E-5) and serogroup WII (strain N-10). When the dot-blot assay was applied to the detection of gonococcal antigen in urethral exudates from 95 men with urethritis it was reactive in 43 of 48 men with culture proven gonorrhoea. The test was also reactive in four of 47 men with negative gonococcal cultures giving a sensitivity and specificity of 91-5 and 89-6%, respectively. A mixture of antisera raised against purified protein I from strains E-5 and N-10 did not improve sensitivity and specificity. As the dot-blot immunoassay is not dependent on viable organisms it would be a useful alternative to culture in areas where there are problems in maintaining the viability of gonococci during transport.

Keywords: gonorrhoea, diagnosis, ELISA, gonococcal antigen.

Introduction

Gram staining of genital secretions remains the only widely accepted routine procedure for making an “on-the-spot” diagnosis of gonococcal infection. In men with symptomatic urethritis the Gram stain has an accuracy of 85–99% in differentiating between gonococcal and non-gonococcal urethritis1-4. However, in asymptomatic men and women the sensitivity (40–60%) of the Gram stain is poor5-8.

Cultural diagnosis is essential in asymptomatic men and women and should also be used to confirm a microscopic diagnosis. Ideally specimens should be plated directly on to selective media and cultures placed at 37°C in a moist carbon dioxide enriched atmosphere. This is not always practicable and many clinics rely on the transport of specimens to a centralised laboratory. Transport media can result in decreased recovery of gonococci: this is more likely when transport exceeds 12 h and when the initial inoculum is small.

Failure to culture gonococci may also be due to hypersensitivity to vancomycin, an antibiotic used in gonococcal selective media3,9,10. Immunological detection of gonococcal antigen is not dependent on viable organisms and could be a useful alternative to culture in providing a confirmatory diagnosis.

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† Correspondence should be addressed to Dr H. Young.
A number of laboratories have attempted to develop their own enzyme-linked immunosorbent assay (ELISA) systems or have evaluated a commercially available ELISA (Gonozyme, Abbott Diagnostics). Evaluations of Gonozyme report sensitivities and specificities of 94–100% and 96%–100%, respectively, for urethral samples from men. The reported sensitivity (78–92%) and specificity (87–98%) are poorer with specimens from women.

Low sensitivity in ELISA tests may be related to poor binding of antigen to the solid phase, usually plastic beads or wells of a microtitre plate. Inability of the antiserum used to detect bound antigen to react with all strains of gonococci is another likely cause of poor sensitivity.

In an attempt to overcome these problems we have (i) used nitrocellulose strips as the solid phase allowing direct spotting; (ii) utilised the reliable coagglutination serogrouping system based on differences in gonococcal protein to make a rational selection of antisera for antigen detection.

As a result of serogrouping studies carried out in this locality we have found that a combination of two antisera will detect 97.2% (694/714) of strains. This paper reports the results of an initial study employing these antisera for detecting gonococcal antigen in male urethral exudates by means of a simple dot-blot ELISA with a nitrocellulose membrane as the solid phase.

**Materials and methods**

*Patients*

Ninety-five male patients attending the Department of Genitourinary Medicine, the Royal Infirmary, Edinburgh, with urethritis were included in the study. Urethral exudate was collected for microscopy and cultured on modified New York City (MNYC) medium. A small cotton tipped chlamydial swab (Mediculture and Equipment Ltd, Corsham, England) was then inserted into the urethra to collect material for the dot-blot ELISA.

Cultures were processed and *Neisseria gonorrhoeae* identified as described previously. Swabs were transported to the laboratory within 4 h. On receipt the swabs were placed in 200 μl of Tris-buffered saline (20 mm Tris, 500 mm NaCl, pH 7.5) and agitated for 60 s with a vortex mixer prior to boiling for 10 min. After boiling the swabs were again vortexed and material expressed before discarding the swab. Specimens of boiled exudate were stored at −20°C until required for testing.

*Stock cultures*

*Neisseria gonorrhoeae* major outer membrane protein (MOMP) reference strains E-5 and N-10 were kindly supplied by Dr D. Danielsson, Department of Clinical Bacteriology and Immunology, Central County Hospital, Orebro, Sweden.

*Preparation of whole cell antisera*

Antisera against whole cell antigen of MOMP strains E-5 and N-10 were raised in New Zealand white rabbits immunised according to the procedure of Sandstrom & Danielsson.
Purification of protein I antigen. Protein I was purified from MOMP E-5 and N-10 by the method of Heckels²⁴ and James & Heckels²⁵. After purification protein I antigen was stored at −20°C until required.

Preparation of antisera against protein I. Antisera against MOMP E-5 and MOMP N-10 protein were raised in New Zealand white rabbits. A test bleed was taken prior to immunisation.

Aliquots of MOMP E-5 and N-10 protein I antigen were thawed and diluted with an equal volume of Freund’s complete adjuvant to give a final vaccine volume of 0.5 ml containing 100 μg of protein I antigen. The rabbits were immunised intramuscularly according to the following procedure: Day 1, two 0.5-ml injections, one in each hind leg; Day 14, two 0.5-ml injections in the back; Day 28, one 0.5-ml injection in each hind leg; Day 42, the rabbits were test-bled prior to receiving a 0.5 ml booster injection. Two weeks later the rabbits were exsanguinated by cardiac puncture.

Dot-blot immunoassay procedure

The following procedure was used throughout the study. Details of the antigen and antisera are described later where appropriate.

Strips of nitrocellulose (Bio-Rad Laboratories Ltd, Watford, England) were cut to the required size, allowing approx 1 cm² per sample, placed in Tris-buffered saline (TBS) (20 mM Tris; 500 mM NaCl, pH 7.5) at a 45° angle and allowed to wet by capillary action. Strips were removed from the TBS, air dried, and antigen, either 1 μl or successive 1 μl amounts, applied to separate sites on the nitrocellulose strips. The strips were air dried and placed in a blocking solution of 3% (w/v) gelatin in TBS and incubated at room temperature with constant shaking for 45 min. The strips were then transferred to antigenococcal antiserum in antibody buffer (1%, w/v, gelatin in TBS containing 0.05% Tween 20) and incubated for 1 h at room temperature with constant shaking. The strips were washed twice in TBS containing 0.05% Tween 20 and transferred to the conjugate solution containing a 1/3000 dilution of anti-rabbit IgG horseradish peroxidase conjugate (Bio-Rad) in antibody buffer.

The strips were incubated with the conjugate solution for 1 h at room temperature with constant shaking. Strips were washed as before, rinsed in distilled water and transferred to the substrate solution. The substrate solution was prepared by adding 60 mg of 4-chloro-1-naphthol (Bio-Rad) in 20 ml of methanol to 100 ml of TBS containing 0.015% ice cold H₂O₂.

The strips were incubated at room temperature for 20 min, with constant shaking. Colour reactions were stopped by placing strips in distilled water for 10 min. Positive reactions resulted in a purple colour appearing on the antigen dots. Colour development on the dots was graded subjectively, by eye, according to the following protocol: 3+, strong positive reaction (very deep purple); 2+, medium positive reaction (purple); +, positive reaction (light purple); +/−, very slight colour development; −, no colour development.

Detection of gonococcal antigen in male urethral specimens

Antigen blotting. Stored clinical specimens were thawed and boiled for 10 min. Each specimen was then blotted on to a separate area of the nitrocellulose strip by placing
successive 1 μl amounts to give a total of 10 μl of antigen per spot: up to nine clinical specimens could be tested on each strip.

**Antigen detection with whole cell antisera.** This comprised 1:1 mixture of anti-E-5 and anti-N-10. An arbitrary dilution of 1/100 for the mixture (1/200 dilution for each individual antiserum) was chosen.

**Antigen detection with anti-protein I antisera.** In the second half of the study 45 specimens were tested in parallel with a 1:100 dilution of a 1:1 mixture of anti-E-5 protein I and anti-N-10 protein I antisera.

**Controls and scoring of results.** Each strip included a negative control containing TBS as well as control antigen suspensions. These comprised MOMP E-5 boiled whole cell antigen at concentrations of \(10^7\), \(1.6 \times 10^5\) and \(8 \times 10^4\) cfu which gave 3+ (strong positive; 1+ (positive) and +/- (very little colour) reactions, respectively.

Clinical specimens were scored positive if they gave a reaction of >1+. Specimens giving very little colour (+/-) reaction or no colour were scored negative.

**Correlation with culture results.** The dot-blot immunoassay results for the clinical specimens were read without prior knowledge of the respective culture results.

**Determination of the minimum amount of antigen detectable.** Nine gonococcal strains isolated from men from whom urethral specimens had been taken for testing with the dot-blot immunoassay were used as antigen. Suspensions of the gonococcal isolates were made in TBS adjusted to an OD of approximately 1.0 (range 0.8-1.1) at 540 nm (approximately 10⁵ cfu). An aliquot of each suspension was boiled for 10 min, and doubling dilutions from 1 in 2 to 1 in 624 were prepared in TBS. One-microlitre amounts of each antigen dilution from undiluted to 1/1024 were applied to specific spots on nitrocellulose strips.

Antigen was detected by a 1:1 mixture of anti-E-5 and anti-N-10 whole cell antisera and by a 1:1 mixture of anti-E-5 protein I and anti-N-10 protein I antisera. A dilution of 1/100 was used for both antisera mixtures.

**CoA serogrouping.** The nine gonococcal isolates were serogrouped by CoA reagents defining CoA serogroups WI, WII and WIII.

**Determination of the minimal amount of purified MOMP E-5 and N-10 protein I detectable**

Purified protein I antigen (50, 10, 5 and 1 ng) from MOMP E-5 and N-10, was applied to separate spots on nitrocellulose strips. Antigen was detected by a 1/100 dilution of a 1:1 mixture of anti-E-5 and anti-N-10 whole cell antisera and by a 1/100 dilution of a 1:1 mixture of anti-E-5 protein I and anti-N-10 protein I antisera.

**Testing of the anti-rabbit IgG peroxidase conjugate for non-specific binding to antigen and urethral specimens for endogenous peroxidase activity**

Control antigen consisted of undiluted (1 x 10⁶ cfu), 1/64 and 1/256 dilutions of MOMP E-5 boiled whole cell antigen and undiluted (1 x 10⁶ cfu) MOMP N-10 boiled whole cell antigen. One-microlitre amounts of each antigen dilution were applied successfully to separate spots on two nitrocellulose strips to give a total of 10 μl of antigen per spot. Three male urethral specimens were also applied to each strip. These were treated as
previously described and successive 1 μl amounts were applied to separate spots on the nitrocellulose strips to give a total of 10 μl of antigen per spot.

**Testing for non-specific binding of the conjugate.** One blocked strip was placed in the conjugate solution (1/3000 dilution of anti-rabbit IgG peroxidase conjugate in antibody buffer) for 1 h with constant shaking, washed, placed in the substrate solution and incubated for 20 min.

**Testing for endogenous peroxidase activity.** The second strip was removed from the blocking solution, washed in distilled water and placed directly into the substrate solution and incubated at room temperature, with constant shaking for 20 min.

After incubation in the substrate solution both strips were removed, washed in distilled water for 10 min and examined for colour development.

**Re-testing of 74 clinical specimens with antisera diluted 1 in 1600**

Anti-E-5 and anti-N-10 whole cell antisera were tested against dilutions of homologous antigen in a standard checkerboard titration. The titre of each antiserum against its homologous antigen was found to be 1 in 1600. A total of 74 clinical specimens were re-tested in parallel against each antiserum at a dilution of 1 in 1600.

**Calculation of predictive values**

Predictive values were calculated according to the formula given by Vecchio.

The predictive value of a positive test (PV*) is the probability that the clinical specimen giving a positive dot-blot result represents infection with *N. gonorrhoeae* and is calculated according to the formula

\[ PV^* = \frac{pa}{pa + (1-p)(1-b)} \times 100. \]

The predictive value of a negative test (PV−) is the probability that a clinical specimen giving a negative dot-blot result is from a non-infected patient and is calculated by the formula

\[ PV^- = \frac{(1-p)b}{(1-p)b + p(1-a)} \times 100, \]

where \( p \) = prevalence of gonococcal infection within the total population studied, \( a \) = test sensitivity, and \( b \) = test specificity.

**Statistical analyses**

The significance of differences in the results were determined by the \( \chi^2 \)-test with Yates' correction.

**Results**

Table 1 shows the correlation of the dot-blot immunoassay results with culture results for 95 male urethral specimens. Antigen was detected in the immunoassay with a 1:1 mixture of anti-E-5 and anti-N-10 whole cell antisera at an arbitrary dilution of 1/100 (1/200 for each specific antiserum in the mixture). At this dilution the antisera mixture
Table 1. Correlation of gonococcal culture results with dot-blot immunoassay results

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Dot-blot immunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>43</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
</tr>
</tbody>
</table>

detected $10^3$ to $10^4$ cfu MOMP E-5 and N-10 whole cell antigen. Overall 48 (51%) and 47 (49%) of the 95 urethral specimens were scored positive and negative, respectively.

Correlation with culture results showed that the dot-blot immunoassay had a sensitivity of 91.5% (dot-blot positive/culture positive specimens) and a specificity of 89.6% (dot-blot negative specimens/culture negative specimens). There were four false negative and five false positive clinical specimens.

Figure 1 shows the dot-blot immunoassay results and demonstrates the colour intensity obtained with clinical specimens 1–19.

Detection of antigen with a mixture of anti-E-5 and anti-N-10 protein I antisera

Table 2 shows the correlation of culture results with the dot-blot immunoassay results for 45 clinical specimens tested with a 1/100 dilution of a 1:1 mixture of anti-E-5 protein I and anti-N-10 protein I antisera. At this dilution the anti-protein I antisera mixture detected $4.8 \times 10^4$ and $1.6 \times 10^4$ cfu of MOMP E-5 and N-10 whole cell antigen, respectively.
ELISA for gonococcal antigen

Table 2. Correlation of culture results with dot-blot immunoassay results using protein I antisera for antigen detection in 45 male urethral specimens

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Dot-blot immunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
</tr>
</tbody>
</table>

The sensitivity and specificity of the immunoassay with the anti-protein I antisera were 84 and 85%, respectively. Four false negative and three false positive results were obtained. The four false negative results occurred with the same specimens giving false negatives with the whole cell antisera. Two of the three false positive specimens were also false positives with the whole cell antisera.

Detection of antigen with anti-E-5 and anti-N-10 whole cell antisera at a 1/1600 dilution

Table 3 shows the correlation of culture results with the dot-blot immunoassay results for 74 male urethral specimens re-tested with each whole cell antiserum at 1/1600 dilution. This test system has a sensitivity and specificity of 85.4 and 69.7%, respectively.

Of the five false positives detected in the first dot-blot immunoassay using a 1/100 dilution of the whole cell antisera (Table 1), four were available for re-testing with the individual antisera at a dilution of 1/1600. All four gave positive results with antisera at this dilution. In addition six new false positives (four positive with both anti-E-5 and anti-N-10 and two with only anti-N-10) were detected.

Of the four false negatives shown in Table 1 three were available for re-testing with anti-E-5 and anti-N-10 at a dilution of 1/1600. All three specimens remained negative. In addition a further three specimens were found to be negative on re-testing with antisera at this dilution.

Table 3. Correlation of gonococcal culture results with dot-blot immunoassay results using anti-E-5 and anti-N-10 at a 1/1600 dilution for antigen detection in 74 male urethral specimens

<table>
<thead>
<tr>
<th>Culture result</th>
<th>Dot-blot immunoassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of clinical specimens</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>35</td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>
There was no significant difference in the sensitivity of the dot-blot assay using individual antisera at a dilution of 1/1600 compared with a mixture of antisera at a dilution of 1/100 ($\chi^2 = 0.321; P > 0.5$). However, the specificity of the assay was significantly poorer when the specimens were re-tested with antisera at a dilution of 1/1600 ($\chi^2 = 3.89; P < 0.05$).

**Determination of the minimum amount of antigen detected in the dot-blot immunoassay and the corresponding CoA serogroups for nine clinical isolates of N. gonorrhoeae**

As shown in Table 4 the amount of gonococcal antigen detected ranged from $4 \times 10^3$ to $3 \times 10^4$ cfu. The mixture of anti-E-5 and anti-N-10 whole cell antisera usually detected a two-fold lower number of gonococci than the mixture of anti-E-5 protein I and anti-N-10 protein I antisera. However, the minimum amount of antigen detected by the two antisera mixtures was identical for gonococcal strains 3 and 5. The antisera mixtures were equally effective in detecting gonococcal isolates belonging to the various serogroups. Isolates from patients whose urethral exudates were negative in the dot-blot immunoassay were detectable at levels similar to those from patients whose exudates were positive.

**Determination of the minimal amount of purified MOMP E-5 and N-10 protein I antigen detected by a mixture of whole cell antisera and by a mixture of anti-protein I antisera**

The mixture of anti-E-5 and anti-N-10 whole cell antisera (1/100 dilution) detected MOMP E-5 and N-10 protein I antigen at a concentration of 1 ng (1+ reaction). The mixture of anti-E-5 protein I and anti-N-10 protein I antisera (1/100 dilution) detected MOMP E-5 and N-10 protein I at a concentration of 5 ng (2+ reaction); a little colour developed (+/- reaction) on the MOMP E-5 protein I spot containing 1 ng of protein but no colour developed with MOMP N-10 protein I at a similar concentration.

**Table 4. Correlation of dot-blot immunoassay results with CoA serogroup and minimum amount of antigen detected**

<table>
<thead>
<tr>
<th>Clinical isolate</th>
<th>CoA serogroup</th>
<th>Minimum amount of antigen (cfu) detected with a 1/100 dilution of:</th>
<th>Corresponding clinical specimen dot-blot result using a mixture of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole cell antisera</td>
<td>Anti-protein I antisera</td>
</tr>
<tr>
<td>1</td>
<td>WI</td>
<td>$4 \times 10^3$</td>
<td>$8 \times 10^3$</td>
</tr>
<tr>
<td>2</td>
<td>WII</td>
<td>$8 \times 10^3$</td>
<td>$1 \cdot 6 \times 10^4$</td>
</tr>
<tr>
<td>3</td>
<td>WII</td>
<td>$8 \times 10^3$</td>
<td>$8 \times 10^3$</td>
</tr>
<tr>
<td>4</td>
<td>WII</td>
<td>$4 \times 10^3$</td>
<td>$8 \times 10^3$</td>
</tr>
<tr>
<td>5</td>
<td>WII</td>
<td>$8 \times 10^3$</td>
<td>$8 \times 10^3$</td>
</tr>
<tr>
<td>6</td>
<td>WII/WIII</td>
<td>$4 \times 10^3$</td>
<td>$8 \times 10^3$</td>
</tr>
<tr>
<td>7</td>
<td>WI</td>
<td>$8 \times 10^3$</td>
<td>$1 \cdot 6 \times 10^4$</td>
</tr>
<tr>
<td>8</td>
<td>WII</td>
<td>$8 \times 10^3$</td>
<td>$1 \cdot 6 \times 10^4$</td>
</tr>
<tr>
<td>9</td>
<td>WII/WIII</td>
<td>$1 \cdot 6 \times 10^3$</td>
<td>$3 \times 10^4$</td>
</tr>
</tbody>
</table>

*P = positive; N = negative.
Non-specific binding of the conjugate to antigen

Some colour developed on the control spots containing undiluted (10^7 cfu) MOMP E-5 and N-10 whole cell antigen and this was scored +/-. A similar amount of colour developed on each of the three spots containing the clinical specimens. The clinical specimens chosen had given high background negative or false positive results when previously tested in the complete dot-blot immunoassay. However, although the conjugate appears to react to some degree with the control antigen at high concentrations and with the clinical specimens, the intensity of the colour which developed with these specimens in the complete immunoassay suggests that the conjugate is not the major contributor to non-specific colour development.

Endogenous peroxidase activity

No colour developed on the antigen spots on the strip incubated with the substrate solution alone. This indicated that there was no residual peroxidase activity in the clinical specimens.

Discussion

The dot-blot immunoassay is based upon the non-covalent binding of proteins to nitrocellulose membranes. Immunoblotting (electrophoretic transfer followed by immunoassay) has been used to examine the antibody response to the pili and protein II of gonococci isolated from individual patients within contact groups. Brooks et al. used a dot-blot immunoassay to detect *Toxoplasma gondii* antigen in laboratory-infected mice and in the serum and cerebrospinal fluid of six infected infants.

Our results show that antigen blotting on nitrocellulose membranes can be used to detect gonococcal infection in men with urethritis. Comparable results were obtained when whole cell antiserum and anti-protein I antiserum were used for antigen detection. The sensitivity and specificity with whole cell antiserum were 91.5 and 89.6%, respectively. When anti-protein I antiserum was used to detect antigen in 45 specimens the sensitivity and specificity were 84 and 85%, respectively. As the four false negative and most of the false positive results occurred during the period of parallel testing the sensitivity and specificity of the assay using the whole cell antiserum were identical for these 45 specimens.

Lack of sufficient antigen on the initial swab specimen seems the most likely reason for false negative results. As shown in Table 4, isolates from two of the four patients with negative antigen detection tests were detectable at levels similar to those from patients whose exudates were positive for antigen. The urethral swab from one of these patients was noted to have very little material on it during the preparation of the specimen. All specimens for antigen detection were taken after specimens had been taken for routine culture and microscopy. Therefore it is possible that some of these tests might have been positive if a single specimen had been taken for antigen detection. A small number of infecting organisms has been suggested as the reason why the performance of the Gonozyme test is poorer in asymptomatic men and women than in men with urethritis.

Five specimens gave a positive antigen detection test with whole cell antiserum but a negative culture. It is unlikely that these were patients with gonorrhoea from whom we failed to isolate the organism: none of the men had known source contacts with
gonorrhoea and none of their secondary contacts developed gonorrhoea. Cross-reaction of the anti-gonococcal detection serum with other genital pathogens or commensal organisms could account for these “false positive” reactions.

Non-gonococcal neisseriae are detectable at 4- to 125-fold higher levels than N. gonorrhoeae. However, organisms such as Neisseria meningitidis and Neisseria lactamica are relatively rare in male urethral specimens. In contrast Bacteroides spp are frequently isolated from the genital tract of both men and women. When Bacteroides bivius, Bacteroides intermedius and Bacteroides asaccharolyticus were examined, the greatest degree of cross-reaction was found with B. bivius which gave a false positive dot-blot result with as little as 6·25 × 10^4 cfu. Two of four “false positive” clinical specimens were negative when re-tested with whole cell anti-gonococcal antiserum absorbed with B. bivius.

Therefore cross-reactions with antigens, possibly lipopolysaccharide, present on organisms such as B. bivius may cause problems in some cases. The use of antiserum against purified protein I does not eliminate these problems. When three of the “false positive” specimens were re-tested with anti-protein I antiserum two remained positive. It is possible that the protein I used to immunise the rabbits contained traces of lipopolysaccharide.

Reducing the antibody concentration of the whole cell antiserum failed to eliminate the “false positive” reactions: all four specimens available for re-testing with a 1 in 1600 antiserum dilution remained positive. Indeed when this dilution was used to re-test 74 clinical specimens that had been stored at −20°C a further six “false positive” reactions were obtained. This represented a statistically significant decrease in the specificity of the assay from 89·6 to 69·7% (χ^2 = 3·89; P < 0·05). Although the sensitivity of the assay was reduced from 91·5% to 85·4% this was not significant (χ^2 = 0·321; P > 0·5). The prolonged storage at −20°C may have had an adverse effect on the nature of the non-covalent binding of antigen to nitrocellulose thus promoting more non-specific antiserum and conjugate interactions. Until the effect of prolonged storage is investigated further it is recommended that specimens should be tested within 1 month of collection.

Overall the results obtained with the dot-blot immunoassay compare favourably with those reported for the commercially available Gonozyme ELISA assay. The predictive value of a positive Gonozyme test was 90·1% and 96·5%, and that of a negative test 96·8% and 99·9%. Using a mixture of anti-E-5 and anti-N-10 whole cell antiserum we obtained a positive predictive value of 89·6% and a negative predictive value of 91·5%.

Because microscopy gives good results in the case of male patients a test such as the dot-blot immunoassay, even if rapid enough, would be of limited value in terms of “on-the-spot” diagnosis. However, as the dot-blot test is not dependent on viable organisms, it could be a useful alternative to culture in providing a confirmatory diagnosis in areas where there are problems associated with maintaining the viability of gonococci during transport of specimens. The poor results with microscopy in female patients means that a dot-blot test with similar sensitivity and specificity for cervical exudates would be of great value as an alternative to culture as well as having potential for “on-the-spot” diagnosis. Inability to determine antibiotic sensitivity remains a limitation of all non-cultural tests for gonococcal infection.

Acknowledgements

This work was funded by a Scottish Home and Health Department research grant K/MRS/50/C594. K.G.R. and D.V.C. also gratefully acknowledge receipt of Faculty of
ELISA for gonococcal antigen

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Medicine Research Scholarships. We thank our colleagues in the Department of Genito-urinary Medicine for sending us patient material.

References


(Manuscript received 5th September 1986)
Ciprofloxacin versus ampicillin and probenecid in the treatment of uncomplicated gonorrhoea in men

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*Department of Genito-Urinary Medicine Royal Infirmary of Edinburgh; †Bacteriology Department, University of Edinburgh, Scotland

Eighty-six men with uncomplicated gonorrhoea were entered into a study comparing the efficacy of a single oral dose of 250 mg of ciprofloxacin with a single oral dose of ampicillin 2 g and probenecid 1 g for urethral gonorrhoea and a course of ampicillin, 500 mg four times per day for five days in rectal and pharyngeal infection. Two patients were excluded. Of the remaining 84, 45 were treated with ampicillin and 39 with ciprofloxacin. In the ampicillin group there were two treatment failures out of 40 urethral infections. Three patients with rectal infection were cured. Only one patient out of three pharyngeal infections was cured. Ciprofloxacin cured all of 34 urethral and three rectal infections including one penicillinase producing strain of Neisseria gonorrhoeae (PPNG). Four of five pharyngeal infections were cured but there was one treatment failure. There were no major side effects in either treatment group.

In conclusion, a single oral dose of 250 mg of ciprofloxacin is an effective treatment for uncomplicated gonorrhoea in men.

Introduction

Members of the 4-quinolone group of antibiotics have recently been evaluated in the treatment of infections due to Neisseria gonorrhoeae. Clinical studies using some of these, such as acrosaxcin (Cohen, Rein & Noble, 1984; Lim et al., 1984) and norfloxacin (Crider et al., 1984), have shown good results with successful treatment of gonorrhoea caused by strains showing either β-lactamase or chromosomally-mediated resistance to penicillin. Their efficacy is bolstered by the fact that they may be administered orally in a single dose.

Ciprofloxacin, a new member of the group, was found to be highly active against N. gonorrhoeae in vitro (Felmingham et al., 1983), and its efficacy has also been confirmed in vivo in an open study (Loo, Ridgway & Oriel, 1985).

We report here on a study comparing a single oral dose of 250 mg of ciprofloxacin with a single oral dose of ampicillin for urethral gonorrhoea and a course of ampicillin, 500 mg four times per day for five days, in rectal and pharyngeal infection in men.

Methods

Eighty-six patients who were entered into the study attended the University department of Genito-Urinary Medicine at Edinburgh Royal Infirmary between October 1984 and September 1985.
Routine diagnostic tests for gonorrhoea included microscopic examination of Gram's stained smears of urethral exudate and culture from the urethra and throat. Rectal cultures were taken whenever there was a risk of homosexually acquired infection. Material was plated directly onto modified New York City (MNYC) medium (Young, 1978) and transported to the laboratory within four hours. After incubation for 24 h at 37°C in a carbon dioxide enriched (10%) atmosphere, plates were examined for oxidase-positive Gram-negative cocci. N. gonorrhoeae was identified by sugar utilisation and coagglutination (Young & McMillan 1982).

A presumptive diagnosis of gonococcal urethritis was made if Gram-negative diplococci were seen in a urethral smear. A diagnosis of gonococcal proctitis or pharyngitis was made by culture alone. All gonococcal infections were uncomplicated.

A computer-selected rota randomly assigned patients to treatment with a single oral dose of 250 mg ciprofloxacin or 2 g ampicillin plus 1 g probenecid. Ampicillin given in an oral dose of 500 mg four times per day for five days was used in rectal and pharyngeal infections. Urine was examined for the presence of protein, blood and sugar, and blood was taken for routine haematological and biochemical tests. Serological tests for syphilis were performed at the initial clinic attendance and 28 days later.

Antibiotic susceptibility

The minimum inhibitory concentrations (MICs) of penicillin and ciprofloxacin were determined by the agar-plate dilution method. Concentrations (mg/l) of penicillin (0.015, 0.06, 0.12, 0.5 and 1.0) and ciprofloxacin (0.015, 0.03, 0.06 and 0.12) were prepared in MNYC medium lacking selective antibiotics.

Strains of N. gonorrhoeae were grown overnight on MNYC medium. A suspension of the organism was made in nutrient broth such that it was just turbid to the naked eye. Approximately 3 µl of each suspension were then spotted on a series of antibiotic-containing plates and a control plate by a multi-point inoculator. The plates were dried and incubated overnight at 37°C in CO₂. The MIC was read as the lowest concentration of antibiotic that permitted the growth of no more than two colonies.

Follow up

Patients were requested to re-attend on days 3 or 4, 7 and 14 after treatment. On each occasion, patients were asked about symptoms and further sexual intercourse and specimens were taken from previously infected sites for culture of N. gonorrhoeae. Haematological and biochemical tests were repeated at the first follow-up attendance and a further specimen of urine was examined for the presence of protein, blood and sugar.

Post-gonococcal urethritis (PGU) was diagnosed by the presence of ≥ 10 polymorphonuclear leucocytes per high power field (× 100 objective) in a Gram's stained urethral smear.

Patients were designated treatment failures if N. gonorrhoeae was isolated on subsequent culture and the patient denied further sexual intercourse. Patients who admitted further intercourse and had positive cultures were designated re-infections.
Ciprofloxacin for gonorrhoea

Results

Two patients entered into the trial gave positive smears but negative cultures and were excluded from further analysis. Of the 84 patients remaining, 45 were treated with ampicillin and 39 with ciprofloxacin.

MICs were determined for isolates from all 39 patients in the ciprofloxacin group and for isolates from 42 of the 45 patients treated with ampicillin. All isolates including two penicillinase producing *N. gonorrhoeae* (PPNG) were highly sensitive to ciprofloxacin (MIC≤0.015 mg/l).

The distribution of MICs of penicillin for the two treatment groups is shown in Table I.

Overall the organisms were fairly sensitive to penicillin with 89% (72/81) of isolates having an MIC of ≤0.12 mg/l; sensitivity was distributed evenly within the two groups, the corresponding values being 91% (38/42) for the ampicillin group and 87% (34/39) for the ciprofloxacin group.

The number of patients in each group attending for follow-up cultures was comparable. Two patients in each treatment group did not re-attend and were discounted from the evaluation of treatment. Of the remaining 43 patients in the ampicillin group, six attended for one test-of-cure culture, 19 for two test-of-cure cultures and 18 for three test-of-cure cultures. The corresponding numbers for the patients in the ciprofloxacin group were eight, 12 and 17.

As shown in Table II the spectrum of infection was also similar for the two groups.

<table>
<thead>
<tr>
<th>MIC Penicillin mg/l</th>
<th>Number (and percentage) of isolates within each treatment group with corresponding MIC</th>
<th>ampicillin group</th>
<th>ciprofloxacin group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td>15 (35.7)</td>
<td>8 (20.5)</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>17 (40.5)</td>
<td>17 (43.6)</td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>6 (14.3)</td>
<td>9 (23.1)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>3 (7.1)</td>
<td>3 (7.7)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1 (2.4)</td>
<td>2* (5.1)</td>
<td></td>
</tr>
</tbody>
</table>

*Both PPNG.

<table>
<thead>
<tr>
<th>Site(s) infected</th>
<th>No. of patients treated with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ampicillin</td>
</tr>
<tr>
<td>Urethra</td>
<td>37</td>
</tr>
<tr>
<td>Rectum</td>
<td>3</td>
</tr>
<tr>
<td>Throat</td>
<td>0</td>
</tr>
<tr>
<td>Urethra + rectum</td>
<td>0</td>
</tr>
<tr>
<td>Urethra + throat</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
</tr>
</tbody>
</table>
Table III. Results of treatment with ciprofloxacin and ampicillin in urethral, rectal and pharyngeal gonorrhoea

<table>
<thead>
<tr>
<th>Site</th>
<th>Treatment</th>
<th>Number treated</th>
<th>Number cured</th>
<th>Treatment failure</th>
<th>Re-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra</td>
<td>ciprofloxacin</td>
<td>34</td>
<td>31</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>ampicillin</td>
<td>40</td>
<td>37</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Rectum</td>
<td>ciprofloxacin</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ampicillin</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Throat</td>
<td>ciprofloxacin</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>ampicillin</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>88</td>
<td>79</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

There were 46 infected sites in 43 patients treated with ampicillin and 42 infected sites in 37 patients treated with ciprofloxacin. The results of treatment are given in Table III.

There were two treatment failures among 40 urethral infections treated with ampicillin. The penicillin MICs for these isolates were 0.06 mg/l, and >0.12 but <0.5 mg/l. A further patient with a positive urethral culture at first test of cure had had intercourse with an untreated regular partner and was regarded as a re-infection. Single dose treatment was effective in one throat infection with an isolate with an MIC of ≤0.015 mg/l but failed in another two throat infections where the isolates had MICs of 0.12 mg/l. Corresponding urethral infection in these two patients was cured by single dose treatment: both throat infections were cured by a course of ampicillin. All three patients with rectal infection were treated successfully by the allocated course of treatment.

There were no treatment failures among 34 urethral (including one PPNG) infections treated with single dose ciprofloxacin. Of the three patients designated as re-infections, two were negative at first test of cure, but positive in a subsequent test. The third patient was positive at first test of cure, but had had intercourse with an untreated regular partner. Three rectal infections were successfully treated with single dose ciprofloxacin, but one case of pharyngeal gonorrhoea was considered a treatment failure. The MIC for this isolate was ≤0.015 mg/l both before and after treatment, and a corresponding urethral infection in this patient was cured by a single dose. The four successfully treated pharyngeal infection included one PPNG.

There is no statistically significant difference in the outcome of treatment between the two groups ($\chi^2 = 0.57; P > 0.3$).

In patients with appropriate follow-up for urethral infection, PGU occurred in 39% (13/33) of men in the ampicillin group and 35% (8/23) men in the ciprofloxacin group.

There were no serious side effects in any of the patients.

One patient suffered an attack of vertigo while taking his dose of ciprofloxacin. This was probably a vasovagal attack following venepuncture.

One patient in the ciprofloxacin group showed a slight elevation of alanine aminotransferase at 62 u/l (upper limit of normal 40 u/l). This was transient, the value having returned to the normal range seven days later.
One patient developed a mild eosinophilia of $0.7 \times 10^9/l$, following ciprofloxacin therapy. He failed to attend for further follow-up and this abnormality could not be investigated further.

**Discussion**

Ciprofloxacin in a single oral dose of 250 mg successfully treated all urethral and rectal infections with *N. gonorrhoeae*, including one PPNG. This adds further support to the findings of a previous open study (Loo, Ridgway & Oriel 1985). Single dose treatment was also effective in four of five cases of pharyngeal infections.

Previous work has demonstrated that ciprofloxacin in this dosage does not eradicate *Chlamydia trachomatis* from the urethra (Loo et al., 1985). We did not perform routine urethral cultures for this organism. However, the incidence of PGU did not differ significantly between the two treatment groups.

Ciprofloxacin was well tolerated, as was ampicillin and probenecid. One patient complained of dizziness at the time of taking ciprofloxacin but this was probably due to a vaso-vagal attack following venepuncture. There were no haematological or biochemical abnormalities in the ampicillin group. One patient in the ciprofloxacin group, however, showed a transient rise in plasma hepatic transaminases and one patient developed a mild eosinophilia.

In summary, ciprofloxacin is a safe and effective treatment for gonococcal urethritis and proctitis in men. Further studies are required in women and in the treatment of gonococcal infection of the pharynx in men.

**References**


*(Manuscript accepted 12 January 1987)*
Serological classification of *Neisseria gonorrhoeae*. Serovars associated with homosexually acquired infection

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Abstract. Two panels of monoclonal coagglutination reagents were used to classify 464 gonococci isolated from 372 patients. The reagents, developed against gonococcal outer membrane protein I, subdivided gonococci into serovars within their WI and WI1/III serogroups. The overall distribution of WI and WI1/III serogroups was found to be 47% and 53% respectively. A total of 41 gonococci isolated from 32 homosexual men were included. Serogroup WI1/III predominated amongst these strains, accounting for 97% of infections. In comparison, the distribution of serogroups WI and WI1/III strains was 48% and 52% respectively amongst heterosexual men and 57% and 43% amongst women. A total of six Ph-serovars were recognised amongst the 41 isolates from homosexual men. These six serovars accounted for 44% of infections in heterosexual men and 42% of infections in women. Four of the serovars – Bropyt, Bopst, Bpyust and Bpyvut – occurred more frequently amongst homosexual men than heterosexual patients, although the difference was most marked with serovar Bropyt. The eight GS-serovars recognised amongst isolates from homosexual men accounted for 42% of infections in heterosexual men and 30% of infections in women. Six of these serovars – Back, Bcjjk, Bacjk, Bacejk, Bacehjk and Bacehj – occurred more frequently amongst homosexual men, although the difference was most marked with serovar Back. Eight Ph/GS serovar combinations were recognised amongst isolates from homosexual men. The serovar combination Bropyt/Back predominated, accounting for 69% of homosexually acquired infections. This combination was recognised in only 1% of heterosexual men and was absent from women. These findings suggest that serovar determination could prove valuable as an indicator of homosexually acquired infection.

Introduction

In 1980, Sandstrom & Danielsson described a simple, rapid coagglutination scheme for the serological classification of *Neisseria gonorrhoeae*. Using polyclonal antiserum, they were able to divide gonococci into three serogroups – WI, WII and WIII. Two mutually exclusive types of protein I molecule are recognised – Protein 1A molecules which contain epitopes recognised as serogroup WI and Protein 1B molecules which contain the epitopes recognised as serogroups WII and WIII. The value of serological classification using polyclonal antibodies

J. T. Poolman et al. (Eds) Gonococci and Meningococci. ISBN 90-247-3607-2
is limited however, by their inability to further sub-divide W serogroups reliably and reproducibly. Using monoclonal antibodies directed against protein I epitopes, it has been possible to further subdivide the W serogroups into serovars (Tam et al. 1982; Sandstrom et al. 1985a). A number of correlations have been reported between serovar and auxotype (Knapp et al. 1984; Knapp, Sandstrom & Holmes 1985; Kohl et al. 1986) and serovar and antibiotic susceptibility (Bygdeman, Mardh & Sandstrom 1984; Ruden et al. 1985; Bygdeman 1988) but data on correlations between serovars and sexual preference are limited (Backman et al. 1985).

To our knowledge, there are no published reports of the prevalence of serovars anywhere within the United Kingdom. A strong association between serogroup WII and homosexually acquired infection has been reported (Bygdeman 1981a; Morse et al. 1982; Reid & Young 1984). Reid et al. (1985) suggested that the WII isolates found among homosexual men differed from those in the heterosexual population.

The aims of this study were to examine the diversity of gonococcal serovars in the Edinburgh area and to determine whether distinct serovar patterns could be correlated with homosexually acquired infections.

Materials and methods

Clinical isolates

464 isolates of Neisseria gonorrhoeae were collected from 372 patients (32 homosexual men, 193 heterosexual men and 147 women) attending the Department of Genitorinary Medicine at the Edinburgh Royal Infirmary during the eight month period, November 1985 to June 1986 inclusive.

Serovar determination

Antigen preparation. Isolates were subcultured onto clear GC medium (Kellogg et al. 1963), incubated at 37°C for 18–24 h in a moist 10% CO₂ atmosphere and harvested into 1 ml of phosphate buffered saline (PBS) (pH 7.2). The suspension was boiled for 10 min and allowed to cool before testing.

Monoclonal coagglutination reagents. Two panels of monoclonal reagents were kindly supplied by Dr Solgun Bygdeman, Huddinge University Hospital, Stockholm, Sweden. The Pharmacia (Ph-) panel comprised five Protein IA specific reagents (Ar, Ao, As, At, Av) and nine Protein IB specific reagents (Br, Bo, Bp, By, Bv, Bu, Bs, Bt, Bx) and the Genetic Systems (GS-) panel comprised
seven Protein IA-specific reagents (Af, Ae, Ad, Ag, Ak, Al, Ah) and seven Protein IB-specific reagents (Ba, Be, Be, Bg, Bh, Bj, Bk).

*Test procedure.* Antigen preparation (20μl) was added to 20μl of each reagent on defined areas of surface-treated plastic plates (Bioplate® Type II, Biotest Flex Ltd, Moseley, Birmingham, England). The plates were rocked for two minutes and reactions read using oblique light against a dark background. A negative control of antigen preparation alone was used to check for autoagglutination.

Reactions were scored according to the strength of the coagglutination observed after two minutes. Strains were assigned to serovars according to the system of Bygdeman et al. 1985.

**Results**

464 isolates of *N. gonorrhoeae* were cultured from 346 genital (urethra and/or cervix), 75 rectal and 42 throat cultures. Gonococci from different sites in the same patient with the same serogroup and serovar were regarded as one isolate whereas those isolated from the same patient with different serogroups and/or serovars were counted as separate isolates. Three women were infected simultaneously at two sites with gonococci belonging to different serogroups. In addition, multiple isolates belonging to serogroup WI from one female patient had slightly different serovars with the GS-reagents.

**Distribution of serogroups and serovars in the overall population**

The overall distribution of WI and WII/III serogroups was found to be 47% and 53% respectively. Figure 1 shows the distribution of Ph- and GS-serovars amongst the 179 WI isolates and illustrates the correlation between Ph- and GS-serovars. The isolates could be resolved into four Ph-serovars and ten GS-serovars. One Ph-serovar – Arost – and one GS-serovar – Aedgkih – was shown to predominate. A total of 15 Ph/GS-serovar combinations were recognised.

Figure 2 shows the distribution of Ph- and GS-serovars amongst the 198 WII/III isolates and illustrates the correlation between Ph- and GS-serovars. The isolates could be resolved into 21 Ph-serovars and 18 GS-serovars. There were a greater variety of WII/WIII Ph- and GS-serovars than WI serovars. No predominateing serovars were found with either panel of reagents. A total of 34 WII/III Ph/GS-serovar combinations were recognised.
**Fig. 1.** Serovars of WI isolates using two different sets of monoclonal antibody reagents. * Multiple isolates from one female patient gave slightly different serovars with GS-reagents (Cervix = Arst/Adg; Rectum = Arst/Aedg; Fauces = Arst/Aedgk).

<table>
<thead>
<tr>
<th>Ph – serovars</th>
<th>Aedgkh</th>
<th>Aedghi</th>
<th>Aedg</th>
<th>Aegk</th>
<th>Adg</th>
<th>Agk</th>
<th>Aedgh</th>
<th>Aedh</th>
<th>Ae</th>
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<tbody>
<tr>
<td>Arost</td>
<td>132</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>138</td>
</tr>
<tr>
<td>Arst</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td></td>
<td></td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Aros</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Av</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>134</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td>8</td>
<td>179*</td>
</tr>
</tbody>
</table>

**Fig. 2.** Serovars of WI I/I II isolates using two different sets of monoclonal antibody reagents.
Gonococcal serovars in homosexual men

Distribution of 70 serogroups with respect to patient group (%)

* Three women were infected with WI and WII/III strains simultaneously. One woman was infected at three sites with a WI strain of slightly different GS-serovars

Fig. 3. Distribution of serogroups WI and WII/III in homosexual men and the heterosexual population.

Distribution of serogroups and serovars in the homosexual population

These included 41 isolates from 15 rectal, 17 urethral and nine throat cultures. The distribution of WI and WII/III serogroups amongst the homosexual population in comparison with the distribution in heterosexual men and women is illustrated in Fig. 3. Serogroup WII/III accounted for 97% of homosexual male infections and for 52% and 43% of heterosexual male and female infections respectively.

All of the serovars recognised amongst homosexual men and their prevalence in these patients in comparison with heterosexual men and women are illustrated in Table 1. Six Ph-serovars were recognised among isolates from homosexual men. These serovars accounted for 44% of infections in heterosexual men and 42% of infections in women. Four serovars occurred more frequently amongst homosexual men: Bopst was not found in heterosexual patients; Brypvt was found in 69% of homosexual men and 1.8% of heterosexual patients: Bryyst was found in 15.6% of homosexual men and 9.4% of heterosexual patients; and Bpyvut was found in 6.3% of homosexual men and 1.8% of heterosexual patients.

Eight GS-serovars were recognised among isolates from homosexual men – seven WII/III serovars and one WI serovar. These serovars accounted for 42% of infections in heterosexual men and 30% of infections in women. Six of these
Table 1. Serovars recognised amongst homosexual men: their prevalence in homosexual men and heterosexual patients.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Serovars</th>
<th>No. of strains amongst patient populations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Homosexual men</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 32)</td>
</tr>
<tr>
<td>Ph</td>
<td>Bropyt</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Bropt</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bopst</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bpyvut</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Bopyust</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Arst</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32</td>
</tr>
<tr>
<td>GS</td>
<td>Back</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Bajk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Begjk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Baejk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bacejk</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Bacehjk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bahjk</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Aedih</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32</td>
</tr>
<tr>
<td>Ph/GS</td>
<td>Bropyt/Back</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Bropt/Bajk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bopst/Begjk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bpyvut/Baejk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bpyvut/Bacejk</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Bpyvut/Bacehjk</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Bpyvut/Baejk</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Arst/Aedih</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>32</td>
</tr>
</tbody>
</table>

serovars – Back, Baejk, Bajk, Baejk, Bacejk and Bacehjk – were more frequent among homosexual men. The difference was most marked with serovar Back which accounted for 69% of homosexual infections but only 3% and 0.7% of infections in heterosexual men and women respectively.

Eight serovar combinations were recognised amongst isolates from homosexual men. These serovar combinations accounted for 36% and 29% of isolates from heterosexual men and women respectively. Two of the serovar combinations – Bopst/Begjk and Bpyvut/Baejk, were recognised uniquely in homosexually acquired infections. In addition, two of the serovar combinations – Bropyt/Back and Bpyvut/Baejk, were absent in women. Serovar combination Bropyt/Back was recognised amongst 22 (69%) of the strains isolates from homosexual
patients but was recognised in only 2 (1%) of heterosexual men and was absent from women.

Discussion

Using a panel of 14 Ph- and 14 GS-reagents, all isolates reacted with at least one monoclonal antibody. Strains of serogroup WI could be resolved into four Ph-serovars and ten GS-serovars with a total of 15 Ph/GS-serovar combinations. Strains of serogroup WII/III could be resolved into 21 Ph-serovars and 18 GS-serovars with a total of 34 Ph/GS-serovar combinations. A number of studies using selected panels of these monoclonal reagents have been carried out (Bygdeman et al. 1983; Knapp et al. 1984; Sandstrom et al. 1985a, b; Whittington et al. 1985; Kohl et al. 1986).

Serovar patterns within the two major serogroups – WI and WII/III differed markedly. Strains belonging to serogroup WII/III belonged to a greater variety of serovars than WI strains. One predominating serovar combination Arost/Aedgkih which accounted for 74% of WI isolates has been similarly reported in previous worldwide studies (Sandstrom et al. 1985b; Bygdeman 1988). As found in previous studies, no predominating serovar was recognised amongst strains belonging to serogroup WII/III (Sandstrom et al. 1985a, b; Bygdeman 1988).

Reports that it is more difficult to obtain different WI-specific antibodies (Tam et al. 1982; Knapp et al. 1984; Sandstrom et al. 1985a) could explain the small variety of serovars noted amongst WI isolates. Alternatively, the number of different epitopes of Protein IA may be limited, a view supported by the observation of Sandstrom et al. (1985b) that the WI serogroup is more antigenically stable than serogroup WII/III. Auxotype analysis combined with serovar has proved successful in improving the resolution of the serovar system (Knapp et al. 1984; Kohl et al. 1986; Bygdeman 1988).

Serological classification has potential value in the study of gonococcal infection in defined patient groups. In the present study, the distribution of serogroups and serovars of isolates from homosexual men was compared with isolates from the heterosexual population. Serogroup WII/III accounted for 97% of strains isolated from homosexual men and 52% and 43% of strains isolated from heterosexual men and women respectively. This strong association between serogroup WII and homosexually acquired infections has been noted in previous studies (Bygdeman, 1981a; Morse et al. 1982; Reid and Young, 1984; Backman et al. 1985; Reid et al. 1985). These observations illustrate the importance of distinguishing between homosexual males when studying the distribution of serogroups amongst men.

Morse et al. (1982) reported a strong association between strains isolated from
homosexual men and a mutation (mtr) which reduced the cell envelope permeability. Reid et al. (1985) found that the Mtr phenotype was strongly associated with serogroup WII in homosexual men but not in isolates from heterosexual men and women. This finding suggested that the WII strains involved in homosexually acquired infections differed from those causing infections in the heterosexual population: the serovar data reported in this study confirms this.

A total of eight serovar combinations were recognised among isolates from homosexual men. These serovars accounted for 36% and 29% of isolates from heterosexual men and women respectively. Two of the serovar combinations, Bopst/Begik and Bpyvut/Baehik, were absent among strains isolated from heterosexual patients and two additional serovar combinations, Bropty/Back and Bpyust/Baejk, were not found in women. Serovar combination Bropty/Back was recognised amongst 69% of strains isolated from homosexual men. Two heterosexual men were infected with isolates belonging to this serovar combination but insufficient information was available to rule out the possibility that these patients were bisexual. No isolates with this serovar combination were recognised in female patients. The same pressures previously proposed to select the Mtr phenotype and serogroup WII in homosexual men (Morse et al. 1982; Reid et al. 1985) could be selecting this particular subgroup of Protein I molecules detected by the monoclonal reagents as serovar combination Bropty/Back.

Using combinations of the two sets of monoclonal reagents, a better correlation between serovar and homosexually acquired infection was obtained.

Reports of the association between serovar and sexual preference are limited (Backman et al. 1985; Bygdeman 1988). Backman et al. (1985) recognised a larger number of WI infections in homosexual patients than were revealed in the present study. They reported that serovars Ae, Bacek and Bacejk more frequently identified in isolates from homosexual men. Bygdeman (1988) also found that serovars Ae and Bacejk were more frequently isolated from homosexual patients. Although the serovar Bacejk was more frequently recognised amongst homosexually acquired infections in the present study, this association was not nearly so marked as that noted with the serovar Back.

Since the strains isolated from homosexual patients appear to differ according to geographical region, serovar patterns in homosexual and heterosexual patients must be monitored in any particular region if serovar determination is to prove valuable as an indicator of homosexually acquired infection. The recognition of a particular serovar or serovars as a reliable indicator of homosexually acquired infection would enable the epidemiology of gonococcal infection in the homosexual population to be studied and thus aid contact tracing efforts.
Acknowledgements

This work was supported by a grant from the Scottish Home and Health Department (research grant no. K/MRS50/C785). DVC gratefully acknowledges receipt of a Faculty of Medicine Scholarship.

We thank Dr Solgun Bygdeman for supplying the monoclonal reagents and for her most valuable help and advice.

Thanks also to Mrs Marilyn Cole for the typing of the manuscript.

References


Immunological Diagnosis of Sexually Transmitted Diseases

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MARCEL DEKKER, INC. New York and Basel
Foreword

It is gratifying to be invited to write a foreword to this significant book. The editors are colleagues whom we greatly respect; their collaboration is another rewarding example of the constructive association that our departments have enjoyed over the years.

It is now more important than ever that clinicians engaged in the diagnosis and the management of sexually transmitted diseases should have close links with their colleagues in medical microbiological work. The daunting expansion of the range of recognized sexually transmitted diseases has called for very significant development of related laboratory expertise. Moreover, our increasing ability to offer specific and effective treatment to many patients increasingly obliges us to make a definitive diagnosis as promptly as possible and to have continuing liaison with the laboratory in the follow-up work that is often necessary. This relates to the continuing management of the patient and to our joint commitment to epidemiological surveillance.

To some extent, clinical bacteriologists have tended to rely unduly on cultural methods for the identification of pathogens, although Treponema pallidum remains elusive and still obliges us to rely heavily on indirect methods of laboratory
diagnosis to identify syphilis. The advances made in our serological diagnostic work in the recent past indicate how much progress can be made when new technical methods become available. With the more recent development of immunological diagnostic methods, and with many other advances in our clinical and laboratory sciences, we are entering a new era in which the sensitive and specific detection of early antibody may be rivaled by elegant procedures to detect antigen. This optimistic view is endorsed by the quality and the range of the contributions that make this book.

The editors are to be congratulated on recruiting such an authoritative team. The book is a mine of information; it brings together up-to-date accounts of accepted practice and careful assessments of the potential value of new approaches to the diagnosis of sexually transmitted diseases. We join in wishing the book the success that it clearly deserves.

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Sexually transmitted diseases represent a major communicable disease problem on a global scale. The extent of their morbidity and mortality is now known to range from uncomplicated local genital infection to the lethal consequences of the acquired immunodeficiency syndrome (AIDS). Between these extremes lies their significant impact on infertility resulting from pelvic inflammatory disease, the health and well-being of the fetus and neonate, and the possible viral etiology of genital malignancy, particularly cervical carcinoma.

It is now clearly recognized that the control of sexually transmitted diseases requires the highly coordinated and collaborative efforts of many groups of health care personnel, including physicians, nurses, microbiologists, and epidemiologists, as well as those with responsibility for health education. Those in the forefront of health care have been fortunate in being supported by a cadre of enthusiastic and able researchers who have studied the basic biology of the pathogens, the pathophysiology of the diseases, and the development and application of new diagnostic methods.

It is this background that allowed the rapid exploitation of the pioneering work of George Köhler and Cesar Milstein on the production of individual monoclonal antibodies of precise specificity by cloned hybrid cells. The diagnostic
potential of selecting monoclonal antibodies of required specificity was quickly appreciated, thus underlining the particularly close relationship among diagnosis, therapy, and epidemiology of sexually transmitted diseases. Because effective management depends on a specific organismic diagnosis from within a broad spectrum of diverse infectious agents, a wide range of diagnostic methods is employed. These include direct microscopy of exudates, conventional culture and identification, immunological detection of antigen, and the detection of specific antibodies. The rate of progress has been such that monoclonal antibodies have made a major impact on each type of method and are already used routinely in the diagnosis of gonococcal, chlamydial, and herpesvirus infections.

In this text we have not attempted to include every possible sexually transmissible infection but have selected a team of internationally renowned contributors to cover those areas where the monoclonal antibody technology is most advanced and where the development of monoclonal antibodies might be most helpful. We have considered the role of immunological methods in the overall diagnostic strategy and assessed the utility of monoclonal antibody reagents already in routine use, as well as outlining current developments and future applications. We have striven throughout to provide appropriate background information on the choice of antigens and the selection and characterization of monoclonal antibodies, thus allowing the more specialized reader a full appreciation of the significance and diagnostic implications of monoclonal antibody reagents. Therefore, not only will the book be of interest to researchers, microbiologists, and physicians directly involved in the management of patients with sexually transmitted diseases, but by increasing the awareness of improved diagnostic capabilities it should be of value to all professional groups who may also deal with such patients. We hope that we have provided a sound understanding and framework of knowledge into which subsequent advances can be easily assimilated.

Circumstances have necessitated the omission of the chapter on the new and complex issues arising from infection with the human immunodeficiency viruses and AIDS, but we hope that our readers will nevertheless appreciate the focus of the text on other major sexually transmissible viral, bacterial, and protozoal causes of disease.

We are grateful to our expert contributors from many countries who produced texts that were a pleasure to edit. Thanks are also due to Paul Dolgert and William Cary of Marcel Dekker, Inc., who have given us expert advice and guidance throughout this project. Finally, we are indebted to Mrs. Marilyn Cole for dedicated secretarial support.

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Immunological Diagnosis of Gonococcal Infection

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I. INTRODUCTION

Gonorrhea, an infection of the mucosal surfaces of the genitourinary tract, is transmitted mainly by sexual intercourse and is common in developed as well as in developing countries. The global incidence of approximately 200 million cases a year clearly indicates the need for improved control. Prompt and accurate diagnosis followed by effective treatment is an important component of any gonorrhea control program. This chapter first outlines the nature of the infection and discusses this in relation to diagnostic strategies before dealing with the immunological diagnosis.

A. Disease Entities

1. Genital Infection

Acute anterior urethritis is the most common manifestation of gonococcal infection in men and results in a purulent discharge. The incubation period is, on average, 3-4 days with a range of 2-14 days. Before effective antimicrobial agents were available most patients had symptomatic resolution within 8 weeks and 95% were free of symptoms within 6 months (Holmes, 1974).

The columnar epithelium of the endocervix is the primary site of infection in women. The cervix is involved in 85-90% and the urethra in 65-75% of the patients (Robertson et al., 1988). The most common symptoms among women infected with Neisseria gonorrhoeae are abnormal or increased vaginal discharge, abnormal uterine bleeding, dysuria, and urinary frequency and urgency (Handsfield, 1977). Most women with endocervical gonococcal infections, however, develop very slight or nonspecific symptoms and do not seek medical attention.

Asymptomatic urethral infections are also found in men. Overall, 10% of men acquire infections in which few or no symptoms develop (Handsfield et al., 1980), although a figure of 40% was reported for men who were known contacts of women with gonorrhea. John and Donald (1978) found that 17% of 203 cases of urethral gonorrhea were asymptomatic and a further 7% of cases had symptoms so mild that they failed to seek medical attention. All of these cases were brought to attention by contact tracing.

a. Complications of Local Genital Infection. Local spread of urethral gonococcal infections in men may result in prostatitis, seminal vesiculitis, epididymitis,
inflammation, and abscess formation in the urethral glands (Robertson et al., 1988). Urethral strictures and fistulae, once common in the preantibiotic era are now rare in the Western world. In West Africa, and probably also in other developing countries where medical facilities are inadequate and improper self-treatment with antibiotics occurs, all of the mentioned complications are frequently seen (Osoba and Alausa, 1976).

Local complications in women include involvement of the paraurethral and greater vestibular (Bartholin’s) glands. Pelvic inflammatory disease (PID) occurs in up to 10% of untreated patients, with 20% of such women developing impaired fertility (Eschenbach and Holmes, 1975). It has been estimated that the cost of PID in the United States is 700 million dollars per year (U.S. Department of Health and Human Services, 1981), thus there is a strong economic incentive to control gonorrhea. The World Health Organization (WHO, 1978) considers that gonorrhea is a major cause for the high prevalence of infertility in parts of Africa.

2. Rectal Infection

Rectal infections occur in both men and women. Anorectal infections in men result from receptive anal intercourse. In 1977, 10.9% of men with gonorrhea had acquired their infection as a result of homosexual contact (British Cooperative Clinical Group, 1980). The majority of male patients with rectal gonorrhea produce no symptoms (Bygdeman, 1981; McMillan and Young, 1978), and the proctoscopic appearance of the rectal mucosa is normal in 84% of these men (McMillan et al., 1983).

The rectum is involved in approximately 25-50% of women with gonorrhea (Handsfield et al., 1980; Wilcox, 1981). Rectal infection is mainly due to contamination of the anus by infected vaginal discharge (Wilcox, 1981) but may also result from rectal coitus (Cornthwaite et al., 1974; Kinghorn and Rashid, 1979).

3. Pharyngeal Infection

Lack of correlation between pharyngeal colonization and symptoms of pharyngitis (Wiesner, 1975; Young and Bain, 1983), combined with the reluctance of many patients to admit to orogenital contact (fellatio and, less commonly, cunnilingus), make it difficult for the clinician to use these criteria in selecting patients from whom to take throat cultures. Although the prevalence of pharyngeal infection is highest in homosexual men, there has been a marked increase in heterosexually acquired pharyngeal infection in Edinburgh in recent years (Robertson et al., 1988): during 1985, 9% of heterosexual men, 14.6% of women, and 27.8% of homosexual men with gonorrhea had pharyngeal infection. Pharyngeal transfer of gonococci by kissing is very rare (Tice and Rodriguez, 1981). Oral-to-genital transmission is rare, although it has been reported in relation to subsets of prostitutes in Southeast Asia who specialize in oral sex (Soendjojo, 1983).
4. Disseminated Infection

In a small percentage of untreated cases of mucosal gonorrhea, systemic spread gives rise to the entity termed disseminated gonococcal infection (DGI), often referred to as the arthritis-dermatitis syndrome. Clinically, DGI can be divided into two stages: an initial bacteremic stage associated with fever, leukocytosis, and skin lesions, closely followed by a second stage associated with tenosynovitis or septic arthritis (Brooks, 1985). Occasionally the two stages overlap (Brogadir et al., 1979), and rarely, DGI may be followed by the more severe manifestations of endocarditis or meningitis (Masi and Eisenstein, 1981).

Disseminated infection is more likely to occur in women than in men, possibly because a greater proportion of women are symptomless (Holmes et al., 1971). Barr and Danielsson (1971) found that the overall prevalence of septic gonococcal dermatitis was 1.9%, 3% for women and 0.7% for men. The incubation period for DGI ranges from seven to 30 days, but it is difficult to determine accurately as most cases result from asymptomatic infection. The disseminated form occurs most commonly in women at the time of menstruation when the incubation period may represent the time between the acquisition of infection and the onset of menstruation.

5. Infection in Infants and Children

Gonococcal conjunctivitis of the newborn (ophthalmia neonatorum) was the single largest cause of blindness during the preantibiotic era. Nowadays this condition is uncommon in developed countries owing to general improvements in antenatal care and the detection and treatment of gonococcal infection. Gonococcal conjunctivitis in older children and in adults is usually acquired by contact with fingers and/or moist towels contaminated with fresh pus. Although genital and rectal infection in young children under the age of puberty may result from accidental contamination of the child with discharge when sleeping with an infected parent, sexual contact and abuse probably occur much more frequently than is generally supposed (Alexander et al., 1984; Sgroi, 1982).

B. Epidemiology

1. Prevalence of Gonorrhea

Differences and inadequacies in reporting systems make absolute comparison of data between countries difficult. The available data, however, give a useful guide about trends. In many countries, as illustrated by data from the United Kingdom (Fig. 1), there was a sharp rise in the incidence of gonorrhea in the late 1950s and throughout the 1960s, reaching a peak of 65,997 recorded cases in 1973. Numbers stabilized and declined thereafter with 53,802 cases recorded in 1984. Most infections occur in the 20- to 25-year-old group.
Fig. 1 Clinic returns for gonorrhea in the United Kingdom 1925-1984. Data not available for Northern Ireland before 1958 (Source: reproduced from Communicable Diseases Scotland Weekly Report 86/31 by permission of Dr. J. Emslie, Medical Editor and Dr. N. S. Galbraith, Director PHLS Communicable Disease Surveillance Centre, Colindale, London).

In England in 1982, the reported incidence rate was 111.4/100,000 population and the male/female ratio was 1.7:1 (Extract from the report of the Chief Medical Officer, 1985). In the United States in 1981 990,864 cases were reported (435/100,000 population): 92% of cases occurred in those under the age of 35. The male/female ratio decreased from 2.4:1 in 1960 to 1.4:1 in 1981 (U.S. Department of Health and Human Services, 1982). The decrease in the male/female ratio is in part due to public health service programs of more culture screening to detect infected women who are often symptomless.

In developing countries in Asia, Africa, Central and South America the prevalence of gonorrhea is not known because of inadequacies in the reporting systems. Data from several studies from Africa suggest that there are very high levels of infection. In Nairobi 17.5% of women attending a family planning clinic had gonorrhea (Hopcraft et al., 1973); in rural Uganda infection was found in 9% of men and 18% of women randomly selected for testing (Arya et al., 1973); and in Salisbury, Zimbabwe, 35% of women attending an STD clinic were infected (Latif, 1981).

2. Properties of the Organism

Apart from behavioral factors of the host, the spread of gonorrhea is to some extent dependent upon properties of the organism. Gonococcal typing and the asso-
ciation of certain strains with specific clinical and epidemiological situations is considered in detail in Chap. 4. Certain of these organismal factors, may, however, affect diagnosis and hence the spread of infection.

Strains that require arginine, hypoxanthine, and uracil for growth (the AHU\(^-\) auxotype) are associated with asymptomatic infection in men (Crawford et al., 1975), thus allowing these strains a greater opportunity to disseminate within the community. In addition, difficulty may be experienced in detecting these strains by culture as a result of their antibiotic hypersensitivity: there is a significant correlation between the AHU\(^-\) auxotype and sensitivity to vancomycin, one of the antibiotics used in certain gonococcal selective media (Mirrett et al., 1981).

Provided that virulence is not adversely affected, antibiotic-resistant gonococci that are associated with treatment failure have a greater opportunity to spread. Currently recommended dosages of penicillin and ampicillin/amoxycillin tend to be ineffective in the treatment of infections with isolates with a minimum inhibitory concentration (MIC) \(\geq 1\) mg/L.

Strains showing chromosomal antibiotic resistance that is mediated through changes in the cell envelope and resistance that is mediated through plasmid-encoded \(\beta\)-lactamase (penicillinase) enzymes are now prevalent in many areas. There is considerable geographical variation in the proportion of strains showing significant levels of resistance to penicillin. Strains from Southeast Asia tend to be most resistant. In Singapore 64% of the strains have MICs \(\geq 0.5\) mg/L resulting from chromosomally mediated resistance (Sng et al., 1984). Similar levels of resistance are common in parts of Africa (Sparling, 1977).

In one large London clinic the prevalence of strains with a penicillin MIC of \(\geq 1\) mg/L was 8.5% (Easmon, 1985). In the United States chromosomally mediated resistant gonococci have been reported in 23 states (Rice et al., 1986). As prevalence data were not given, it is impossible to gauge the size of the problem.

Penicillinase-producing \(N.\) gonorrhoeae (PPNG) were first reported simultaneously in the United Kingdom (Phillips, 1976) and the United States (Ashford et al., 1976); the respective isolates were linked epidemiologically with West Africa and Southeast Asia. The PPNG now occur throughout the world and have become endemic in many countries, albeit at a low level. The prevalence of PPNG decreased in the United States during 1983 and in some parts of the United Kingdom during 1984 (Easmon, 1985). Early diagnosis, effective therapy and intensive contact tracing have undoubtedly contributed to the decrease in PPNG. Certain serogroups and serovars of gonococci are correlated with antibiotic resistance (Chap. 4).

II. DIAGNOSTIC STRATEGIES

The main aim of diagnosis is to determine whether or not \(N.\) gonorrhoeae is present in a specimen and, if present, whether or not the strain produces \(\beta\)-lactamase.
The greater the number of sites examined, the better will be the chance of detecting gonococcal infection.

A. Multiple-Specimen Versus Single-Specimen Screening

In women seen at genitourinary medicine clinics, specimens for culture should be taken from the urethra, endocervix, rectum, and if the contact of a man with gonorrhea, from the pharynx. Smears should be taken from the endocervix for examination after Gram staining. In studies involving repeated examinations of multiple sites, the proportion of infected women detected at their first clinic attendance has been reported as 91% (Chipperfield and Catterall, 1976), 97% (Barlow et al., 1976), and 98% (Young et al., 1979).

The effectiveness of screening by a single endocervical culture varies widely with reported values as disparate as 40% (Norins, 1974) and 90% (Young et al., 1979). In terms of a screening schedule, more infections are detected by testing additional sites than by rescreening by endocervical culture: 97.6% of 451 infections were detected by testing multiple sites compared with only 92.4% by three endocervical cultures. One in three infected women is likely to be missed if a high vaginal swab is the only specimen taken (Bhattacharyya et al., 1973).

Multiple infections are also common in homosexual men. In a study of 278 cases of homosexually acquired infection, the urethra, rectum, and throat were infected in 60.8%, 41.0%, and 8.3% of the patients, respectively (McMillan and Young, 1978). If only one set of tests had been relied upon, 7% of patients with rectal and 26% of patients with pharyngeal gonorrhea would not have been identified. Detection of rectal infection in homosexual men is particularly important because the biological properties of gonococci associated with homosexually acquired infection tend to make them more refractory to treatment. Rectal infection in women is also linked with treatment failure, although to a lesser extent.

Pharyngeal gonorrhea in both sexes requires a higher penicillin dosage than does uncomplicated infection. There is, however, little risk associated with pharyngeal gonorrhea, either to the individual, or to their sexual partner(s). Consequently, the detection of pharyngeal gonorrhea will have little impact on the overall control of infection and has low priority compared with the detection of endocervical infection, anorectal infection in homosexual men, and urethral gonorrhea in males. Anogenital examination is also important in diagnosing DGI because most patients, including those without genital symptoms, are likely to yield gonococci from an anogenital site (Barr and Danielsson, 1971).

Although repeated examination of multiple sites should be the goal within genitourinary medical clinics, there is also an important role for widespread screening based on the examination of a single specimen. Screening with a single endocervical specimen has the potential to detect up to 90% of infected women, many of whom would be symptomless. Provided that “target groups” are properly selected, even a low positivity rate would have an amplified effect in decreasing
the level of infection within the community at large. Whereas examination of a single urethral specimen is highly effective in heterosexually acquired urethral gonorrhea, single-specimen screening is unsuitable for rectal infection in homosexual men and for pharyngeal infection in both men and women.

B. Spectrum of Neisserial Colonization

As the spectrum of colonization with neisseriae other than the gonococcus varies markedly between different anatomical sites, this also has an important bearing on the utility of identification methods. Genital sites that form the mainstay of single-specimen noncultural immunological diagnosis are least subject to interference by related neisseriae such as *N. meningitidis* and *N. lactamica* (Sect. III.C.1.a).

III. NONIMMUNOLOGICAL DIAGNOSIS

This section provides an overview of conventional diagnostic methods as a background with which to compare the advantages and disadvantages of immunological diagnosis.

A. Immediate Diagnosis

Gram staining of genital secretions remains the only widely accepted routine procedure for making an immediate diagnosis of gonorrhea. In men, Gram-stained smears of urethral discharge provided an immediate differential diagnosis between gonococcal and nongonococcal urethritis in 85% of patients (Jacobs and Kraus, 1975). The sensitivity and specificity in relation to urethral smears from symptomatic men ranges from 83 to 96% and 95 to 99%, respectively (Goodhart et al., 1982). Although Gram staining is a simple method, the experience of the observer is of paramount importance. The probability of gonorrhea in men whose smears were reported as containing typical intracellular gram-negative diplococci (GNDC) dropped from 94.8 to 53.9% when an inexperienced technician interpreted smears from men with urethritis (Goodhart et al., 1982). The Gram stain method is unsuitable for men without clinical features of urethritis. In a symptomless population in which the prevalence of gonorrhea was 2%, the probability of infection in patients whose smears were reported positive by an experienced observer was only 34.9% (Goodhart et al., 1982).

The sensitivity and specificity of the Gram stain for cervical smears ranges from 23 to 65% and 88 to 100%, respectively (Goodhart et al., 1982). The poorer performance in women is related to the asymptomatic nature of the infection as well as the difficulty of differentiating GNDC within the normal vaginal flora. The experience of the observer is even more important than in the male.

The quality of the culture system used as a standard influences the observed sensitivity and specificity of microscopy. A poor culture system that fails to detect
Infections with low numbers of gonococci, i.e., those most likely to be missed by microscopy, will result in a Gram stain that gives falsely high sensitivity. Conversely, specificity will be falsely poor if vancomycin-sensitive gonococci are missed with a culture medium containing vancomycin. Patients who have taken antibiotics before examination are also likely to yield positive smears and negative cultures.

Because of the complexity of the gut flora, rectal smears are not routinely taken. Rectal smears may be of value in homosexual men with proctitis if pus or mucopus can be collected by proctoscopy (William et al., 1981). A Gram stain has no place in the diagnosis of pharyngeal infection.

Simplified Staining Procedures

Single stains, such as methylene blue or safranin, or a single reagent, such as methyl-green pyronin, have been investigated as a means of shortening the time required to stain smears. The rapid exclusion of gram-positive organisms, however, saves more technician time than does shortening of the staining procedure (Oxtoby et al., 1982).

B. Culture

With over 70% of the laboratories in England and Wales using some form of selective medium (Adler et al., 1978), its importance is firmly established. A combination of a selective and a nonselective medium has been recommended by some workers. While this may be theoretically desirable, it is too time-consuming and technically demanding, and it is not cost-effective in areas where the prevalence of antibiotic-sensitive strains is low (Bonin et al., 1984). Most selective media contain a rich nutrient base supplemented with blood, partially lysed by heat (chocolate agar) or completely lysed by saponin; an antimicrobial cocktail is added to inhibit microorganisms other than pathogenic neisseriae. The following are the most widely used selective media.

1. Thayer-Martin Medium and Its Modifications

The original selective medium of Thayer and Martin (1966) contains the antimicrobials vancomycin, colistin, and nystatin. Although widely used in many laboratories Thayer-Martin (TM) medium has been criticized because a proportion of gonococcal strains are inhibited by vancomycin; growth is slow and colonies are small on TM medium; Proteus spp. and yeasts are not suppressed effectively. These problems were overcome to some extent with the introduction of modified TM medium (Martin et al., 1974). Modified TM medium contained double the concentration of agar, glucose (0.25%) was added to promote more rapid growth, and trimethoprim added to inhibit Proteus spp. Unfortunately, modified TM medium does not overcome the problem of vancomycin sensitivity. As
vancomycin sensitivity varies geographically and has been reported to be as high as 30% (Windall et al., 1980), this is more important in some areas than in others.

2. Martin-Lewis Medium

Martin-Lewis medium is similar to modified TM medium but contains anisomycin in place of nystatin to inhibit yeasts (Martin and Lewis, 1977). Anisomycin is more stable than nystatin and is useful in providing commercially prepared selective media with longer shelf-lives. Smeltzer et al. (1979) found the enhanced inhibition of yeasts on Martin-Lewis medium made screening plates for gonococcal colonies much easier.

3. New York City Medium and Its Modification

New York City (NYC) medium was devised originally by Faur et al. (1973) to provide a luxuriant growth of pathogenic neisseriae after incubation for 24 hr. The NYC medium essentially consists of a proteose peptone-corn starch agar-buffered base to which is added a hemoglobin solution prepared from fresh horse erythrocytes, horse plasma, yeast dialysate, glucose, and vancomycin, colistin, amphotericin B, and trimethoprim lactate. This medium is inconvenient for many service laboratories to prepare, and the modified NYC (MNYC) medium described by Young (1978a) may be more suitable for routine use. The MNYC medium uses a commercially prepared gonococcal base, completely lysed whole blood in place of plasma and hemoglobin solution made from fresh horse erythrocytes, and lincomycin in place of vancomycin.

In comparison with TM medium, MNYC medium improved the overall isolation rate and enabled a larger percentage of isolates to be identified after 24 hr. Svarva and Maeland (1979) and Hookham (1981) also noted an improvement in culture results with MNYC medium and adopted it for routine use. It is, however, important to examine cultures after 24 hr of incubation. Because lincomycin is less inhibitory than vancomycin, if rectal cultures are not examined until after 48 hr of incubation, contaminants may mask small numbers of gonococcal colonies. Lawton and Koch (1982) compared commercially available NYC and Martin-Lewis media and found the NYC medium to be much superior.

Comparing smear and culture results is an essential part of the quality control of gonorrhea diagnosis. If the proportion of patients giving positive smears but negative cultures is high, or increases, the culture procedures should be investigated. In Atlanta, Georgia, the proportion of cervical specimens yielding typical positive smears but negative cultures on selective media containing vancomycin varied from 2.6% (Oxtoby et al., 1982) to 5.6% (Goodhart et al., 1982). Young et al. (1979), by using MNYC medium containing lincomycin, showed that only 1% of infections in women gave typical positive smears that were not confirmed by culture.
C. Identification

1. Introduction

The use of a selective medium aids in the identification of *N. gonorrhoeae* because commensal neisseriae are inhibited. *Neisseria meningitidis* and *Branhamella catarrhalis*, however, are two organisms most often confused with *N. gonorrhoeae* (Arko et al., 1982). As *N. meningitidis* grows well on selective media it is important to know its prevalence in the population of GNDC isolated from anatomical sites examined for gonococcal infection. Because the spectrum of nongonococcal neisseriae (including *B. catarrhalis*) (NGN) varies markedly with anatomical site, identification methods that are of acceptable sensitivity and specificity for one site may be unsuitable for another site.

a. Spectrum of Neisserial Colonization. The prevalence of NGN isolated by routine culture of various anatomical sites on MNYC medium from patients attending the Department of Genito-Urinary Medicine, Edinburgh between 1978 and 1985 is shown in Tables 1 to 4.

The results shown in Table 1 for pharyngeal cultures illustrate the effectiveness of selective media in inhibiting nonpathogenic neisseriae. The mucosal membranes of the oropharynx are the natural habitat of commensal neisseriae, yet such organisms accounted for only 6.2% of all GNDC, or 2.4% if *N. lactamica* is excluded. Although *N. lactamica* is regarded as a commensal organism, it grows well on selective media with a colonial morphology similar to *N. meningitidis*. It is obviously important to have reliable methods for differentiating the gonococcus from the meningococcus when examining pharyngeal cultures, as almost 70% of the GNDC isolated are meningococci. As GNDC isolated from anogenital sites in heterosexual patients have a high probability of being gonococci, less accurate

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of isolates</th>
<th>% of total pharyngeal cultures</th>
<th>% of oxidase positive GNDC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>632</td>
<td>9.8</td>
<td>24.7</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>1763</td>
<td>27.4</td>
<td>69.1</td>
</tr>
<tr>
<td><em>N. lactamica</em></td>
<td>97</td>
<td>1.5</td>
<td>3.8</td>
</tr>
<tr>
<td><em>N. flava</em></td>
<td>3</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><em>N. perflava</em></td>
<td>32</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td><em>B. catarrhalis</em></td>
<td>23</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Unidentified</td>
<td>3</td>
<td>&lt;0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Total</td>
<td>2553</td>
<td>39.6</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 2  Anogenital Isolation of Nongonococcal Neisseriae\(^a\) (NGN) in Women (Edinburgh 1978-1985)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of patients with anogenital GNDC</th>
<th>Number of isolates of NGN from following sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urethra</td>
<td>Cervix</td>
</tr>
<tr>
<td>1978</td>
<td>456</td>
<td>0</td>
</tr>
<tr>
<td>1979</td>
<td>424</td>
<td>0</td>
</tr>
<tr>
<td>1980</td>
<td>404</td>
<td>1(^c)</td>
</tr>
<tr>
<td>1981</td>
<td>333</td>
<td>0</td>
</tr>
<tr>
<td>1982</td>
<td>336</td>
<td>1(^d)</td>
</tr>
<tr>
<td>1983</td>
<td>325</td>
<td>0</td>
</tr>
<tr>
<td>1984</td>
<td>266</td>
<td>2(^f)</td>
</tr>
<tr>
<td>1985</td>
<td>332</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2876</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\)N. meningitidis unless denoted otherwise.
\(^b\)Includes one B. catarrhalis.
\(^c\)Same patient.
\(^d\)Same patient.
\(^e\)Includes two B. catarrhalis.
\(^f\)Both B. catarrhalis.

Table 3  Heterosexually Acquired Genital Nongonococcal Neisseriae\(^a\) (NGN) in Men (Edinburgh 1978-1985)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of urethral GNDC</th>
<th>Number (%) shown to be NGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>614</td>
<td>3 (0.49)</td>
</tr>
<tr>
<td>1979</td>
<td>544</td>
<td>1 (0.18)</td>
</tr>
<tr>
<td>1980</td>
<td>519</td>
<td>2 (0.39)</td>
</tr>
<tr>
<td>1981</td>
<td>477</td>
<td>0</td>
</tr>
<tr>
<td>1982</td>
<td>458</td>
<td>1 (0.22)</td>
</tr>
<tr>
<td>1983</td>
<td>420</td>
<td>3 (0.71)</td>
</tr>
<tr>
<td>1984</td>
<td>419</td>
<td>1 (0.24)</td>
</tr>
<tr>
<td>1985</td>
<td>451</td>
<td>4(^b) (0.89)</td>
</tr>
<tr>
<td>Total</td>
<td>3902</td>
<td>15 (0.38)</td>
</tr>
</tbody>
</table>

\(^a\)N. meningitidis unless denoted otherwise.
\(^b\)Includes one B. catarrhalis.
Table 4  Homosexually Acquired Anogenital Nongonococcal Neisseriae\(^a\) (NGN) (Edinburgh 1978-1985)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of patients with urethral GNDC</th>
<th>Number (%) shown to be NGN</th>
<th>Number of patients with rectal GNDC</th>
<th>Number (%) shown to be NGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>50</td>
<td>0 (4.0)</td>
<td>46</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>1979</td>
<td>25</td>
<td>1 (4.0)</td>
<td>31</td>
<td>1(^b) (3.2)</td>
</tr>
<tr>
<td>1980</td>
<td>32</td>
<td>0 (0)</td>
<td>35</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td>1981</td>
<td>32</td>
<td>0 (0)</td>
<td>41</td>
<td>4 (9.8)</td>
</tr>
<tr>
<td>1982</td>
<td>42</td>
<td>1(^e) (2.4)</td>
<td>75</td>
<td>9(^e)(^d) (12.0)</td>
</tr>
<tr>
<td>1983</td>
<td>46</td>
<td>1 (2.2)</td>
<td>65</td>
<td>8 (12.3)</td>
</tr>
<tr>
<td>1984</td>
<td>38</td>
<td>3 (7.9)</td>
<td>54</td>
<td>9(^e) (16.7)</td>
</tr>
<tr>
<td>1985</td>
<td>27</td>
<td>0 (0)</td>
<td>38</td>
<td>6 (15.8)</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
<td>6 (2.1)</td>
<td>385</td>
<td>44 (11.4)</td>
</tr>
</tbody>
</table>

\(^a\)N. meningitidis unless denoted otherwise.
\(^b\)N. perflava.
\(^c\)Same patient with B. catarrhalis.
\(^d\)One N. perflava.
\(^e\)Two B. catarrhalis, one N. lactamica.

methods may be sufficient. The NGN accounted for only 0.7% of anogenital GNDC from women (Table 2) and 0.4% of GNDC from urethral cultures from heterosexual men (Table 3). Depending upon the sexual orientation of the patient, the same site may have a different spectrum of colonization. For example, in homosexual men, NGN are much more common, accounting for 2.1% of urethral isolates and 11.4% of rectal isolates of GNDC (Table 4). Neisseria meningitidis and B. catarrhalis accounted for 84.5% and 11.9%, respectively, of the 84 NGN isolated from anogenital sites (see Tables 2-4).

2. Presumptive Identification

The data presented in Table 2 show that in a female population in whom the prevalence of gonorrhea is around 10%, a presumptive identification of N. gonorrhoeae made on the basis of oxidase-positive GNDC growing on selective medium is approximately 99.8% accurate for genital cultures and 99.4% accurate for rectal cultures. Smeltzer et al. (1980) evaluated a low-prevalence population (1.4%) and showed that 98.5% of the presumptive positive cervical isolates available for confirmation were identified as N. gonorrhoeae. Presumptive identification is 99.6 and 98% accurate for urethral GNDC from heterosexual men (see Table 3) and homosexual men (see Table 4), respectively. Accuracy of presump-
tive identification is poor (89%) for male rectal cultures and is totally unsuitable for throat culture because most GNDC isolated are meningococci (see Table 1).

3. Carbohydrate Utilization Tests

A definitive diagnosis has traditionally been made on the basis of carbohydrate utilization tests.

a. Conventional Tests. In conventional tests for carbohydrate utilization, a solid medium containing the appropriate carbohydrate and pH indicator is inoculated with the test organism. Although widely used, these tests are unsuitable because a positive reaction is dependent upon adequate growth of the test organism, which may take up to 72 hr (Pollock, 1976).

Failure to support gonococcal growth is a major problem with the commercially available cystine-trypticase agar (CTA) system widely used in the United States. Inconclusive results and other problems with CTA medium accounted for 44% of 141 gonococcal cultures sent to the Centers for Disease Control (CDC), Atlanta, for confirmation (Arko et al., 1982). The poor performance of CTA medium has led to the acceptance of “glucose-negative” gonococci, a concept that may cause diagnostic problems. Knapp et al. (1984) described an asaccharolytic diplococcus that had been isolated from the cervix of a patient with arthritis. The isolate was presumptively identified as a glucose-negative gonococcus but later identified as N. cinerea.

Certain meningococci fail to give a positive reaction with maltose in the CTA system owing to deficiencies in maltose permease or phosphorylase (Saiz-Nieto et al., 1982). Colonization with “maltose-negative” meningococci has been misdiagnosed as pharyngeal gonorrhea (Noble and Cooper, 1979).

The excellent growth-supporting capacity of MNYC medium has been utilized in the commercially available MNYC carbohydrate medium, which was shown to provide more accurate and reliable results than CTA medium (Simms and Lue, 1982). Knapp and Holmes (1983) developed a semisolid medium which they termed modified oxidation-fermentation (MOF) medium. This medium proved much more reliable than CTA medium. Up to 24 hr of incubation may be required to obtain results.

b. Rapid Carbohydrate Utilization Test. In the rapid carbohydrate utilization test (RCUT) preformed enzyme is measured by adding a suspension of the overnight growth of the suspect organism to a buffered, nonnutrient solution containing the sugar to be tested and a pH indicator (Young et al., 1976). Apart from rapidity, the ability to identify other neisseriae and confirm N. gonorrhoeae within 24-72 hr of seeing the patient, the RCUT, in measuring preformed enzyme, has the advantage of being independent of growth. Results of the RCUT are usually available after 1-3 hr incubation at 37°C.
If the test reagents are properly quality controlled and suspensions are prepared with young 18 to 24-hr cultures grown on medium containing glucose, RCUT methods are more accurate than conventional tests (Tapsall and Cheng, 1981). It is important to use extra pure maltose. Many batches of maltose are contaminated with glucose, which may result in gonococci giving false-positive reactions. In our experience, glucose-negative gonococci are not encountered, and meningococci found to be maltose-negative in the CTA test utilize maltose in the RCUT (Wood and Young, 1986).

The RCUT methods have the additional advantage that isolates can be characterized, including penicillinase testing, from the primary isolation plate (Young, 1978b). Considerable saving in time and reagents can be made by performing the test in microtiter plates. Plates containing reagents can be prepared in batches and stored at −20°C for several months.

Commercially available adaptations of the RCUT method include the Mini-tek system (BBL Microbiology Systems) and Oxoid Neisseria Identification Discs. Recently a 1-hr test was developed by incorporating chloroform in the incubation mixture (Lairscey and Kelly, 1985), presumably to increase bacterial permeability.

c. Other Carbohydrate Utilization Methods. Yong and Prytula (1978) described a modified RCUT that used both preformed enzymes and enzymes formed as a result of growth in a superenriched medium. The combined action of the two sources of enzyme make this method suitable for very small inocula. Because of possible contamination by other bacteria, the method cannot be used to characterize isolates directly from primary isolation cultures.

The BACTEC Neisseria differentiation kits are available commercially. They are designed to identify N. gonorrhoeae, N. meningitidis, and N. lactamica in 3 hr by detecting [14C]CO₂ resulting from the metabolism of [14C] sugars by a bacterial suspension in a closed bottle. Apart from the disadvantages of working with radiolabeled material and the expense of monitoring equipment, the system is oversensitive and gives positive results with N. cinerea (Boyce et al., 1985). This resulted in proctitis associated with N. cinerea being misdiagnosed as a gonococcal infection in an 8-year-old boy (Dossett et al., 1985).

4. Biochemical Identification Systems

A number of neisserial identification systems such as the RapID NH System (Innovative Diagnostics Systems Inc.) and Gonocheck (E-Y Laboratories) are now available commercially (Philip and Garton, 1985).

a. RapID NH System. The RapID NH system is a 4-hr test designed to differentiate Neisseria spp., Branhamella, Moraxella, and Haemophilus spp. and is based on both sugar utilization tests and single-substrate chromogenic biochemical tests. A suspension of the test organism is added to reconstituted dehydrated reagents
in the appropriate wells of a plastic tray. In addition to sugar utilization, tests such as β-D-galactosidase, aminopeptidase, indole production, urea hydrolysis, and phosphate and nitrate degradation, are included. Robinson and Oberhofer (1983) found that there were considerable problems associated with the use of this system. Branhamella catarrhalis could not be distinguished from species of the gram-negative coccobacilli Moraxella and Kingella; 44% of gonococcal isolates tested did not utilize glucose, and the other tests had to be relied upon to give definitive identification. Similar problems were found with isolates of N. meningitidis. This system also failed to differentiate accurately between N. gonorrhoeae and N. cinerea (Boyce and Mitchell, 1985).

b. Gonocheck. Gonocheck is a single-tube, multiple chromogenic substrate test designed to identify N. gonorrhoeae, N. meningitidis, N. lactamica, and B. catarrhalis. The enzymes produced by N. gonorrhoeae, N. meningitidis, and N. lactamica are proline aminopeptidase, γ-glutamyl aminopeptidase, and β-galactosidase, respectively; B. catarrhalis does not produce these enzymes.

Oxidase-positive GNDC are added to the reconstituted tubes which are then incubated at 35°C for 30 min. A blue coloration designates N. lactamica; yellow designates N. meningitidis. If there is no color development a diazonium salt derivative (EY 20) is added. If the tube subsequently turns red, the isolate is a gonococcus. If no color develops after the addition of EY 20, then the isolate is B. catarrhalis.

Welborn et al. (1984), comparing the Gonocheck with RapID NH and the RCUT system of Young et al. (1976), found that Gonocheck compared well with the RCUT provided that the tubes were read carefully for color production before the addition of the EY 20 reagents. The addition of EY 20 to tubes that had developed a slight blue would produce false-positive red, resulting in N. lactamica being falsely identified as a gonococcus. The RapID NH performed poorly and was not recommended.

Wood and Young (1986) found that some meningococci produced very low levels of γ-glutamyl aminopeptidase and very little or no yellow developed. This resulted in the misidentification of three strains, two as N. gonorrhoeae and one as B. catarrhalis on first testing from primary culture plates. After 24-hr subculture these strains were identified correctly. Gonocheck persistently misidentified one gonococcal strain as B. catarrhalis. Boyce and Mitchell (1985) reported that Gonocheck could not differentiate between N. gonorrhoeae and N. cinerea.

These newer test systems have been evaluated with only small numbers of isolates. The evaluation of the RapID NH System by Robinson and Oberhofer (1983) included 28 meningococci, while the Gonocheck evaluations of Welborn et al. (1984) and Wood and Young (1986) included only six and 65 meningococci, respectively. The reliability of differentiating gonococci and meningococci on the basis of enzyme profiles remains to be established in large scale "in-use"
IMMUNOLOGICAL DIAGNOSIS OF GONORRHEA

evaluations. Throat meningococci form a more heterogeneous group and have lower mean γ-glutamyl aminopeptidase levels and higher mean hydroxyproline activity than systemic strains (Ison et al., 1982). Unlike systemic strains that are almost always groupable, a large proportion (36.5%) of meningococci isolated from the throat of patients with gonorrhea are nongroupable (Young et al., 1983a).

c. Miscellaneous Identification Methods. These include identification using lectins and the superoxol test. Lectins are plant proteins that react with carbohydrates: certain lectins agglutinate gonococci and can be used in a slide test. Curtis and Slack (1981) tested 168 gonococci and 96 meningococci with wheat germ lectin and found a sensitivity of 94.6% and a specificity of 78.1%. The poor specificity is caused by lectin agglutination of nongroupable meningococci. Lectins fail to agglutinate groupable meningococci because the capsular polysaccharide blocks the N-acetylglucosamine receptor. Lectin agglutination has also been used in conjunction with chromogenic peptidase assays (Yajko et al., 1984).

The superoxol test (Saginur et al., 1982) is essentially a catalase test performed with 20-30% hydrogen peroxide in place of 3%. A positive superoxol test reaction is of limited diagnostic value because a considerable number of meningococci and some commensal neisseriae give a positive reaction (Young et al., 1984). In contrast, an isolate is almost certainly not a gonococcus if it gives a negative superoxol test result, as all but one of 596 gonococcal isolates tested gave positive results. Odugbemi and Arko (1983) found a negative superoxol test result valuable in differentiating N. gonorrhoeae from Kingella denitrificans. The latter has a colonial morphology similar to the gonococcus, and on Gram staining may appear as coccoid or short rod-shaped.

D. Antibiotic Susceptibility Tests

Detection of PPNG is the most important aspect of sensitivity testing, and all isolates should be screened immediately. Because most patients with gonococcal infection who attend genitourinary medical clinics will have been treated on the basis of a positive Gram-stained smear, susceptibility tests, other than those to detect PPNG, are of little help in the initial management of the patient. Routine testing of at least a proportion of isolates is worthwhile, however, as it provides epidemiological data on which to plan rational therapy.

1. Penicillinase-Producing Neisseria gonorrhoeae

Screening for PPNG can be performed very simply by inoculating several colonies from the primary isolation plate onto a suitable nonselective medium and placing a 6 μg disk on the well. After overnight incubation, any isolate showing a zone of inhibition of < 20 mm is likely to be a PPNG and should be confirmed by one of the specific penicillinase detection methods (World Health Organization, 1978).
One of the most sensitive and convenient methods uses commercially available paper strips impregnated with a chromogenic cephalosporin. If all isolates are not screened routinely for penicillinase production, it is most important to test all strains isolated after treatment. All strains with decreased susceptibility to penicillin (MIC $\geq 0.125$ mg/L) should also be tested. Recently Taylor et al. (1985) described the detection of PPNG in urethral exudates by measuring the fluorescent end products of $\beta$-lactamase activity on an ampicillin substrate. Although the sensitivity and specificity of this method were 91 and 96%, respectively, there were occasional unresolved technical difficulties with the test.

2. **Minimum Inhibitory Concentration**

A proportion of strains may show chromosomally mediated resistance to penicillin at a level ($\geq 1$ mg/L), which makes failure likely with current penicillin treatment regimens.

These strains that have been common in Southeast Asia for some years (Sng et al., 1984) are now becoming more common in the United Kingdom (Easmon, 1985) and the United States (Rice et al., 1986). Such strains tend to be more resistant to tetracycline and erythromycin.

Although standardization of MIC testing is desirable, most laboratories use disk or agar dilution methods with media and inocula to suit their individual needs and preferences. Disk susceptibility testing has been widely used because it is simple and can give a rapid guide to the sensitivity or resistance of an isolate. Although more accurate results are obtained with an agar dilution method, this can be tedious and time-consuming for small numbers of isolates. Large numbers of isolates can be conveniently tested in batches using multipoint inoculation technology. Much useful information is accumulating on the correlation between gonococcal serovar, as determined with monoclonal antibodies (MAbs) and antibiotic susceptibility (Chap. 4). It may be that in the future, serovar analysis will provide a rapid guide to the antibiotic susceptibility pattern of an isolate.

**E. Transport and Culture Systems**

The most suitable transport and culture system must be related to local constraints imposed on individual geographical areas. Direct plating should be used whenever possible as it produces the best results for specimens from all sites. In comparison with specimens cultured following transport, cultures plated directly yield larger colonies at 18-24 hr. This is important in providing rapid identification, as many of the newer identification methods can be used directly with the growth from primary cultures.

When direct plating and immediate incubation are impractical a conventional nonnutrient transport medium such as Amies’ modification of Stuart’s medium
IMMUNOLOGICAL DIAGNOSIS OF GONORRHEA

(Amies, 1967) or a nutrient transport and culture system based on a selective medium contained in a biological environment chamber (JEMBEC) (Martin and Jackson, 1975) should be used.

Nutrient transport and culture systems are expensive and would seem to offer little advantage over a nonnutrient medium when the transit time is about 3 hr. A significant loss of viability occurs when specimens are in transit for longer than 3 hr (Ebright et al., 1982; Sng et al., 1982; Spence et al., 1983). Amies' medium is more effective than Stuart's in maintaining gonococcal viability (Human and Jones, 1986). When transit times longer than 3 hr are anticipated, it is best to use a transport/culture system and to incubate the container overnight before forwarding to the laboratory. Faur et al. (1977) found that NYC medium in a biological environment chamber was an effective method for the handling, transport, and culture of N. gonorrhoeae, provided the delay in transport did not exceed 24 hr: a delay of 48 hr resulted in 17.5% fewer positive cultures than direct plating and immediate incubation.

Because of the problems associated with maintaining the viability of gonococci during transit, particularly when the inoculum is low, several noncultural methods have been developed. These include both immunological (Sect. IV.C) and nonimmunological methods (Sect. V).

IV. IMMUNOLOGICAL DIAGNOSIS

In detecting gonococcal antigen, immunological diagnosis obviates problems associated with the transport and culture of the gonococcus. Immunological diagnosis is also adaptable and has the potential for immediate diagnosis, the identification of N. gonorrhoeae following culture, and detection of antigen in exudates transported to a central laboratory.

A. Immediate Diagnosis

1. Polyclonal Antibodies

The limitations of the Gram-stained smear (Sect. III.A), particularly in female patients, led to the development of immediate diagnosis based on direct fluorescent antibody (FA) staining of urogenital smears. Although FA staining of smears can provide results in less than 1 hr, there were inconsistencies in test performance when results from several published trials were compared (Hare, 1974). In spite of FA staining being superior to Gram staining in many of the trials, Hare (1974) considered that the direct FA test was unsuitable for routine work but could be useful in the investigation of special cases and in research. A similar conclusion was reached by Danielsson and Forsum (1975) in a detailed discussion of the methodology of FA staining.
Subsequent laboratory procedures have not included routine FA staining thus endorsing these early recommendations. Apart from the need for a highly experienced staff and a fluorescence microscope on site, the reading of FA tests was difficult owing to various degrees of cross-reaction with other organisms. Although reagents were absorbed to remove cross-reacting antibodies, this also tended to decrease the specific staining with gonococci. In addition, the pool of polyclonal antibodies used in preparing the FA reagents were chosen without the benefit of the serogrouping and serotyping system described in Chap. 4.

2. Monoclonal Antibodies

The advent of MAbs reactive with epitopes on protein I of *N. gonorrhoeae* (Chap. 2) has revitalized interest in FA staining. Monoclonal antibody reagents for direct FA staining have been developed jointly by Syva and Genetic Systems Inc. As yet, these reagents are not available for routine use. A preliminary evaluation, however, showed that they were superior to Gram staining for specimens from women but not from men (Ison et al., 1985).

Urethral specimens from 105 men and urethral and cervical specimens from 60 women were examined. For the 45 positive cultures from men, the FA test had a sensitivity of 85% and a specificity of 100%: Gram stains gave values of 94 and 100%, respectively. For the 17 positive cultures from women, the FA test had a sensitivity of 65% and a specificity of 98% for urethral samples; the corresponding values for cervical samples were 72 and 94%, respectively. The sensitivity of Gram stains was 40% for both sites.

The FA staining had no advantage over Gram staining for detecting gonorrhea in men, but the superior sensitivity was a marked advantage when dealing with female patients. Testing duplicate smears increased the sensitivity from 85 to 89% in men and from 72 to 88% for cervical samples. This indicates that the failure of the FA test to detect infected patients was most likely due to specimen collection (sample variation) rather than a failure of the MAbs to recognize epitopes on certain strains of gonococci. Direct FA staining with MAbs awaits widespread evaluation. If sufficiently sensitive and specific, it could be of great value in the rapid diagnosis of rectal gonorrhea in men.

B. Identification of *Neisseria gonorrhoeae*

Advantages of confirming the identity of *N. gonorrhoeae* by immunological methods include rapidity and the use of very small amounts of bacterial growth. These factors, combined with the early difficulties with culture and carbohydrate utilization, stimulated the development of immunological methods.

1. Polyclonal Antibodies

Methods such as direct FA staining and coagglutination (CoA) have been used widely in identifying gonococci. Experience with other systems such as agglutina-
tion with antigonococcal lipopolysaccharide hen serum is extremely limited (Maly-
sheff et al., 1978; Wallace et al., 1978).

Although FA staining was the first method to be used, CoA is simpler to
perform, easier to interpret, and does not require expensive immunofluorescence
equipment (Young and McMillan, 1982). Coagglutination is a rapid slide test that
uses protein A-containing staphylococci with rabbit antigonococcal antibodies
bound by their Fc portion to the protein A (Danielsson and Kronvall, 1974).
When this reagent is mixed with gonococci, a readily visible agglutination is pro-
duced. This reaction is compared with a control in which the staphylococci have
not been coated with specific antibodies.

The sensitivity and specificity obtained with commercial reagents for CoA
(Phadebact Gonococcus Test, Pharmacia) and direct FA staining (Difco Labora-
tories) are comparable (Shanker et al., 1981; Young and McMillan, 1982). These
and other groups (Carlson et al., 1982; Lim and Wall, 1980) reported CoA sensi-
tivities and specificities in the range of 92.6-97.8% and 93.0-96.8%, respectively.
Insofar as they fail to detect a small proportion of gonococcal isolates and fail
to give clear-cut negative reactions with a similar proportion of NGN, both FA
staining and CoA suffer from the same disadvantages. Although CoA testing ef-
effectively replaced the FA method in most laboratories, it was particularly prone
to cross-reaction with *N. lactamica*. This made the test unsuitable for confirming
the identity of pharyngeal isolates.

2. Monoclonal Antibodies

Immunological identification of *N. gonorrhoeae* with MAb CoA reagents has super-
seded both FA and CoA polyclonal antibody tests. Two commercial test test systems
are available.

a. *Gono Gen*. The Gono Gen CoA test (New Horizons Diagnostic Company,
Columbia, MD) consists of a single test reagent prepared from a pool of murine
MAbs reactive with protein I (Tam et al., 1982; see also Chap. 2) and a control
reagent (nonimmune rabbit IgG bound to staphylococci). The test is performed
with growth from the primary isolation plate. A light suspension of organisms is
made in distilled water and boiled for 5 min before mixing with test and control
reagents. A positive reaction is indicated within 1 min by substantially stronger
agglutination with test reagent than with control reagent.

The sensitivity of the Gono Gen test was 99.1% for 110 isolates (Lawton and
Battaglioli, 1983) and 96.6% in evaluations involving 56 (Philip et al., 1984) and
205 gonococcal isolates (Young and Reid, 1984). Lawton and Battaglioli (1983)
tested 57 meningococcal isolates and reported a specificity of 100% as did Philip
et al. (1984) who tested 24 meningococci and three isolates of *N. lactamica*. Young
and Reid (1984) reported one of 52 meningococci reactive when tested from the
primary isolates plate but negative after two subcultures. One of three clinical
isolates of *N. lactamica* reacted with control reagent as well as the test reagent.
Thirteen stock cultures of *N. lactamica* were negative with Gono Gen, whereas seven were positive with the Phadebact Gonococcus Test which used polyclonal reagents. If the absolute specificity of the Gono Gen MAb reagent is confirmed in larger surveys, it would mean that a positive reaction with an isolated from any anatomical site, including the pharynx, could be regarded as a gonococcus without recourse to further testing: a specificity of 100% yields no false-positive reactions, hence the positive predictive value is also 100%.

Unfortunately, the sensitivity of Gono Gen was only 96.6% in the two evaluations reported from the United Kingdom (Philip et al., 1984; Young and Reid, 1984). The 99.1% sensitivity reported by Lawton and Battaglioli (1983) in New York may suggest differences in the antigenic profile of gonococci circulating in these different geographical areas. Knapp (1985) reporting on unpublished observations from the United States noted that a panel of 10 MAb reagents detected 100% of 839 strains and a panel of three of these reagents detected 99.5% (835) of the strains. It may be that MAb pools will have to be “tailored” to suit broad geographical areas. Each time a pool is changed by the addition of a new reagent, however, it is important to ensure that this does not have an adverse effect on the overall performance of the new composite reagent. A sensitivity marginally less than 100% has a very marked effect on the negative predictive value, particularly when testing anogenital isolates from heterosexual patients. The low prevalence of NGN in anogenital sites means that a negative Gono Gen result is more likely to be due to a nonreactive gonococcus than to NGN: this is reflected in the poor negative predictive value (10.68%) indicating the need to confirm Gono Gen-negative anogenital isolates by biochemical tests (Young and Reid, 1984). Conversely, because of the preponderance of NGN, mainly meningococci among pharyngeal isolates, a negative Gono Gen result has a high predictive value (99.13%) for NGN.

### b. Phadebact Monoclonal GC Test

The Phadebact Monoclonal GC test contains two separate reagents, WI and WII/III, prepared with different pools of MAbs reactive with protein IA and protein IB, respectively (Sandström et al., 1985). The pools of MAbs are selected from the Ph-antibodies used in serovar determination (Chap. 4).

The test is performed on suspect gonococci (oxidase-positive GNDC) from primary isolation cultures. It is important to follow the manufacturer’s instructions to make a light suspension in 0.9% saline and boil for 5 min. When the boiled antigen is cooled it is tested against both the WI and WII/III reagents: a substantially stronger reaction in either the WI or WII/III reagent constitutes a positive result and provides instant serogrouping. Positive results tend to be stronger and appear more quickly than with those with polyclonal reagents.

The Phadebact Monoclonal GC test was shown to be 100% sensitive and specific when tested against 550 gonococcal and 197 nongonococcal GNDC including
*N. meningitidis*, *N. lactamica*, *N. cinerea*, and *B. catarrhalis* (Blomqvist et al., 1985).

A prolonged "in-use" evaluation (Young, unpublished results) has confirmed the absolute specificity and extremely high sensitivity. All 861 NGN (784 *N. meningitidis*, 46 *N. lactamica*, 18 *N. perflava*, 8 *N. flava*, and 5 *B. catarrhalis*) identified by the rapid carbohydrate utilization test gave unequivocal negative reactions. Of 1017 gonococci tested 1014 were unequivocally positive, giving an overall sensitivity of 99.7%. An analysis of the results with the individual reagents showed that all 480 serogroup WI strains were reactive, whereas three of the 537 serogroup WII strains were nonreactive, giving a sensitivity of 99.4%. All three nonreactive WII/III strains were of the extremely rare serovar Bj/Bro (see Chap. 4) and were isolated over a restricted period.

Because of the occurrence, albeit very rarely, of nonreactive gonococci, it is advisable to confirm the identity of any nonreactive anogenital isolate but particularly those from heterosexual patients.

Using the formula of Vecchio (1960) the spectrum of neisserial colonization given in Tables 1 to 4 can be used to calculate predictive values for results on isolates from various anatomical sites. As shown in Table 5 a small decrease in sensitivity is translated into a marked decrease in the predictive value of a negative test.

### Table 5  Effect of Decreasing Sensitivity on Negative Predictive Value (NPV) for GNDC Isolated From Various Anatomical Sites

<table>
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<tr>
<th>Sensitivity</th>
<th>Heterosexual male urethra</th>
<th>Female anogenital</th>
<th>Homosexual male Urethra</th>
<th>Rectum</th>
<th>Throat (all groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
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</tr>
<tr>
<td>99.90</td>
<td>80.06</td>
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<td>95.55</td>
<td>99.23</td>
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<td>97.72</td>
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<td>58.50</td>
<td>81.10</td>
<td>95.94</td>
<td>99.84</td>
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<td>70.44</td>
<td>93.46</td>
<td>99.71</td>
</tr>
<tr>
<td>98.00</td>
<td>16.72</td>
<td>26.06</td>
<td>51.75</td>
<td>86.55</td>
<td>99.35</td>
</tr>
</tbody>
</table>

Specificity 100%: Positive Predictive Value 100% for all groups.

- Proportion of gonococcal GNDC = 0.996 (Table 3).
- Proportion of gonococcal GNDC = 0.993 (Table 2).
- Proportion of gonococcal GNDC = 0.979 (Table 4).
- Proportion of gonococcal GNDC = 0.886 (Table 4).
- Proportion of gonococcal GNDC = 0.247 (Table 1).
This is most noticeable for sites, such as the male urethra, where NGN are rarely isolated. For example, at a sensitivity between 99.5 and 99.7% the negative predictive value is around 50%, indicating that a negative test result is as likely to be due to a nonreactive gonococcus as the NGN. Even at a sensitivity of 99.9% two of 10 negative test results from male urethral isolates will be false-negative. Because NGN are isolated more frequently from homosexual patients, negative predictive values are higher. When dealing with isolates from the throat, the negative predictive value remains high, even at a sensitivity of 98%.

The absolute specificity of the test makes it suitable for confirming the identity of throat isolates. In our experience this is particularly useful when characterizing isolates from primary cultures. Cultures with apparently characteristic meningococcal growth have, on several occasions, given a positive Phadebact Monoclonal GC test. On careful investigation, these cultures were shown to be a mixed infection with gonococci and meningococci. Likewise the test is useful in the rapid identification of gonococci in rectal cultures that may be contaminated with organisms other than neisseriae.

An omnireagent combining protein IA and IB MAb pools is also available in certain areas. As agglutination of the test reagent must be compared with a control reagent, the test is not any simpler to perform. In addition, an omnireagent is less flexible, and it would be more difficult to "modify" the MAb pool to cover nonreactive strains should the proportion of such strains increase.

C. Detection of Antigen in Exudates

A reliable method for the detection of gonococcal antigen in patient exudates would remove the need to maintain the viability of the gonococcus during transport, as well as overcoming problems associated with biochemical identification methods. Although a few groups have attempted to develop antigen detection systems based on enzyme-linked immunosorbent assay (ELISA) methods (Sarafian and Young, 1982; Young et al., 1983b) most published studies have evaluated the commercially available Gonozyme test (Abbott Diagnostics).

1. Gonozyme Test

The Gonozyme test is performed with urethral or cervical exudate collected on a swab and transported to the laboratory in a special transport tube containing a preservative. The swab should be processed to extract antigen within 5 days. Polystyrene beads (pretreated in an unspecified manner) are used as the solid phase to capture antigen, which is detected by polyclonal antibodies. Initially, the test was based on 15-min incubation periods and results could be obtained in 90 min. The test was later modified by extending the incubations to 45 min which increased the time taken to obtain results to over 3 hr.

a. Diagnosis from Urethral Swabs from Men. As shown in Table 6, the sensitivity, specificity, positive, and negative values are high in the case of symptomatic
### Table 6 Detection of Gonococcal Antigen in Male Urethral Specimens by Gonozyne

<table>
<thead>
<tr>
<th>Author</th>
<th>Study population</th>
<th>Prevalence of gonorrhea by culture (%)</th>
<th>Culture system</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PVP</th>
<th>PVN</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aardoom et al. (1982)</td>
<td>Symptomatic (n = 52)</td>
<td>67</td>
<td>Direct plating: TM and chocolate agar</td>
<td>100 ((90-100)^c)</td>
<td>100 ((81-100)^c)</td>
<td>–</td>
<td>–</td>
<td>Same swab; plate inoculated first</td>
</tr>
<tr>
<td>Demetriou et al. (1984)</td>
<td>Symptomatic (n = 57)</td>
<td>42</td>
<td>Direct plating: JEMBEC with Martin-Lewis medium</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>Same swab; plate inoculated first</td>
</tr>
<tr>
<td>Papasian et al. (1984)</td>
<td>Symptomatic (n = 208)</td>
<td>54</td>
<td>Direct plating: Martin-Lewis or MTM medium</td>
<td>97.3</td>
<td>95.8</td>
<td>96.5</td>
<td>96.8</td>
<td>Two swabs; plate inoculated with first</td>
</tr>
<tr>
<td>Stamm et al. (1984)</td>
<td>Symptomatic (n = 465)</td>
<td>36</td>
<td>Direct plating: TM and chocolate agar biplate</td>
<td>95</td>
<td>98</td>
<td>97</td>
<td>97</td>
<td>Same swab used in order: Gram stain, biplate, Gonozyne</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic (n = 664)</td>
<td>2</td>
<td>Direct plating: TM and chocolate agar biplate</td>
<td>67</td>
<td>98</td>
<td>30</td>
<td>99</td>
<td>Same swab used in order: Gram stain, biplate, Gonozyne</td>
</tr>
<tr>
<td>Granato and Roefaro (1985)</td>
<td>Symptomatic (n = 217)</td>
<td>33</td>
<td>Direct plating: JEMBEC with Martin-Lewis medium</td>
<td>95.3</td>
<td>99.4</td>
<td>97.6</td>
<td>98.8</td>
<td>Culture and Gonozyne performed with eluate from a single swab</td>
</tr>
</tbody>
</table>

*aPVP, predictive value for a positive test. 
\(b\)PVN, predictive value for a negative test. 
\(c\)95% confidence intervals given as probability percentages.
Table 7: Detection of Gonococcal Antigen in Cervical Specimens by Gonozyme

<table>
<thead>
<tr>
<th>Study population</th>
<th>Prevalence of gonorrhea by culture (%)</th>
<th>Culture system</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aardoom et al. (1982)</td>
<td>Contacts of gonorrhea suspects (n = 54)</td>
<td>Prostate STD control screen</td>
<td>Direct plating: TM and chocolate</td>
<td>86.7 (60.98, 97.97)</td>
<td>Same swab; plate inoculated first</td>
</tr>
<tr>
<td>Demetriou et al. (1984)</td>
<td>Symptomatic, high and low risk (n = 102)</td>
<td>Martin-Lewis medium</td>
<td>Direct plating: JM and chocolate</td>
<td>87.5</td>
<td>Same swab; plate inoculated first</td>
</tr>
<tr>
<td>Papian et al. (1984)</td>
<td>Symptomatic, asymptomatic STD clinic (n = 252)</td>
<td>MTM medium</td>
<td>Direct plating: JM and chocolate</td>
<td>78</td>
<td>Two swabs; plate inoculated with first</td>
</tr>
<tr>
<td>Stamm et al. (1984)</td>
<td>Attenders</td>
<td>MTM medium</td>
<td>Direct plating: JM and chocolate</td>
<td>76</td>
<td>Two swabs; plate inoculated with first</td>
</tr>
<tr>
<td>Granato et al. (1985)</td>
<td></td>
<td>JEMBEC with biplate</td>
<td>Direct plating: JM and chocolate</td>
<td>100</td>
<td>Culture and Gonozyme performed with eluate from a single swab</td>
</tr>
<tr>
<td>Regeero et al. (1985)</td>
<td></td>
<td>MTM medium</td>
<td>Direct plating: JM and chocolate</td>
<td>100</td>
<td>Same swab used in order: Gram stain, Gonozyme, and biplate culture</td>
</tr>
</tbody>
</table>

PVPA, predictive value for a positive test; PVNB, predictive value for a negative test.

95% confidence intervals given as probability percentages.

Results after retesting equivocal specimens.

Excluding 30 specimens from contacts of infected men and test-of-cure specimens.
patients. The test is not reliable, however, for diagnosing infection in men without urethral discharge (Stamm et al., 1984); although the specificity was the same for both symptomatic and asymptomatic patients, the reduction in sensitivity from 95 to 67% was significant ($p < .001$) as was the reduction in positive predictive value from 97 to 30%. In symptomatic patients Gram staining, which is both inexpensive and simple to perform, correlated well with Gonozyme (Demetriou et al., 1984; Granato and Roefaro, 1985; Stamm et al., 1984). Consequently, when examining male urethral specimens there would seem to be little need for the more expensive Gonozyme assay.

b. **Diagnosis from Cervical Swabs.** In comparison with the results from men, antigen detection from cervical swabs (Table 7) is generally less sensitive and less specific. Demetriou et al. (1984) found the difference in sensitivity was significant ($p = .03$). There was no significant difference in sensitivity among the various subgroups of female patients (Demetriou et al., 1984). The lowest values for sensitivity were obtained by Stamm et al. (1984) who used the same specimen to make a Gram-stained smear and to inoculate a biplate before testing for antigen. It is likely that progressive loss of antigen during these procedures contributed to the lower sensitivity found in this study. Nevertheless, the sensitivity of Gonozyme (78%) was significantly greater than that of the Gram stain (48%). Unfortunately, as the test takes about 3-4 hr to perform, a result cannot be obtained when the patient is at the clinic.

The poorer sensitivity in women compared with men most probably results from less antigen in certain specimens. On the basis of semiquantitative counts, Demetriou et al. (1984) were able to show that cultures from six of 12 women with false-negative antigen assays had fewer than 100 colony-forming units (CFU) compared with 9 (10.8%) of 83 for whom quantitation was available in the true positive group ($p = .006$). The number of gonococci recovered from cervical swabs varies widely. Young et al. (1983b) reported a range of $5.0 \times 10^3$ to $8.0 \times 10^6$ CFU/ml (mean $1.0 \times 10^6$ CFU/ml) in 37 infected women. The numbers in combined cervicovaginal washings from 52 women ranged from $4.0 \times 10^2$ to $1.8 \times 10^2$ (mean $1.45 \times 10^5$) (Lowe and Kraus, 1976).

Papasin et al. (1984) considered the poorer sensitivity in women may also be related to the more complex microbial flora of the female genital tract. If nongonococcal antigens adsorb onto the bead they could compete with gonococcal antigen for binding sites. Adsorption of nongonococcal antigens combined with cross-reactivity of the polyclonal detection antiserum may also account for the poorer specificity in women. Demetriou et al. (1984) found the difference in positive predictive value between male and female patients was statistically significant ($p = .02$). Because the detection antiserum may cross-react with other neisseriae, such as *N. meningitidis* and *N. lactamica*, the Gonozyme test is not recommended for pharyngeal or rectal specimens.
Unless a test exhibits absolute specificity, its positive predictive value will decline rapidly as the prevalence of the disease in the population decreases. Using the sensitivity and specificity values shown in Table 7 Demetriou et al. (1984) calculated that in a population with a 2% prevalence rate, as typically found in many family-planning clinics, the positive and negative predictive values would be 47.2 and 99.7%, respectively. Under such circumstances, the Gonozyme test could be used only for screening: as over one-half of the positives would be false-positive, culture confirmation under optimum conditions is essential. The high negative predictive value, however, means that screening with Gonozyme is likely to be more reliable for excluding gonococcal infection than culture techniques after prolonged transport. Martin et al. (1984) evaluated 510 patients (282 women and 140 men) with Gonozyme and Transgrow, containing Martin-Lewis medium, shipped to the laboratory after overnight incubation. The prevalence of gonorrhea by culture was approximately 15%. When specificity and sensitivity were calculated on the basis of clinical, epidemiological, and on-site laboratory data, Gonozyme had a sensitivity of 95% and a specificity of 99%. Transgrow had a sensitivity of only 69% but was 100% specific. More extensive evaluation of Gonozyme is required, however, for low-prevalence populations.

c. Diagnosis from First-Voided Urine. Schachter et al. (1986) compared the Gonozyme test, performed on centrifuged urinary sediment, with conventional urethral culture in 196 men attending an STD clinic. The men were either asymptomatic, requesting routine investigation, or were named contacts. The prevalence of infection was 14% by culture. The sensitivity and specificity of Gonozyme were 93% (25 of 27) and 99% (167 of 169), respectively, and the negative and positive predictive values were 99 and 93%, respectively. The ability to detect gonococcal antigen in urinary sediment may provide the basis for a noninvasive method of screening for male infection. Again, evaluation of the test in the low-prevalence populations is required.

Detection of antigen in urine is ultimately to provide the optimum screen for infected women as urethral infection occurs in only around 75% (Young et al., 1979). In a direct comparison, Chapel and Smeltzer (1975) found that culture of urinary sediment was only 80% as productive as cervical culture.

d. Detection of Antigen in Treated Patients. Granato and Roefaro (1985) considered that almost all of their false-positive Gonozyme test results occurred in either test-of-cure samples or specimens from women who were recent contacts of infected men. Because of the possible persistence of antigen in endocervical secretions, the Gonozyme test was not recommended for evaluating test-of-cure specimens. In a more detailed evaluation Stamm et al. (1984) tested 10 men and 10 women daily until both culture and immunoassay results became negative. On the first day after treatment all 10 men were culture-negative and specimens from seven of the men were also antigen test-negative. Two men did not become
immunoassay-negative until the second day after treatment, while one man yielded persistent positive immunoassays, despite negative cultures, for 5 days after treatment. All 10 women yielded negative cultures on the first day after treatment and were immunoassay-negative by the second day.

D. Detection of Antibody

1. Humoral Antibody

The gonococcal complement fixation test (GCFT) is the only test that has been used to any extent in routine diagnosis. The test has fallen into disrepute over the years and it should not be relied upon either to detect or exclude uncomplicated infection. The GCFT has been withdrawn from most laboratories. As genital examination does not lend itself to large-scale-screening programs there is a real need for a sensitive and specific serological test to screen large groups of individuals, particularly asymptomatic women. Modern approaches using radioimmunoassay (RIA) and ELISA techniques with highly purified antigens, such as gonococcal pilus protein or outer membrane protein, have failed to provide a suitable test. The main problem is that antibody levels in infected and noninfected individuals overlap to such an extent that it is impossible to define an antibody level that gives a reliable indication of infection. Monoclonal antibodies by defining antigens specific for gonococcal infection, may aid the development of a suitable serological test(s). The serological diagnosis of gonococcal infection is reviewed in detail by Donegan (1985).

2. Local Antibody

The production of secretory IgA can be detected in cervical secretions by an indirect immunofluorescent (IF) method (McMillan et al., 1980). The use of exudates collected on swabs detected antigonococcal IgA in 72% of 78 infected women, but it was detected in only 5% of 490 noninfected women. Although this test could be developed further, it suffers from the disadvantage of requiring a genital specimen and must therefore be considered in relation to other non-cultural methods.

V. NONCULTURAL, NONIMMUNOLOGICAL DIAGNOSIS

The following nonimmunological methods are available for detecting gonococcal components. These methods, however, have not been evaluated, or exploited commercially, to the same extent as immunological methods.

A. Endotoxin

The limulus amebocyte lysate (LAL) assay is a test to detect endotoxin. It can be used to detect gonococcal endotoxin in urethral and cervical exudates. The
assay is not specific for gonococcal endotoxin but depends upon the absence of other bacteria producing endotoxin in amounts that would give a positive result. Because of the qualitative and quantitative differences in the genital tract flora of men and women the LAL assay performs best with male urethral exudates. Results with cervical exudates vary widely depending upon the dilution of exudate used in the test (Spagna et al., 1982).

Prior and Spagna (1985) evaluated the LAL assay on 200 men with varying quantities of urethral discharge. Pyrogen-free Dacron swabs were used for sample collection and a chromogenic substrate was used for visible endpoint determination after incubation for 10 min. The prevalence of gonorrhea by culture was 40% and the sensitivity and specificity of the LAL assay were 95 and 97%, respectively. There was no statistical difference between these results and those of Gram-stained smears read by an experienced microscopist. Five of 80 men with culture-proven gonorrhea and 52 of 120 men with nongonococcal urethritis had a minimal amount of urethral discharge and could not be evaluated by the LAL assay. Although the test is described as a simple, fast, and accurate method for the evaluation of exudative urethritis in an office setting, great care is required to ensure that all materials are pyrogen-free. The LAL assay is not suitable for populations with a low prevalence of gonorrhea (Judson et al., 1985).

B. Genetic Transformation

Gonococcal DNA can be detected in clinical specimens by transforming auxotrophic indicator gonococci to protrophy (Janik et al., 1976). This assay is extremely sensitive and can detect as few as 50 CFU of donor gonococci. One of the main limitations of this method is that clinical isolates of *N. gonorrhoeae* may themselves be auxotrophic for the same marker as the indicator strain and thus unable to transform it. The proportion of such auxotrophs will vary geographically. Although it may be possible to overcome these problems by using a temperature-sensitive mutant as indicator (Zubrycki and Weinberger, 1980), the test remains difficult to perform and interpret.

C. DNA Hybridization

Gonococcal DNA has been detected in urethral exudates by hybridization with the 2.6 megadalton (Md) gonococcal cryptic plasmid as a radiolabeled $[^{32}\text{P}]$ probe (Totten et al., 1983). Again, this test is very sensitive, detecting about 100 CFU of gonococci or 0.1 pg of gonococcal cryptic plasmid DNA. Unfortunately, certain strains of gonococci such as the PCU$^+$ auxotroph that are common in certain geographical areas, especially Canada (Chap. 4), lack the 2.6 Md cryptic plasmid and would not be identified by this assay.

Perine et al. (1985) used both the 2.6 Md plasmid and the 4.4 Md β-lactamase-encoding plasmid as probes for the detection of African and Asian strains of *N.*
gonorrhoeae in men with urethritis. With use of the 2.6 Md plasmid, the sensitivity and specificity of the hybridization method were 96% (180 of 187) and 93% (27 of 29), respectively. The 4.4 Md probe also detected infection with strains carrying the 3.2 Md plasmid and gave an overall sensitivity of 91% (59 of 65) and a specificity of 98% (136 of 139) compared with the chromogenic cephalosporin assay on cultured gonococci. Because organisms such as Haemophilus influenzae and Escherichia coli may contain β-lactamase-encoding plasmids that would react with the gonococcal plasmid probe, the hybridization method could not be used for specimens from the cervix, rectum, and throat. As the test takes at least 3 days to perform, it is useful only for epidemiological purposes. The use of biotin-labeled probes coupled with an avidin detection system provides the potential for results within 1 hr making the test useful in diagnosis.

VI. CONCLUSIONS

The accurate diagnosis of gonorrhea is particularly challenging because infection may involve a variety of anatomical sites with marked quantitative and qualitative differences in normal flora. Because of the medicolegal and social aspects of the disease, however, the level of accuracy required of tests to identify N. gonorrhoeae is greater than that accepted for many other organisms. Unless tests have a specificity in the region of 99% and a sensitivity greater than 99.9% they cannot be used reliably for confirming and excluding N. gonorrhoeae from all anatomical sites.

Monoclonal antibodies have an important role in the simple and reliable identification of gonococci. The new coagglutination identification reagents employing pools of MAbs offer 100% specificity and by careful selection and monitoring of MAb pools, approach a sensitivity of 99.9%. Serovar determination with MAbs as outlined in Chap. 4 is important in monitoring the antigenic profile of circulating gonococci and in constructing appropriate pools of MAb reagents.

Much smaller numbers of isolates have been evaluated using the newer biochemical identification methods, and their ability to give comparable levels of accuracy remains to be established. Biochemical methods do have the advantage, however, of identifying NGN to species level rather than simply differentiating between gonococci and NGN.

The use of MAbs in the direct detection of gonococci in genital exudates remains to be evaluated as an “on-the-spot” test for use in the clinic setting. It also remains to be shown that MAbs are useful for detecting gonococcal antigen in transported genital exudates. Gonozyme, the only immunological antigen detection method currently available commercially, uses a mixture of polyclonal antibodies. The levels of sensitivity and specificity of the Gonozyme test make it more suitable for excluding, rather than confirming, gonococcal infection. By
combining polyclonal antibodies for antigen capture with MAbs for specific antigen recognition it should be possible to develop more specific, and possibly more sensitive, tests.

Because of the variety of diagnostic problems, some of which have been outlined in this chapter, there is an impetus for the increased use of immunological detection for the large-scale screening of genital specimens. Before this happens a more comprehensive evaluation of the performance of antigen detection tests in populations with a low prevalence of gonorrhea is required. Provided that such tests perform well in low-prevalence populations, and provided that screening programs are effective in reaching women with asymptomatic infection, they should have a marked effect on gonorrhea control. Although screening will be expensive, cost/benefit analysis must take into account the enormous cost of hospitalization because of PID resulting from untreated infection. Immunological detection is now best regarded as screening rather than diagnostic. Whenever possible, patients with positive antigen tests should be fully investigated by culture of the appropriate sites. Apart from confirming the diagnosis, culture will identify antibiotic-resistant strains.

REFERENCES


IMMUNOLOGICAL DIAGNOSIS OF GONORRHEA


IMMUNOLOGICAL DIAGNOSIS OF GONORRHEA


Genital gonorrhoea in women: a serovar correlation with concomitant rectal infection

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Accepted for publication 18 August 1988

Summary

Strains of gonococci isolated from 383 episodes of infection in women were classified serologically by means of two independently developed panels (Pharmacia (Ph) and Genetic Sytems (GS)) of monoclonal coagglutination reagents. Strains isolated from two groups of patients – those with concomitant genital and rectal infection (Group R) and those with genital infection in the absence of rectal infection (Group G) – were compared in order to determine whether certain strains of gonococci are isolated more often from women with concomitant rectal infection. Group R patients accounted for 126 (33%) episodes and Group G patients accounted for 223 (58%) episodes. Strains belonging to serogroup WII/III accounted for 61 (48%) Group R infections and 86 (39%) Group G infections. The difference was not statistically significant ($P > 0.05$). Strains of serogroup WI could be resolved into 7 Ph-serovars and 10 GS-serovars while strains of serogroup WII/III could be resolved into 19 Ph-serovars and 14 GS-serovars. One GS-serovar, Bajk, was isolated from 34 (27%) Group R patients compared with 39 (17%) Group G patients. This difference was statistically significant ($P = 0.05$). Compared with non-Bajk isolates, Bajk strongly correlated with reduced susceptibility to penicillin: 60 (92%) Bajk isolates had minimum inhibitory concentrations (MIC) $\geq 0.06$ mg/l penicillin compared with 81 (33%) non-Bajk isolates ($P < 0.001$). The GS-serovar Bacejk, however, was significantly less susceptible to penicillin than was serovar Bajk: 26 (90%) Bacejk isolates had MICs $\geq 0.12$ mg/l penicillin compared with 29 (44%) Bajk isolates ($P < 0.001$). Therefore decreased susceptibility to penicillin does not lead in itself to rectal colonisation.

It was concluded that certain gonococcal strains are more likely to cause concomitant rectal infection than others and that their reduced susceptibility to penicillin suggests that rectal test-of-cure cultures are essential in those women treated for anogenital infection.

Introduction

The gonococcus primarily infects the mucosa of the lower genital tract but can infect a diverse array of mucosal surfaces, including the rectum and pharynx. Rectal gonorrhoea is most prevalent among homosexual men and heterosexual women. Unlike homosexual men who acquire gonococcal infection by rectal intercourse, most rectal infections in women are considered to result from the inoculation of the anorectal mucosa with infectious vaginal discharge.\(^1\) Rectal infection has been reported in 30–60% women with gonorrhoea; 2–9% may be infected at the rectal site alone.\(^2\) The rectum is relatively rich in inhibitory hydrophobic molecules since faeces contain 4–5% lipid consisting of fatty acids, sterols and bile acids (Konrad, cited in McFarland et al.\(^9\)).

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The gonococcal outer membrane is particularly permeable to fatty acids. Rectal isolates from homosexual men and heterosexual women, however, are more resistant to faecal lipids than cervical or urethral isolates. A correlation between the mtr mutation, a chromosomal locus which confers partial resistance to various antibiotics, dyes, detergents and fatty acids and gonococcal strains isolated from homosexual men has been reported but this correlation was not observed in rectal isolates from women. McFarland et al. proposed that mtr-independent mechanisms were involved in the increased resistances noted.

These studies suggest that the host environment plays a role in the selection of gonococcal strains. Particular gonococcal strains might therefore be expected to predominate in the female rectum. Gonococcal strains of serogroup WII are associated with homosexually acquired rectal infection. Unfortunately, these serogrouping studies, based on polyclonal antibodies, did not allow a reliable subdivision within the serogroups. Development of coagglutination reagents containing monoclonal antibodies directed against Protein I enabled the W serogroups to be subdivided into serovariants or serovars. The finer discrimination obtained with monoclonal antibodies disclosed correlations between serovars and homosexually acquired infection. To our knowledge, there are no published reports correlating serogroup or serovar with rectal infection in women. This study reports our use of serovar analysis in order to determine whether certain strains of gonococci are isolated more often from women with concomitant rectal infection than from those in whom infection has not spread from the genital tract to the rectum.

Materials and methods

Clinical isolates

Gonococcal isolates were obtained from 383 episodes of infection in female patients attending the Department of Genito-urinary Medicine at the Edinburgh Royal Infirmary during the period of 24 months from October 1985 to September 1987 inclusive. Initial culture was on modified New York City medium (MNYC). All isolates were identified as Neisseria gonorrhoeae by the rapid carbohydrate utilisation test and the ‘Phadebact’ monoclonal GC test which differentiates between serogroup WI and serogroups WII/III. Isolates identified as Neisseria gonorrhoeae were subcultured on MNYC and clear gonococcal (GC) agar which were incubated for 18–24 h at 37 °C in a moist atmosphere containing 10% carbon dioxide. The resulting bacterial growth was used for serovar and penicillin MIC determination.

Storage of isolates

All gonococcal isolates were cultured overnight on MNYC medium and the resulting growth transferred to Trypticase Soya Broth (TSB) containing 6% lactose for short-term storage at −20 °C, as described in a previous study. Isolates with rare or distinctive serovars were lyophilised for long-term storage.
Identification of serovars

Identification of serovars was performed as described previously\textsuperscript{26} by means of two different sets of monoclonal coagglutination reagents developed by Pharmacia (Ph) and Genetic Systems (GS).\textsuperscript{27}

Determination of penicillin minimum inhibitory concentration (MIC)

The MICs of penicillin were determined by the agar plate dilution method. Concentrations of penicillin of 0.015, 0.06, 0.12, 0.5 and 1.0 mg/l were prepared in MNYC medium lacking selective antibiotics. Strains of \textit{N. gonorrhoeae} were grown overnight on MNYC medium and a light suspension, just turbid to the naked eye, was made in nutrient broth. By use of a multipoint inoculator, a drop (approximately 3 µl) of each suspension was inoculated on a series of antibiotic-containing plates of medium and a control plate. The plates were allowed to dry before being incubated overnight at 37 °C in a moist atmosphere containing 10% carbon dioxide.

The MIC was defined as the lowest concentration of penicillin which completely inhibited growth of the inoculum. Occasionally, a spot was observed which had two or three colonies or a very thin film of growth and this was scored as negative.

Statistical analysis

The $\chi^2$ test with Yates' correction was used throughout.

Results

Anatomical sites involved in gonococcal infection in the study population

Altogether, 383 episodes of gonococcal infection were detected by diagnostic tests. In 26 (7%) of infections, gonococci were isolated from the rectum in the absence of genital infection. These isolates were excluded from the study since they might have been acquired by rectal intercourse. One (0.3%) patient with pharyngeal infection alone was also excluded from the study. Genital infection was present in the remaining 356 (93%) infections, with concomitant rectal infection present in 132 (34%) and absent in 224 (58%) of all cases. Six patients with concomitant rectal infection and one without were infected with different serovars simultaneously at different sites and were excluded from the study.

The final study population consisted of 349 (91%) episodes of infection. Concomitant rectal infection was present in 126 (36%) of these episodes (Group R) and absent in 223 (64%) (Group G).

Distribution of serogroups

The distribution of serogroups in the patients in Group G and Group R is given in Table I.

The overall prevalence of WI and WII/III strains in the final study population was 202 (58%) and 147 (42%) respectively. Serogroup WI strains accounted for 137 (61%) of group G isolates compared with 65 (52%)
Table I Distribution of serogroups of gonococci in women with (Group R) and without (Group G) concomitant rectal infection

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. (%) infected with</th>
<th>Serogroup WI</th>
<th>Serogroup WII/III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group G</td>
<td>137 (61)</td>
<td>86 (39)</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Group R</td>
<td>65 (52)</td>
<td>61 (48)</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>147</td>
<td>349</td>
<td></td>
</tr>
</tbody>
</table>

Table II Distribution of Pharmacia serovars of gonococci in women with (Group R) and without (Group G) concomitant rectal infection

<table>
<thead>
<tr>
<th>Pharmacia serovar</th>
<th>No. (%) episodes in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group G</td>
</tr>
<tr>
<td>WI</td>
<td></td>
</tr>
<tr>
<td>Arost</td>
<td>114 (51)</td>
</tr>
<tr>
<td>Aros</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Arst</td>
<td>15 (7)</td>
</tr>
<tr>
<td>Ars</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Ar</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Aro</td>
<td>—</td>
</tr>
<tr>
<td>Av</td>
<td>—</td>
</tr>
<tr>
<td>WII/III</td>
<td></td>
</tr>
<tr>
<td>Bropt</td>
<td>36 (16)</td>
</tr>
<tr>
<td>Broput</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Brop</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>Bropyst</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Bropyt</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Bopyt</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Brot</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Bot</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Bopu</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>Brpyust</td>
<td>23 (10)</td>
</tr>
<tr>
<td>Bpyst</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Brpyut</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Bpvyut</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Bpvt</td>
<td>—</td>
</tr>
<tr>
<td>Bro</td>
<td>—</td>
</tr>
<tr>
<td>Bropv</td>
<td>—</td>
</tr>
<tr>
<td>Bpyust</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Av/Bx</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>223</td>
</tr>
</tbody>
</table>

Group R isolates whereas serogroup WII/III strains accounted for 61 (48 %) Group R isolates compared with 86 (39 %) Group G isolates. This association between serogroup WII/III and Group R isolates is not statistically significant at the 5 % level ($\chi^2 = 2.8:0.1 > P > 0.05$).
Table III Distribution of Genetic Systems serovars of gonococci in women with (Group R) and without (Group G) concomitant rectal infection

<table>
<thead>
<tr>
<th>Genetic Systems serovar</th>
<th>Group G</th>
<th>Group R</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aedgkih</td>
<td>109 (49)</td>
<td>53 (42)</td>
</tr>
<tr>
<td>Aedgki</td>
<td>1 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>Aedgk</td>
<td>2 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Aedgih</td>
<td>2 (1)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Aedg</td>
<td>5 (2)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Aegk</td>
<td>4 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Agk</td>
<td>1 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>Afc</td>
<td>1 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>Aedih</td>
<td>11 (5)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Ae</td>
<td>1 (0.5)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>WII/III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bajk</td>
<td>39 (17)</td>
<td>34 (27)</td>
</tr>
<tr>
<td>Bacjk</td>
<td>2 (1)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Baj</td>
<td>3 (1.5)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Bj</td>
<td>1 (0.5)</td>
<td>3 (2.5)</td>
</tr>
<tr>
<td>Back</td>
<td>7 (3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Bagk</td>
<td>1 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>Bak</td>
<td>1 (0.5)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Bacejk</td>
<td>20 (9)</td>
<td>11 (9)</td>
</tr>
<tr>
<td>Bacehjk</td>
<td>2 (1)</td>
<td>—</td>
</tr>
<tr>
<td>Bacej</td>
<td>2 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Bacek</td>
<td>1 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>Beghjk</td>
<td>1 (0.5)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Behj</td>
<td>5 (2)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>Bcegjk</td>
<td>1 (0.5)</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>223</td>
<td>126</td>
</tr>
</tbody>
</table>

Distribution of serovars

*Pharmacia* serovars

The distribution of Ph-serovars in Group G and Group R patients is given in Table II. Serogroup WI strains could be resolved into seven serovars with five present in each group. One serovar, Arost, predominated in each of these groups accounting for 114 (51%) Group G infections and 54 (43%) Group R infections. Serogroup WII/III strains could be resolved into 18 serovars with 15 present in Group G and 14 in Group R. The most common serovar, Bropt, accounted for 36 (16%) Group G infections and 29 (23%) Group R infections. No statistically significant correlations were observed between Ph-serovar and concomitant rectal infection.

*Genetic systems* serovars

The distribution of GS-serovars in Group G and Group R patients is given in Table III. Serogroup WI strains could be resolved into ten serovars, all of
Table IV  Relationship between minimum inhibitory concentration (MIC) of penicillin and gonococcal serogroup

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>(\leq 0.015)</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1.0</th>
<th>&gt; 1.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WI</td>
<td>164</td>
<td>10</td>
<td>9</td>
<td>2</td>
<td>—</td>
<td>1</td>
<td>186</td>
</tr>
<tr>
<td>WII/III</td>
<td>10</td>
<td>42</td>
<td>44</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>134</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>52</td>
<td>53</td>
<td>38</td>
<td>1</td>
<td>2</td>
<td>320</td>
</tr>
</tbody>
</table>

Table V  Relationship between minimum inhibitory concentration (MIC) of penicillin and GS-WII/III serovar

<table>
<thead>
<tr>
<th>Genetic systems serovar</th>
<th>(\leq 0.015)</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1.0</th>
<th>&gt; 1.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bajk</td>
<td>5</td>
<td>32</td>
<td>24</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>66</td>
</tr>
<tr>
<td>Bak</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Bacej</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3</td>
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<tr>
<td>Bacejk</td>
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<td>—</td>
<td>7</td>
<td>19</td>
<td>—</td>
<td>—</td>
<td>29</td>
</tr>
<tr>
<td>Back</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Behj</td>
<td>—</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Bajck</td>
<td>1</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Baj</td>
<td>—</td>
<td>7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Bagk</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Baj</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>Bacehjk</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Bacek</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Bcegjk</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Beghjk</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>43</td>
<td>43</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>134</td>
</tr>
</tbody>
</table>

which were present in Group G infections and seven in Group R. One serovar, Aedgkih, predominated in each of these groups, accounting for 109 (49%) Group G infections and 53 (42%) Group R infections. Serogroup WII/III could be resolved into 14 serovars, all of which were present in Group G infections and ten in Group R. The most common serovar, Bajk, accounted for 39 (17%) Group G infections and 34 (27%) Group R infections. This difference is statistically significant \((P=0.05)\). No other GS-serovar correlated with concomitant rectal infection.

Relationship between gonococcal serogroup and penicillin susceptibility

Minimum inhibitory concentrations (MIC) of penicillin were unavailable for ten strains in Group G and five strains in Group R. In 14 episodes of infection in Group R patients strains from different anatomical sites had penicillin
MICs which differed by one dilution or more. No association between site and penicillin MIC was noted and these strains were excluded from the study. Table IV illustrates the relationship between serogroup and susceptibility to penicillin in the remaining 320 (92%) of the Group G and Group R patients. A correlation between serogroup WII/III isolates and decreased susceptibility to penicillin (MICs ≥ 0.06 mg/l) was observed. This correlation was statistically significant ($\chi^2 = 201.3, P < 0.001$).

Relationship between gonococcal serovar and penicillin susceptibility

The relationship between gonococcal WII/III GS-serovar and penicillin susceptibility is given in Table V. Sixty-one (92%) Bajk isolates had MICs ≥ 0.06 mg/l penicillin compared with 85 (33%) non-Bajk isolates. This association between serovar Bajk and decreased susceptibility to penicillin is statistically significant ($\chi^2 = 71.6, P < 0.001$). However, the GS-serovar Bacejk was significantly less susceptible to penicillin than the serovar Bajk: 26 (90%) Bacejk isolates had MICs of ≥ 0.12 mg/l penicillin compared with 29 (44%) Bajk isolates and this difference is statistically significant ($\chi^2 = 32.8, P < 0.001$).

Discussion

By use of monoclonal antibody coagglutination reagents, the overall distribution of WI and WII/III serogroups in the population was 58% and 42% respectively. This distribution is consistent with previously reported serogroup patterns which showed that WI strains were isolated more often from women.26,28 Strains belonging to serogroup WI could be resolved into seven Ph-serovars, ten GS-serovars and 16 Ph/GS-serovar combinations while strains belonging to serogroup WII/III could be resolved into 19 Ph-serovars, 14 GS-serovars and 29 Ph/GS-serovar combinations. In a world-wide epidemiological study, 12 WI Ph-serovars, 21 WI GS-serovars, 39 WII/III Ph serovars and 62 WII/III GS-serovars were reported with a total of 27 WI Ph/GS-serovar combinations and 93 WII/III Ph/GS-serovar combinations.29 In previous localised studies, a more limited variety of serovars was recognised,21,26 similar to that noted in this study. Since the distribution of isolates among the various serovars was also similar, it seems likely that the serovars found in this study population reflect the variety of serovars circulating locally.

The prevalence of rectal infection in our study is similar to that found elsewhere.7,8,30 Rectal infection was detected in 42% of 383 episodes of gonorrhoea in female patients and in 7% the rectum was the only site infected. When the distribution of serogroups was analysed with respect to rectal infection, WI and WII/III strains accounted for 61 and 39% respectively Group G (genital only) infections and 52 and 48% respectively of group R (genital + rectal) infections. Although serogroup WII/III strains were more often isolated from Group R patients than from Group G patients, this association was not statistically significant at the 5% level. The variety and pattern of serovars recognised in Group G and Group R patients was similar, the greater variety of serovars in Group G probably being due to the larger size of this group. Strains with the GS-serovar Bajk were isolated from 27%
Group R patients compared with 17% Group G patients, a difference which is statistically significant at the 5% level \((P = 0.05)\). No single Ph-serovar was associated with concomitant rectal infection. However, strains belonging to GS-serovar Bajk corresponded to seven Ph-serovars (Bropt, Broput, Bropyt, Brpt, Brot, Bopt and Bot). When these seven serovars were considered together, they accounted for 21% (46) infections in Group G patients and 33% (41) infections in Group R patients: this association is statistically significant \((0.01 > P > 0.02)\).

These correlations are less strong than the serovar correlation found in homosexually acquired infection.\(^{21}\) The serovars correlated with homosexually acquired infection – GS-Back and Ph-Bropyt- are only rarely encountered among women with rectal infection and accounted for 1 and 2.5% episodes respectively.

The different serovars associated with homosexual and heterosexual rectal infection are not surprising in view of the earlier differences postulated between serogroup WII strains in these two groups of patients. Morse et al.\(^{13}\) observed a correlation between the mtr phenotype and WII strains in homosexual men but this correlation was not observed among WII strains from heterosexual women.\(^{14}\) The difference between strains associated with rectal gonorrhoea in homosexual men and heterosexual women possibly relates to the different modes of transmission of infection. Survival in the rectum is essential for the propagation of gonococci among homosexual men whereas in heterosexual women, most rectal infections are considered to be secondary to genital infection. Survival in the rectum is not therefore essential for spread of infection.

While the properties required of strains to enable them to survive in the hostile rectal environment are basically the same in both sexes, selective pressures are much stronger in the case of rectal infection in homosexual men. By contrast in women, although certain strains have properties which enable them to colonise the rectum and establish rectal infection more effectively, this does not lead to their selective propagation in the heterosexual pool of gonococcal infection.

Various factors have been implicated in the survival of gonococci in the rectum including the mtr phenotype. The reduced membrane permeability associated with the mtr phenotype is possibly disadvantageous in sites other than the rectum since it may reduce the uptake of essential nutrients, preventing mtr strains from competing successfully with non-mtr strains. Eisenstein and Sparling\(^{31}\) reported that the mtr mutation consistently resulted in reduced rates of exponential growth in enriched broth cultures and that partial restoration to normal growth rates \(in vitro\) was observed when the env mutation, a mutation which increases the membrane permeability and completely suppresses the phenotypic expression of the mtr mutation, was introduced by transformation.

Therefore, in heterosexual women, in whom most rectal infections are thought to be secondary to genital infection, the absence of mtr strains is not surprising. McFarland et al.\(^{9}\) demonstrated that strains isolated from the rectum of homosexual men and heterosexual women were more resistant to faecal lipids than strains isolated from the cervix or from the urethra of
heterosexual men and proposed the existence of a non-\textit{mtr} resistance mechanism.

Protein I type has also been associated with rectal infection. Serogroup WII has been correlated with strains isolated from homosexual men\textsuperscript{13,16,17,21} and in this study, WII/III strains were observed more often in women with concomitant rectal infection than in those without. Protein I functions as a porin, enabling the selective passage of molecules into the cell from the environment.\textsuperscript{32} Furthermore, since the pore size of Protein IA and of Protein IB, which represent serogroups WI and WII/III respectively, differ (Black, personal communication cited by Barrera and Swanson\textsuperscript{33}) permeability to hydrophobic molecules may differ also. This could explain the association between serogroup and colonisation of the rectum.

A strong correlation between serogroup WII/III strains and decreased susceptibility to penicillin was noted in this study. Such an association is consistent with previous reports of a correlation between serogroup WII and multiple antibiotic resistance.\textsuperscript{34} The serovar associated with concomitant rectal infection in women in this study, namely Bajk, was also associated with decreased susceptibility to penicillin. Of all Bajk isolates, 92\% had MICs $\geq 0.06$ mg/l penicillin compared with 33\% non-Bajk isolates. Serogroup specificity and reduced susceptibility to antibiotics are genetically linked.\textsuperscript{35} Therefore the correlation between increased penicillin resistance and isolates associated with anogenital infection may be coincidental. This is supported by the observation that although strains with the serovar Bacejk are correlated with greater resistance to penicillin than Bajk isolates, unlike the latter they are not associated with concomitant rectal infection in women. It is therefore unlikely that decreased susceptibility to penicillin in itself leads to rectal colonisation in women. Serovar Bacek (corresponding to Bacejk), however, was associated with isolates from homosexual men.\textsuperscript{20} In a previous localised study, Bacejk strains were the second most common isolates from homosexually acquired infection, accounting for 9\% infections in homosexual men.\textsuperscript{21} Furthermore, the prevalence of these strains among homosexual men is increasing in the Edinburgh area (D. V. Coghill, unpublished observation) thus suggesting that the antibiotic resistance of these strains may confer a selective advantage in the rectum of homosexual men at least.

The reduced penicillin susceptibility of Bajk isolates and those of serogroup WII/III in general, as well as the association of these strains with concomitant rectal infection in women, supports the view that rectal infection may be more difficult to cure than genital infection. The effectiveness of the standard single-dose penicillin therapy for treating rectal gonorrhoea in women is controversial. Some workers have reported higher treatment failure rates in rectal infections,\textsuperscript{2,36,37} whereas others have found rectal infection no more difficult to cure than endocervical infection.\textsuperscript{3} These differences may be related to the prevalence of serogroup WII among the various populations.

Why certain strains are more likely than others to cause concomitant rectal infection in women is unclear. Since most cases of rectal gonorrhoea in women are thought to result from inoculation of the rectal mucosa with infectious vaginal secretions, the amount of vaginal discharge may be important in development of rectal infection. In heterosexual men, urethral discharge was
more common and contained greater numbers of leucocytes in patients infected with WII/III strains than in those infected with WI strains. A similar study on the ability of different strains of gonococci to produce vaginal discharge in heterosexual women would therefore be valuable.

(This work was supported by a Scottish Home and Health Department grant (research grant no. K/MRS/50.C785). D.V.C. gratefully acknowledges receipt of a Faculty of Medicine Scholarship. We thank Dr Solgun Bygdeman for supplying the monoclonal reagents and Mrs Marilyn Cole for typing the manuscript.)

References

Serovars and female rectal gonorrhoea


Mackie & McCartney
Practical Medical Microbiology

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THIRTEENTH EDITION
Volume 2 of Medical Microbiology

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1989
Neisseria: Acinetobacter: Branhamella

There are two genera of medically important aerobic Gram-negative diplococci, Neisseria and Branhamella. The distinguishing features of these are shown in Table 21.1. The meningococcus N. meningitidis and the gonococcus N. gonorrhoeae are the important pathogens in the genus Neisseria; other Neisseria species are commonly found as commensals in the upper respiratory tract. N. meningitidis gives rise to septicaemia and meningitis with or without septicaemia; it is normally carried in the throat but may be found in other sites such as the genital tract, where its presence is not usually of pathological significance. N. meningitidis gives rise to disseminated gonococcal infection, characterized by arthritis with or without skin lesions.

Branhamella catarrhalis is the only important member of the genus Branhamella, which may now be considered as a subgroup of Moraxella (Bovre. 1984). This organism is normally an upper respiratory tract commensal but occasionally gives rise to respiratory infection, usually as an opportunist pathogen. B. catarrhalis was formerly classified in the genus Neisseria but differs from Neisseria in DNA base content, fatty acid composition, and inability to produce acid from carbohydrates (Catlin 1970).

Neisseria and Branhamella are recognized in a clinical specimen by the appearance of oval Gram-negative diplococci with flattened or concave opposing edges and long axes parallel, either lying free in the specimen or, in the case of N. meningitidis and N. gonorrhoeae, often inside polymorphonuclear leucocytes. The colonies of neisseriae differ according to the species, but all are oxidase positive. Those of the pathogenic N. meningitidis and N. gonorrhoeae species are small and mucoid, whereas those of other species tend to be larger, may be smooth or rough and tend to be sticky so that colonies adhere to a wire loop. In general, gonococci and meningococci may be distinguished from other neisseriae by their ability to grow on appropriate selective media. However N. lactamica, which is

Table 21.1 Distinguishing characters of Neisseria and Branhamella species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pigment</th>
<th>Growth at 22°C</th>
<th>Requirement for blood or serum</th>
<th>Growth on selective media (e.g. MNYC)</th>
<th>Oxidation of carbohydrate(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. gonorrhoeae</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>-</td>
<td>-</td>
<td>+*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>±/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>N. pharyngis</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N. polysaccharaeae</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>B. catarrhalis</td>
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* However, N. meningitidis will grow on Mueller Hinton medium without the addition of blood or serum.
commonly found in the throat and resembles \textit{N. meningitidis} closely on culture, grows well on the selective media, as does \textit{N. polysacchareae}; some strains of \textit{B. catarrhalis} may also grow on the selective media.

\textit{N. meningitidis} and \textit{N. gonorrhoeae} must be identified accurately both for general medical and for medico-legal purposes: the sugar utilization reactions that distinguish the species of \textit{Neisseria} from each other are shown in Table 21.2. Odugbemi et al (1978) described a simple and inexpensive manganous chloride and Congo red disk method which could increase the efficiency of distinguishing between pathogenic \textit{Neisseria} in laboratories with limited facilities: \textit{N. meningitidis} is resistant to manganous chloride and Congo red whereas \textit{N. gonorrhoeae} is sensitive.

\textbf{Morphology and staining}

Oval Gram-negative diplococci, with flattened or concave opposing edges and the long axes parallel; about 0.8 $\mu$m in diameter; typically seen in large numbers inside polymorphonuclear leucocytes. Films from cultures show more rounded cocci and some pleomorphism with irregular staining. Capsules are not ordinarily evident; non-spore forming; non-motile.

\textbf{Cultural characters}

Aerobe, but primary cultures grow better in an atmosphere containing 5–10\% CO$_2$. Temperature range $25$–$42^\circ$C, optimum $35$–$36^\circ$C. Optimum pH 7.0–7.4. Strains will grow on Mueller Hinton medium without the addition of blood or serum but grow poorly if at all on most unenriched media. After incubation in 5–10\% CO$_2$ in air for 24 h at $37^\circ$C colonies on blood agar are 1–2 mm in diameter, convex, grey and translucent. After 48 h colonies are larger with an opaque raised centre and thin transparent margins which may be crenated. No haemolysis on blood agar. Colonies are slightly larger on heated blood (chocolate) agar than on ordinary blood agar.

\textbf{Biochemical reactions}

Oxidase reaction: quickly positive when the reagent is flooded onto agar cultures. Utilize glucose and maltose but not lactose or sucrose. Occasional strains are found that utilize only glucose or maltose on primary isolation.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
\textbf{species} & \textbf{Glucose} & \textbf{Maltose} & \textbf{Fructose} & \textbf{Sucrose} & \textbf{Lactose} \\
\hline
\textit{N. gonorrhoeae} & - & - & - & - & - \\
\textit{N. meningitidis} & - & - & - & - & - \\
\textit{N. lacamena} & - & - & - & - & - \\
\textit{N. pharyngis} & - & - & - & - & - \\
\textit{N. vitius} & - & - & - & - & - \\
\textit{N. perilava} & - & - & - & - & - \\
\textit{N. seca} & - & - & - & - & - \\
\textit{N. polysacchareae} & - & - & - & - & - \\
\textit{B. catarrhalis} & - & - & - & - & - \\
\hline
\end{tabular}
\caption{Sugar utilization reactions of \textit{Neisseria} and \textit{Branhamella} species.}
\end{table}
repeated subculture of these strains may be necessary before they utilize both sugars. Non-maltose-utilizing strains of *N. meningitidis* may be mistaken for gonococci. This could be of medico-legal importance: if there is any doubt about the identity of the *Neisseria* in this situation it should be forwarded to a reference laboratory for further examination. Recently an organism with the growth and biochemical characteristics of *N. meningitidis* and the serological characteristics of *N. gonorrhoeae* has been isolated from a vaginal swab (Hodge et al. 1987). This underlines the importance of seeking expert advice with atypical neisseriae.

**Sensitivity to physical and chemical agents**

Dies within a few days at room temperature but cultures may be maintained on Dorset's egg medium or heated blood agar slopes in screw-capped bijou bottles for several weeks. Colonies emulsified in peptone water will survive at −70°C or in liquid nitrogen for months, but freeze-drying is preferable for long-term storage. Killed at 55°C in 5 min. Readily killed by disinfectants at their correct use-dilution.

**Antibiotic sensitivity**

*N. meningitidis* is sensitive to a wide variety of antibiotics, of which benzylpenicillin, chloramphenicol and rifampicin are the most important; it is also sensitive to the more recent cephalosporins, e.g. cefuroxime and cefotaxime. 10–20% of strains are resistant to sulphonamides.

**Serogrouping**

The serogroups of meningococci of pathological importance whose polysaccharide antigen structure has been determined are: A, B, C, X, Y, Z, Z₁ (29E) and W135. Further serogroups H, I, K and L have also been described but their pathological significance is not yet clear. A group D was described by Branham (1958) but no capsular polysaccharide specific for this group has yet been demonstrated. Meningococci of groups A and C are those principally associated with epidemics; group B meningococci are the common inter-epidemic strains but recently epidemics due to these organisms have been described in Scandinavia. Non-serogroupable strains are commonly found in carriers, but rarely in disease.

Outer membrane protein and lipoprotein serotypes occur within groups B, C, Y and W135 and may be identified by reference laboratories for epidemiological purposes. A detailed identification of a meningococcus by group, type and subtype antigens, e.g. a Group B meningococcus of type 15 with the protein subtype antigen P1.1b would be shown as B:15:P1.1b (Frasch et al. 1985). Types 2, 15, and recently 4, have been particularly associated with Group B meningococcal disease.

**Animal pathogenicity**

Intravenous inoculation of viable meningococci into rabbits may be fatal within 24 h but animal tests are not used for diagnosis.

**Laboratory diagnosis of meningococcal infection**

Specimens may include cerebrospinal fluid (CSF), blood for culture (which may come from a patient with meningitis, a haemorrhagic rash or pyrexia of uncertain origin), aspirate from skin lesions or pus from an infected joint. Throat, nasopharyngeal and, in certain circumstances, genital swabs may be collected from suspected carriers. Swabs are plunged into transport medium (e.g. Stuart's) for forwarding to the laboratory; all specimens where meningococcal infection is suspected must be submitted to the laboratory immediately.

**Cerebrospinal fluid**

1. Perform a cell count (see Ch. 39); the exudate in meningococcal meningitis is typically polymorphonuclear.

2. Centrifuge the remaining CSF. Make a smear of the centrifuged deposit and stain with Gram stain. Stain a second film with methylene blue to determine the cell type; occasionally, diplococci may be seen more easily with this stain. CSF from a typical case of meningococcal meningitis will show Gram-negative diplococci.
inside a limited proportion of the pus cells; many are extracellular. If fluorescein isothiocyanate-coupled antiserum is available, a smear of the deposit may be examined for the direct identification of the meningococcal serogroup responsible for infection (Fallon 1983).

5. Divide the supernatant CSF into two aliquots - one to be kept if necessary for biochemical examination, the other to be examined for the presence of meningococcal polysaccharide antigen by counter-immunoelectrophoresis, latex agglutination* or coagglutination.

4. Plate out the specimen on both blood and heated blood agar and incubate at 37°C in 5-10% CO₂ for 24 h. Add Robertson’s cooked meat broth to the deposit, incubate overnight and subculture in the same way. Examine colonies appearing after incubation by Gram stain and oxidase reaction.

5. Set up the rapid carbohydrate utilization test (RCUT)* or inoculate a set of Flynn & Waitkins sugar media* for utilization reactions. In addition to glucose, maltose, lactose and sucrose, inoculate an ONPG tube; the ONPG medium base used is Mueller Hinton broth. This latter test will identify strains of N. lactamica where lactose utilization on solid media may be slow.

Forward cultures of organisms identified as meningococci by sugar utilization reactions to an appropriate reference laboratory for determination of sulphonamide sensitivity, serogrouping and, where appropriate for epidemiological purposes, serotyping (Dr D. M. Jones, Public Health Laboratory, Withington Hospital, Manchester M20 8LR; Dr R. J. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Bilsland Drive, Glasgow G20 9NB).

6. Set up antibiotic sensitivity tests. Strains resistant to penicillin have not yet been described although for a small proportion of strains the MIC is as high as 0.25 mg/litre; this antibiotic should be tested along with chloramphenicol and rifampicin. Appropriate concentrations are benzylpenicillin 1.2 μg (2 units)/disk, chloramphenicol 10 μg, rifampicin 10 μg. Disk testing for sulphonamide sensitivity is satisfactory provided a 25 μg sulphadiazine disk is used in a Stokes technique with a control sensitive meningococcus. Preferably, test by the agar dilution method using Mueller Hinton agar as described by Fallon (1979). The inoculum should be a 1 in 50 dilution of a shaken overnight broth culture and a suitable range of concentrations of sulphadiazine is 0.1, 1, 10, 50 and 100 mg/litre. Strains resistant to 10 mg/l are resistant to sulphonamide therapy; strains resistant to 1 mg/l may not be eradicated from the nasopharynx if sulphonamides are used for chemoprophylaxis.

7. Serogrouping is performed by slide agglutination with hyperimmune sera (Wellcome). Care must be taken to test a strain against antiserum to each serogroup as fine non-specific agglutination may occur which may be thought to be specific unless the reaction with all antisera has been checked. If difficulty is experienced in serogrouping, the culture may be plated on Mueller Hinton agar containing about 5% (depending on the strength of the serum) group-specific antiserum. A halo of precipitation appearing in the medium round the colonies after 24-48 h incubation is a positive reaction (Craven & Frasch 1979).

**Blood cultures**

Subculture to blood agar and heated blood agar. Incubate cultures in 5-10% CO₂ for 24 h and examine oxidase positive colonies of Gram-negative diplococci as above.

**Pus, aspirates and swabs**

Examine Gram stained films and inoculate pus, aspirates and swabs on to selective heated blood agar* or Modified New York City (MNYC) medium* in addition to media normally used for the examination of pus from any source.

**Serological diagnosis**

Paired sera may be tested for the presence of complement-fixing antibodies (Ross & Stevenson 1962). This test is helpful in cases where no organisms have been isolated or in obscure pyrexias, which may be due to chronic menin-
gonococcal septicemia. Specific antibodies to capsular polysaccharide may be demonstrated by a haemagglutination test (Edwards & Driscoll 1967).

**NEISSERIA GONORRHOEAE**

Morphology and staining of *N. gonorrhoeae* are identical to those of *N. meningitidis* (see above). The main character that distinguishes the gonococcus from the meningococcus is the ability to produce acid from glucose but not maltose.

**Cultural characters**

A delicate organism with exacting nutritional and environmental requirements. Aerobe, but most strains have an absolute requirement for CO₂. Optimum pH 7.0–7.4. Recommended culture media contain a rich nutrient base supplemented with blood, either partially lysed by heat (chocolate agar) or completely lysed by saponin; unlysed blood agar is not recommended for diagnostic cultures. Selective media are valuable in isolating gonococci from heavily contaminated sites such as the rectum or pharynx.

After incubation for 24 h in a moist aerobic environment enriched with 5–10% CO₂ colonies on Modified New York City (MNYC) medium are small (c. 1 mm), grey and convex; after 48 h the colonies are larger (1.5–2.5 mm), sometimes with a crusted margin and an opaque raised centre. Considerable variation in size occurs with gonococcal colonies and on most culture media the colony outline is irregular, unlike the circular colonies of *N. meningitidis*. On Thayer Martin medium growth is slower; although colonies are similar to those on MNYC medium they are usually smaller.

Naturally occurring variants with specific requirements for particular amino acids, bases or vitamins may be detected; this technique (auxotyping) has been applied for epidemiological typing (Catlin 1973).

**Biochemical reactions**

The gonococcus is oxidase positive and utilizes glucose but not maltose, sucrose, lactose or fructose. The rapid carbohydrate utilization test (RCUT; Young 1978a) measures preformed enzymes and provides a quicker and more reliable identification than conventional growth-dependent sugar tests using solid or semi-solid media (Tapsall & Cheng 1981).

**Sensitivity to physical and chemical agents**

Readily killed by drying, soap and water, and many other cleansing or antiseptic agents at their correct use-dilution. Organisms may remain viable for a day or so in pus containing linen or other fabrics. Cultured gonococci die in a few days at room temperature. Survival can be ensured for several months by harvesting an overnight plate culture into 1 ml of tryptone soy broth containing 6% lactose and freezing at −20 or −70°C. Freeze-drying is the most reliable method for long-term storage of gonococci but storage at −70°C or in liquid nitrogen may be more convenient for intermediate storage.

**Antibiotic sensitivity**

The gonococcus is usually sensitive to many antibiotics including penicillin, cefuroxime, cefotaxime, spectinomycin, cotrimoxazole, tetracycline, erythromycin and streptomycin. However, sensitivity of isolates may vary geographically making it important to base antibiotic policies on the sensitivity of local isolates.

A definite progression towards decreased sensitivity to various antibiotics has occurred over the past three or four decades. Because of its central role in the treatment of gonorrhoea, sensitivity to penicillin has been studied most widely. By convention, strains with an MIC of 0.125 mg/litre or more are defined as less sensitive or relatively resistant; around 20% of strains in the UK fall into this category. The majority of strains isolated in the UK are fully sensitive to penicillin (MIC <0.125 mg/l). Multiple antibiotic resistance sometimes occurs and may be due to a mutation giving reduced permeability of the outer membrane (Maness & Sparling 1973). The most significant development in recent years has been the emergence of penicillinase-producing *N. gonorrhoeae* (PPNG) giving
Resistance to high levels of penicillin tests for \( \beta \)-lactamase production should always be performed.

Mutations also exist which increase the permeability of the membrane, making isolates hypersensitive to certain antibiotics: hypersensitivity may include vancomycin (Exner et al. 1982) and therefore influence choice of selective media.

Serogrouping

Antigenic heterogeneity has been demonstrated in purified surface components of \( N. gonorrhoeae \), including pilus (fimbrial) protein, lipopolysaccharide and outer membrane proteins, and this has made it difficult to devise a standard scheme for dividing the species into serogroups as in the case of the meningococcus. The coagglutination serogrouping system of Sandstrom & Danielsson (1980) which is based on differences in the principal outer membrane protein (Pr1) is gaining widespread acceptance; using specifically absorbed polyclonal antisera the gonococcus can be divided into two major groups termed WI and WII/WIII. More recently, serogroups W1 and WII/WIII have been subdivided into serovars on the basis of their reaction patterns with panels of monoclonal antibodies reactive with epitopes on Pr1A and Pr1B respectively. The biological and epidemiological significance of gonococcal serogroups and serovars is reviewed in detail by Bygdemar (1988).

\( Neisseria \) gonorrhoeae ssp. \( kochi \). Mazloum et al (1986) suggested that an unusual neisseria isolated from conjunctival cultures in rural Egypt and originally described by Robert Koch as an atypical gonococcus merits subspecies status. These isolates do not react with the monoclonal antibodies currently used in the serological classification of gonococci.

Laboratory diagnosis of gonorrhoea

The main task of the bacteriologist is to determine whether or not \( N. gonorrhoeae \) is present in a specimen and, if present, whether or not the strain produces \( \beta \)-lactamase. Since the management of gonorrhoea includes the tracing of infected contacts, laboratory diagnosis is best carried out in association with a special department of genitourinary medicine.

The greater the number of sites examined the better will be the chance of detecting gonococcal infection. Details of specimens required for bacteriological diagnosis are given by Robertson et al. (1981). In men urethral samples usually suffice (with rectal cultures in homosexual males), but in women urethral, cervical and rectal specimens should always be examined. Although repeated sampling of multiple sites is ideal, a single well taken endocervical swab will detect approximately 90% of gonococcal infections in women. A high vaginal swab is not suitable and, if this is the only specimen taken, 1 in 3 infected women is likely to be missed.

Throat infection also occurs and should be sought where appropriate. In suspected disseminated gonococcal infection (DGI), specimens may include blood, swabs of skin lesions, or pus aspirated from a joint. Occasionally conjunctival material is examined, particularly in neonatal ophthalmia. Any urine specimen showing Gram-negative diplococci in a Gram stain should be cultured on an appropriate selective medium.

The gonococcus is very fastidious and care is needed in the collection of specimens and their transport to the laboratory. Best results are achieved by the direct inoculation of culture plates with patients’ secretions, followed by immediate incubation at 36-37°C in a moist atmosphere containing 5-10% CO\(_2\). When direct plating and immediate incubation is impracticable several transport and culture systems are available. These consist of a selective medium, usually present in a small chamber containing CO\(_2\) or a CO\(_2\)-generating system, e.g. Transgrow or Jemtec (Martin & Jackson 1975). The media can be inoculated directly from the patient and transported to the laboratory either before or after incubation. Such systems are expensive and it is more usual to send a charcoal-coated swab in Stuart’s medium, or preferably Amies’s modification of Stuart’s medium (see Ch. 6) in which case a plain swab is adequate. Dry swabs should not be sent as the gonococcus is very susceptible to drying.
1. Examine Gram stained smears of urethral discharge from men and urethral and cervical secretions from women. The observation of characteristic kidney-shaped Gram-negative diploccci lying within polymorphonuclear leucocytes with a few extracellular organisms is typical of gonococcal infection and the smear is reported as positive. If Gram-negative diploccci are seen only extracellularly, the result of the smear examination is equivocal; a diagnosis should not be made on this basis. If no Gram-negative diploccci are seen, report the smear as negative.

Approximately 95% of infected men and 55-66% of infected women will yield a positive smear: if the smear is examined while the patient is at the clinic immediate treatment can be given.

2. Plate out the specimen on selective culture media and, in the case of specimens from normally sterile sites, on the same medium lacking antibiotics; incubate immediately in a moist CO₂-enriched aerobic atmosphere at 37°C.

The original selective medium of Thayer & Martin (TM medium) contains the antibiotics vancomycin, colistin and nystatin. Although widely used in many laboratories TM medium has been criticized because 3-10% of gonococcal strains are inhibited by vancomycin (Mccrett et al 1981). A modified TM medium (Martin et al 1974) gives superior results; however, MNYC medium* is preferred because it gives better growth and the use of lincomycin as selective agent avoids the problem of vancomycin sensitivity.

3. Examine plates after 24 h incubation and test suspect colonies by touching with a cotton bud soaked in oxidase reagent: oxidase positive bacteria turn the contact area of the bud purple within 5-15 s. If oxidase positive, Gram stain an identical colony. Incubation of primary isolation plates is continued for 48 h and cultures are re-examined by the above procedures before any specimen can be reported negative.

A presumptive diagnosis of gonorrhoea made on the basis of oxidase positive Gram-negative diploccci growing on selective medium is approximately 99% accurate for specimens taken from the male urethra and female urethra, cervix or rectum. A presumptive diagnosis of gonorrhoea is much less reliable in the case of rectal cultures from homosexual males. Particular attention must be paid to throat cultures where gonococcal and meningococcal colonies may coexist: Gram-negative diploccci isolated from the throat are most likely to be meningococcal.

4. If there is sufficient growth on the primary isolation plate set up the rapid carbohydrate utilization test (RCUT)*, including a tube to detect β-lactamase production. Otherwise subculture on antibiotic-free medium and incubate overnight to obtain sufficient material for the test.

5. Recently a coagglutination test* using monoclonal antibodies reactive with epitopes on Prf has become available and this test may be used in place of the RCUT (Young & Reid. 1988) for identification of an isolate. These reagents, which are 100% specific, do not cross-react with N. lactamica or meningococci and provided that the test is properly controlled, a positive reaction is a reliable indicator of gonococcal infection at any site. Although the sensitivity is very high (99.7%) it is prudent to confirm the identity of any non-reactive genital isolate by RCUT. Biochemical confirmation is also recommended whenever medico-legal proceedings may be involved.

6. Inoculate a suitable non-selective medium (e.g. the isolation medium lacking antibiotics) with the growth from several colonies and place a 6 μg penicillin disk on the well. If the zone of inhibition is less than 20 mm after overnight incubation test for β-lactamase by the filter-paper acidometric method* or by the chromogenic cephalosporin method (Ch. 9).

Since the majority of patients with gonococcal infection will have been treated on the basis of a positive smear, antibiotic tests other than those to detect β-lactamase are of little help in the initial management of the patient. However, they are important in planning rational therapy for use in the geographical area concerned: it is sufficient to test only a proportion of isolates in a laboratory with a high number of positive cultures. The sensitivity of all gonococci isolated from complicated and disseminated infections or after treatment failure should be tested.

If antibiotic sensitivity tests are indicated
prepare a turbid suspension of organisms in peptone water for use as inoculum in the agar dilution or disk diffusion sensitivity test. Diffusion tests using disks of several strengths can be made to give acceptable results but an agar dilution method is preferable (Jephcott 1981). The Adatab system available commercially (Mast), provides tablets containing suitable quantities of a wide variety of antibiotics. This system, suitably controlled, makes accurate agar dilution testing a practical proposition for most laboratories.

7. In suspected disseminated gonococcal infection, set up blood cultures with a biphasic medium (Jephcott 1981) and incubate in a CO₂ incubator with standard closures replaced by cotton-wool plugs. Immunofluorescence staining may be of value in examining exudate from skin lesions (Tronca et al 1974). Culture on non-selective media may be advisable.

Quality control

Reliable diagnosis or exclusion of gonococcal infection depends upon high microbiological standards. Procedures must be subjected to proper quality control and the efficiency of culture carefully monitored. The percentage of microscopy-positive specimens that fail to yield gonococci on selective medium (possibly because of antibiotic sensitivity) should be determined by correlating the results of microscopy and culture; a figure of 1-2% is acceptable.

Serological diagnosis

The low sensitivity and specificity of existing serological tests, and the persistence of antibody due to past infection, limit their value in clinical practice. Serological tests are not suitable for screening for gonococcal infection and should not be used in this way to diagnose or exclude gonorrhoea. The gonococcal complement fixation test (GCFT) is the only serological test for gonococcal infection that has been much used in clinical practice. This test may occasionally be useful in conjunction with culture in suspected disseminated gonococcal infection but the GCFT should not be performed as a routine.

COMMENSAL NEISSERIAE

These organisms occur on various mucous surfaces of the body; they are regularly found in the throat, nose and mouth and, less frequently, on the genital mucosae. When inflammatory or other pathological conditions affect these mucous membranes, Gram-negative diplococci may constitute a prominent feature of the bacterial flora and may possibly act as secondary infecting agents in such conditions. The commensal neisseriae are much less well characterized than the important human pathogens described above.

*Neisseria lactamica*. Carriage of this organism occurs more frequently in infants and young children than in adults. Morphology and staining is similar to *N. meningitidis* but it differs in its relative lack of virulence and ability to utilize lactose. It grows readily on selective media and some strains cross-react with antisera raised against gonococci and meningococci. Whenever Gram-negative diplococci are isolated from the throat, biochemical tests are required to provide accurate differentiation between *N. lactamica* and the meningococcus and gonococcus.

*Neisseria pharyngis*. The classical nasopharyngeal commensals *N. subflava*, *N. flava*, *N. perflava* and *N. sicca* are included in the umbrella species *N. pharyngis*; their main characters are given in Tables 21.1 and 21.2. Most of these nasopharyngeal commensals produce moist pigmented colonies and utilize glucose, maltose, fructose and sucrose when tested by the RCUT. Although *N. sicca* utilizes the same sugars, colonies are dry, tough, adherent to the medium and opaque.

*Neisseria polysaccharae* (which resembles the meningococcus in appearance) grows on selective media, utilizes glucose and maltose (Riou et al 1983), and is found in the nasopharynx of healthy carriers (Boquete et al 1986). Unlike *N. meningitidis* it produces polysaccharide when grown on medium containing 5% sucrose and lacks gamma-glutamyl aminopeptidase activity.

*Neisseria cinerea* has been isolated as a commensal, frequently from the oropharynx and
less commonly from genital sites (Knapp et al. 1984). Occasionally it is isolated on selective medium, when it resembles N. gonorrhoeae. Although considered to be asaccharolytic it may utilize glucose in certain biochemical test systems (Dossett et al 1985). N. cinerea is non-reactive in the coagglutination test for the gonococcus. Lack of DNase helps to differentiate it from B. catarrhalis.

*Neisseria flavescens.* This organism was described in 1930 as the causative pathogen in a group of cases of meningitis in America but has not with certainty been isolated since. It resembled the meningococcus in morphology but on blood agar produced golden-yellow colonies. Initially the isolates did not utilize carbohydrates but later developed the ability to produce acid from glucose, maltose and sucrose. It may be biologically related to *N. pharaonis*.

*Neisseria mucosa* differs from other members of the group in being definitively capsulate and producing mucoid colonies. It has been isolated only sporadically from cases of meningitis, endocarditis and also opportunistic infections (Gini 1987). Its carbohydrate utilization reactions are similar to *N. sicca* but it reduces nitrates and, like *N. polysaccharae*, it synthesises polysaccharide. There is much variation in the characteristics of the organisms described under this name (Brodie et al. 1971; Johnson 1983).

**BRANHAMELLA CATARRHALIS**

The characters of *B. catarrhalis* that are useful in identification include oxidase positivity, inability to produce acid from sugars and ability to grow on medium lacking blood. *B. catarrhalis* is antigenically distinct from commensal *neisseriae*; there are no recognized serogroups.

**Morphology and staining**

Oval Gram-negative cocci about 0.8 μm in diameter. Sometimes organisms are single, but more often in pairs with adjacent sides flattened; occasionally found in groups of four as a result of characteristic division in two successive planes at right angles to one another. On occasion they may be found inside polymorphonuclear leucocytes.

**Cultural characters**

Aerobe with optimum temperature about 37°C but growth of many strains occurs at 22°C. Although CO₂ may enhance growth there is no absolute requirement. Most strains grow on nutrient agar. After incubation for 24 h colonies on blood or heated blood agar are 1–2 mm in diameter, non-haemolytic, often friable, white or greyish, convex with an entire margin later becoming irregular. After 48 h colonies are larger, more elevated with a raised opaque centre. Most strains do not grow on media selective for pathogenic *neisseriae*.

**Biochemical reactions**

Oxidase positive; does not produce acid from glucose, maltose, sucrose, lactose or fructose; reduces nitrate to nitrite.

**Sensitivity to physical and chemical agents**

Appears to be more resistant than the meningococcus or gonococcus. Cultures may remain viable for several months at 20°C if prevented from drying. May survive in sputum for 3–4 weeks.

Susceptible to a wide range of antibiotics but many strains produce β-lactamase and are resistant to penicillin and ampicillin. Sensitivity tests can be carried out by disk or agar dilution methods as for meningococci and gonococci.

**Laboratory diagnosis**

*B. catarrhalis* is normally considered to be a harmless commensal of the upper respiratory tract and is most often encountered when examining throat swabs and specimens of sputum. The finding of a few colonies of *B. catarrhalis* in a mixed culture containing other upper respiratory tract commensal organisms is probably of little or no significance. However, in patients with compromised lung function, *B. catarrhalis* may be a pathogen of the lower respiratory tract. In
these patients a relatively pure growth of B. catarrhalis is often obtained from sputum and other specimens such as transtracheal aspirates.

Specimens should be cultured on blood agar and a selective medium. After overnight incubation in 5–10% CO₂ in air cultures are examined by the oxidase test and, if positive, Gram stained. Oxidase positive Gram-negative diplococci are then tested by the RCUT for their ability to utilize sugars and to produce β-lactamase. If the isolate grows well on selective medium it should also be shown to be immunologically distinct from N. gonorrhoeae and N. meningitidis by the tests described previously. Recent reports (Doern & Morse 1980) suggest that clinically significant isolates of B. catarrhalis grow well on modified TM medium, produce β-lactamase and do not grow on nutrient agar at 22°C. The extent of the correlation between pathogenicity, β-lactamase production and ability to grow on selective media remains to be elucidated.

**MORAXELLA, KINGELLA AND ACINETOBACTER**

Organisms classified in the family Neisseriaceae include *Moraxella*, *Kingella* and *Acinetobacter* spp.

*Moraxella*. The genus *Moraxella* includes *Moraxella lacunata* which causes a form of purulent conjunctivitis classically presenting as an angular blepharoconjunctivitis. The moraxellas occur as components of the normal flora of the upper respiratory tract, the conjunctiva, the skin and the genital tract. Moraxellas may be involved in opportunist infections in compromised patients.

The moraxellas are stout Gram-negative cocci or short stout rods; they typically occur in pairs and may simulate gonococci. They are strict aerobes, non-capsulate, non-motile. *M. lacunata* and *M. atlantae* require serum for growth but some other species are less demanding. Loeffler medium is pitted by colonies of *M. lacunata* and the variant *M. liquefaciens*. *M. lacunata* cannot grow on MacConkey agar but some other species can. The moraxellas are relatively inactive in biochemical tests. Moraxellas are oxidase positive and usually catalase positive. They do not ferment sugars and do not produce indole or H₂S. *M. lacinata* produces a gelatinase. Sensitivity to penicillin has been regarded by some workers as a feature of the moraxellas that distinguishes them from acinetobacters.

*Kingella*. These are Gram-negative rods and the genus contains three species that differ from *Moraxella* in being saccharolytic and catalase negative. They are of low pathogenicity but, as they grow on Thayer Martin medium and are oxidase positive, they could be mistaken for pathogenic neisseriae.

*Acinetobacter*. This genus contains strictly aerobic short, stout, often capsulate, non-motile Gram-negative (or Gram variable) bacilli or cocobacilli (often diplococci-bacilli) that grow well on simple media. They are usually free-living saprophytes in soil and water. The genus contains only one species, *Acinetobacter calcoaceticus*, which embraces two variants; *A. calcoaceticus* var. *anitratus* produces acid oxidatively from dextrose whereas *A. calcoaceticus* var. *lwofii* does not. This terminology supersedes earlier terms such as *Herellea vaginicola* and *A. anitratus* which correspond to the *anitratus* variant, and *Mima polymorpha* and *Moraxella lwofii* which correspond to the *lwofii* variant. In the past *Achromobacter species* names were also assigned to these variants.

Acinetobacter organisms occur frequently as components of the commensal flora of man and animals and are therefore regular contaminants of the hospital environment. They are increasingly recognized as opportunistic pathogens associated with infections that range from bronchopneumonia to septicaemia in compromised patients. Predisposing factors include the presence of a prosthesis, endotracheal intubation, intravenous catheters, and prior antibiotic therapy in a seriously ill patient in hospital.

*A. calcoaceticus* is oxidase negative, catalase positive, and indole negative. Some strains produce urease. Acinetobacter organisms do not reduce nitrates and do not ferment sugars. The *anitratus* variant produces acid from dextrose and other sugars oxidatively but the *lwofii*
variant does not. Colonies are white or cream coloured, smooth, circular with an entire edge, sometimes raised and opaque, and may show surface spreading. Some strains are haemolytic on blood agar. Some strains liquefy gelatin slowly. All strains are penicillin resistant. Hospital strains of Acinetobacter are often resistant to many other antibiotics. Most strains are resistant to sulphonamides, penicillins including ampicillin, the cephalosporins, erythromycin, the tetracyclines and chloramphenicol. They are often resistant to gentamicin and other aminoglycosides (see Bergogne-Berezin & Jolv, 1985). It is essential to guide antimicrobial management by antimicrobial sensitivity tests.

Hospital strains can be traced by a combination of biotyping, antibiograms, serotyping, bacteriocin typing and immunofluorescence tests, but these approaches to tracing have limitations at present (see Stone & Das, 1986).

METHODS

Modified New York City (MNYC) medium
(Young, 1978b)

Preparation of yeast dialysate. Mix 908 g baker’s yeast to a smooth paste with 2.5 litres distilled water. Autoclave at 110°C for 10 min and dialyse against 2 litres distilled water for 48 h at 4°C. Dispense the dialysate (material outside sac) into 25 ml amounts and autoclave at 121°C for 15 min. Store at −20°C.

Ingredients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC Medium Base (Difco)</td>
<td>36 g</td>
</tr>
<tr>
<td>Yeast dialysate</td>
<td>25 ml</td>
</tr>
<tr>
<td>Human or horse blood (100 ml) lysed with 5 ml of 10% saponin</td>
<td>105 ml</td>
</tr>
<tr>
<td>Glucose (10%) sterilized at 115°C for 10 min</td>
<td>10 ml</td>
</tr>
<tr>
<td>Colistin (6 mg/litre)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Lincomycin (1 mg/l)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Trimethoprim (5 mg/l)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Amphotericin B (1 mg/l)</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Method. Dissolve the GC agar base in 856 ml distilled water and autoclave at 121°C for 15 min. Allow to cool and hold at 50°C. Add lysed blood, glucose, yeast dialysate and the antibiotics. Mix and pour plates.

Set up quality control cultures by inoculating plates from each batch of medium with the following cultures: N. gonorrhoeae (recent clinical isolate); N. meningitidis; S. pneumoniae; S. aureus; E. coli; P. mirabilis; C. albicans. Incubate plates in a CO2 incubator as for gonococcal cultures. Release a batch of plates for routine use only if the medium supports good growth of the pathogenic neisseriae while inhibiting completely the growth of N. pharyngis and the other test organisms.


Selective heated blood agar

Heated blood (chocolate) agar may be made selective for the pathogenic neisseriae by the addition of vancomycin 3 mg/litre, colistin 7.5 mg/l and nystatin 12 500 units/l (or use Oxoid VCN Antibiotic Supplement, SR101). This medium facilitates the isolation of pathogenic neisseriae in mixed cultures; appearances are the same as on non-selective heated blood agar.

Rapid carbohydrate utilization test (RCUT) and penicillinase test (Young, 1978a)

In the RCUT, preformed enzyme is measured by adding a suspension of the overnight growth of the test organism to a buffered (non-nutrient) solution containing the sugar to be tested and a pH indicator. β-lactamase can be detected by substituting ampicillin for sugar. Acid production resulting from sugar utilization or from the splitting of ampicillin to penicilloic acid is detected by a colour change of the pH indicator from red to yellow.

Buffer-salt solution (BSS). Prepare by mixing the following solutions: 40 ml 0.1 mol/litre
Sugars. Prepare 100 ml of 10% w/v solutions in distilled water of glucose, maltose, sucrose, fructose and lactose; filter sterilize, dispense in 4 ml amounts and store at −20°C.

Note: Some batches of maltose contain excessive amounts of glucose and give false positive results; Extra Pure Maltose (BDH) gives satisfactory results. Commercial carbohydrate disks are available for preparation of RCU T substrates (Neisseria Identification Disks, Oxoid DD7).

Ampicillin solution. Dissolve the contents of a 500 mg vial of sodium ampicillin (Beecham) in 2 ml BSS to give a concentration of 250 mg/ml. Dispense in 200 μl amounts and store at −20°C.

Method. 1. Each week (or more often if required) thaw one bottle of BSS, one of each sugar, and one of ampicillin and store at 4°C for daily use.
2. Set out 5 tubes (70 × 10 mm) for each suspect gonococcal isolate from a genital site, and 7 tubes for each non-genital or atypical isolate. Add 20 μl of glucose, maltose, sucrose and ampicillin respectively to 4 individual tubes for the genital isolates; also add 20 μl of fructose and lactose respectively to the extra 2 tubes for non-genital isolates.
3. Add 100 μl of BSS to each of the tubes containing sugar or ampicillin and 300 μl of BSS to the remaining tube.
4. Harvest growth from a 16–24 h culture with a cotton-tipped swab and make a thick suspension in the 300 μl of BSS. Transfer 30 μl of the suspension to each sugar-containing tube and to the ampicillin tube. Shake tubes and incubate at 37°C in a waterbath.
5. Examine tubes after 30–60 min and make a definitive reading after 3 h. A yellow colour (occasionally yellow-orange) is positive; red is negative.

Each day set up a β-lactamase-producing N. gonorrhoeae and a strain of N. meningitidis as controls.

The RCU T can also be conveniently performed in microtitre plates. The appropriate reagents are dispensed in wells and the plates stored at −20°C until required.

Serum-free agar sugars. Flynn & Watkins (1972).

Supplement. Solution A: dissolve 1 g of L-glutamine in 90 ml distilled water. Solution B: dissolve ferric nitrate 0.05 g in 10 ml distilled water. Prepare the supplement by adding 90 ml of solution A to 10 ml of solution B.

Sugars. 10% solutions of glucose, sucrose or maltose, sterilized by filtration.

Method. Boil 36 g of GC Medium Base (Difco) in 970 ml distilled water and when clear add 20 ml of the supplement and 10 ml of phenol red (0.2% stock solution). Adjust to pH 7.6 with NaOH (1 mol/litre), and distribute in 90 ml volumes in screw-cap bottles. Autoclave at 121°C for 10 min. Cool to 50°C. Add 10 ml of the appropriate sugar solution (aseptically) to 90 ml of medium to give a final concentration of 1% sugar. Dispense 3 ml amounts into sterile 5 ml screw-cap bottles and allow to set as slopes.

For use, slopes should be inoculated heavily and incubated at 37°C in an atmosphere containing 5–10% CO₂, the screw caps of the containers being loosened. A positive result (colour change to yellow) should be obtained after overnight incubation, although cultures should routinely be kept for 48 h. With very small inocula a longer period of incubation may be necessary.

Filter-paper acidometric test for β-lactamase production (Sng et al 1981)

Penicillin solution. Prepare 0.05 mol/litre phosphate buffer, pH 8.0, by dissolving 37.5 mg KH₂PO₄ and 842 mg Na₂HPO₄·2H₂O in 100 ml distilled water. Dissolve 5% crystalline benzylpenicillin (buffer-free) and 0.2% bromocresol purple in this buffer. Divide into small aliquots
Coagglutination test

This is a rapid (10 min) coagglutination slide test which uses protein-A-containing staphylococci with murine monoclonal antibodies bound by the Fc portion to the protein A. The test uses two reagents, WI and WI/III, composed of separate pools of monoclonal antibodies reactive with PrIA and PrIB respectively. When a test sample containing gonococci, usually a primary culture of an isolate, is mixed with the reagents, the specific monoclonal antibodies react with the appropriate PrI antigen. A coagglutination lattice is formed which is visible to the naked eye. Kits are available from Pharmacia. The test should be performed according to the manufacturer’s instructions, with a light suspension of the organism boiled in 0.9% saline. The unit volume of reagents may be reduced to 15 μl for economy.

Polysaccharide antigen of N. meningitidis Groups A, B, C, Y and W135 can be detected in CSF (treated to remove non-specific reactions) by coagglutination with a kit available from Pharmacia.

Latex agglutination

The polysaccharide antigens of meningococci can be detected in CSF, urine (concentrated if necessary) or serum, by agglutination of antibody-coated latex particles. Body fluids must be heated or centrifuged to remove non-specific reactions. The group B reagent will cross-react with Escherichia coli K1 antigen which may be found in neonatal meningitis. Kits are available from Wellcome.

REFERENCES


CHLAMYDIA TRACHOMATIS INFECTION IN SEXUALLY ACTIVE WOMEN WITH NO KNOWN SEXUAL CONTACTS WITH URETHRITIS

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Abstract: During the period from March 1985 to March 1986, 216 consecutive patients with vaginal symptoms were screened for cervical infection with Chlamydia trachomatis and other sexually transmitted organisms. Eleven (5%) yielded C. trachomatis without a history of recent contact with males with urethritis.

Key words: Vaginal discharge; C. trachomatis; non-symptomatic contact.

Introduction

UNTREATED cervical infection with C. trachomatis has been shown to be a major cause of pelvic inflammatory disease1,2,3 and infertility in later years4,5,6.

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Routine screening by isolation for C. trachomatis in a general practice setting has not been widely practised due to the lability of the organism and the necessity for rapid, chilled transport of specimens to the laboratory. The alternative antigen detection tests which overcome this problem use commercial reagents and are more costly. A low index of suspicion of such infection is, however, the
Results

A total of 216 patients were entered into the study from March 1985 to March 1986. The age range of the patients in the study was biased towards the 20-25-year age groups as seen in Table I. This reflects the predominantly student population of the practice.

The results of the microbiological investigation are given in Table II. As expected, the most common organism isolated was a yeast, but, perhaps surprisingly, *Trichomonas vaginalis* was found in only two cases.

Gram-negative bacterial infections were second most common and notoriously difficult to eliminate except in the short term, usually with metronidazole. In no case was *Neisseria gonorrhoeae* detected.

Eleven patients (5%) were infected with *C. trachomatis* with no recent history of contact with a male with symptoms of urethritis.

Thirty-five of the swabs sent for chlamydial culture were reported as toxic for tissue culture and had to be repeated. Further experience has shown that immediate refreezing of specimens after sampling will prevent this toxicity in all but a few. Repeat swabs one month later were negative for *C. trachomatis* in seven cases, two have defaulted from follow-up, one returned to the USA for follow-up and in one case the repeat swab was positive: her boyfriend had failed to obtain treatment and further intercourse had ensued. After both partners were treated, the woman's follow-up swab was negative for *C. trachomatis*.

Some 30% of patients were found to have no detectable microbial infection. Symptoms in many of these resolved with no treatment, but some proved to have intractable problems and were referred for gynaecological or psychosexual assessment.

Discussion

A comparison of the detection rate of micro-organisms in high vaginal and endocervical swabs with those of other published work is difficult due to the exclusion of known contacts from this study. In a North American study where they examined 430 patients they had a similar isolation rate of *Beta-haemolytic streptococci* (18%), fewer yeasts (26%), a low level of *T. vaginalis* (2%) and a higher incidence of *G. vaginalis* (32%). This may be just a reflection of the patients studied. In the Department of Genito-urinary Medicine in Edinburgh, there has been a fall in the number of cases of *trichomiasis* detected from 237 (10.5%) in 1979 to 66 (2.4%) in 1987 of all new female cases. This decreasing prevalence reported here may have been influenced by the widespread use of metronidazole to which the protozoan is extremely sensitive. In both this study and those reported from North America the isolation rate of chlamydiae was 5%.

There were 11 cases of cervical infection with *C. trachomatis*, most of whom had not knowingly had sexual intercourse with a man with urethritis. One woman had a regular boyfriend who one year previously had had non-specific urethritis. On screening elsewhere at that time, the woman was not found to be harbouring *C. trachomatis*, so only the boyfriend was treated. As he has shown no further symptoms it is uncertain whether the woman harboured the organism over the year or was recently infected. However, both partners have now been treated with antibiotics and the follow-up of the woman has revealed no infection.

The importance of contact tracing and treatment of regular partners of cases is illustrated by the previous case
and by the one woman from whom the organism was isolated from the follow-up specimen.

We had hoped that this study might help to distinguish those women who should be screened for C. trachomatis infection. With this in mind, symptoms and signs were noted initially to see if it might be possible to pinpoint those at risk. Unfortunately, the vaginal symptoms were often so vague and imprecise and most women complained of both discharge and soreness. Obviously, a sexual history should be taken of all women with vaginal symptoms, but in this study none of the cases of C. trachomatis infection were expected from the history at the initial presentation. It would seem from this experience that cases of genital C. trachomatis infection would be missed if routine screening were not conducted.

In this study it was possible to screen these patients by attempting to isolate C. trachomatis in tissue culture. The transport of the specimens (although they were best to be rapidly refrozen) to the laboratory was easy due to the proximity of this general practice to the laboratory. This allowed the laboratory to employ the test which is cheapest in terms of consumables cost — tissue culture. For general practitioners who are further removed from the laboratory, antigen detection tests would require to be used. Both immunofluorescence and ELISA detection involve commercial reagents and so the cost of testing would be greater.

This study indicates that lower genital tract infection with C. trachomatis would be missed in a proportion, albeit small, of women with vaginal symptoms but no recent history of sexual contact with a male with symptomatic urethritis. Within the context of this general practice, it has been feasible to continue to screen women routinely for such infection along with more usual checks for yeasts and bacterial infections.

If general practitioners were to screen routinely for C. trachomatis, the increase in cost and workload at the microbiology laboratory would be considerable and may not be tolerable in these times of cutbacks and economies. However, screening only of those who give a history of sexual contact with symptomatic males would undoubtedly miss a few cases who may in the long term suffer from pelvic inflammatory disease and infertility. This dilemma between ideal screening and cost of such work is a common one in medicine and is not easy to resolve.

ACKNOWLEDGEMENT: We wish to thank the nursing staff of the University Health Centre, technical staff of the Sexually Transmitted Diseases and virology laboratories of University of Edinburgh and Dr A. McMillan for help with preparation of the manuscript.

REFERENCES
Fluorescent monoclonal antibody test for the confirmation of *Neisseria gonorrhoeae*

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(Accepted 12 August 1988)

The Microtrak* fluorescence labelled monoclonal antibody test for the culture confirmation of *Neisseria gonorrhoeae* was evaluated with 157 clinical isolates of oxidase-positive Gram-negative diplococci, including 110 strains of *N. gonorrhoeae*. Seventy-eight stock cultures of neisseria were tested to confirm the results from clinical samples. The test gave a specificity of 100%, and sensitivity of 98%. Only four strains of the rare serovar Behj were non-reactive. As the prevalence of this serovar influences the utility of the test, the rarity of this serovar in different geographical locations is discussed.

**Key words:** Fluoresceins. Monoclonal antibody. *Neisseria gonorrhoeae.*

### Introduction

Present methodology for the identification of *Neisseria gonorrhoeae* includes conventional and rapid sugar degradation tests, chromogenic substrate identification systems, and immunological methods.1

Difficulties have been experienced in many laboratories with carbohydrate utilization tests, *Neisseria meningitidis* and *Branhamella catarrhalis* causing the major problems.2 Difficulties have also been experienced with the newer commercially available biochemical identification methods such as Gonocheck3,4 and the RapID N.H. system.4,5

The advantages of immunological methods for confirming the identity of *N. gonorrhoeae* include rapidity and the use of very small amounts of bacterial growth. Immunological tests based on polyclonal antibodies were hampered by cross-reactions with non-gonococcal neisseria, particularly *N. lactamica*.6 Monoclonal antibodies which recognise epitopes on gonococcal protein I7 have now superseded polyclonal antibodies in test systems, resulting in reagents with a potential absolute specificity and high sensitivity. Because of the antigenic variation in protein I, a single monoclonal antibody can not react with all strains of gonococci, and the actual performance of monoclonal antibody-based tests will depend on the appropriate selection of individual monoclonal antibodies for inclusion in the reagent pools. Similar monoclonal antibodies can be used to sub-divide isolates of *N. gonorrhoeae* into serovars; a serovar is defined as the pattern of reactivity of each strain with a panel of individual monoclonal antibody reagents.8

In this study we evaluated the sensitivity and specificity of the Syva MicroTrak* fluorescent monoclonal antibody test9 for the routine identification of *N. gonorrhoeae*. An analysis was made of the test reactivity in relation to the serovar of the isolate, and a comparison drawn with the sensitivity and specificity of the Phadebact* monoclonal G.C. test.10

### Materials and methods

To evaluate the test in routine use, material from anogenital and pharyngeal sites from patients attending the Department of Genito-urinary Medicine, Royal Infirmary, Edinburgh, was inoculated on to modified New York City medium.11 After overnight incubation at 37°C in an aerobic atmosphere containing 5% carbon dioxide, cultures were examined for oxidase-positive Gram negative diplococci (GNDC).

Suspect colonies were tested by the Micro-
Trak® and Phadebact® Monoclonal GC test, according to the manufacturers’ instructions. Whenever GNDC were isolated from more than one site in the same patient, genital (urethral or cervical), rectal and throat isolates were tested separately. If there was insufficient material on the primary isolation plate a subculture was made and the isolate identified the following day.

The MicroTrak® results were scored subjectively depending on the degree of fluorescence:

- positive = strong fluorescence
- weak positive = weak or moderate fluorescence
- equivocal = borderline fluorescence
- negative = no fluorescence

The identity of each isolate was confirmed by the rapid carbohydrate utilization test, modified for performance in microtitre trays. The serovar of the isolate was determined with a panel of monoclonal antibodies developed by Genetic Systems.

To confirm the sensitivity and specificity found with clinical isolates a further 78 stock cultures of gonococci and nine stock cultures of non-gonococcal neisseriae were also examined by the MicroTrak® test.

The statistical significance of the differences in the results was determined by the $\chi^2$ test with Yates’ correction.

**Results**

**Clinical isolates**

GNDC were isolated from 157 cultures from 46 female and 74 male patients. The isolates were identified biochemically as: *N. gonorrhoeae* 110 (genital 79, rectal 17, throat 14); *N. meningitidis* 44 (rectal 1, throat 43); *N. lactamica* 2 (throat); and *B. catarrhalis* 1 (throat). All 47 non-gonococcal GNDC were negative with both the MicroTrak® and Phadebact® Monoclonal GC tests.

Fifty-one (46.4%) of the 110 gonococcal isolates reacted with the Phadebact® Monoclonal GC test serogroup WI reagent, and 59 (53.6%) with the WII/III reagent.

The 51 serogroup WI isolates represented six different protein 1A serovars (Table 1). Although all 51 isolates were reactive in the MicroTrak® test, only nine were scored positive while 42 gave a weak positive (or equivocal) reaction.

The 59 serogroup WII/III isolates represented eight different protein 1B serovars (Table 2). The majority of the isolates (39) were scored positive, while 18 were weak positive (or equivocal) and two negative. The two MicroTrak® test negative isolates were the only strains of the rare serovar Behj, and were isolated from a male patient and his female partner.

Performance data based on our ‘in use’ evaluation of the MicroTrak® test gave a specificity of 100% (47/47) and a sensitivity of 98.2% (108/110).

Weak positive (or equivocal) reactions were significantly more common with protein 1A serovars (82.4%) than with protein 1B serovars (31.6%); $P < 0.001$.

**Stock cultures**

These results confirmed the findings with clini-

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**Table 1. Serovar and Microtrak reactivity of 51 serogroup WI clinical isolates of gonococci**

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates</th>
<th>P</th>
<th>WP</th>
<th>E</th>
<th>NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aeddki</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aeddgh</td>
<td>37</td>
<td>5</td>
<td>31</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aeddhih</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aegk</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Afeddk</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>9</td>
<td>41</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 2. Serovar and Microtrak reactivity of 59 serogroup WII/III clinical isolates of gonococci**

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates</th>
<th>P</th>
<th>WP</th>
<th>E</th>
<th>NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacej</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bacek</td>
<td>17</td>
<td>14</td>
<td>3</td>
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<td>0</td>
</tr>
<tr>
<td>Baj</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bajk</td>
<td>23</td>
<td>21</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Behj</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>39</td>
<td>14</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
cal isolates. All non-gonococcal GNDC (N. flava 2, N. perflava 2, N. lactamica 1, N. meningitidis 1 and B. catarrhalis 3) gave a negative MicroTrak® result.

Twenty-five (32%) of the 78 gonococcal stock cultures reacted with the Phadebact® Monoclonal G.C. test serogroup WI reagent, 49 (63%) with the WII/III reagent, while four isolates (5%) failed to react with either reagent.

The 25 serogroup WI isolates represented four protein 1A serovars (Table 3) one of which (Aedgk) was not found in the fresh clinical isolates. Again, the majority (84%) of these protein 1A serovars gave weak positive reactions in the MicroTrak® test.

The 53 serogroup WII/III isolates represented 11 protein 1B serovars (Table 4), six of which (Bak, Bajk, Becgjk, Beghjk, Begjk and Bcegjk) were not found in our sample of clinical isolates. Isolates belonging to these additional serovars were all reactive in the MicroTrak® test. The only non-reactive isolates belonged to the rare serovar Behj, thus confirming our findings with clinical isolates.

All four Phadebact® Monoclonal GC test negative isolates of serovar Bj were reactive in the MicroTrak® test.

Again, weak positive (or equivocal) reactions were significantly more common with protein 1A serovar (84%) than with protein 1B serovars (47%); P<0.05.

### Discussion

The high specificity and sensitivity of the Microtrak® test make it ideally suited for screening suspect GNDC colonies from primary cultures. The absolute specificity of the test means that a positive result provides a reliable identification of N. gonorrhoeae from any anatomical site. This represents an improvement over polyclonal antibody-based tests, which are unsuitable for confirming the identity of pharyngeal isolates. However, because of the occurrence of a small number of non-reactive isolates, MicroTrak® negative GNDC isolates from anogenital sites should be confirmed by biochemical tests.

Although 21 different serovars were represented in the isolates tested the non-reactive isolates belonged to a single serovar, Behj (equivalent to serovar 1B24 (Bh) using the nomenclature and antibody panel of Knapp et al.16). This serovar accounts for only two (1.8%) of the 110 unselected consecutive isolates in our study. The occurrence of minor serovars can vary, and is influenced markedly by local outbreaks. In a previous study involving 289 unselected strains isolated during autumn 1985 and spring 1986, 28 different serovars were found and the serovar Behj accounted for 14 (4.8%) of the isolates. During 1987 serovar Behj was isolated on nine occasions, accounting for approximately 1.8% of all isolates tested. Although its prevalence in other areas is not known, seven isolates of serovar Behj were found in Sweden in 1985, three in Norway in 1984,8 and only one isolate was found among 1433 strains examined in a world-wide survey.15 The rarity of serovar Behj (1B24) is emphasised by its absence in the following studies involving: 181 unselected isolates in Seattle, USA;19 253 beta-lactamase producing gonococci isolated in Sweden;20 320 strains from southern Florida and 598 strains.

### Table 3. Serovar and Microtrak reactivity of 25 serogroup WI stock cultures of gonococci

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates</th>
<th>Microtrak reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedgk</td>
<td>2</td>
<td>P:0  WP:2  E:0  NEG:0</td>
</tr>
<tr>
<td>Aedgkjh</td>
<td>18</td>
<td>P:4  WP:14  E:0  NEG:0</td>
</tr>
<tr>
<td>Aedh</td>
<td>4</td>
<td>P:0  WP:4  E:0  NEG:0</td>
</tr>
<tr>
<td>Aegk</td>
<td>1</td>
<td>P:0  WP:1  E:0  NEG:0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>P:4  WP:21  E:0  NEG:0</td>
</tr>
</tbody>
</table>

### Table 4. Serovar and Microtrak reactivity of 53 serogroup WII/III stock cultures of gonococci

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates</th>
<th>Microtrak reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacejk</td>
<td>17</td>
<td>P:15  WP:2  E:0  NEG:0</td>
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<tr>
<td>Bacjk</td>
<td>6</td>
<td>P:1  WP:5  E:0  NEG:0</td>
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<tr>
<td>Back</td>
<td>8</td>
<td>P:5  WP:3  E:0  NEG:0</td>
</tr>
<tr>
<td>Bahj</td>
<td>2</td>
<td>P:2  WP:1  E:0  NEG:0</td>
</tr>
<tr>
<td>Bajk</td>
<td>6</td>
<td>P:4  WP:2  E:0  NEG:0</td>
</tr>
<tr>
<td>Bak</td>
<td>1</td>
<td>P:1  WP:1  E:0  NEG:0</td>
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<td>4</td>
<td>P:1  WP:3  E:0  NEG:0</td>
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<tr>
<td>Total</td>
<td>53</td>
<td>P:27  WP:23  E:1  NEG:2</td>
</tr>
</tbody>
</table>

from Des Moines, Iowa (USA); 395 serogroup W11/111 strains from Thailand, Singapore and Korea; 141 isolates from Scandinavia, Australia, Auckland, Bangkok, Singapore and Korea belonging to the more unusual serovars; 377 strains chosen to reflect the distribution of auxotypes seen in Canada; 188 strains isolated in Jamaica.

Serovar Behj is reactive in the Phadebact® Monoclonal GC test, and in a study in this locality involving 1080 unselected isolates the Phadebact® Monoclonal GC test was 100% specific and 99.7% sensitive. All three non-reactive isolates in this case were of the rare serovar Bj (equivalent to serovar 1B17 [Bb1]), using the nomenclature and antibody panel of Knapp et al. As shown in Table 4, serovar Bj is reactive in the MicroTrak® test. In most geographical areas serovar Bj is extremely rare, and it was absent in the studies of Dillon et al., Knapp et al. and Bygdeman et al. described above. It was also absent in five isolates from Des Moines, Iowa (USA), but two isolates were found among 320 strains from southern Florida, two in the 395 isolates from Thailand, Singapore and Korea, three (Helsinki 1, Singapore 2) among the 141 unusual W11/111 serovars from Scandinavia, Australia, Auckland, Bangkok, Singapore and Korea, and two among the 1433 strains examined in a worldwide study. However, as with serovar Behj, local outbreaks will occur and in Glasgow 1% (15/1509) of isolates were of serovar Bj.

The MicroTrak® test uses a single pool of monoclonal antibodies and differences in the strength of fluorescence reaction between serovars can be detected. Serovar 1A isolates give significantly weaker fluorescence reactions than serovar 1B isolates (P < 0.001). This difference held for both fresh clinical isolates and stock cultures. As there is a greater number of 1B than 1A serovars it is likely that the antibody pool contains more antibodies against 1B serovars than against 1A. Differences in the degree of fluorescence, however, could not be used to discriminate between 1A and 1B isolates. The Phadebact® Monoclonal GC test uses two pools of antibodies and can easily distinguish between 1A and 1B serovars, allowing the identification of a double infection with more than one serogroup. The detection of double infections is important in the overall control strategy of gonococcal infection, enabling effective contact tracing of patients with multiple partners.

The MicroTrak® test is rapid, easy to perform and only a few suspect colonies are required to perform this test. When there is poor growth on the primary isolation plate this allows immediate identification without awaiting subculture. On the other hand, however, a small test sample resulting in the selection of one colony type could lead to a false negative result in the case of pharyngeal cultures with mixed populations of gonococcal and non-gonococcal neisseria. The density of the sample is important, as thick suspensions cause clumping and uneven staining which may lead to misinterpretation.

In conclusion, there is no appreciable differences in overall sensitivity and specificity between the MicroTrak® and Phadebact® Monoclonal GC test. However, account must be taken of the geographical distribution and prevalence of serovars and the limitations of the confirmatory system known. In areas with a high prevalence of serovar Bj the MicroTrak® test would perform better than the Phadebact® Monoclonal GC test. The converse would apply in those areas with a high prevalence of serovar Behj.

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9 Syva Company, Arastradero Road, Palo Alto, California, USA.

10 Pharmacia diagnostics AB, Uppsala, Sweden.


Clinical Practice in Sexually Transmissible Diseases

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SECOND EDITION

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1989
Preface to the Second Edition

The objectives of this second edition continue to be those outlined in the preface to the first. The nine years that have passed have brought many scientific advances and it has been our fascinating task to incorporate these and develop the text, which has been rewritten virtually in its entirety.

In Chapter 1 we have described the pattern of human sexual behaviour as the background to infection and assembled information which sheds light on its nature and evolution. In Chapter 2 we have given attention to the taxonomy and classification of the infective organisms or agents referred to in the book. We hope these chapters will help clinicians to develop or renew their understanding of these subjects. A new chapter has been devoted to the problems arising from infection with the human immunodeficiency viruses (HIV), and other persistent virus infections have been given fuller attention. The opportunistic pathogens which are such an important feature of HIV infection are considered in Chapter 2 and again from the clinical point of view in Chapter 31. Much new and important information has been assembled relating to the classical sexually transmitted pathogens and in particular to Neisseria gonorrhoeae and Treponema pallidum. Epidemiological, psychosocial and educational matters are touched upon in Chapters 3-5 and an approach to the clinical investigation of the patient is outlined in Chapter 6. The book covers those sexually transmissible diseases in which the traditional clinical services have been very effective in specific diagnosis, effective specific treatment and contact tracing, activities where secondary prevention is of prime importance as a method of control. Much of the book, however, is devoted to persistent virus infections in which primary prevention provides only a weak and uncertain means of control. In this connection the impact of the human immunodeficiency viruses has set in train extremes of suffering for the patient and testing questions for society. Far-reaching complexities also surround papillomavirus infection, not least in the organization of life-long follow-up of women.

Sound knowledge of the sexually transmissible diseases and firm application of principles developed in this subject remain among the indispensable essentials of medical practice.

Edinburgh, 1989

D.H.H.R.
A.M.
H.Y.
This book has been written primarily for those who are actively engaged in the increasingly demanding clinical practice of venereology and who, by so doing in the United Kingdom at least, now tend to undertake so large a part of the primary care of adolescents and young adults. We have attempted to bring together information which is widely scattered in the literature and to present an appreciation of clinical and laboratory aspects of the various subjects in a way which we believe will be useful to our colleagues. It is our hope that the book will be of value, as a reference in teaching and also to the wider range of clinicians, for instance gynaecologists and urologists, who participate as we do in the practice of sexual and reproductive medicine and will therefore often require to consider the full range of sexually transmitted diseases. General practitioners and other clinicians, who are involved in clinics set up for counselling and for giving contraceptive and kindred advice, will find some answers to their questions, and physicians, who may not ordinarily look after adolescents and young adults, may find the inclusion of sexually transmissible infections in their differential diagnosis a rewarding exercise. It is clear that when precise information about the organisms involved is regarded as an essential discipline in the diagnosis of genito-urinary and pelvic inflammatory disease, the importance of transmission by sexual intercourse will become better appreciated and the application of isolation methods, more searching than conventional bacteriological investigations, will help in developing more rational care of patients.

Although this book is primarily for medical readers it is hoped that those involved in nursing or counselling patients, tracing contacts or in health education will be able to obtain some of the factual information which they require. Barriers between disciplines are tending to become inappropriate and those who share objectives in patient care will require to pool their knowledge to obtain the best results possible.

There is more to be achieved by those concerned than a technical understanding of one aspect of clinical medicine and microbiology because psychological and social barriers intrude and hamper at every level. Some aims of this text are primarily intellectual and are fundamental to practice in a subject which involves deep personal feelings to such an extent that patient, doctor and society may appear not infrequently to be bewitched. The reader will be encouraged to adopt a logical attitude essential in clinical practice and an approach to the subject based on an acceptance of human diversity.

Edinburgh, 1980

D.H.H.R.
A.M.
H.Y.
Acknowledgements

We wish to acknowledge the advice, help and discussion given by many friends and colleagues in the Royal Infirmary of Edinburgh and University of Edinburgh. We wish to give our appreciation and thanks also to: Professor R. V. Short, Professor of Reproductive Biology, Monash University, Melbourne, Australia, and the publishers, Australian Academy of Science, for permission to use Figs. 1.1 and 1.2; Professor J. M. Tanner, Professor of Child Health and Growth at the Institute of Child Health, University of London, and Honorary Consultant Physician at the Hospital for Sick Children, Great Ormond Street, London, and the publishers, Open Books Publishing Ltd., London, for permission to use Figs. 1.3 and 1.4; Professor D. C. G. Skegg, Professor of Preventive and Social Medicine, University of Otago, Dunedin, New Zealand, and the Lancet, 7 Adam Street, London, for permission to use Fig. 1.6. Fig. 1.7 is derived from data which is Crown copyright and is reproduced with permission of the Controller of Her Majesty's Stationery Office. The authors also wish to give their appreciation and thanks to: Ms Kathleen E. Kiernan for permission to use data from which Fig. 1.8 is derived; Ms Audrey C. Brown of the Office of Population Censuses and Survey and Ms Kathleen E. Kiernan, Centre for Population Studies, London School of Hygiene and Tropical Medicine, for permission to use data from which Fig. 1.9 is derived; Dr Philip Blumstein and Dr Pepper Schwartz of the University of Washington, USA, for permission to use data from their book American Couples, William Morrow and Co., New York (1983), from which Figs. 1.10, 1.11 and 1.13 have been constructed; Professor A. P. Bell and Professor M. S. Weinberg of Indiana University, USA, for permission to use data from their book Homosexuality: A Study of Diversity among Men and Women, Simon and Schuster, New York (1978), from which Figs. 1.12 and 1.14 and Table 1.5 have been constructed; Dr H. Alzate of the University of Caldas, Manizales, Colombia, and the publisher of Archives of Sexual Behaviour, Plenum Publishing Corporation, New York, for permission to include the data shown in Table 1.3; Professor Harold T. Christiansen, Professor Emeritus of Sociology, Purdue University, Lafayette, Indiana, for permission to include data in Table 1.1; Professor B. Roizman, Joseph Regenstein Distinguished Service Professor and Chairman, Department of Molecular Genetics and Cell Biology, The University of Chicago, for his courtesy and help in connection with matters relating to the taxonomy of human herpes viruses; Dr Mary P. English, formerly Consultant Mycologist, Bristol Royal Infirmary and Research Fellow, University of Bristol and Dr P. M. Stockdale, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, for their help with taxonomy: The World Health Organization, Geneva, for permission to include Figures 3.1 and 3.8; The Office of Health Economics, 195 Knightsbridge, London SW7, for permission to use Figs. 3.2, 3.3 and 3.4; Dr D. B. L. McClelland, Regional Director, Edinburgh, and South East Scotland Regional Blood Transfusion Centre, Royal Infirmary of Edinburgh, for helpful discussion and information in connection with the precautions used against HIV infection; The Health Education Council, 78 New Oxford Street, London, and Mr Colin Forbes, Marketing Director of Forbes Publications Limited, 120 Bayswater Road, London W2 3JH, for permission to include material from The Schools Council's Project on Health Education as detailed in Chapter 4; Dr M. S. R. Hutt, Geographical Pathology Unit, Department of Histopathology, St Thomas's Hospital Medical School, London, and the British Medical Bulletin (Churchill Livingstone, Publishers) for permission to use data on the proportional frequencies of endemic (African) Kaposi's sarcoma given in Chapter 5; Mrs Catherine Harrison, Research Associate, Department of Genito-Urinary Medicine and Mr Ray Harris of the Drummond Street Reprographics Unit, University of Edinburgh, for their help in the preparation of the world map in Figures 5.1, 13.1, 13.2 and 31.3; The Organizer of National External
Quality Assessment Scheme. Dr. J. J. S. Snell of the Division of Microbiological Reagents and Quality Control, Public Health Laboratory Service, 61 Colindale Avenue, London NW9 5HT, for permission to publish the data in Table 8.3; Ms Anne B. Prasad, Executive Editor, British National Formulary, for her patient help and correspondence which encouraged the present authors to try to bring reason into statements of penicillin dosage in Chapter 10 particularly; Dr Clive Osmond and other members of the MRC Environmental Epidemiology Unit, University of Southampton, for their permission to use diagrams on cervical cancer in Chapter 25, namely Figs. 25.1 and 25.2 (these figures are Crown copyright, reproduced with permission of the Controller of Her Majesty’s Stationery Office); Wellcome Foundation Ltd. for permission to use information from recent data sheets on Zovirax (acyclovir) and Retrovir (zidovudine) respectively in Chapters 26 and 31; Professor L. G. Whitby, Professor I. W. Percy-Robb, Dr A. F. Smith and Blackwell Scientific Publications, who have kindly agreed to allow us to use extracts from their book Lecture Notes on Clinical Chemistry, 3rd edition, 1984, as a note to our chapter on Viral Hepatitis (Note 30.1); Dr B. G. Gazzard, Dr D. C. Shanson and the Lancet for permission to include Fig. 31.2: Mr Paul E. Bishop, Research Associate, Department of Genito-Urinary Medicine, University of Edinburgh, for his kindness in preparing Note 31.4; Professor Kenneth Mellanby, Monks Wood Experimental Station, Abbots Ripton, Huntingdon, for permission to include Fig. 36.1: Dr J. F. Peutherer and Dr I. W. Smith, Senior Lecturers, Department of Bacteriology, University of Edinburgh, for much help and advice on virus disease; Dr R. Heyworth, Senior Biochemist, Department of Clinical Chemistry, Royal Infirmary of Edinburgh, for his help and advice.

The contribution of members of the Department of Medical Illustration of the University of Edinburgh is gratefully acknowledged. The authors wish to thank Mrs J. M. Gilbertson for her skill in applying modern technology in the preparation of the manuscript and for her patience and help.

The authors are also indebted to the staff of Churchill Livingstone, Edinburgh, our publishers, for their encouragement and help.

Edinburgh, 1989

D.H.H.R.

A.M.

H.Y.
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Infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women with lower abdominal pain admitted to a gynaecology unit

G. R. SCOTT, C. THOMPSON, I. W. SMITH, H. YOUNG

**Summary.** One hundred and sixty-five women admitted to a gynaecology unit with lower abdominal pain were screened for infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis* by members of a department of genitourinary medicine. *C. trachomatis* alone was detected in 21 patients. *N. gonorrhoeae* alone was isolated from five patients, and dual infection was present in six patients, giving a total of 32 (19%) patients in whom a sexually transmitted disease (STD) was diagnosed. The combination of an endocervical swab placed in Amies transport medium for gonococcal isolation and an endocervical slide for immunofluorescent detection of chlamydiae proved to be a simple and accurate method of screening for STD. As a result of contact tracing, 16 sexual partners of women in whom STD was detected were examined. Three cases of gonococcal and nine cases of non-gonococcal urethritis were diagnosed. None of the sexual partners had symptoms suggestive of genitourinary infection.

Lower abdominal pain in young women is a common cause of admission to gynaecology units. The differential diagnosis includes pelvic inflammatory disease (PID), ectopic pregnancy, abortion and complications of ovarian cysts (Beard et al. 1986). Cysts and the complications of pregnancy can usually be excluded by the combination of an ultrasound examination and estimation of the plasma level of the beta subunit of human chorionic gonadotrophin (β-hCG) (Kadar et al. 1981). The management of possible PID may be more haphazard, particularly with regard to diagnosing infection by *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, both of which are important causes of salpingitis (Mardh 1980). Diagnostic methods that would be employed in genitourinary medicine departments such as direct plating of material on to modified New York City (MNYC) medium which is selective for gonococcal isolation (Young 1978), and inoculation of McCoy cells for chlamydial culture (Evans & Woodland 1983) may not be readily available, and undue reliance may be placed on a less sensitive method such as a high vaginal swab (Battacharyya et al. 1974).

The aim of this study was to develop liaison between the departments of genitourinary medicine and gynaecology at the Royal Infirmary of Edinburgh in the management of women suspected of PID, both by improving diagnostic methods and also by demonstrating the value of contact tracing and examination of sexual partners.
Patients and methods

The study included 165 patients investigated between July 1985 and August 1986 whose prime complaint was of lower abdominal pain when admitted as emergencies to the Royal Infirmary of Edinburgh under the care of the gynaecologists. Routine investigation consisted usually of a white blood count, ESR, midstream urine specimen, dry high vaginal swab, estimation of β-hCG and an ultrasound examination. Laparoscopy was performed on 50 patients with more severe symptoms or in whom there was continuing diagnostic uncertainty. Patients thought likely to have PID on the basis of standard criteria were referred to two of the authors (either G.R.S. or C.T.) for detailed bacteriological screening. Patients who had received antimicrobial therapy within the previous 2 weeks were excluded.

The system was designed to work along the following lines. A minority of the patients were admitted during office hours, and these patients were screened within 3 h of admission. A more typical patient with lower abdominal pain was admitted as an emergency during the evening and overnight. If the presumptive diagnosis was PID, the gynaecology registrar or senior registrar decided whether immediate antimicrobial therapy was necessary. Patients given immediate therapy were excluded from further study. If the patient’s condition was judged sufficiently mild, she was simply observed overnight and given analgesia if appropriate. The next morning, either G.R.S. or C.T. contacted the overnight waiting team for a list of such patients. Specimens for bacterial culture were obtained in the gynaecology wards.

The following specimens were obtained using cotton-wool-tipped applicator sticks. Urethral, cervical and rectal material was plated directly on to MNYC medium (Young 1978). Within 30 min the plates were placed in an incubator in a carbon dioxide enriched (10%) atmosphere at 37°C. After 24 h incubation, the presence of oxidase-positive Gram-negative cocci was sought. N. gonorrhoeae was identified by sugar utilization and coagglutination (Young & McMillan 1982). An endocervical swab was also placed in Amies transport medium and transported to the laboratory within 4 h. Material from the swab was then plated on to MNYC medium, and N. gonorrhoeae was identified.

For chlamydial detection, an endocervical swab was expressed into 1 ml of 2SP transport medium which was placed in a freezer at −70°C within 30 min. Care was taken to ensure that the cervix had been cleaned of vaginal exudate before material was obtained. Isolation was attempted in McCoy cells which were incubated in growth medium for 72 h. Cells were then stained with iodine, and C. trachomatis was considered to be present if characteristic intracytoplasmic inclusions were seen (Evans & Woodland 1983). A further specimen from the cervix was wiped on a teflon slide. The material was fixed with acetone and transported to the laboratory within 4 h. The slide was stained with a fluorescein-labelled monoclonal antibody (Chlamyset, Orion Diagnostica, Espoo, Finland) and examined for chlamydial elementary bodies using a fluorescence microscope. Chlamydial infection was diagnosed if ≥10 elementary bodies could be detected. In 122 patients, serological testing for chlamydiae was performed using an immunofluorescence test. Blind antimicrobial therapy could be instituted at this stage if felt appropriate, the standard regimen being doxycycline and metronidazole if pregnancy had been excluded.

Results of gonococcal cultures were always available within 24 h, and it was intended that the result of the Chlamyset would also be available within that time, although sometimes pressure of work on the laboratory resulted in delays. Results of chlamydial culture took approximately 7 days. Where positive results were found before the woman left the hospital, she was interviewed again by G.R.S. or C.T., the diagnosis was explained and the importance of examining sexual contacts was emphasized. Appropriate antimicrobial therapy was instituted and further follow-up with tests of cure and contact tracing was continued in the outpatient clinic of the genitourinary medicine department at the Royal Infirmary of Edinburgh. If the patient had been discharged from hospital before the discovery of positive results, she was contacted by telephone (if feasible, or letter if not) by the gynaecology firm, who informed the patient of the diagnosis and gave an appointment for follow-up and contact tracing at the genitourinary medicine department.

Results

A total of 165 patients with a mean age of 23·9 years (range 16–42 years) was screened. C. tra-
Table 1. Age groups of patients

<table>
<thead>
<tr>
<th>Age groups</th>
<th>No. of patients</th>
<th>No. in whom STD was detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–19</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>20–24</td>
<td>54</td>
<td>14</td>
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<td>25–29</td>
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<td>30–34</td>
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<td>35–39</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>&gt;40</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td>32</td>
</tr>
</tbody>
</table>

*chlamatis* alone was detected in 21, *N. gonorrhoeae* alone was detected in five and dual infection was present in six patients, giving a total of 32 (19%) patients in whom a sexually transmitted disease (STD) was detected. The age groups of the patients plus numbers in whom STD was detected are presented in Table 1. It can be seen that the bulk of positive results was found in women aged 16–29. There were, however, two women aged 37 in whom chlamydial infection was diagnosed.

The clinical diagnosis was made in 158 patients; the case notes of the other seven could not be traced, and therefore they could not be placed in any diagnostic category. A precise diagnosis was only possible in 50 (32%) patients who underwent diagnostic laparoscopy. In these patients PID was diagnosed with certainty in 12 and was thought probable in three, based on standard diagnostic criteria (Jacobson & Westrom 1969). Of the other 35, ectopic pregnancy was diagnosed in eight, ovarian cyst in seven, endometriosis in three and no pathology was found in 17 patients. Of the remaining 108 patients, PID was strongly suspected in 30, probably present in 28, and very doubtful in 50. Again these observations were based on standard diagnostic criteria (Hare 1986), and categorization was made without knowledge of the results of bacteriological investigation. A comparison of probable clinical diagnosis versus detection of STD is presented in Table 2. It can be seen that of the 42 patients with PID strongly suspected on laparoscopic or clinical grounds, STD was diagnosed in 17 (40%). Alternatively, if one considers that all the patients had PID apart from those with normal laparoscopic findings, then 30/123 (24%) had an STD. The true rate of STD in PID in this group will clearly lie within these two percentages. It is of interest that chlamydial infection in two patients was thought not to be related to the patient’s symptoms.

**Comparison of diagnostic methods**

In all 11 women with gonococcal infection, *N. gonorrhoeae* was isolated following direct plating on to MYNC medium. One cervical specimen placed in Amies transport medium was unfortunately discarded before culture could be attempted, but *N. gonorrhoeae* was isolated from the other 10 specimens. Gonococcal isolation was achieved from only six of 11 dry high vaginal swabs.

The results for chlamydial detection were more complex. In two specimens, chlamydial culture alone was positive. One of these two had insufficient cellular material on the immunofluorescence slide for diagnostic purposes, the second test was definitely negative. In 10 cases both cultures and the immunofluorescence tests were positive. In 15, the immunofluorescence test alone was positive.

**Follow-up and contact tracing**

Of the 32 patients with STD, 24 were followed up in the genitourinary medicine department. Repeat tests for *N. gonorrhoeae* and *C. trachomatis* were negative in all of them. Sixteen sexual partners were also traced and examined subsequently in the department. Of the five women with gonococcal infection alone, one contact was seen and culture from the urethra was negative on two occasions. He denied the

Table 2. Clinical diagnosis of PID and laboratory detection of STD in 158 patients

<table>
<thead>
<tr>
<th>Laparoscopic diagnosis</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PID+  PID±  PID−</td>
<td>PID+  PID±  PID−</td>
</tr>
<tr>
<td>No. of patients</td>
<td>12  3  35</td>
</tr>
<tr>
<td>No. of patients with STD</td>
<td>2  1  0</td>
</tr>
</tbody>
</table>

STD, Sexually transmitted disease; PID, pelvic inflammatory disease; +, classical; ±, probable; −, improbable.
recent use of antibiotics. Of the six women with dual infection, three contacts were examined. N. gonorrhoeae was isolated in all three. Chlamydial culture was negative in one, and unfortunately was not performed in the other two. Of the 21 women with chlamydial infection alone, 12 contacts were examined. Although chlamydia was not cultured from the urethra in any of the 12, nine patients were found to have non-gonococcal urethritis (NGU), defined as >10 pus cells per high-power field (×100) in a Gram-stained smear of urethral exudate. In addition, one contact with NGU had bilateral conjunctivitis, with C. trachomatis being cultured from both eyes. Of 13 women in whom chlamydia was detected by immunofluorescence alone, seven contacts were examined, of whom five had NGU.

None of the sexual contacts had symptoms suggestive of genitourinary infection. The only male with any symptoms at all was the individual with conjunctivitis, for which he had been attending the Eye Pavilion where topical treatment had been prescribed with no response. His conjunctivitis resolved following a course of doxycycline, and repeat chlamydial cultures from both eyes were negative.

Discussion

This was not a study of the prevalence of infection with N. gonorrhoeae and C. trachomatis in women with PID. The degree of patient selection and lack of routine laparoscopy to confirm the diagnosis preclude that possibility. Rather we have attempted to show that young women admitted to gynaecology units with lower abdominal pain represent a high-risk group for STD, and we believe that similar results would be obtained in any gynaecology unit in the UK. We would encourage that such patients should be routinely screened for STD in the same way that patients are routinely screened for ectopic pregnancy and ovarian cysts. It seems unrealistic to expect that standard genitourinary methods for diagnosis would be employed, and in their place we would suggest the combination of an endocervical swab placed in Amies transport medium for gonococcal isolation and an endocervical slide for immunofluorescence detection of chlamydia.

For gonococcal isolation, direct plating on to MNYC in this setting has proved impractical in our experience, due to difficulties with the appropriate handling of culture plates before their use, and to the relative difficulty in obtaining access to an incubator with a CO₂-enriched atmosphere. In screening for gonococcal infection, it is advocated that material should also be obtained from the urethra and rectum in addition to the cervix (Young et al. 1979). However, we feel that in the setting of PID where infection ascends from the cervix, a specimen from this site alone is adequate for diagnostic purposes.

It is interesting that immunofluorescence testing and chlamydial culture gave such disparate

<table>
<thead>
<tr>
<th>Index patient</th>
<th>Chlamydia culture</th>
<th>Chlamydial serology</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Toxic</td>
<td>NK</td>
<td>Repeat chlamydial culture positive</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>1:512</td>
<td>Contact diagnosed as having NGU</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>1:256</td>
<td>Laparoscopic diagnosis of PID</td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>1:16</td>
<td>Laparoscopic diagnosis of PID</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>NK</td>
<td>Concomitant gonococcal infection</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>1:256</td>
<td>Laparoscopic diagnosis of PID</td>
</tr>
<tr>
<td>7</td>
<td>Toxic</td>
<td>1:256</td>
<td>Nil</td>
</tr>
<tr>
<td>8</td>
<td>Negative</td>
<td>1:32</td>
<td>Repeat immunofluorescence test negative</td>
</tr>
<tr>
<td>9</td>
<td>Toxic</td>
<td>1:8</td>
<td>Contact diagnosed as having NGU</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td>Negative</td>
<td>Concomitant gonococcal infection</td>
</tr>
<tr>
<td>11</td>
<td>Negative</td>
<td>1:128</td>
<td>Nil</td>
</tr>
<tr>
<td>12</td>
<td>Negative</td>
<td>Positive*</td>
<td>Contact had chlamydial conjunctivitis</td>
</tr>
<tr>
<td>13</td>
<td>Negative</td>
<td>NK</td>
<td>Course of co-trimoxazole 3 weeks previously</td>
</tr>
<tr>
<td>14</td>
<td>Negative</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td>15</td>
<td>NK</td>
<td>NK</td>
<td>Nil</td>
</tr>
</tbody>
</table>

NK, Not known; NGU, non-gonococcal urethritis; PID, pelvic inflammatory disease.

* No titre available.
results. We believe that the positive immunofluorescence results were genuine, and Table 3 shows corroborative evidence (if any) in these cases which supports this belief. A recent study has also produced evidence that immunological methods for detecting chlamydiae may be more sensitive than cell culture in women with PID (Mahony et al. 1987).

For the detection of chlamydial infection, immunofluorescence offers many advantages over culture. First it is quicker, with results theoretically available within 30–60 min. Handling of the specimen is simpler and does not require access to a −70°C freezer, as is the case for specimens taken for chlamydial culture (Evans & Woodland 1983). Although it is impossible to define precisely the accuracy of the test, most workers are satisfied with the specificity and the sensitivity (Thomas et al. 1984).

As with any STD, contact tracing is mandatory, and in keeping with previous studies (Jacob et al. 1987), we found a significant number of infected male partners. The fact that all the sexual partners with gonococcal or non-gonococcal urethritis were symptomless makes it unlikely that they would have sought medical attention, thus allowing them to act as a potential source of reinfection.

Women with lower abdominal pain will continue to be managed in gynaecology units, and this should continue to be the case in view of the frequency with which operative intervention is required. However, in view of the high frequency of STD in such a population it is essential that gynaecologists familiarize themselves with and implement the basic principles of management of STD with particular regard to diagnosis, treatment and contact tracing. Optimal patient care requires joint management, and a burden therefore falls upon genitourinary physicians to be available for consultation.

Acknowledgments

We thank the consultants, junior staff, sisters and nurses of the gynaecology unit of the Royal Infirmary of Edinburgh for their invaluable help, and Mr Angus MacAulay of the department of bacteriology who looked at the immunofluorescence slides.

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Received 25 April 1988
Accepted 7 June 1988
Mackie & McCartney
Practical Medical Microbiology

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THIRTEENTH EDITION

Volume 2 of Medical Microbiology

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1989
against the microorganism and labelled with a dye that fluoresces under UV light.

The second level at which antigen-antibody combination can be detected depends on the development, after primary union, of certain changes in the physical state of the complex, resulting in precipitation or agglutination of the components or, alternatively, in the activation of non-antibody components such as serum complement or histamine from mast cells. Reactions of this type occurring subsequent to primary union are termed secondary phenomena. This discussion is concerned with the principles of a few of these secondary phenomena which are in common use.

Before considering these reactions individually, it is important to be aware of the difficulties in interpreting results of such tests. The initiation and development of the secondary phenomena constitute a complicated series of events involving many variables such as the type of antibody taking part, the relative proportions of antibody and antigen, characteristics of the antigen molecule, presence of electrolytes, inhibitory substances and unstable components.

Despite these formidable difficulties, the widely and long used secondary phenomena such as precipitation, agglutination and complement fixation have an important role to play as aids in the diagnosis of disease and in the identification of microorganisms.

The secondary phenomena, as already indicated, can bring about several readily observable changes when carried out in vitro and these are used in tests to demonstrate the presence of antibody in the sera of patients suffering from infectious disease, or producing an antibody response to cell antigens as might, for example, occur after incompatible blood transfusion, tissue grafting or in autoimmune states.

Reactions of this type can also be used to identify antigens in the tissue or body fluids and, for example, would be utilized for blood grouping, tissue typing or the identification of microorganisms.

Among the most important of these reactions are precipitation, which occurs between antibody and antigen molecules in soluble form; agglutination, in which the antibodies directed against surface antigens of particulate materials such as microorganisms or erythrocytes link them together in large clumps or aggregates; and complement fixation in which antibody molecules, after reaction with antigen, activate the complex blood components which make up serum complement.

In addition to these widely used serological tests, a number of other effects of antigen-antibody interaction are of medical importance. These include neutralization tests used for example in virus identification, immobilization tests with bacteria and protozoa and skin tests for the reaginic antibody characteristic of anaphylactic states.

The advent of monoclonal antibodies, many of which are now available against microbial antigens, is having an increasingly important impact in the detection and identification of these antigens. As these antibodies become more readily available they are likely to replace many of the conventional antisera used in serological diagnosis (see Winstanley & Blackwell 1986).

Quantitative tests: dilutions and titres

In diagnostic serology it is often necessary to determine the amount of a specific antibody in the patient's serum in order to distinguish between the presence of a large amount of antibody produced in response to a current infection and that of a small amount of 'natural' or cross-reacting antibody unrelated to the patient's illness. In routine tests it is impracticable to isolate the specific antibody and measure its mass. Instead, the amount of the antibody is estimated by determining the greatest degree to which the serum may be diluted without losing the power to give an observable effect in a mixture with the specific antigen. Different dilutions of the serum are tested in mixtures with a constant amount of antigen and the greatest reacting dilution is taken as the measure, or titre, of the concentration of antibody in the undiluted serum.

Dilutions may be expressed in one of two ways: (1) they may be expressed in terms of the way in which they are made, e.g. a dilution of '1 in 8' is a dilution made by mixing one volume...
Fig. 10.2 The composition of an antigen-antibody precipitate is determined by the proportions or ratios of the components in the reaction.

up with antibody, so that very little linking can take place between the complexes (as in tube 3 of Fig. 10.2).

The precipitin test can be carried out in a quantitative manner by estimating the protein content of the precipitate at optimal proportions. The qualitative test is much more widely used and is of considerable value in detecting and identifying antigens, having applications in the typing of streptococci or pneumococci. This is done by layering an extract of the organism over antiserum in tubes. After a short while, a ring of precipitate forms at the interface (this is called the ring test). The technique is also used in forensic studies and in detecting adulteration of foodstuffs.

A modification of the test in which precipitation is allowed to occur by double diffusion in agar gel is very widely used for detecting the presence of antibody in serum or antigen in unknown preparations, and is valuable for showing the identity of different antigen preparations (Fig. 10.3). A concentration gradient forms in the gel, the concentration of a substance decreasing as the molecules diffuse away from the well in which they were placed. Precipitin bands form in the gel in the position where the

Fig. 10.3 Immunodiffusion or gel diffusion test. Wells are cut in a layer of agar in a Petri dish. Antiserum and antigen solutions are placed opposite each other in the wells and after allowing a few days for diffusion to take place precipitin bands will form where antibody and antigen meet in suitable proportions (optimal proportions). No reactions take place with antigens C and D as the antiserum in the central well contains antibodies only for antigens A and B. Lines of identity as formed between the two A wells enable the technique to be used for identifying unknown antigens.
Fig. 10.4 Diagram of viewing box for immunodiffusion.

Fig. 10.5 Commonly observed patterns found in double diffusion plates where two antigen solutions are compared using antiserum as the analytic agent.
The preparation is examined at intervals with the viewing box depicted in Figure 10.4. Any precipitin bands formed can be recorded by drawing the pattern obtained on a piece of paper or the preparation can be photographed. Staining of the bands with a protein stain improves the clarity of the patterns and may even show up precipitin bands which cannot be seen in the unstained preparation. Such stained preparations can conveniently be dried and kept for reference. A suitable procedure for staining and drying is as follows:

1. The unprecipitated protein is washed out of the agar by immersion of the slide for 24 h in the buffer used to make up the agar solution.
2. The slide is then washed for 15 min in 1% acetic acid to remove excess salts.
3. Staining is carried out with a protein stain such as naphthalene black made up to a 1% solution in a solvent containing glacial acetic acid 1 ml, distilled water 49 ml, and methylated spirit 50 ml. Staining should be carried out for about 30 min with this stain.
4. Excess stain is washed out with the solvent to give a preparation with dark blue precipitin bands on a clear background.
5. The preparation is finally soaked in 1% acetic acid containing 1% glycerol for 15 min and dried at 37°C in an incubator.

**Crossed immunoelectrophoresis (CIE)**

As with immunoelectrophoresis the proteins in the antigen under examination are first separated by agarose gel electrophoresis after which they are electrophoresed at right angles to the original direction into an antibody-containing agarose gel. The precipitates that form show up as sharp peaks (rockets), the height of the peak being determined by the concentration and mobility of the protein. The method also shows up heterogeneity of antigens or identity of various components by fusion or overlapping of patterns. The usual procedure (see Fig. 10.7) is to use $5 \times 7.5$ cm glass slides coated with a layer of 1% (w/v) agarose ($4 \text{ ml}$) in barbitone buffer. The agarose is cut into 5 strips of $5 \times 1.2$ cm with a razor blade and the strips slightly separated from one another. As in immunoelectrophoresis a small well is bored in each strip about 1 cm from one end and in this case close to one of the longitudinal sides of the strip. After placing the sample under test (2–5 μl) into the wells electrophoresis is carried out at 6 V/cm for 1–2 h. Each strip is then transferred to another $5 \times 7.5$ cm slide, precoated with 0.5% agar (0.2 ml/cm²) and dried overnight at room temperature (or 4–6 h at 40°C). Agarose to which antibody has been added is then poured on to the slide using a level table and adding between 3 and 3.5 ml of the mixture per slide.

**Countercurrent immunoelectrophoresis**

In countercurrent immunoelectrophoresis antiserum is placed in one well and the antigen
Fig. 10.9 Principle of hormone assay by agglutination inhibition. Red cells coated with hormone are agglutinable by anti-hormone antibody. The addition to the antiserum of a test sample containing free hormone will block the antigen-binding sites and prevent agglutination. The test can be carried out quantitatively by comparing the activity of a known standard hormone preparation with the test sample.

Fig. 10.10 Virus haemagglutination inhibition test. Above: cell agglutination is brought about by a variety of viruses (see text). Below: this can be inhibited by mixing the virus with anti-viral antibody as shown in the diagram. The test can be quantitated by comparison of serial dilutions of virus alone and virus-antibody mixture.
Agglutination is carried out with undiluted serum, e.g. in typing pneumococci or typing streptococci by Griffith's method, and it is necessary to use as small a quantity as possible. The method may be applied likewise for identifying organisms of the salmonella and dysentery groups. Slide agglutination is practicable only when the clumping of organisms occurs within a minute or so; it is not suitable where the mixture of organisms and serum has to be incubated.

The procedure can be carried out quite readily on an ordinary slide, but where a number of agglutination tests have to be made it is more convenient to use a piece of 60 mm polished plate glass c. 5 × 15 cm. A long horizontal line is ruled with a grease pencil through the middle of the glass from end to end and then a number of lines are ruled at 1 cm intervals at right angles to this line, thereby dividing the glass into a series of divisions.

A drop of saline is placed in one of the divisions and a small amount of culture from a solid medium emulsified in it by means of an inoculating loop. It is then examined through a hand lens (× 8 or × 10), or the low-power microscope, to ascertain that the suspension is even and that the bacteria are well separated and not in visible clumps. With a small loop, 1.5 mm diameter, made from thin platinum wire take up a drop of the serum and place it on the slide just beside the bacterial suspension. Mix the serum and bacterial suspension and examine with the hand lens, or place on the stage of the microscope. Agglutination when it occurs is rapid and the clumps can be seen with the naked eye, but the use of some form of magnification is an advantage. For control purposes, two drops of saline can be placed in adjacent divisions and bacterial culture emulsified in both, one only being mixed with the serum. With streptococci a broth culture is used, and methods for obtaining suitable suspensions for the agglutination test are described in Chapter 17. Two drops of suspension are placed on the slide and a small loopful of the serum mixed with one of them and examined as described above.

While the slide agglutination test is rapid and convenient, its limitations must be realized. In order to obtain rapid agglutination the serum is used undiluted or in low dilutions. In consequence, it may contain normal agglutinins which give non-specific agglutination with organisms other than that against which the serum was prepared. Thus, with regard to the Salmonella group particularly, slide agglutination with its high concentration of agglutinins may show low-titre reactions with organisms outside the group which may also have somewhat similar biochemical reactions. It is important therefore to confirm the slide test by quantitative tests in tubes, particularly when any doubt arises or where precise results from agglutination tests are desired. In these tube tests, the demonstration that the organism is agglutinated by the stated maximum effective dilution (titre) of the diagnostic serum confirms the specificity of the reaction and excludes the possibility that low-level cross-reactions are responsible for the positive slide test.

Agglutinin-absorption tests

Agglutinins, like other antibodies, combine firmly with their homologous antigens, and by treating an agglutinating antiserum with the homologous bacteria and then separating the organisms by centrifuging, it is found that the agglutinin has been 'absorbed' or removed by the organisms from the serum.

In certain cases, to prove the serological identity of an unknown strain with a particular species, it may be necessary to show not only that it is agglutinated by a specific antiserum to approximately its titre but also that it can absorb from the serum the agglutinins for the known organism. This becomes necessary owing to the fact that, on immunizing an animal with a particular bacterium, 'group antibodies' for allied organisms are developed, and in some cases these may act in relatively high titre. Absorption with a heterologous strain would remove only the group agglutinins without affecting the specific agglutinin. These effects are exemplified in the Salmonella and Brucella groups.

The general method of carrying out such absorption tests is to mix a dense suspension of
Fig. 10.12 Schematic representation of coagglutination reaction. Presence of antigen demonstrated by cross-linking of specific antibody attached to *Staphylococcus aureus*.

with the control reagent. Absence of reaction with the test reagent indicates a negative result irrespective of any reaction with the control reagent. Occasionally a COA test may be non-interpretable owing to a reaction of similar strength with the test and control reagents. Reagents are stable at 4°C although IgG may slowly leach away from protein A resulting in a loss of sensitivity. Sensitivity may sometimes be restored to a suspension by washing.

Reagents for the identification of *Neisseria gonorrhoeae* (Ch. 21) and serogrouping of *Streptococcus pyogenes* A, B, C, D or G (Ch. 17) are available commercially and are used widely. Reagents are also available commercially for detecting meningococcal, pneumococcal and haemophilus antigen in CSF. COA may be more sensitive than CIE for detecting bacterial antigen in CSF (Jones 1979). However, because protein A staphylococci agglutinate non-specifically with human IgG it is necessary to absorb the CSF with stabilized staphylococci before testing for antigen.

**Passive agglutination**

In passive agglutination antigens or antibodies are non-specifically absorbed to a carrier such as latex polystyrene beads of uniform diameter (0.8 μm) or erythrocytes. Latex polystyrene beads coated with denatured human IgG were first used to detect rheumatoid factor in serum.

Latex particles coated with globulin from antisera to meningococci, *Haemophilus influenzae* type b or from pneumococcal omni-serum can be used to detect the corresponding antigen in cases of pyogenic meningitis (Fallon 1983). Antigen in the CSF will cause agglutination of the appropriate particles within a few minutes. For the test, two drops of CSF are placed in each of four rings of a glass agglutination plate. One drop of one of the types of coated latex particle is placed in each ring. The slide is gently rocked for up to 3 min when the pattern of agglutination is read. If more than one suspension of latex is agglutinated the result is regarded as a false positive. Reagents are reasonably stable and sensitized latex particles retain their sensitivity for at least 4–6 months if kept at 4°C.

One of the most widely used passive agglutination tests employing erythrocytes is the *Trepomonema pallidum* haemagglutination (TPHA) test for the serological diagnosis of treponemal infection (Ch. 40). Sheep or avian erythrocytes are first treated with formaldehyde and tannic acid before being sensitized with a sonicate of pathogenic *T. pallidum*. When this reagent (available commercially) is mixed with patient's serum and allowed to stand for a few hours a diffuse carpet of haemagglutination is formed if anti-treponemal antibody is present. In the absence of specific antibody the sensitized erythrocytes settle to form a compact button of cells.
LABELLED ANTIBODIES

Fluorescence labelled antibody

The precise localization of tissue antigens or the antigens of infecting organisms in the body, of anti-tissue antibody and of antigen-antibody complexes was achieved by the introduction of the use of fluorochrome-labelled proteins. The absorption of UV light between 290 and 495 nm by fluorescein and its emission of longer wavelength green light (525 nm) is used to visualize protein labelled with this dye. The technique is more sensitive than precipitation or complement fixation techniques, and fluorescent protein tracers can be detected at a concentration of the order of 1 μg protein/ml body fluid.

It is advisable to use high avidity antibody from which the immunoglobulin fraction has been purified by salt fractionation (see Johnstone & Thorpe 1982). Antibody (conventional or monoclonal) against individual immunoglobulin classes and subclasses can be obtained commercially conjugated with fluorescein isothiocyanate. If preparing conjugates as described below, conjugates with a satisfactory labelling ratio of dye to protein have an optical density ratio at 495 nm and 280 nm approaching unity. Values below 0.5 indicate low labelling and over 1.5 are likely to result in non-specific staining. A suitable dilution of conjugate to test for obtaining this ratio is usually about 1 in 40. Performance assessment will need to be carried out to establish an optimal working dilution in the test. A useful way to establish specificity of an anti-immunoglobulin is to stain bone marrow cells obtained from patients with a myeloma of known paraprotein type. Conjugates should be freed of non-attached dye by chromatography on Sephadex G-25, and stored in aliquots at -20°C.

It is recommended that prior to examining smears of microorganisms obtained from a patient for attached antibody it is necessary to remove 'blocking' proteins by glycine-HCl buffer, pH 2.4, prior to staining with the fluorescein-conjugated anti-immunoglobulin. When looking for antibody in patient's serum, immunoglobulin of one class can prevent the detection of immunoglobulin of another class by saturating the antigenic sites on a microorganism. To avoid this, prefractionation of the serum will be required.

Many of the problems of non-specific staining are simply overcome by using diluted conjugates. The appropriate dilution will have been obtained by performance assessment as described above. Recommendations for the testing of fluorochrome-labelled anti-immunoglobulin antibodies are given in the MRC Working Party Report on the use of immunochemical reagents (MRC 1971). For further details of these methods see Johnson & Holborow (1986) and Johnstone & Thorpe (1982).

Some of the uses to which the technique has been put include the localization of the origin of a variety of serum protein components, for example immunoglobulin production by plasma cells and other lymphoid cells. The demonstration and localization in the tissues of antibody globulin in a variety of autoimmune conditions has been shown, including an antinuclear antibody in the serum of patients with systemic lupus erythematosus and thyroid autoantibodies in the serum of patients with Hashimoto's thyroiditis. In the diagnostic field most human pathogens can be demonstrated by immunofluorescence and a tentative diagnosis may be made much sooner than by cultivation; the fluorescent method at present can be used to supplement rather than replace conventional methods.

There are two main procedures in use, the direct and indirect methods (Fig. 10.14). The direct method consists of bringing fluorescein-tagged antibodies into contact with antigens fixed on a slide (e.g. in the form of a tissue section or a smear of an organism), allowing them to react, washing off excess antibody and examining under the UV light microscope. The site of union of the labelled antibody with its antigen can be seen by the apple-green fluorescent areas on the slide. The indirect method can be used both for detecting specific antibodies in sera or other body fluids and also for identifying antigens. This method differs from the direct method in the use of a non-labelled antiserum which is layered on first, in the same way as described above. Whether or not this antiserum has reacted with the material on the slide is
(made up fresh before use) so that the final mixture contains 10% buffer.

2. The solution is chilled to 4°C and 0.05 mg of fluorescein isothiocyanate (BDH) per mg of protein is added. During the addition of the fluorochrome and for the next 18 h the mixture should be stirred continuously, e.g. with a magnetic stirrer, and kept at 4°C.

4. Following conjugation excess fluorescein is dialysed away against phosphate buffered saline (0.01 mol/litre potassium phosphate buffer, pH 7.3; see Ch. 5) in the cold. Changing the buffer frequently until the dialysate contains no further dye.

5. The conjugate is finally centrifuged for 45 min at 3000 g at 4°C to remove any precipitated denatured protein.

**Conjugation with lissamine rhodamine B (RB200).** The fluorochrome is used as the sulphonyl chloride prepared by grinding 0.5 g of RB200 with 1 g of PCl₃ in a mortar (using a fume cupboard). After mixing, 5 ml of acetone is added with stirring for a few minutes and the mixture filtered. This solution should be used within 48 h of preparation. The conjugation process is similar to that for fluorescein isothiocyanate except that 0.2 ml of the solution is used for every 100 mg of protein and after mixing with the globulin stirring need only be continued for 30 min.

**Storage of conjugates.** Conjugates may be stored at 4°C with the addition of preservative, e.g. merthiolate 1 in 10,000; alternatively they may be stored at -20°C or freeze dried. Prior to use non-specific fluorescence will require to be absorbed from the conjugate.

**Enzyme labelled antibody**

Similar principles are used as described above except that the antibody is conjugated with an enzyme such as horseradish peroxidase. The technique has the advantage that after addition of the enzyme substrate the stained preparation can be stored. Fluorescein preparations in contrast fade after exposure to UV light. The techniques are fully described in Johnstone & Thorpe (1982) and Weir et al (1986, Ch. 27) and reagents are available commercially (see also Enzyme immunoassay below).

**Fluorescence polarization**

Fluorescein-labelled compounds are also used in fluorescence polarization immunoassay (FIA). FIA is becoming increasingly popular for measuring antibiotic levels in serum. The method, which is extremely rapid, combines competitive protein binding with fluorescence polarization to give a direct measurement without the need for a separation process. The test is based on the principle that antibiotic in the patient sample will compete with fluorescein-labelled antibody for a limited number of binding sites on antibodies specific for the antibiotic being measured. The concentration of unlabelled antibody from the patient sample will determine how much fluorescein-labelled antibody can bind to the specific antibody. A sophisticated optical detection system (Fluorescence Polarization Analyser) is used to measure the increase in the polarization of fluorescent light which results when the fluorescein-labelled antibody binds to antibody. The concentration of antibiotic in the patient's serum is determined from an instrument-stored calibration curve of polarization values versus drug concentration. Reagents are available commercially (Abbott) for measuring antibiotics such as gentamicin, amikacin and tobramycin. FIA systems have not yet been used for detection of microbial antigens.

**Enzyme immunoassay**

Enzyme immunoassays are of two types - the homogeneous type without washing stages (the EMIT system, Syva), and the heterogeneous types known as enzyme-linked immunosorbent assays (ELISA). In the homogeneous assays inactivation of the enzyme label by antibody makes it unnecessary to separate bound from unbound compound as in other immunoassay methods. Homogeneous assays are very rapid but are not very sensitive and are only suitable for the measurement of small molecules.

Competitive protein binding assays such as
assay. For further details of radioimmunoassay methods see Bolton & Hunter (1986).

MISCELLANEOUS TESTS

The following tests may also find application in some laboratories.

Immobilization. This test depends on the principle that antibody combining with an antigen on a locomotor organ (flagellum, cilium) results in inhibition of motility. The classical application of this reaction is the Treponema pallidum immobilization test for the serological diagnosis of syphilis (Ch. 40).

Complement lysis. In the presence of complement antibody combines with a microbial surface antigen causing lysis of bacterium or enveloped virus. Used mainly in research with organisms such as Vibrio cholerae, Escherichia coli and Neisseria gonorrhoeae.

Immune electron microscopy. Antibody combining with viral or surface antigen causes clumping of virus particles visualized by electron microscopy. Used mainly in virus research. The procedures are described in Weir et al (1986, Ch. 35).

'Capsule swelling'. Mixing capsulate bacteria (e.g. pneumococci, Klebsiella) with homologous antibody makes possible the direct, microscopic visualization of capsules. At one time the antibody was thought to make the capsule visible by increasing the size of the capsule, i.e. the 'Quellung' or swelling reaction; there is some evidence that swelling can occur when the reaction with antibody takes place in the presence of complement, but whether to a visible extent is doubtful (Ch. 18, Methods).

Toxin neutralization. Homologous antibody prevents the biological effect of toxin as observed with experimental animals (e.g. intracutaneous or intravenous administration of a mixture of Clostridium perfringens toxin and antitoxin to guinea-pigs or mice) or with special culture media (e.g. Nagler reaction, Ch. 37).

Infection with Streptococcus pyogenes (Ch. 17) or Staphylococcus aureus (Ch. 16) may result in antibody against bacterial haemolysin appearing in the serum. These antibodies are detected by incubating the serum with standard amounts of active haemolysin and noting the degree of inhibition of haemolysis when erythrocytes are added to the mixtures.

Detection of immune complexes. Complexes of antigen and antibody have been demonstrated in many infections and are sometimes responsible for pathological changes, e.g. focal nephritis and endocarditis in streptococcal infections and liver damage and polyarthritis in hepatitis B infections. Many methods are available to detect immune complexes including cryoprecipitation, precipitation by polyethylene glycol and detection of complement components in complexes. A commercial test (Pharmacia) is available for use in diagnosis of bacterial endocarditis. A range of techniques and their limitations are described in Weir et al (1986, Ch. 128).

Table 10.1 Immunological methods used in investigation of microbial and parasitic infections. (For details see appropriate sections in Weir et al, 1986, Vol. 4, Chs 119–123.)

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<td>Crossed immunoelectrophoresis (CIE) and modifications for identification of antigens of Neisseria meningitidis, N. gonorrhoeae, Pseudomonas, Clostridium difficile, Spiroplasma, Salmonella.</td>
</tr>
<tr>
<td>Virology</td>
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<td>Double diffusion in agar and CIE for identification and quantitation of many viral antigens, e.g. adenovirus and vaccinia virus. Radioimmunoprecipitation for characterization of antigens of membrane-bound viruses that are difficult to purify, e.g. measles, respiratory syncytial virus. Also in routine use to define antigenic specificity of monoclonal antibodies.</td>
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<td>Mycology</td>
</tr>
<tr>
<td>Immunodiffusion for detection of precipitins to Aspergillus, Candida, Micromonospora. WHO reference method for antibodies to histoplasmin; serodiagnosis of other systemic mycoses, e.g. coccidioidomycosis. CIE and modifications for detection of immunological responses to many fungi.</td>
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Utility of monoclonal antibody coagglutination to identify *Neisseria gonorrhoeae*

H Young, A Moyes
Utility of monoclonal antibody coagglutination to identify *Neisseria gonorrhoeae*

**H Young, A Moyes**

From the STD Diagnostic Laboratory, Department of Bacteriology, University Medical School, Edinburgh

**Summary** The Phadebact monoclonal gonococcus coagglutination test was evaluated with 1367 (996 anogenital and 371 pharyngeal) neisserial isolates from patients who gave positive diagnostic test results for *Neisseria gonorrhoeae*. The overall correlation with carbohydrate utilisation was 99.7%. The Phadebact test had a specificity of 100% (286/286) and a sensitivity of 99.7% (1077/1080). The three non-reactive strains were epidemiologically linked and were of a very unusual serovar. Unlike polyclonal antibody based tests, the monoclonal antibody test provides reliable identification of gonococci from anogenital and pharyngeal sites. Because non-reactive strains are rare, however, negative anogenital isolates from heterosexual patients should be tested biochemically. The use of two reagents comprising separate pools of monoclonal antibodies against gonococcal protein IA and IB permitted the identification of an appreciable number of double infections, which would otherwise have been missed. Genital, rectal, and pharyngeal isolates from the same patient should be identified individually.

The microbiological diagnosis of gonorrhoea is particularly challenging as several anatomical sites may be infected. In general, the greater the number of sites examined the better will be the chance of detecting gonococcal infection. The range of colonising neisserials other than the gonococcus varies widely between different anatomical sites, however, which has an important bearing on the utility of identification methods. There is currently a wide array of identification tests, which include conventional and rapid sugar degradation tests, new chromogenic substrate identification systems, and immunological methods. The precise role of the various identification methods remains to be assessed.

Many laboratories experience difficulties with carbohydrate utilisation tests, organisms such as *Neisseria meningitidis* and *Branhamella (Moraxella) catarrhalis* being a major cause of diagnostic problems. The failure of many biochemical identification systems to differentiate between *N cinerea* and *N gonorrhoeae* has serious implications as it may lead to misdiagnosis. Although *N cinerea* gave negative results in the Phadebact polyclonal antibody coagglutination test, occasional cross reactivity with *N meningitidis* and *N lactamica* limited the use of this test to isolates from genital sites. The use of reagents prepared from monoclonal antibodies that recognise epitopes on gonococcal protein I, provided that the reagents are sufficiently sensitive, should overcome problems of specificity. Immunological identification should thus be reliable for isolates from all anatomical sites.

This study analyses the utility of the Phadebact (Pharmacia Diagnostics, Sweden) monoclonal gonococcus coagglutination test to identify routinely *N gonorrhoeae* cultured from genital, rectal, and pharyngeal sites.

**Patients and methods**

We studied all patients examined for gonococcal infection at the department of genitourinary medicine of Edinburgh Royal Infirmary in September 1985 to December 1986. Material from anogenital and pharyngeal sites was inoculated directly on to modified New York City medium. After being incubated overnight at 37°C in an aerobic atmosphere enriched with 5% carbon dioxide, cultures were examined for oxidase positive Gram negative diplococci (GND). Suspect neisserial organisms were then tested by the Phadebact monoclonal gonococcus coagglutination test. Whenever GND were isolated from more than one site in the same patient, genital (urethral or cervical), rectal, and pharyngeal isolates were tested separately.
Utility of monoclonal antibody coagglutination to identify Neisseria gonorrhoeae

PHADEBACT MONOCLONAL GONOCOCUS
COAGGLUTINATION TEST
This test kit contains two separate coagglutination reagents, WI and WII/III, which are prepared with pools of monoclonal antibodies reactive with protein IA and protein IB, respectively. A suspension of suspect organisms was prepared in 0-9% saline and boiled for 10 minutes. After cooling, the boiled antigen was tested against the WI and WII/III reagents (unit volume 20 µl): the respective reagents were mixed thoroughly with the antigen by rocking and tilting the slides. Results were read within one minute: a reaction with either the WI or WII/III reagent constituted a positive result and provided instant serotyping.

If there was insufficient material on the primary plate after overnight incubation a subculture was made and the isolate identified next day.

CONFIRMATORY IDENTIFICATION
A rapid carbohydrate utilisation test, modified for performance in microtitre trays, was used to confirm the coagglutination results and to identify non-reactive GNDC.

SEROTYPING
Isolates were serotyped with two panels of monoclonal antibody coagglutination reagents as described previously.

STATISTICAL ANALYSIS OF RESULTS
The significance of differences in the results was assessed by the χ² test with Yates’s correction. Predictive values were calculated according to the formula given by Vecchio.

Results
A total of 888 patients (341 women and 547 men) gave positive diagnostic test results, and 43 yielded positive test of cure cultures for Neisseria gonorrhoeae. Infection at more than one site occurred in 147 (43-2%) of the 341 women, which resulted in 504 isolates for identification (table 1): 496 of these isolates were tested by coagglutination as well as the rapid carbohydrate utilisation test. The remaining eight isolates from five women (two with genital and rectal infection, one with genital and throat infection, and two with genital infection only) were not available for coagglutination testing.

Infection at more than one site occurred in 10 (17-5%) of the 57 homosexual men and in 34 (6-9%) of the 490 heterosexual men, and resulted in 592 isolates for identification (table 2): 584 of these isolates were tested by both coagglutination and the rapid carbohydrate utilisation test. The remaining eight isolates, which were from men with urethral infection only, were not available for coagglutination testing.

Throat cultures were taken from 795 (89-5%) of the 888 infected patients. GNDC were isolated from 348 (43-8%) of the 795 patients (table 3).

Excluding the 101 gonococcal throat isolates included in tables 1 and 2, but allowing for the 23 patients with dual isolation of neisseriae, a further 270 coagglutination tests were performed. Coagglutination tests were also performed on 17 anogenital isolates (table 4) confirmed as being N meningitidis by the rapid carbohydrate utilisation test.

CORRELATION BETWEEN COAGGLUTINATION AND RAPID CARBOHYDRATE UTILISATION TESTS
The results for the coagglutination and rapid carbohydrate utilisation tests applied to the 1367 (996 anogenital and 371 pharyngeal) isolates described above are given in table 5. The overall agreement between coagglutination and the rapid utilisation tests was 99.8%. Coagglutination had a specificity of 100% and a sensitivity of 99-7%. The three non-reactive strains were isolated during a restricted time, were epidemiologically linked, and were of the very unusual serovar combination Bj/Bro.

The predictive value of positive coagglutination test

<table>
<thead>
<tr>
<th>Pattern of infection*</th>
<th>No of patients</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital</td>
<td>Rectal</td>
<td>Pharyngeal</td>
</tr>
<tr>
<td>Positive Positive Positive</td>
<td>16</td>
<td>48</td>
</tr>
<tr>
<td>Positive Positive Negative</td>
<td>108</td>
<td>216</td>
</tr>
<tr>
<td>Positive Negative Negative</td>
<td>175</td>
<td>175</td>
</tr>
<tr>
<td>Positive Negative Positive</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>Negative Positive Negative</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Negative Negative Positive</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>341</td>
<td>504</td>
</tr>
</tbody>
</table>

*Results of culture for Neisseria gonorrhoeae.
Genital = urethral or cervical, or both.

Table 2 Pattern of infection in 57 homosexual (and 490 heterosexual) men with positive diagnostic test results

<table>
<thead>
<tr>
<th>Pattern of infection*</th>
<th>No of patients</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethral Rectal Pharyngeal</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Positive Positive Positive Positive</td>
<td>17 (448)</td>
<td>17 (448)</td>
</tr>
<tr>
<td>Positive Positive Negative Negative</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Negative Positive Positive Positive</td>
<td>3 (8)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Positive Negative Positive Positive</td>
<td>5 (34)</td>
<td>10 (68)</td>
</tr>
<tr>
<td>Negative Negative Positive Positive</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>57 (490)</td>
<td>68 (524)</td>
</tr>
</tbody>
</table>

*Results of culture for Neisseria gonorrhoeae.
Table 3  Range of Gram negative diplococci (GNDC) cultured from the throats of 795 patients

<table>
<thead>
<tr>
<th>Organism(s)</th>
<th>Patient group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterosexual men (n = 461)</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>33</td>
</tr>
<tr>
<td>N gonorrhoeae and N meningitidis</td>
<td>9</td>
</tr>
<tr>
<td>N meningitidis</td>
<td>148</td>
</tr>
<tr>
<td>N meningitidis and N lactamica</td>
<td>2</td>
</tr>
<tr>
<td>N meningitidis and N perflava</td>
<td>0</td>
</tr>
<tr>
<td>N lactamica</td>
<td>5</td>
</tr>
<tr>
<td>N flava</td>
<td>1</td>
</tr>
<tr>
<td>N perflava</td>
<td>0</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>0</td>
</tr>
<tr>
<td>Not identified*</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>199</strong></td>
</tr>
</tbody>
</table>

*Failed to grow on subculture.

results was 100% for GNDC isolated from any site. The predictive value of negative coagglutination test results (PV−) varied depending on anatomical site, and it decreased as the proportion of gonococci in the total population of GNDC increased. The PV− was 99.9% for GNDC isolated from the throat, where the incidence of gonococci was 27.2% (101/371, table 3). The PV− was 98.9% for GNDC isolated from the rectum of men, where the incidence of gonococci was 78.4% (29/37, tables 2 and 4), and 96.5% for GNDC isolated from the urethra of homosexual men, where the incidence of gonococci was 92.3% (24/26, tables 2 and 4). The PV− was 66.8% for GNDC isolated from the urethra of heterosexual men, where the incidence of gonococci was 99.4% (482/485, tables 2 and 4) and 75.2% for GNDC isolated from anogenital sites in women, where the incidence of gonococci was 99.1% (460/464, tables 1 and 4).

DISTRIBUTION OF SEROGROUPS IN ISOLATES FROM DIAGNOSTIC TESTS AND TEST OF CURE CULTURES

Table 6 shows the distribution of serogroups in 872 patients with positive diagnostic test results. Three of six women with double infection had genital infection with a serogroup WI isolate and rectal infection with serogroup WII/III; the converse occurred in the remaining three. The man with a double infection had throat infection with serogroup WI and rectal infection with serogroup WII/III. This represents infection with more than one strain of gonococcus in 4.1% (6/147) of women and one in 10 homosexual men who were infected at more than one site.

Fifteen isolates (shown as serogroup WII/III) reacted with both the WII/III and WI reagent pools, although the reaction was stronger with the WII/III reagent. These isolates were of the highly unusual serovar Av/Bx.11

As shown in table 7, WII/III isolates predominated in positive test of cure cultures. In heterosexual patients significantly fewer (χ² = 9.4; p < 0.01) isolates from diagnostic tests (48.5%, 395/815) than test of cure cultures (75%, 30/40) were of serogroup WII/III.

DIRECT SMEAR RESULTS IN RELATION TO SEROGROUP OF INFECTING ISOLATE

Of the 341 women with positive diagnostic tests, 285 had valid smear and serogroup results: no smear

Table 5  Correlation between results of rapid carbohydrate utilisation and Phadebact monoclonal gonococcus coagglutination tests

<table>
<thead>
<tr>
<th>Biochemical identification</th>
<th>Coagglutination results:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>1077</td>
<td>1080</td>
</tr>
<tr>
<td>N meningitidis</td>
<td>0</td>
<td>268</td>
</tr>
<tr>
<td>N lactamica</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>N perflava</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Branhamella catarrhalis</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Not identified*</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1077</strong></td>
<td><strong>290</strong></td>
</tr>
</tbody>
</table>

*One throat isolate failed to grow on subculture.
Table 6  Distribution of serogroups in 872 patients with positive diagnostic test results

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No (%) of isolates with serogroup:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Women</td>
<td>333*</td>
</tr>
<tr>
<td>Heterosexual men</td>
<td>482†</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>872</td>
</tr>
</tbody>
</table>

*Of the 341 infected women, isolates from five were not available for coagglutination testing and three were infected with coagglutination negative isolates.
†Of the 490 infected heterosexual men urethral isolates from eight were not available for coagglutination testing.

Results were available for 42 patients, isolates from five patients were not serogrouped, six patients had a mixed infection, and three were infected with coagglutination negative isolates. The incidence (51.9%) of serogroup WI in 129 isolates from smear positive patients was not significantly different from the incidence (57.7%) in 156 isolates from smear negative patients (data not shown).

Dual isolation of Neisseria gonorrhoeae and Neisseria meningitidis from the throat

As shown in table 3, gonococci were isolated alone in 81 (10.2%) of the 795 throat cultures examined, meningococci alone in 231 (29.1%), and both gonococci and meningococci in 20 (2.5%) of patients. Based on the 81 positive gonococcal cultures and the 231 positive meningococcal cultures, the theoretical isolation rate for both organisms together is 2.9%: that is not significantly different from the observed coexistence of 2.5%.

Discussion

The high specificity (100%) and sensitivity (99.7%) of the Phadebact monoclonal gonococcus coagglutination test, combined with the simplicity of its performance, makes it an ideal first line screening test for characterising neisseriae from primary cultures. In contrast to earlier coagglutination tests with polyclonal antibodies, the absolute specificity of the monoclonal test makes it reliable for differentiating between gonococcal and non-gonococcal neisseriae isolated from any anatomical site. Because of the rare occurrence of a non-reactive serovar, however, coagglutination negative isolates from anogenital sites in heterosexual patients should be confirmed by a biochemical test.

A sensitivity of 99.7% is probably as high as can be expected, given the immunological diversity of gonococcal isolates. By combining the results of two independently prepared panels of monoclonal antibodies, Sandstrom et al recognised a total of 27 WI serovars and 92 WII/III serovars in isolates collected in worldwide epidemiological studies. During a six month period at the beginning of the study we recognised 15 WI and 33 WII/III serovars, although this increased to 22 and 58 respectively during the total study period (Coghill and Young, unpublished observations).

The use of separate monoclonal antibody pools to detect isolates bearing protein IA and IB epitopes may help in providing high sensitivity. Evaluations of a coagglutination reagent containing a different set of protein IA and IB monoclonals in a single pool gave poorer results, with sensitivities ranging from 99.1% to 96.6%. In addition, separate antibody pools would simplify the "tailoring" of reagents to provide optimum detection in distinct geographical regions should that be required. Although our evaluation included most known serovars, a local outbreak caused by a rare non-reactive serovar could substantially alter the performance of the test. The ability to show that non-reactive isolates are of the same rare serovar, however, should aid contact tracing and help prevent non-reactive strains becoming endemic in a given locality.

The use of two separate reagent pools also enabled us to detect infection with more than one strain of gonococcus in 4.1% of 147 women and one in 10 homosexual men with infection at more than one site. As each case of double infection represented infection at genital and rectal (or pharyngeal) sites with different serogroups, we consider it worthwhile to identify genital, rectal, and pharyngeal isolates from the same patient as separate isolates. Backman et al found two strains of gonococci with different serovars isolated from two (1%) of 218 women, two (7%) of 28 heterosexual men, and three (25%) of 12 homosexual men who yielded two or more gonococcal isolates on the same occasion. Only two of the seven patients were infected with strains of different serogroups at the same site. The remaining five patients were infected with two strains of the same serogroup but with
different serovars, which were isolated from different sites. The problems and importance of recognising double infections have been discussed by Bygdeman.15

The serogroup of the infecting strain of gonococcus has been correlated with signs and symptoms of urethritis, men infected with serogroup WI strains having fewer leucocytes per high power field than those infected with WII/III strains (p < 0.05).17 A similar correlation in female patients might have an important influence on the sensitivity of detecting infection by Gram staining of cervical exudates. This did not appear to be the case. Although serogroup WI isolates were more common (57-7%) in smear negative patients than smear positive patients (51-9%), the difference was not significant. The serogroup of the infecting strain did influence the outcome of treatment, however, with WII/III isolates predominating in positive test of cure cultures (p < 0.01). This finding is in keeping with the decreased susceptibility to antibiotics of WII/III isolates.11

The absolute specificity of the monoclonal coagglutination test makes it particularly reliable for excluding non-gonococcal neisseriae and therefore of particular value in clinics where screening for pharyngeal gonorrhoea is performed routinely on patients with anogenital infection.

By performing the coagglutination test direct from the primary isolation plate we were able to detect small amounts of gonococcal antigen in a predominantly meningococcal growth, which enabled us to isolate meningococci and gonococci together as often as expected theoretically. Previous analysis of the isolation of neisseriae from the pharynx showed a significant difference (p < 0.001) between the observed and theoretical levels of coexistence of gonococci and meningococci in the pharynx.18

Although biochemical test systems such as Gonocheck have the potential to identify non-gonococcal neisseriae to species level, their accuracy remains to be established in large scale clinical trials using direct testing from primary isolation plates. Whereas Brown and Thomas found Gonocheck 100% specific for 28 isolates of N meningitidis, mainly taken direct from the primary isolation plate,19 initial testing of 55 meningococci from primary cultures yielded an accuracy of only 94.5%. In general, the accuracy of Gonocheck is increased by repeat testing, and when this is taken into account the test has a sensitivity in the range of 98-9%20 to 100%21-25 for N gonorrhoeae. This suggests that Gonocheck would be a useful test for confirming the identity of immunologically non-reactive GNDC strains from anogenital sites. When used in this way, however, Gonocheck may misidentify N cinerea, which is non-reactive with monoclonal antibodies against gonococcal protein I, as a gonococcus.5

We conclude that the Phadebact monoclonal gonococcus coagglutination test is a valuable first line test to identify gonococci from all potentially infected sites. Because of temporal and geographical differences in the antigenic profile of circulating gonococci, however, it is important for specialised centres to monitor the serovars of circulating gonococci in representative geographical areas. The identity of all coagglutination negative isolates from anogenital sites in heterosexual patients should be confirmed by biochemical tests, and the serovar of the isolate should be identified. The recent finding of a neisserial isolate with antigenic characteristics of a gonococcus and biochemical properties of a meningococcus24 highlights the importance of accurate and reliable identification methods.

References


Utility of monoclonal antibody coagglutination to identify Neisseria gonorrhoeae

Screening for treponemal infection by a new enzyme immunoassay

H YOUNG, A MOYES, A McMILLAN, D H H ROBERTSON
Screening for treponemal infection by a new enzyme immunoassay

H YOUNG,* A MOYES,* A MCMILLAN,† D H H ROBERTSON†

From the *Department of Bacteriology, University of Edinburgh Medical School, and the †Department of Genitourinary Medicine, Royal Infirmary, Edinburgh, and Genitourinary Medicine Unit, Department of Medicine, University of Edinburgh, Edinburgh

SUMMARY A new enzyme immunoassay (EIA, Captia Syphilis-G) for detecting IgG antibodies against Treponema pallidum was evaluated as a screening test for syphilis. When serum samples were tested at a dilution of 1 in 20 (EIA_{20}), the overall agreement between the IgG EIA and serological status based on the T pallidum haemagglutination assay (TPHA) and the fluorescent treponemal antibody absorption (FTA-ABS) test was 99-2% (1310/1321). The sensitivity of the EIA_{20} was 98-4% (60/61) and the specificity 99-3% (1251/1260). Discrimination between patients with and without treponemal infection was good: the mean EIA_{20} absorbance ratios (patient/mean low titre positive control results) were 0-49 for antibody negative patients, 3-30 for patients with positive Venereal Diseases Research Laboratory (VDRL) test and TPHA results, and 1-77 for patients with negative VDRL but positive TPHA results. The cut off point for excluding treponemal infection was taken as 0-9. Specimens with ratios of more than 0-9 should be confirmed by the FTA-ABS test and evaluated for specific IgM antibodies to treponemes. When serum samples were tested at a 1 in 50 dilution (EIA_{50}) the sensitivity was lower (80-3%) but the specificity was absolute. The reduction in sensitivity correlated with low absorbance ratios in the patients who were VDRL negative and TPHA positive.

The screening performance of the IgG EIA_{20} is thus comparable with that provided by a combination of the VDRL test and TPHA. The potential for automation makes the EIA an attractive alternative, particularly in larger centres. Alternatively, the test can be performed at a 1 in 50 dilution (EIA_{50}), at which level it is ideally suited for confirming the treponemal status of antibodies in serum samples preselected by positive cardiolipin antigen screening test results.

Serological screening is important in detecting syphilis. Because of the present low prevalence of syphilis, large numbers of specimens must be tested to detect a relatively small number of antibody positive patients. A screening combination comprising the Venereal Diseases Research Laboratory (VDRL) test and Treponema pallidum haemagglutination assay (TPHA) as suggested in 1974 is now used widely and is generally considered to provide an effective screen for all stages of syphilis. Unfortunately the VDRL and TPHA test combination does not lend itself readily to automation, and results are usually read subjectively and recorded manually. A single test that could be readily automated and had comparable screening performance, in terms of reading and recording results, would be an attractive proposition to larger laboratories.

The potential for fully automated tests was appreciated by Veldkamp and Visser, who described an enzyme linked immunosorbent assay (ELISA) for serodiagnosing syphilis as early as 1975. Several workers have since developed "in house" ELISAs using T pallidum, the axial filament of T phagedenis biotype Reiter, or cardiolipin, cholesterol, and lecithin as antigen. In contrast, commercially available tests have been slow to appear, and published evaluations have focused on the Bio-Enza Bead test.

We report a preliminary evaluation of the Captia Syphilis-G test (Mercia Diagnostics, Guildford, Surrey), a recently developed novel enzyme immunoassay (EIA) to detect IgG antibodies to treponemes.
Patients and methods

SERUM SAMPLES AND SEROLOGICAL SCREENING
To assess the screening performance of the IgG EIA we used 1280 unselected serum samples submitted to the department of bacteriology for serological tests for syphilis. The specimens were from patients attending the following: a genitourinary medicine (GUM) clinic (764), an antenatal clinic (302), a general hospital, including a blood transfusion service (142), and general practitioners (72).

Blood specimens were allowed to clot at room temperature, and a portion of the serum was separated, usually without centrifugation. The original blood tubes were stored at room temperature. Serum samples were inactivated at 56°C for 30 minutes before being screened for antibody to cardiolipin with the VDRL carbon antigen test (Wellcome Diagnostics), the TPHA (Fujirebio), and the IgG EIA. In the case of samples giving a positive or equivocal reaction in the VDRL test or TPHA, or both, a further portion of serum was separated from the original blood tube and the specimen tested again quantitatively and also examined by the fluorescent treponemal antibody absorption (FTA-ABS) test.

CAPTIA SYPHILIS-G TEST
This is an indirect IgG EIA that uses a microtitre plate of 6 x 16 wells coated with a sonicate of T pallidum. The test was carried out according to the manufacturer's instructions. Basically, a 1 in 20 dilution of serum was made by adding 20 µl serum to 380 µl dilution buffer (PBS containing 0.05% Tween (polysorbate) 20 and 0.01% thiomersal), pH 7.0-7.2. Samples of diluted serum (100 µl) were then added to designated wells, and the plate was incubated at 37°C for one hour. After incubation the wells were aspirated and washed with buffer using a Titrertek S8/12 microplate washer set on a standard five rinse cycle. Tracer complex (100 µl), comprising biotinylated monoclonal antibody against human IgG and streptavidin conjugated with horseradish peroxidase, was added to each well and the plate was incubated at 37°C for a further hour. The wells were then aspirated, washed as above, and 100 µl of substrate (tetramethylbenzidine in dimethylsulphoxide) was added to each well. The plate was allowed to incubate at room temperature for 30 minutes, and the reaction was stopped by adding 25 µl 2 mol/l sulphuric acid to each well. On gently tapping the plate the blue colour of reactive samples changed to a uniform yellow colour, the absorbance of which was read at 450 nm using a Titrertek Multiskan MCC/340 plate reader blanked on air.

Test validation and interpretation
The test results were validated by running negative and low titre positive (LTP) kit controls in duplicate. The run was considered valid if: the mean absorbance of the negative control was less than 0.25 and the mean absorbance of the LTP control was more than 1.5 times that of the negative control.

The cut off point in the EIA was determined in relation to the absorbance of the LTP control. Results were scored as follows: test absorbance < 0.9 x mean LTP = negative; test absorbance 0.9 - 1.1 x mean LTP = equivocal; test absorbance > 1.1 x mean LTP = positive.

Specimens with equivocal or positive results were tested by the FTA-ABS test and tested again by the IgG EIA at dilutions of 1 in 20 (EIA\textsubscript{20}) and 1 in 50 (EIA\textsubscript{50}). These additional tests were performed alongside the quantitative VDRL and TPHA tests.

Specific IgM antibody to treponemes was also shown by the Captia Syphilis-M (Mercia Diagnostics), an IgM capture EIA.

STATISTICAL ANALYSIS
The significance of differences in the results was analysed by the \( \chi^2 \) test with Yates's correction.

Results
Table 1 shows the pattern of serological results obtained on screening 1280 samples. The overall correlation between the three screening tests was 94.6% (1211/1280). Eighty two (6.4%) specimens required further investigation. The number (and percentage) of specimens requiring further investigation as a result of screening with individual tests was: VDRL 22 (1.7%), TPHA 41 (3.2%), and IgG EIA\textsubscript{20} 60 (4.7%). Our current screening combination of VDRL.

<table>
<thead>
<tr>
<th>VDRL</th>
<th>TPHA</th>
<th>IgG EIA\textsubscript{20}</th>
<th>No of specimens with corresponding pattern of results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>Equivalent</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>1198</td>
<td>17</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1220</td>
<td>19</td>
</tr>
</tbody>
</table>

IgG EIA\textsubscript{20} = IgG enzyme immunoassay of serum samples diluted 1 in 20. Results determined by absorbances compared with that of low titre positive (LTP) control in kit (negative = absorbance < 0.9 x mean LTP; equivocal = absorbance 0.9-1.1 x mean LTP; positive = absorbance > 1.1 x mean LTP).

VDRL = Venereal Diseases Research Laboratory (test).

TPHA = Treponema pallidum haemagglutination assay.

*Positive result includes equivocal reactions.

\( \dagger \) = One specimen gave prozone reaction in the VDRL screening test; repeat quantitative testing gave reaction at titre of 1/64. The EIA\textsubscript{20} gave strongly positive reaction.
Table 2  Productive and non-productive results in specimens positive on routine screening

<table>
<thead>
<tr>
<th>Test</th>
<th>No tested again</th>
<th>Screening result confirmed as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDRL</td>
<td>22</td>
<td>Positive</td>
</tr>
<tr>
<td>TPHA</td>
<td>41</td>
<td>Positive</td>
</tr>
<tr>
<td>IgG EIA&lt;sub&gt;20&lt;/sub&gt;</td>
<td>19</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

For meanings of abbreviations see table 1.
*One specimen positive in VDRL and TPHA; one positive in TPHA only; testing both again gave positive results in IgG EIA<sub>20</sub>.

Productive = confirmed by positive fluorescent treponemal antibody absorption (FTA-ABS) test results and history or current clinical diagnosis of treponemal infection.
Non-productive = negative on repeat testing or FTA-ABS test, or both, and no history or current clinical evidence of syphilis.

and TPHA tests necessitated the further investigation of 50 (3.9%) specimens, which was not significantly different from the number produced by IgG EIA<sub>20</sub>

Table 2 shows the 82 positive screening test results divided into productive (confirmed by a positive FTA-ABS test result and history or current clinical diagnosis of treponemal infection) and non-productive (negative on repeat testing or FTA-ABS negative, or both, and no history or clinical evidence of syphilis). Of the nine specimens giving non-productive VDRL test results, four remained positive for cardiolipin antibody only and five were negative when tested again quantitatively. Of the 12 specimens giving non-productive TPHA test results, five gave non-specific agglutination reactions and seven gave negative reactions when tested against control cells. The outcome of retesting the 28 samples giving productive and 32 giving non-productive IgG EIA<sub>20</sub> results is given in tables 3 and 4, respectively.

Table 3  Results of retesting 28 specimens yielding productive results in IgG EIA<sub>20</sub> screening tests

<table>
<thead>
<tr>
<th>Test</th>
<th>IgG EIA results:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>EIA&lt;sub&gt;20&lt;/sub&gt; screen</td>
<td>0</td>
</tr>
<tr>
<td>EIA&lt;sub&gt;20&lt;/sub&gt; repeat</td>
<td>41</td>
</tr>
<tr>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
<td>8</td>
</tr>
</tbody>
</table>

*Both specimens gave positive results on retesting in the EIA<sub>20</sub>.
Two specimens positive on screening gave equivocal results on retesting in the EIA<sub>20</sub>.
For definitions of results of IgG EIA see table 1.

Table 4  Results of retesting 32 specimens yielding non-productive results in IgG EIA<sub>20</sub> screening tests

<table>
<thead>
<tr>
<th>Test</th>
<th>IgG EIA results:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>EIA&lt;sub&gt;20&lt;/sub&gt; screen</td>
<td>0</td>
</tr>
<tr>
<td>EIA&lt;sub&gt;20&lt;/sub&gt; repeat</td>
<td>23</td>
</tr>
<tr>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
<td>32</td>
</tr>
</tbody>
</table>

For definitions of results of IgG EIA see table 1.

Regarding the 28 productive results, no cases of treponemal infection were detected by the VDRL test that were not also detected by the TPHA and the IgG EIA<sub>20</sub>. Of the 29 patients with syphilis, one positive in the TPHA at a titre of 1/80 and giving a weak positive FTA-ABS reaction gave an IgG EIA<sub>20</sub> value of 0.69 (cut off value = 0.9). Table 3 shows that the results were reproducible with serum samples from the remaining 28 patients with treponemal infection. Although there was some interchange between the equivocal and positive categories, none of the specimens had results below the cut off point. In contrast, retesting at 1 in 50 dilution (IgG EIA<sub>20</sub>) was

Table 5  Combined results for 1321 specimens tested by IgG EIA at two dilutions

<table>
<thead>
<tr>
<th>VDRL</th>
<th>TPHA</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG EIA:</td>
<td>Dilution</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>NT</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative Negative</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>NSA</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative Positive</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive Positive</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
<td>1252</td>
</tr>
<tr>
<td></td>
<td>EIA&lt;sub&gt;20&lt;/sub&gt;</td>
<td>31</td>
</tr>
</tbody>
</table>

NSA = Non-specific agglutination. For meanings of other abbreviations see table 1.
NT = Not tested apart from 42 specimens positive or equivocal on screening (five in VDRL, seven in TPHA, 15 EIA<sub>20</sub> equivocal, and 15 IgG EIA<sub>20</sub> positive). All 42 specimens were FTA-ABS negative and EIA<sub>20</sub> negative.
*Untreated primary syphilis.
Screening for treponemal infection by a new enzyme immunoassay

Fig 1 Distribution of absorbance ratios for 1321 patients: (a) ratios less than 1.69, (b) ratios above the cut off point of 0.9.
insensitive, and eight specimens had results below the cut off value.

On retesting the 32 specimens from non-infected patients (table 4), 23 gave a negative reaction in the IgG EIA, and all were negative in the IgG EIA. Of the nine remaining positive specimens four were from patients attending the department of genitourinary medicine, three were from antenatal patients, one was from a potential semen donor, and the final patient was a man aged 85 who had severe dementia.

**COMBINED PERFORMANCE BY SCREENING AND TESTING ADDITIONAL TREPONEMAL AND NON-TREPONEMAL SERUM SAMPLES**

To confirm the sensitivity of the test, we tested an additional 32 treponemal serum samples by the IgG EIA at 1 in 20 (EIA) and 1 in 50 (EIA). To confirm the specificity an additional nine non-treponemal serum samples (eight with antibody to cardiolipin only, and one non-specific agglutination reaction in the TPHA) were also tested at both dilutions. Table 5 shows the results from testing those samples combined with the screening results.

From the combined results (table 5) the overall agreement between the IgG EIA results and serological status based on TPHA and FTA-ABS test results was 99-2% (1310/1321). The overall sensitivity of the EIA was 98-4% (60/61) and its specificity 99-3% (1251/1260). IgG EIA testing showed absolute specificity, but resulted in a sensitivity of only 80-3% (49/61), which was significantly lower ($\chi^2 = 9-7; p < 0-01$) than that of the IgG EIA.

An accurate history was available for 37 patients with positive serological test results who attended the GUM clinic. Unfortunately such an accurate history was not available from the other patients. One of the 37 GUM patients with positive serological test results was diagnosed as having untreated early latent syphilis; the remaining 36 were diagnosed as having treated syphilis. The number of patients treated at each stage of infection and the date when treated (range in years) were as follows: six with primary (1947–1986), 12 with secondary (1976–1984), two with early latent (1984), 10 with latent (1967–1987), one with cardiovascular (1966), three with neurological (1967–1987), and two with congenital syphilis (1977 and not known).

Positive IgM capture EIA results were given by the patient with untreated early latent syphilis and a patient with untreated primary syphilis referred from another source. Two of the treated patients gave a positive IgM capture EIA result: one woman had been treated for primary infection 11 months earlier and had a positive result in the TPHA at a titre of 1/80 and a weak positive FTA-ABS reaction, and the second patient, who had been treated for neurosyphilis in 1976, gave positive results in the VDRL at a titre of 1/16 and in the TPHA at a titre of 1/5120. He was assessed for further treatment.

The patient with the negative IgG EIA result was a woman aged 30 considered to have latent syphilis who had coincidentally been treated previously; she gave weakly reactive TPHA (at a titre 1/80) and positive FTA-ABS test results on two separate occasions.

Figure 1 shows that good discrimination was seen between patients with and those without treponemal infection. Of the eight patients with absorbance ratios of more than 4 (fig 1b), four had values between 4-0 and 6-45, three between 4-5 and 4-99, and one between 6-0 and 6-49. The mean IgG EIA absorbance ratio was 0-49 for antibody negative patients, 3-30 for patients with positive VDRL and TPHA test results, and 1-77 for patients with a negative VDRL but positive TPHA results. The mean IgG EIA absorbance ratios for the latter two groups were 2-48 and 1-49 respectively. These differences are reflected in table 5, which shows that equivocal EIA and negative EIA reactions were associated with VDRL negative, TPHA positive patients but not those VDRL and TPHA positive. Those VDRL negative and TPHA positive contained most of the patients with low (1/80 or 1/160) TPHA titres. The “best-fit” line (fig 2) resulting from plotting absorbance ratio against log TPHA titre confirms that low absorbance ratios tended to be associated with low TPHA titres and that a few infected patients gave absorbance ratios in the equivocal range (0-9–1-1). The cut off point of 0-9 therefore seems to be correct, as 47 patients without treponemal antibody yielded absorbance ratios in the range 0-8 to 0-89 (fig 1a).

![Fig 2 Correlation between absorbance ratios in IgG enzyme immunoassay (EIA) and titres in Treponema pallidum haemagglutination assay (TPHA).](image-url)
Discussion

In the United Kingdom, during the five years 1981-85 new cases of syphilis decreased by 36% from 3228 to 2054 in men and by 32% from 983 to 669 in women. This represented an age specific rate for people aged 20-24 of about 12 per 100 000 population. Despite decreases in syphilis and gonorrhoea, clinic workloads have been substantially increased since national publicity about AIDS.16 This trend towards an increase in the number of patients to be screened for treponemal infection increases the need for more highly automated centralised serological testing, to which the EIA technology is ideally suited.

Our "in use" evaluation of the new IgG EIA showed that the test provided good discrimination between antibody positive and antibody negative patients. It also confirmed that the cut off point for the test was established correctly for screening purposes. The overall sensitivity of 98.4% (60/61) and specificity of 99.3% (1251/1260) compares very favourably with published results on the commercially available Bio-Enza Bead test.

The sensitivity of the Bio-Enza Bead test has been reported as 75%, 99.5%, and 95% respectively in studies of 42, 218, 219 and 202 patients with syphilis. These differences in sensitivity may be related to differences in the serological status of the respective test populations. For example, the sensitivity was 75% when evaluated in patients of whom 72.4% reacted in the VDRL, 99.5% when all 218 serum samples were VDRL reactive, and 95% when 94% (190/202) were VDRL reactive. Our finding that the mean IgG EIA ratio was 3.3 for patients with positive VDRL and TPHA results and 1.77 for patients with a negative VDRL but positive TPHA results supports the above interpretation. Considering that only 41% (25/61) of our infected patients were VDRL positive, the sensitivity of 98.4% suggests that the IgG EIA is likely to be more sensitive than the Bio-Enza Bead test in detecting late, including latent, infection irrespective of treatment status. False negative Bio-Enza Bead test results occurred in 10.5% (9/86) of patients with late latent syphilis and in 2.6% (1/38) of patients in whom the stage could not be assessed. The only non-reactive sample in another study was from a patient with treated late latent disease.

The ability of a new serological test to detect VDRL negative treponemal infection is of vital importance as about 30% of cases of untreated late syphilis may fall into this category.2 Detection of VDRL negative serum samples is also important in assessing whether a patient has been treated for syphilis in the past. The mean period of VDRL reactivity after treatment is four months for primary syphilis, 13 months for early latent disease, 17 months for the secondary stage, and 60 months for latent infection of indefinite duration.2 The positive IgG EIA result for all seven of our patients with treated primary syphilis, even those treated up to 40 years previously, confirms the very high sensitivity of this test in detecting treated infection.

Serological detection of untreated early infection is obviously important in the overall control of disease. The VDRL test has been regarded as having particular value in detecting early primary infection because of the poor sensitivity of the TPHA at this stage of the disease.1 The variable sensitivity of the TPHA in primary syphilis has been attributed to variation in the capacity of the reagents to bind IgM.2 The first demonstrable sign of humoral response to infection is the production of IgM class antibodies, which can be detected in the serum towards the end of the second week of infection. IgG production begins soon after that of IgM, and IgG becomes detectable in the serum around the fourth or fifth week after infection.2 Theoretically, therefore, there is a small "window" of two to three weeks during which very early primary infection may escape serological detection when screened with an EIA that uses antibody to human IgG conjugate. Perhaps a more important diagnostic limitation exists in centres that rely solely on VDRL testing. About 1% of patients with secondary syphilis will have a negative result if undiluted serum is used in the test procedure, and the test becomes positive on further dilution of the serum; the so called prozone phenomenon.

In practice it is very unlikely that the "negative window" in very early infection would present a problem. Other workers have found that, even in primary syphilis, IgG was the main immunoglobulin present.12 Although only one patient in our study had untreated primary syphilis, the serum was reactive. The Bio-Enza Bead test (which also utilises an antibody to human IgG conjugate) was reactive in five of six11 and all three12 patients with untreated primary infection. Clearly the sensitivity of the IgG EIA in detecting untreated primary syphilis requires further study. Taking into account the results outlined above, however, and the present very low incidence of primary infection, the need for further data on test performance in untreated primary infection does not detract from the use of the IgG EIA for screening purposes, particularly as detecting most cases of very early primary infection depends on a high level of clinical suspicion. In this context newer methods of direct immunofluorescence staining with monoclonal antibodies specific for the pathogen should prove helpful in examining suspect early lesions.18

Apart from sensitivity, the specificity of a test has an important bearing on its suitability for screening. The specificity of the IgG EIA was 99.3% (97.4% without
repeat testing) for our “in use” evaluation of over 1300 patients representative of the population screened for syphilis. This included over 750 patients attending a GUM clinic, many with other sexually transmitted diseases. The specificity of 99-3% compares very favourably with that found in more selective evaluations of the Bio-Enza Bead test. The latter test gave a specificity of 98% for 100 sera from “normal” people, 98-3% for 60 sera reactive in non-treponemal tests but non-reactive in the FTA-ABS test, and 98% for 304 VDRL non-reactive premartial samples.

With reference to the above data on sensitivity and specificity, we disagree with the view that the main role of the EIA, as has been suggested for the Bio-Enza Bead test, is as an alternative to the FTA-ABS test. The Bio-Enza Bead test, because it lacks sensitivity in late and latent disease, can only be used as a confirmatory test when non-treponemal tests are used for screening and serum samples have been selected by reactivity in a cardiolipin antigen test. In our view it is desirable to screen with treponemal antigen tests to detect treated or partially treated, as well as untreated, treponemal infection. We therefore advocate that the EIA should be considered as a replacement for the present screening combination of VDRL and TPHA. The potential for automation could also be of some cost benefit.

The sensitivity and specificity of the IgG EIA when performed at a 1 in 20 dilution, make it ideal for screening purposes; positive reactions can be confirmed by the FTA-ABS test and evaluated for specific IgM antibodies to treponemes. By performing the test at a 1 in 50 dilution, however, the sensitivity and specificity are ideally suited to confirming the treponemal nature of serum samples preselected on the basis of VDRL reactivity. The only exception might be cases of very early untreated primary syphilis, but this limitation could be overcome by testing VDRL reactive sera in parallel with the IgM capture EIA, which is performed at a recommended serum dilution of 1 in 50. In general, if the IgG EIA is used for screening, reactive serum sample should be tested for specific IgM antibodies to treponemes to detect reinfection and to monitor the efficacy of treatment.

We thank Mercia Diagnostics, Guildford, Surrey, for providing the reagents for this evaluation and Dr Anton F H Luger, Hofrat professor and head of the Ludwig Boltzmann Institut für Dermatovenerologische Serodagnostik, University of Vienna, for providing a specimen from a patient with untreated primary syphilis.

References
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Practical Medical Microbiology

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THIRTEENTH EDITION

Volume 2 of Medical Microbiology

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1989
Treponema: serological tests for syphilis

The general term spirochaete is often used to embrace Treponema species and organisms of similar spiral morphology belonging to the genera Borrelia and Leptospira (see Ch. 41).

Syphilis is an infectious venereal disease caused by Treponema pallidum. A primary lesion or chancre tends to appear on the genitalia at the site of entry of treponemes. The disease is systemic from the onset and the natural course of infection may span several decades (Robertson et al 1988a). T. pallidum is the only pathogenic treponeme indigenous to Britain. Other morphologically indistinguishable treponemes that are pathogenic for man include T. pertenue, the cause of yaws, a non-venereal but communicable disease found in tropical countries, and T. carateum, the cause of pinta, a mild contagious disease similar to yaws but confined to Central and South America.

These pathogenic treponemes cannot be cultured in vitro, either alone or with mammalian cells of various types. In contrast commensal treponemes can be cultured successfully in artificial media. Many commensal species occur in the mouth (e.g. T. macrodentium and T. microdentium) and on the mucous surfaces of the genitalia (e.g. T. calligyrum, T. gentilis) where their differentiation from T. pallidum is of importance in the diagnosis of primary syphilis.

TREPOEMENA PALLIDUM

Morphology and staining

A very delicate, spiral filament 6–14 µm (average 10 µm) by 0.2 µm, with 6–12 coils which are comparatively small, sharp and regular; the length of the coils is about 1 µm and the depth 1–1.5 µm; the ends are pointed and tapering. A capsular or slime layer has been observed occasionally on the surface of T. pallidum and may explain the lack of serological reactivity of organisms freshly isolated from animal tissues. A multilayered membrane, referred to as the outer envelope or outer membrane, encloses the cell; the viability of spirochaetes is dependent on an intact outer envelope.

Inside the outer envelope lie the axial filaments or internal flagella (Johnson 1977); these are presumed to be responsible for motility although there is no direct evidence for this. Spirochaetes show rotary corkscrew-like motility and also movements of flexion; angulation, with the organism bending almost to 90° near its centre, is highly characteristic of T. pallidum. Its progression is relatively slow compared to that of many motile bacteria.

T. pallidum is feebly refractile and darkground illumination is normally used to visualize the organism. It cannot be seen by ordinary staining methods; special techniques such as silver impregnation may be used to demonstrate the organism, particularly in tissue, but this tends to alter the morphology. Immunofluorescent methods can now be used to detect treponemes in tissues and body fluids.

Cultivation

The Nichols strain of T. pallidum was isolated in 1913 from the CSF of a patient with neurosyphilis; it is still virulent for man and is main-
tained in rabbits by intratesticular inoculation and weekly passage. It divides by binary fission approximately once every 30 h when environmental conditions are favourable. It is generally agreed that pathogenic \(T. pallidum\) has not been cultivated in artificial media, embryonated eggs or tissue cultures. However, there is now a better understanding of the conditions that permit prolonged survival of treponemes in vitro, with retention of their pathogenicity for animals. Pathogenic treponemes (unlike cultivable non-pathogens) attach themselves to mammalian cells in culture. The discovery that \(T. pallidum\) is microaerophilic rather than a strict anaerobe has aided the prolonged survival of these microorganisms in vitro.

The genetic relationships between \(T. pallidum\) and cultivable treponemes have been studied by reassocation assays with \(^{125}\)I-labelled treponemal DNA (Miao & Feldsteel 1978). Three groups were distinguished: virulent Nichols strain of \(T. pallidum\); \(T. phagedenis\) and its biotype Reiter; and \(T. refringens\). Features such as the diameter and amplitude of the spiral, the number of axial filaments and the presence of intracytoplasmic microtubules can be used to differentiate between the pathogenic non-cultivable treponemes, which have similar if not identical morphology, and the cultivable treponemes (Hovind-Hougan 1976).

**Sensitivity to physical and chemical agents**

\(T. pallidum\) is so feeably viable outwith its host that syphilis is ordinarily acquired only by sexual intercourse. The organism dies rapidly in water and is very sensitive to drying. However, it can remain viable and maintain its virulence in necropsy material for some time at room temperature, and in serum kept in sealed capillary tubes it remains motile for several days. It is readily killed by heat, e.g. 41.5°C for 1 h. When infected blood is stored at 5°C in citrate anticoagulant, infectivity is lost in 120 h or less. Treponemes survive for only a few days or weeks at −10 to −20°C but remain viable for extended periods at −45°C and for an indefinite period when stored at −78°C. Freezing followed by desiccation (freeze-drying) kills the organism.

**Antibiotic sensitivity**

Penicillin remains the drug of choice in treating syphilis. Therapeutic regimens for early syphilis should aim to maintain a minimum serum penicillin concentration of 0.03 units/ml for a period of 10–15 days. Tetracycline or erythromycin appear to be effective in patients who are hypersensitive to penicillin but results have not been evaluated as fully as for penicillin.

Although \(T. pallidum\) is extremely sensitive to penicillin (healing of lesions occurs rapidly and treponemes disappear from early stage lesions), biological cure (i.e. eradication of treponemes) is difficult to prove since \(T. pallidum\) cannot be cultured in vitro. In a few patients who have been adequately treated with penicillin, residual \(T. pallidum\) has been detected in CSF, lymph nodes, etc. by electron microscopy: in a very few cases some of these treponemes were inoculated into rabbits and produced typical lesions. In such cases the surviving treponemes remained penicillin sensitive and these patients may be considered to indicate treatment failure, probably due to abnormal penicillin metabolism in the patients ('quick penicillin secretors'), rather than acquired drug resistance.

**Animal pathogenicity**

Intratesticular injection leads to a syphilitic orchitis in rabbits; intradermal inoculation also produces lesions. Experimentally infected rabbits have been widely used to test various anti-syphilitic drugs and to study the immune response to \(T. pallidum\). Monkeys and anthropoid apes can also be infected experimentally. At present the only source of \(T. pallidum\) for preparing antigens and for experimental work is from the testes of infected rabbits. Recently, however, \(T. pallidum\) antigens have been expressed in *Escherichia coli* after gene cloning (Dallas et al 1987).

**Laboratory diagnosis of syphilis**

The clinical diagnosis of syphilis is confirmed in the laboratory by (1) demonstrating \(T. pallidum\)
in the exudates from the lesions, or (2) demonstrating antibodies in the serum.

**Darkground microscopy**

The infectious stages of treponemal infections can usually be diagnosed most quickly and effectively by the demonstration of motile treponemes in wet preparations of serous exudate expressed from suspected primary and secondary lesions. Where topical antibiotics have been used, examination of material obtained by lymph gland puncture may prove useful.

As there is a serious risk of infection it is important to use gloves when obtaining material for darkground microscopy. After cleansing the surface of the lesion with a swab soaked in sterile saline, serum is squeezed by gentle pressure from the depth of the lesion. This serum may be collected directly on a glass coverslip or, if this is difficult, in a glass capillary tube. One end of the capillary is heated to expel fluid neatly on to the centre of a coverslip, which is then positioned on a slide. After firmly pressing the coverslip and slide between pieces of filter paper, the preparation can be examined by darkground illumination using the oil immersion objective.

*T. pallidum* is recognized by its slender structure, characteristic slow movements and angulation. It must be carefully distinguished from other treponemes that may occur in genital ulcers, but these tend to be surface organisms and are not found in the depth of the lesions. If the initial test is negative the procedure should be repeated daily for at least 3 days; antibiotics should be withheld during this period although sulphadimidine and local saline lavage may be used to reduce local sepsis. Since many commensal treponemes occur in the mouth, darkground microscopy is not suitable for examining oral lesions. Organisms are not easily found in skin lesions of secondary syphilis except those in moist skin areas.

A more definite approach to diagnosis is provided by **immunofluorescence staining**. A smear of the material to be tested is made on a glass slide, fixed and sent to the laboratory. The smear is then stained with fluorescein-labelled antibody specific for *T. pallidum* and examined by fluorescence microscopy (Daniels & Ferneyhaugh 1977). More recently, fluorescein-labelled pathogen-specific monoclonal antibodies have been used to identify *T. pallidum* in lesions (Lukehart & Baker-Zander 1988).

**Sero logical diagnosis of syphilis**

There is no demonstrable immunological difference between the treponemes responsible for syphilis, yaws or pinta. Although this should not often give rise to problems in the UK the possibility should be borne in mind with patients from areas where these diseases are endemic.

The various methods used to measure antibody responses in treponemal infection can be divided into two major categories: (1) tests to measure antibodies produced against non-specific treponemal antigens, i.e. the cardiolipin or lipoidal antigen tests, formerly referred to as 'reagin' tests; and (2) tests to measure antibodies against antigens specific for pathogenic treponemes, i.e. the *T. pallidum* haemagglutination assay (TPHA) and the fluorescent antibody absorbed test (FTA-ABS).

**Cardiolipin antigen tests.** Cardiolipin, a complex diphospholipid, is widespread in nature and can be isolated from many mammalian tissues as well as from treponemes. Cardiolipin for use as antigen is traditionally prepared from mammalian tissues such as beef heart. Only a few of the numerous tests for detection of antibodies to cardiolipin antigen are still widely used. The classical Wassermann complement fixation test has decreased markedly in popularity as it offers no advantage over the modern flocculation tests, such as the Venereal Diseases Research Laboratory (VDRL) test.* The World Health Organization (1982) now recommends that complement fixation tests should be discontinued; the VDRL test is simpler, quicker and cheaper to perform and should be adopted as standard.

In the rapid plasma reagin (RPR) test,* VDRL antigen is suspended in choline chloride and mixed with finely divided carbon particles.

* Refer to Methods at end of this chapter.
enabling the test to be performed on unheated serum or plasma and read with the naked eye. A fingerprint blood sample can be tested on plastic or paper cards, making the RPR test particularly suitable for use in field studies in developing countries. The test can also be automated for use in centres where large numbers of specimens must be tested.

Because of their simplicity and accuracy, the cardiolipin antigen tests are used as screening or first-line procedures for both routine diagnosis and mass screening programmes. These tests usually become positive 10–14 days after the appearance of the chancre, the titre gradually increasing. The titre diminishes and the test tends to become negative after treatment. In late or latent syphilis the cardiolipin antigen tests are often negative.

Since these tests detect antibodies against a non-specific antigen shared by treponemes and mammalian tissues a positive result is sometimes obtained with sera from healthy individuals or patients without clinical evidence of syphilis; these reactions are termed Biological False Positives (BFP). Tests using specific T. pallidum antigen are required to distinguish between positive cardiolipin antigen tests resulting from BFP reactions and those due to treponemal infection.

T. pallidum haemagglutination assay. The TPHA is very simple to perform and was the first of the specific tests suitable for routine screening. It is often negative in untreated primary syphilis (possibly owing to variability in the IgM-binding capacity of the TPHA reagent). For all other stages of syphilis, the sensitivity of the TPHA is comparable to that of the FTA-ABS (see below). Occasionally, false positive haemagglutination may result from heterophile antibody in the serum of patients with infectious mononucleosis. (This occurs only if the control cells fail to agglutinate, otherwise a non-specific agglutination reaction would be recorded.) In certain tropical countries a small percentage of BFP reactors have also given apparent false positive TPHA results; because of the sensitivity of the test, these could represent the residue of previous infection with endemic treponematoses.

Fluorescent antibody absorbed test. In the FTA-ABS test, binding of specific antibody by T. pallidum is demonstrated by the indirect immunofluorescence technique. The FTA-ABS is an accepted reference test and is highly specific and sensitive at all stages of syphilitic infection although a small percentage of false positive reactions occur, e.g. in patients with systemic lupus erythematosus and other connective tissue diseases.

Other serological tests. The T. pallidum immobilization (TPI) test (Nelson & Mayer 1949) was the first to use specific treponemal antigen but it has been superseded by the TPHA and FTA-ABS tests (Rein et al 1980; Sprott et al 1982). Because the TPI test employs live treponemes it is time-consuming, expensive and technically demanding. A few reference laboratories still perform the TPI test on selected sera for research purposes.

The Reiter protein complement fixation (RPCF) test detects antibodies produced against a group-specific treponemal antigen shared by pathogenic and commensal treponemes. Since the Reiter treponeme can be grown in relatively simple media sufficient antigen can readily be obtained for large scale screening. The RPCF test has now been superseded by the TPHA. The role of the enzyme-linked immunosorbent assay (ELISA) in the serological diagnosis of syphilis remains to be evaluated (Veldkamp & Visser 1975; Pope et al 1982).


Interpretation of serological tests

The pattern of results obtained with the VDRL, TPHA and FTA-ABS tests may give valuable information as to the stage of infection. Table 40.1 provides a guide, but it is important to remember that each case must be interpreted individually in the light of available clinical and epidemiological data. Because of the serious social and medical implications, a diagnosis of syphilis should never be made from the results of a single blood specimen.
of specific anti-treponemal IgM will denote active syphilis. Several methods for demonstrating specific IgM antibodies have been investigated.

One of the most widely used methods relies on the detection of anti-treponemal IgM with monospecific fluorescein-labelled anti-human immunoglobulin in the FTA-ABS test. The performance of this test with unfractionated serum (the IgM-FTA-ABS test) is unsatisfactory as false positive and false negative results are common. Reliable results are obtained when 19S (IgM) antibodies are separated from those of the 7S (IgG) class by gel filtration before performing the test; this test is known as the 19S-IgM-FTA. However this is a time-consuming and technically demanding procedure and is normally restricted to research and reference laboratories. Simpler methods of detecting specific IgM by haemagglutination and enzyme-linked immunosorbent assays are being investigated (Luger 1988).

Serological screening
The continuous serological screening of pregnant women, blood donors and 'at risk' groups is helpful in the detection and control of syphilis. When used together, the VDRL and TPHA tests provide a highly efficient screen for the detection or exclusion of treponemal infection; both are simple to perform and can be readily quantitated. Their activity is complementary; the VDRL test is more sensitive than the TPHA in the detection of very early syphilis while the TPHA is more sensitive than the VDRL in the detection of latent and late infection. The FTA-ABS is not suitable for screening large numbers of sera but should be used as a confirmatory test when one of the screening tests is positive. When both screening tests are unequivocally positive further testing is not essential but a confirmatory FTA-ABS test is usually done if available.

Congenital syphilis
Early-stage congenital syphilis is a rarity in the UK and there are few up-to-date serological data available. Since IgM antibodies do not cross the

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**Table 40.1 Pattern of results of serological tests in different stages of acquired syphilis.**

<table>
<thead>
<tr>
<th>VDRL</th>
<th>TPHA</th>
<th>FTA-ABS</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>False positive reaction: repeat to exclude primary infection</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Primary infection: dark-ground investigation of lesion may be positive</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Untreated (or recently treated); probably beyond primary stage</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Treated or partially treated at any stage: untreated latent or late</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>History of treated syphilis</td>
</tr>
</tbody>
</table>

Quantitative test results may also prove helpful. During the primary stage of infection the VDRL titre rises to 8 or 16. VDRL tests with titres of 16–128 are commonly found in secondary syphilis and active cardiovascular or neurosyphilis. After the secondary stage the VDRL titre declines and eventually becomes negative in c. 30% of untreated latent and late cases.

The TPHA test is often negative in early primary syphilis but may become positive at low titre (80–320) towards the end of the primary stage. Titres rise sharply during the secondary stage and commonly reach 5120 or greater. The TPHA titre declines during the latent stage but invariably remains positive at low titre (80–640).

Response to treatment. The cardiolipin antigen tests primarily reflect disease activity. These tests tend to become negative after treatment, particularly in early syphilis. Serial quantitative VDRL testing provides the best means of measuring response to treatment in most stages of treponemal infection.

Differentiation of treated, partially treated and untreated syphilis. The TPHA and FTA-ABS tests usually remain positive for life, even in those who have been fully treated with adequate doses of penicillin. On the premise that detection
intact placenta a reliable method for demonstrating specific IgM antibodies should indicate active infection of the neonate. Also, antibody titres will rise if a baby has been infected, whereas in the absence of infection, e.g., when the mother has been treated during pregnancy, passively transferred antibody detected by the VDRL will decrease in titre and the test will become negative in approximately 3 months; owing to their greater sensitivity treponemal antigen tests usually take slightly longer to become negative.

Diagnosis of neurosyphilis: examination of CSF

The use of CSF for routine screening tests in patients in whom there is no clinical suspicion of syphilis is unjustified; a negative TPHA test on the blood will virtually exclude active neurosyphilis and is a better screen for the detection of all forms of late syphilis.

However, in cases selected on clinical grounds backed by a positive TPHA test on blood, investigations should be carried out on the CSF to detect early invasion of the central nervous system (CNS). A total volume of 8–10 ml is usually sufficient to carry out the necessary tests; note that contamination of the CSF specimen with even a small amount of blood can give misleading results.

Investigation of the CSF should include a cell count, estimation of total protein and estimation of IgG and IgM. Cell counts exceeding 5 cells/mm³ (5 x 10⁶/litre) and total protein values above 40 mg/100 ml are signs of inflammation but are non-specific as indicators of syphilitic involvement of the nervous system. Specific tests such as the VDRL, TPHA and FTA-ABS should also be performed.

The VDRL test alone is not a reliable indicator of CNS involvement since it is non-reactive in 30–60% of patients with active neurosyphilis. However, a negative TPHA test in CSF excludes neurosyphilis. A positive TPHA or FTA-ABS test in CSF does not necessarily indicate active disease, since reactivity may be caused by transudation of immunoglobulins from the serum into the CSF. The TPHA index, which relates CSF TPHA titre to the albumin quotient (CSF albumin concentration x 10⁵/serum albumin concentration), should help exclude errors associated with disturbed function of the blood-brain barrier. The TPHA index and methods for the demonstration of specific IgM antibodies in CSF are being evaluated as indicators of active neurosyphilis (Luger 1988).

Thus, although active neurosyphilis can be excluded reliably and simply by a negative TPHA test result on the CSF, unequivocal serological evidence of CNS involvement is essentially a procedure for a specialized laboratory.

METHODS

The Venereal Diseases Research Laboratory (VDRL) test

This simple flocculation test of high sensitivity can be performed as a tube test but is more widely employed as a micro-slide test. A macroscopic test using carbon antigen is increasing in popularity.

Preparation of the specimen. Transfer a portion of serum from 5–10 ml of clotted blood into a clean tube. Heat in a waterbath at 56°C for 30 min and allow to return to room temperature before testing. Sera that are excessively haemolysed, grossly contaminated with bacteria, or very turbid are unsatisfactory for testing. Specimens to be tested more than 4 h after the original heating period should be reheated at 56°C for 10 min. Sera need not be heated for testing with carbon antigen.

Preparation of the antigen. VDRL antigen is a colourless alcoholic solution containing cardiolipin (0.03%), lecithin (0.2%) and cholesterol (0.9%). The antigen is widely available commercially in a variety of volumes; vials containing 0.5 ml are most convenient for the majority of laboratories. Buffered saline diluent is also available commercially and contains: 37% (w/v) formaldehyde neutral reagent, 0.5 ml; Na₂HPO₄ (anhydrous), 0.037 g; KH₂PO₄, 0.15 g; NaCl, 10 g; distilled water, 1000 ml. The diluent has a pH of 6.0 ± 0.1.
1. Pipette 0.4 ml of buffered saline into a 30 ml round bottle.
2. Add 0.5 ml of antigen (from the lower half of a 1 ml pipette graduated to the tip) directly on to the saline while continuously but gently rotating the bottle on a flat surface; the antigen should be added over a period of approximately 6 s.
3. Blow the last drop of antigen from the pipette without the pipette touching the diluent and continue to rotate for 10 s.
4. Add 4.1 ml of buffered saline from a 5 ml pipette.
5. Replace the top on the bottle and mix well by inverting approximately 30 times in 10 s.

The antigen suspension must be used only on the day that it is prepared; the amount (5 ml) is sufficient for approximately 250 tests. Check each batch of antigen with sera known to give negative, weak positive and positive reactions as defined below.

**Carbon antigen (RPR)** which requires no prior preparation can be obtained commercially (RPR Card Test Antigen, Gibco). It should be stored at 4°C but it is essential to allow the antigen to reach room temperature before use.

**VDRL screening test**

Screening tests can be carried out on glass slides (10 × 5 cm), each with six paraffin-ringed or ceramic-ringed areas; slides with concavities, wells, or glass rings are not recommended. Oxford-type pipettes and disposable plastic tips are convenient for measuring volumes.

1. Add 60 μl of serum to a clean glass slide.
2. Add 20 μl of antigen to the serum.
3. Mix with a wooden stick and rotate slide for 4 min; mechanical rotators should be set at about 180 rpm.
4. Read and test microscopically with a ×10 eyepiece and ×10 objective immediately after rotation.

Read the screening test as follows: negative, smooth homogeneous particles of antigen; weak positive, small clumps of antigen with little or no background clearing; positive, large clumps of antigen with marked background clearing. Any specimen giving a weak positive or positive reaction should then be tested quantitatively. Occasionally a test result will be intermediate between negative and weak positive; any serum giving this "rough" type of reaction should also be tested quantitatively in case of a prozone reaction (Ch. 10).

The test with **carbon antigen** is usually performed on disposable plastic cards (12.5 × 7 cm), each with 10 clearly defined test areas. Volumes of antigen and serum remain the same but the shaking time is extended to 8 min and the test is read macroscopically.

**Quantitative VDRL tests**

When performing quantitative tests it is a useful check to return to the original blood tube and remove additional serum. This should be heated at 56°C for 30 min as before.

1. Place 100 μl of 0.9% saline in five tubes (7 × 1 cm) for each serum to be tested.
2. Add 100 μl of serum to the first tube and double dilute to give a range of dilutions from 1 in 2 to 1 in 32.
3. Test the serum and dilutions as for the screening test (see above). Any serum giving a positive reaction at a dilution of 1 in 32 should be further diluted and retested to determine the endpoint.

Test results are reported as follows: **borderline reaction**, a weak positive reaction with undiluted serum and a negative reaction with all dilutions; **positive undiluted serum**, a positive reaction with undiluted serum and a weak positive or negative reaction with dilutions; **positive 2, 4, 8, etc.**, titre reported as the last serum dilution to give a positive reaction.

**The Treponema pallidum haemagglutination (TPHA) test**

In this test sheep erythrocytes coated with an extract of *T. pallidum* are agglutinated by antibody from the serum of patients with syphilis. To eliminate non-specific reactions, sera are first absorbed with a special diluent containing sonicated cell membranes from sheep and ox erythrocytes.
rocytes, normal rabbit testicular extract, sonicated Reiter treponemes, normal rabbit serum, Tween 80 and aceta powder. The following method employs commercial reagents supplied by the Fujizoki Pharmaceutical Company, Tokyo, Japan (distributed in the UK by Mast). Each kit comprises lyophilized test (antigen-coated) and control cells, absorbing diluent and a reactive control serum. (Reagents based on low erythrocytes are also available commercially from Whitley and from Wellcome.)

Preparation of the specimen. Prepare heat-inactivated serum as for the VDRL test (the TPHA may be performed without heat inactivation but the pattern of haemagglutination is more distinct with heated serum).

Preparation of the reagents. Kits with different volumes of reagents are available and enable approximately 100–500 screening tests to be carried out.

1. Rehydrate test and control cells with the specified volume of sterile distilled water, mix thoroughly and allow to stand at room temperature for 1 h.

2. Prepare the working dilutions of test and control cells by adding 1 volume of the rehydrated suspension to 5.5 volumes of absorbing diluent.

3. Rehydrate reactive control serum with 1 ml of sterile distilled water.

Reconstituted reagents should be stored at 2–10°C and used within 5 days. Working dilutions of test and control cells should be stored at 2–10°C and used within 1 day.

TPHA screening test

All sera are screened at a 1 in 80 final serum dilution against sensitized (test) cells only. Specimens giving positive or doubtful reactions are then tested quantitatively and also checked for non-specific agglutination with non-sensitized (control) cells. By using only the first 10 wells of alternate rows of a microtitre plate, 50 specimens are conveniently tested on a single plate, leaving sufficient space for appropriate controls.

1. Prepare a 1 in 20 dilution of inactivated serum in a tube by mixing 10 μl serum and 190 μl absorbing diluent. Leave for 30 min at room temperature to absorb.

2. Transfer 20 μl of absorbed serum to the 1st well of a U-type microtitre plate.

3. Transfer 60 μl of test cells to each absorbed serum; the final serum dilution is now 1 in 80.

4. Set up a negative control by treating a known non-reactive serum as above.

5. Determine the titre of the reactive control serum. Place 20 μl of diluent in wells 2–6 of a row. Add 20 μl of reactive control serum to wells 1 and 2. Double dilute from well 2 through to well 6, discarding the last 20 μl. Add 60 μl of test cells to wells 1–6. (Since the positive serum is prediluted to 1 in 80 the final serum dilutions range from 1 in 320 to 1 in 10,240.)

6. Set up a reagent control well containing 20 μl test cells and 60 μl absorbing diluent.

7. Mix the contents of each well by shaking the plate gently. Alternatively place the plate on a Microtiter Microshaker (Denley) for 20 s. Leave plates undisturbed at room temperature for at least 30 min; overnight incubation is often convenient.

Examine individual wells and record the degree of haemagglutination, if any, as follows: ++++, smooth mat of agglutinated cells covering more or less the entire bottom of the well; +++, smooth mat of agglutinated cells with a narrow red circle near the perimeter of the agglutination; +, small smooth mat of agglutinated cells with a thicker red circle near the perimeter of the agglutination; ±, a slightly enlarged ring of cells surrounding a rough margin; —, definite compact button of cells in the centre of the well, with or without a very small ‘hole’ in the centre. Results for a batch of screening tests are valid only if the negative control serum and the reagent control set up with test cells give a negative reaction and the reactive control serum gives a positive (+) reaction within one doubling dilution of 1 in 2560.

Although specimens giving + to +++ reactions are considered positive they must be tested against control cells to confirm the specific
nature of the haemagglutination. This is normally carried out at the same time as the quantitative test. Specimens giving doubtful (+) reactions should also be tested quantitatively.

Quantitative TPHA test

Allow one row of a microtitre plate for each quantitative test.

1. Prepare a 1 in 20 dilution of inactivated serum by mixing 10 μl of serum and 190 μl of absorbing diluent.
2. Add 20 μl of absorbing diluent to wells 2-10, but miss out well 9.
3. Transfer 20 μl of the 1 in 20 diluted serum to wells 1, 2 and 10. Make a series of doubling dilutions from well 2 through to well 8 and discard 20 μl of diluted serum from well 8. Allow to absorb for 30 min at room temperature.
4. Add 60 μl of test cells to wells 1-8 and 60 μl non-sensitized (control) cells to well 10.
5. Set up controls for the sensitized (test) cells and non-sensitized (control) cells by mixing 20 μl of absorbing diluent and 60 μl of cells.
6. Set up a negative control and titrate the positive serum as for the screening test.
7. Allow to stand undisturbed at room temperature for 4 h and examine for haemagglutination.

Results for a batch of quantitative tests are valid only if cell, positive, and negative controls give the expected reactions (as defined for the screening tests). Individual test results are valid only if there is no agglutination of the control cells. Test results are reported as follows: positive 80, 160, 320, etc., titre reported as the last serum dilution to give a positive (+) reaction (see above); negative, any specimen giving a – or ± reaction at 1 in 80; borderline reaction, used for the very occasional specimen that gives a reaction intermediate between ± and +; non-specific agglutination (test invalid), any specimen causing agglutination of non-sensitized (control) cells.

Fluorescent treponemal antibody absorbed (FTA-ABS) test

In this test the patient's serum is absorbed with an autoclaved supernate from cultures of Reiter treponemes in order to remove group-specific antibody. Binding to T. pallidum of antibody specific for pathogenic treponemes is then demonstrated by the indirect immunofluorescence method.

Reagents. The following reagents for this test are widely available commercially (e.g. Wellcome).

1. Phosphate buffered saline (PBS) pH 7.6 (NaCl 8.5 g; Na₂HPO₄ 1.28 g; NaH₂PO₄.2H₂O 0.156 g; distilled water 1000 ml).
2. Treponemal antigen (lyophilized suspension of the Nichols strain of T. pallidum).
3. Known strongly reactive serum.
4. Known minimally reactive serum.
5. Non-specific serum: a non-syphilitic serum which is reactive when diluted in PBS but negative when diluted in sorbent.
7. Fluorescent anti-human immunoglobulin reagent.
8. Acetone.
9. Buffered glycerol mounting fluid (1 volume PBS, pH 7.2 + 9 volumes glycerol). A commercial product. Bacto FA Mounting Fluid (Difco) may also be used.

Preparation of antigen smears. PTFE-coated 'multispot' slides with a dark background containing 12 wells, each 3 mm in diameter, are available from Hendley.

1. Place eight slides in a staining dish and cover with absolute alcohol for 1 h to remove grease; pour off alcohol and allow slides to dry.
2. Reconstitute a vial of treponemal antigen with 1 ml sterile distilled water. Break up any clumps in the antigen suspension by repeated aspiration into a syringe fitted with a 25 gauge needle.
3. Transfer 10 μl of antigen suspension to each well of the multispot slides.
4. Allow smears to stand for 5 min at room temperature, remove excess liquid from each well and allow to dry completely for a further 10-15 min.
5. Place slides in a staining dish and fix by covering with acetone for 10 min.
6. Pour off acetone and allow smears to dry in air.

This procedure should give antigen smears with 50–100 evenly distributed treponemes per field when viewed at a magnification of × 400. Slides will keep for at least 6 months if stored at −20°C in airtight containers. Do not thaw and refreeze smears.

Method for the FTA-ABS test

1. Calculate how many antigen wells will be required for tests and controls and remove the appropriate number of slides from the freezer.
2. Inactivate test and control sera at 56°C for 30 min. If sera have already been heat treated, reheat for 10 min at 56°C.
3. Label one test tube to correspond to each serum being tested and place in a rack. Dilute test sera and control sera known to give strong, weak and negative reactions. 1 in 5 in sorbent (10 μl serum + 40 μl sorbent).
4. Transfer 10 μl of each serum to an antigen well on the multispot slide; specimens can be identified on the slide by writing on the black background with a lead pencil.
5. Incubate at 37°C for 30 min in a humid atmosphere.
6. Place slides in slide carrier and rinse off excess serum with PBS. Wash slides in four changes of PBS for a total of 20 min. Rinse in distilled water, blot gently and allow to dry in air.
7. Remove an aliquot of fluorescein-conjugated anti-human globulin from the freezer. Allow to thaw and dilute with PBS to give the predetermined working dilution (see below); add 10 μl to each antigen well.
8. Repeat steps (5) and (6).
9. Add a small drop of buffered glycerol mounting fluid to each well and apply a coverslip.

Results. Examine slides as soon as possible. (If a delay is necessary place slides in a darkened room and read within 4 h.) Locate treponemes using a high-power dry objective and darkground illumination before assessing the degree of fluorescence with a suitable optical system, e.g., a microscope fitted with an HBO 200 W lamp.

BG12 primary filter and OG4 and GG9 secondary filters. Positive reactions are shown by fluorescent treponemes, in contrast to almost invisible non-fluorescent organisms in negative reactions. Results are scored and reported as shown in Table 40.2.

The following additional controls should be set up when testing new batches of reagents (new reagents should be tested in parallel with the existing reagents): (a) weak positive serum diluted 1 in 5 in sorbent and 1 in 5 in PBS. These should give equivalent fluorescence, demonstrating the failure of sorbent to remove specific antibody which would give rise to false negative results with weak positive samples. (b) Non-specific staining controls comprising antigen + PBS + conjugate, and antigen + sorbent + conjugate. The non-specific serum should give a positive reaction (++) when diluted in PBS but a negative reaction when diluted in sorbent, demonstrating the efficacy of sorbent in inhibiting non-specific staining. Non-specific staining controls should always be negative.

Preparation of the working dilution of conjugate. An economical working dilution of the fluorescent antibody is the highest dilution which will give intense specific staining with negligible background staining. The approximate range of working dilutions of conjugate for use in the FTA-ABS test is stated by the manufacturer. Variations in the optical and test systems used in different laboratories make it desirable to

Table 40.2 Reporting of results in FTA-ABS test for antibody to T. pallidum.

<table>
<thead>
<tr>
<th>Appearance of treponemes</th>
<th>Fluorescence score</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brilliant green fluorescence</td>
<td>+++</td>
<td>Positive</td>
</tr>
<tr>
<td>Moderate to bright green fluorescence</td>
<td>++</td>
<td>Positive</td>
</tr>
<tr>
<td>Weak but definite uniform green fluorescence, equivalent to weakly reactive control</td>
<td>+</td>
<td>Weak positive</td>
</tr>
<tr>
<td>Weak but definite fluorescence, less than weakly reactive control</td>
<td>±</td>
<td>Borderline reaction</td>
</tr>
<tr>
<td>No fluorescence, treponemes vaguely visible or completely invisible</td>
<td>–</td>
<td>Negative</td>
</tr>
</tbody>
</table>
confirm the most suitable working dilution by

1. Rehydrate the contents of a vial of
fluorescein-labelled anti-human globulin with
1 ml sterile distilled water. Mix the contents
thoroughly by swirling gently and allow to
dissolve slowly; vigorous action is not
recommended.

2. Prepare a series of doubling dilutions of
conjugate in PBS to cover the range of approxi¬
mate working dilutions, e.g. 1 in 10 to 1 in 2560.

3. Test each conjugate dilution, and the
existing batch of conjugate at its working
dilution, with the strong positive, weak positive
and negative sera by the standard FTA-ABS
procedure.

4. Include a non-specific staining control
(antigen + PBS) for each conjugate dilution.

5. Read the slides and determine the endpoint
of the titration, i.e. the highest dilution of
conjugate giving strong (++) fluorescence.
The working dilution of the new conjugate is one
doubling dilution below the endpoint. This
dilution should give acceptable fluorescence
(+) with the weakly reactive serum. If it does
not, repeat the titration with a series of inter¬
mEDIATE dilutions, e.g. 1 in 15 to 1 in 1920. The
conjugate must not stain non-specifically at 3
doubling dilutions below the working dilution.

6. Aliquot undiluted conjugate in amounts
suitable for 1 day's testing when diluted to the
working dilution and store at -20°C. Do not
refreeze after thawing.

Note: Commercial kits containing all of the
necessary reagents and controls, pre-tested and
ready for use, are now available.

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First coitus before menarche and risk of sexually transmitted disease

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HUGH YOUNG YASMIN JAMIL SOHRAB DAROUGHAR

The prevalence of sexually transmitted disease (STD), pelvic inflammatory disease (PID), and cervical cancer, and the relation between these conditions were studied in 2111 Ethiopian women. Early sexual activity was associated with an increase in prevalence rates of STD and PID; possible aetiological factors include physical and immunological immaturity of the female genital tract and the number of sexual partners.


Introduction

Cervical cancer is an important cause of cancer mortality among women in Ethiopia and in much of the rest of Africa, where patients often present with advanced disease. In high incidence areas, age at first coitus, specifically before age 21, 20, or 17 years is the most important risk factor,1–4 with a doubling of the risk in those having first coitus at age 16 or less.4 The association between cervical cancer and the number of sexual partners is well established: in married women with cervical cancer who claim not to have had a sexual partner other than their husband, the number of sexual partners of their husbands was a significant risk factor for cervical cancer.4 This male promiscuity has been called the "male factor." 5

Only one study has reported an association between cervical cancer and chronic pelvic inflammatory disease (PID) and sexually transmitted disease (STD).6 A study in Ethiopia in 1975 showed that STD (particularly syphilis and gonorrhoea) was highly prevalent; prevalence rates for other STD were unknown, although acute and chronic PID and their complications were the most important cause of morbidity and mortality in women.6,7 An international collaborative study was therefore planned to investigate the prevalence of STD and cervical cancer in an unscreened population, the causal factors of cervical cancer to define an "at risk" group, and possible associations between STD and cervical cancer.

Patients and methods

2111 unselected Ethiopian women were studied; half were outpatients with gynaecological symptoms, and half were healthy women attending family planning, antenatal, and postnatal clinics in Addis Ababa. The collection of data, cytology slides, and sera, and the VDRL test were done in 1975 and 1976 while tribal, religious, and other factors could still be assessed independently of population migration (before the Ethiopian revolution). It was not until 1977 and 1978 that the data and specimens could be despatched from the country, although full permission had been given by the Ethiopian Ministry of Health. The patients' data, used with their consent, were as follows:

Personal details were obtained by questionnaire (completed by an Ethiopian female assistant), and included age, hospital clinic attended, address (origin: town or rural), tribe, religion, parity, total number of pregnancies, "marital status" (single, married, divorced, widowed, single, married, divorced, widowed, "single, married, divorced, widowed, domestic worker, prostitute, "talla" [local beer seller, maid], monthly family income, age at first marriage and first coitus and

ADDRESS: Department of Bacteriology, Edinburgh University, Edinburgh, UK (M. E. Duncan, MD); J. F. Peutherer, MD, P. Simmons, PhD, H. Young, PhD; Liége University, Belgium (G. Tibaux, MD); Statens Serum Institut, Copenhagen, Denmark (K. Reimann); and Institute of Ophthalmology, London, UK (Y. Jamil, MD; Prof S. Daroughar, MD). Correspondence to Dr M. E. Duncan, "Ahlaine", Cardrona, Peebles, Edinburgh EH45 9HX, UK.

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Fig 1—Prevalence of STD and cervical cancer (CC) in relation to first coitus.

Open columns, early coitus—in, first coitus before the menarche (49.9%); shaded columns, late coitus—in, first coitus after the menarche (50.1%).

*p < 0.001; tp < 0.005; t = not significant.

relation to the menarche, number of husbands-consorts, years married, duration of sex life, contraception, history of STD, and main complaint/reason for clinic attendance.

Clinical data about vaginal discharge, state of the cervix and uterus, evidence of previous/present pelvic infection, and diagnosis were recorded. Cytological data were obtained from Papanicolaou stained cervical smear preparations. Serology tests were done for syphilis in Ethiopia (VDRL) and in Edinburgh (TPHA), for hepatitis B virus (HBV) in Edinburgh, for *Chlamydia trachomatis* (D-K) and herpes simplex virus in London, and for *Neisseria gonorrhoeae* in Copenhagen.

Statistical analysis was done with *t*-test for association of the various factors.

Results

All but 13 women (0.6%) were virgins at the time of their first marriage in keeping with Ethiopian culture. Child marriage was still practised, especially in rural areas. Since first marriage could precede first coitus by several months or even years, the data presented here are for age at first coitus.

Half the women had first coitus before the menarche, the mean age of which was 15 years. A high proportion of Ethiopian women are exposed to various STD pathogens. There was a significant increase in the prevalence rates of STD and PID in those who were sexually active before the menarche (fig 1), especially in those who started their sexual activity aged 12 or less (table). The rate of cervical cancer was higher in those sexually active before the menarche (fig 1) than in those sexually active after the menarche.

Analysis of the socioeconomic data (M.E.D., G.T., A.P., unpublished) indicates that the younger the age of first marriage and first coitus, the shorter the duration of that marriage, and the greater the likelihood of the woman being divorced, possibly remarried, or leaving home; thus, she would be more likely to support herself as a maid or by prostitution, in either event leading to more sexual partners and more STD, PID, infertility, and cervical cancer. 23.7% of those sexually active by the age of 12 supported themselves by prostitution compared with 7% of those who had had first coitus after the age of 15.

Detection of gonococcal antibodies (GA) provides a good marker of sexual activity. In married women who had only one husband there was a significant decrease in the prevalence of GA as the age at first coitus increased (fig 2). By contrast, in women who had had more than five husbands/sexual partners GA titers tended to be higher and prevalence of these antibodies did not decrease with increasing age at first coitus. The significantly higher GA seropositivity in women with more than five partners in all age groups shows that the influence of the number of partners was greater than that of the age of first coitus.

Discussion

Prevalence rates for exposure to STD in Ethiopia from this study are considerably higher than those reported elsewhere in Africa, and in Ethiopia. Factors that may account for this are: the limitations (in earlier studies) of bacteriological culture techniques for isolation of *N. gonorrhoeae* because of previous antibiotic therapy; our use of newly available highly sensitive serological tests, which indicate exposure to pathogen rather than evidence of disease; and bias because of a degree of patient self-selection in a hospital/clinic population. In particular, the GA test cannot distinguish between past and present infection and is thus unsuited for diagnosis of current gonorrhoea. However, since the antibodies are specific and stable, the test is useful as an epidemiological tool to show differences of

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**PREVALENCE OF STD AND CERVICAL CANCER**

<table>
<thead>
<tr>
<th>Age at first coitus (yr)</th>
<th>Disease</th>
<th>&lt;13 (n = 422)</th>
<th>13-15 (n = 871)</th>
<th>&gt;15 (n = 725)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis (VDRL)*</td>
<td>53.9%</td>
<td>45.4%</td>
<td>34.7%</td>
<td></td>
</tr>
<tr>
<td>Syphilis (TPHA)*</td>
<td>46.9%</td>
<td>39.5%</td>
<td>23.9%</td>
<td></td>
</tr>
<tr>
<td>Gonorrhoea*</td>
<td>68.8%</td>
<td>63.2%</td>
<td>42.2%</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B+</td>
<td>44.5%</td>
<td>36.8%</td>
<td>33.6%</td>
<td></td>
</tr>
<tr>
<td>Chlamydia D-K*</td>
<td>57.5%</td>
<td>55.8%</td>
<td>44.5%</td>
<td></td>
</tr>
<tr>
<td>Pelvic infection†</td>
<td>43.3%</td>
<td>40.9%</td>
<td>33.5%</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer†</td>
<td>16.7/1000</td>
<td>20.7/1000</td>
<td>9.7/1000</td>
<td></td>
</tr>
</tbody>
</table>

Data for 93 women unavailable. *p < 0.001; tp < 0.005; t = not significant.
† Not significant (p < 0.1 for rates in those sexually active <15 yr vs >15 yr); data for 1 woman unavailable.
The prevalence of gonorrhoea in various groups. The prevalence of GA in married women with one husband is similar to that in Danes born between 1900 and 1960 (23% vs 10–25%), whereas the prevalence of GA in Ethiopian women with more than five partners is similar to that in women with a history of gonorrhoea attending a Danish STD clinic (86% vs 96%).

Our findings show that there are three possible reasons/factors for our observations that early sexual activity leads to an increase in prevalence of STD and (non-significantly) cervical cancer. Firstly, early sexual debut is associated with patterns of sexual behaviour that influence numbers of sexual partners. The significant association of GA and (early) age at first coitus reflects socioeconomic status (76% of the very poor had first coitus before the menarche compared with only 20% of the richest, who were better educated), and the social consequences of early sexual experience. In particular, prevalence of STD, which was lowest in married women with one husband, increased with two husbands and was highest in bargirls, prostitutes, and tala sellers.

Secondly, at puberty, oestrogen secretion leads to the development of secondary sexual characteristics, and to increasing maturation of the epithelium of the lower genital tract which becomes stratified squamous epithelium. Progesterone stimulation causes the cells of this multilayered epithelium to fill with glycogen, which sustains the population of lactobacilli. The defence role of cervical mucus under the influence of cyclic progesterone, as yet undeveloped, awaits the onset of regular ovulation, which may be some months after the menarche. Little is known of the immunology and defence factors of the female genital tract before the menarche. Complement factors C3 and C4 are well developed by the age of 1 year. Concentrations of secretory IgA are unknown but serum IgA is lower in children; they have only 60% of the adult level by puberty, and this level increases slowly thereafter. Young children, gonococcal disease frequently cause a severe lower genital tract infection because of the immaturity of the single cell layer epithelium. Costal injury in the young (child brides) may be very severe. The role of traumatic first coitus in the spread of STD pathogens other than human immunodeficiency virus (HIV) is unknown. The effect of human papillomavirus on cervical cancer was not assessed in this study because of the lack of facilities for DNA hybridisation and the absence of a proven serological test. However, koliocytes were not a common feature of the cervical cytology smears.

Thirdly, without a male partner, women with one sexual partner should not have GA. For very young women who marry older men, a male partner contribution is virtually inevitable. Further evidence for male promiscuity is the 80% exposure rate for HBV in a predominantly male population, which is double that for women of similar age reported here.

Although the high prevalence of STD and cervical cancer is important, these findings must have serious implications in the wider context of the possibility of HIV entering the community.

We thank Sister Leterbirhan Mebr and Dr Philippa Wilson for their help in compiling the clinical data; Dr P. L. Perine and Ato Mebratu Tesfahun-Mike for help with VDRL testing; and Dr I. Smith, Dr C. Blackwell, and Prof D. M. Wear for constructive criticism of the text. This study was funded in part with research grants from Allied Medical Group for serological testing and salary for P. S., from the Ethicon Foundation for travel expenses for M. E. D., and from the Wellcome Trust for travelling and secretarial expenses for M. E. D.
ORIGINAL ARTICLE

Is coexisting chlamydial infection more common in gonococcal infections with serogroup WI?

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Summary: Over a 4-year period a total of 8974 women were screened for Neisseria gonorrhoeae and Chlamydia trachomatis. There were 489 cases of cervical gonorrhoea, 261 serogroup WI and 228 serogroup WII/III. A total of 169 (34.6%) cases had a dual infection with C. trachomatis, 92 from the WI serogroup and 77 from the WII/III. Using Fisher’s exact test, no statistically significant difference was observed in the rates of chlamydial carriage between the two serogroups (P=0.39). These findings are at odds with previously reported data, which suggested a biological interaction resulting in a positive correlation between colonization with serogroup WI and C. trachomatis. Possible reasons for the difference between the findings are discussed.

Keywords: Chlamydia trachomatis, Neisseria gonorrhoeae, coexistence

INTRODUCTION

There is a widely accepted association between gonococcal and chlamydial infections. It is unclear however, to what extent the basis of this association is explained by epidemiological factors or by a true biological interaction between the two organisms. Ruden et al.¹ reporting from Stockholm noted a positive correlation between Chlamydia trachomatis and Neisseria gonorrhoeae serogroup WI in heterosexual men. Preliminary evidence was also presented for a similar correlation in women. In three other Swedish geographical locations, chlamydial infection was more common among both heterosexual men and women infected with WI strains than among those infected with WII/WIII strains but the differences were significant in only two of the three regions². It is possible that these results could be explained by a variation in the epidemiological factors which contribute to the levels of gonococcal and chlamydial infection within the respective populations of these various areas. The aim of this study was to determine whether there was an association between C. trachomatis and N. gonorrhoeae serogroup WI in female patients in our locality before initiating more detailed prospective studies on chlamydial reactivation by gonococci.

PATIENTS AND METHODS

All female patients who attended the Department of Genito-Urinary Medicine, Royal Infirmary of Edinburgh, during the 4-year period 1986–1989, were considered for inclusion in the study. An analysis of laboratory reports identified a total of 8974 women who had been screened for N. gonorrhoeae by culture and C. trachomatis. Gonococcal culture was performed by direct inoculation of material on to modified New York City medium. Cultures were incubated at 37°C in a carbon dioxide enriched aerobic atmosphere for up to 48 h. Suspect gonococcal cultures were identified and serogrouped by the Phadebact Monoclonal GC Test⁴.

Chlamydia trachomatis was identified by culture on cycloheximide-treated McCoy cells. After 3 days' incubation at 35°C, the coverslips were fixed and stained with iodine to detect the glycogen-containing inclusion bodies. When diagnosis by culture was not possible an ELISA swab was examined by Chlamydiazyme (Abbott). All positive ELISA specimens were further examined either by staining the deposit by immunofluorescence (Chlamyset, Orion Diagnostica) or by blocking with the Abbott blocking reagent. Hence only confirmed positive results are recorded. Cultures were not received from high risk patients but fixed smears were examined by immunofluorescence using the Orion reagent.
The significance of the different rates of chlamydial carriage between serogroups WI and WII/III was assessed using Fisher's Exact Test.

RESULTS
A total of 489 patients were positive for cervical gonorrhoea over the 4-year period. Two hundred and sixty-one (53.3%) belonged to serogroup WI and 228 (46.7%) belonged to serogroup WII/III. In the same period, a total of 979 were positive for C. trachomatis, 884 (90.3%) by culture, 33 (3.4%) by immunofluorescence (IF) alone, and 62 (6.3%) by ELISA (Abbott). Thirty-nine of these were confirmed by IF and 23 by antibody blocking (Abbott).

In total, there were 169 cases of dual infection; of these, C. trachomatis was diagnosed by culture in 158 (93.5%), in 8 (4.7%) by IF and by confirmed ELISA in 3 (1.8%).

The frequency of distribution of chlamydial infection within the gonococcal serogroups is presented in Table 1. Using Fisher’s Exact Test the rate of chlamydial carriage between the two serogroups was not significant (P=0.39). When the analysis was repeated using only the figures obtained by culture, again no significant difference was evident.

Table 2 compares the prevalence of C. trachomatis in patients with coexisting infection with N. gonorrhoeae in our population to that of the Swedish studies. The overall prevalence of combined infection was higher in Edinburgh women (28%) than Swedish men (20%). When the data concerning the prevalence of C. trachomatis within the gonococcal serogroups was further analysed, identical carriage rates were noted within the WI group of both populations (30%) but the Swedish males had a reduced prevalence at 16%.

Further variations in gonococcal serogroup prevalence per se are known to exist in different populations, and this was again found in our study, where the prevalence of WI in Edinburgh women (53%) is significantly different from that (30%) in Swedish men (P<0.001).

DISCUSSION
Dual infection of the genital tract with C. trachomatis and N. gonorrhoeae is well described. Occurrence of this association could result from either epidemiological factors alone, biological interaction between the two organisms or a complex interplay of both factors. Several workers have suggested that gonococcal infections can reactivate latent chlamydial infection. Some indirect clinical evidence exists to support this view. However, care must be taken when trying to derive proof for further biological interaction from what are purely epidemiological data. The initial report of association between C. trachomatis, WI serogroup of N. gonorrhoeae was highly significant (P<0.01). However, this held specifically for Swedish heterosexual men; of the 312 men with gonorrhoea the ratio of WI to WII/III was 30:70. When however, another population is considered, either in a different area within the same country or a different national location the relative proportions of WI and WII/III are known to vary widely: in Edinburgh of the 489 women with gonorrhoea 47% of the infections were of the WII/III serogroup. A further problem is the variation in the underlying prevalence of C. trachomatis infections in the gonococcal serogroups within the two locations studied. (Table 2). To what extent these initial intrinsic differences have resulted in the association described in Sweden and our failure to repeat this in Edinburgh is open to speculation. However, our findings would tend to suggest that epidemiological factors have an important influence.

Danielsson8 considered a possible explanation for the major decrease of gonorrhoea in Sweden and the other Scandinavian countries was the strong motivation during the last few years by venereologists, gynaecologists and general practitioners to diagnose, treat and manage chlamydial infections from an epidemiological point of view. The treatment of index cases and their consorts for 7-10 days with tetracycline or erythromycin is standard practice and will presumably also eradicate a proportion of gonococcal infections. Danielsson8 found some indirect evidence for this in that gonococcal and chlamydial infection rates were lowest in those areas of Sweden where the epidemiological treatment of genital and chlamydial infections first started. As serogroup WI strains are significantly more susceptible to antibiotics than serogroup WII/III strains2, the epidemiological treatment for chlamydial infection is likely to eradicate more WI strains than WII/WIII. This could be a contributing factor in the high proportion of WII/WIII strains found in Sweden.

One further consequence of widespread epidemiological treatment would be a reduction in the
absolute number of C. trachomatis infections generally, with a small number of treatment failures. However with the greater reduction in the WI strains there would be an apparently greater proportion of combined chlamydial and gonococcal WI infections within the treatment failures. This could provide a further basis to explain the Swedish observations.

In Edinburgh the epidemiological treatment of chlamydial infection is rare in patients who have not been screened by culture for gonococcal infection. This will have the effect of minimizing the selective enhancement of coexisting chlamydia and serogroup WI infection, possibly accounting for our failure to show a significant association. However, our data does not exclude a biological interaction between C. trachomatis and N. gonorrhoeae. Sixbey et al. reported that gonococcal infection enhanced the formation of chlamydial inclusions in cervical explants in tissue culture. Batteiger et al. cited unpublished observations that there was increased shedding of C. trachomatis from the endocervix of women with gonorrhoea as opposed to those without and considered that this was consistent with their hypothesis that gonococcal infection could reactivate latent chlamydial infection. Even if the hypothesis of reactivation of latent chlamydial infection by gonococci is accepted to explain the Swedish data, it would have to be shown that WI strains were more effective than WII/WIII strains. Clearly further prospective studies involving detailed gonococcal and chlamydial serotyping analysis applied to different national populations are required to clarify the biological and epidemiological factors in dual infection with these two organisms.

Acknowledgements: We would like to acknowledge the generous help given by Dr R A Elton, Medical Statistics Unit, University of Edinburgh in the preparation of the paper.

References

(Accepted 5 June 1990)
CASE REPORT

Influence of human immunodeficiency virus infection on treponemal serology, in patients who have been treated for syphilis

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Accepted for publication 17 February 1990

Summary

Sera from 20 homosexual men who were infected with the human immunodeficiency virus (HIV) and had been treated previously for syphilis, were examined for cardiolipin and treponemal antibodies by the Venereal Diseases Research Laboratory (VDRL) test, and the Treponema pallidum haemagglutination assay (TPHA) and fluorescent treponemal antibody absorbed test. In only one case was probable reactivation of syphilis, as judged by rising titres in the VDRL test, noted.

Introduction

In developed countries, men who have had homosexual anal intercourse have been at increased risk from both syphilis and human immunodeficiency virus (HIV) infection. For example, the sera of 60% HIV infected men who attended a Sexually Transmitted Diseases (STD) Clinic in west London gave positive results for treponemal antibodies.1

Although it is known that only about one third of patients with untreated syphilis will develop late manifestations of the infection, the factors that determine progression of the disease are uncertain. Cellular immune responses, however, are probably important.2 In rabbits treated with drugs that suppress cell mediated, but not humoral, immunity, disseminated infection occurs after the intradermal inoculation of Treponema pallidum. By contrast untreated animals develop typical lesions only at the challenge site.3 Although treponemes can persist in tissues, particularly nervous tissue,4 reactivation of human infection has been described only rarely in immunocompetent patients who have been treated with adequate doses of penicillin. Because infection with HIV is associated with a progressive impairment of cellular immunity that can result in reactivation of latent infections such as Toxoplasma gondii and herpes viruses,5,6 it is of concern that HIV may also result in reactivation of syphilis. In this study the results of serological testing of a group of HIV infected homosexual men who had been treated previously for syphilis are presented.

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Patients and methods

Patients
The study group comprised 20 homosexual men who attended the Department of Genito-Urinary Medicine, Edinburgh Royal Infirmary, who were seropositive for HIV and who had been identified as having had syphilis previously. Nineteen men had been given IM procaine penicillin in a dosage of 600 mg once daily for 14 days; the other patient, who had penicillin hypersensitivity, had been treated with doxycycline 300 mg daily by mouth for 21 days.

When HIV infection was diagnosed, and at subsequent attendances, a history was taken from each patient and a detailed clinical examination was performed. At each clinic visit a sample of blood was taken for serological tests for syphilis and in some cases for serum immunoglobulins and lymphocyte subset analysis. Whenever possible, and with the patient’s informed consent, sera that had been obtained at previous clinic attendances as part of a study of homosexually acquired infections and stored at $-70\,^\circ C$, were examined for antibodies against HIV (anti-HIV) to determine the approximate duration of that infection.

Laboratory methods
The Venereal Diseases Research Laboratory (VDRL) test, *T. pallidum* haemagglutination assay (TPHA) and the fluorescent treponemal antibody absorbed (FTA-Abs) tests were performed according to standard methods. The Captia Syphilis-M test (Mercia Diagnostics) was used for the detection of serum antitreponemal IgM.

Commercial enzyme linked immunosorbent assays (ELISA) were used to screen for antibody to HIV. The assay used was a competitive assay (Wellcome). Positive samples were confirmed if they reacted positively in a different assay of the antiglobulin type (Pasteur or Du Pont). A few sera required further investigation by immunoblotting. All assays were performed according to the manufacturer’s instructions.

Serum immunoglobulins were determined by radial immunodiffusion and the numbers of CD4+ and CD8+ cells by flow cytofluorimetry.

Results
Table I shows the age of each patient, the clinical features of HIV infection at the most recent clinic attendance, most recent CD4+ cell count and serum immunoglobulin concentrations. In eight cases (nos 3, 4, 11, 12, 15, 17, 18, 19) analysis of stored sera showed that the treponemal infection had preceded the acquisition of HIV. Case no. 16 had probably acquired both infections simultaneously while visiting the U.S.A. in 1983 and case no. 7 had acquired HIV in 1981, 2 years before he presented with early latent syphilis (Table II): the time of infection with HIV in the other cases was uncertain.

The results of serological tests for syphilis are shown in Table II. Sera from each of the 13 patients who were available for study 12 months after treatment for syphilis gave negative or weakly positive results to the VDRL test and with
Table I  Clinical and immunological findings at the most recent clinic attendance of HIV infected men who had been treated previously for syphilis

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Clinical features of HIV infection</th>
<th>Serum immunoglobulin concentration (g/l)*</th>
<th>Number of CD4+ and CD8+ cells in peripheral blood (per mm³)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgG</td>
<td>IgA</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>Dementia</td>
<td>14.8</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>PGL</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>PGL</td>
<td>15.4</td>
<td>2.7</td>
</tr>
<tr>
<td>4</td>
<td>37</td>
<td>PGL</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>PGL</td>
<td>28.0</td>
<td>4.8</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>None</td>
<td>16.8</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>Pneumocystis pneumonia</td>
<td>30.4</td>
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</tr>
<tr>
<td>8</td>
<td>30</td>
<td>PGL</td>
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<td>3.9</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>PGL</td>
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<td>N.A.</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>PGL</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>11</td>
<td>29</td>
<td>PGL</td>
<td>16.4</td>
<td>2.2</td>
</tr>
<tr>
<td>12</td>
<td>26</td>
<td>None</td>
<td>13.6</td>
<td>2.4</td>
</tr>
<tr>
<td>13</td>
<td>36</td>
<td>Wasting illness‡</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>14</td>
<td>32</td>
<td>PGL</td>
<td>16.0</td>
<td>2.8</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>PGL</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
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<td>54</td>
<td>Kaposi's sarcoma</td>
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</tr>
<tr>
<td>17</td>
<td>32</td>
<td>PGL</td>
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<tr>
<td>18</td>
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<td>14.0</td>
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<tr>
<td>20</td>
<td>43</td>
<td>Cerebral toxoplasmosis</td>
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<td>6.1</td>
</tr>
</tbody>
</table>

* Normal ranges: IgG 5.0-13.0 g/l; IgA 0.5-4.0 g/l; IgM 0.3-2.2 g/l.
† Normal ranges: CD4+ 500-1500 per mm³; CD8+ 250-750 per mm³.
‡ Deceased.
N.A. = Data not available.
PGL = Persistent generalised lymphadenopathy.

one exception (case no. 12) it remained negative or only weakly positive for the duration of follow up. Seropositivity for treponemal antibodies (TPHA and FTA-Abs tests) persisted in most cases but significant increases in antibody titres were not noted. Specific anti-treponemal IgM was not detected in any post treatment serum.

As the VDRL titre increased significantly in case no. 12 (Fig. 1) a brief report on this patient is given. A corresponding increase in the TPHA titre cannot be discounted as 1 in 5120 was the final serum dilution tested.

**Case report**

This patient first attended the clinic in 1981. Six months previously he had been treated for secondary syphilis with procaine penicillin in standard dosage.
Table II Results of serological tests for syphilis in HIV infected homosexual men who had been treated previously for syphilis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at diagnosis of syphilis (years)</th>
<th>Stage of syphilis (years of treatment)</th>
<th>Estimated year of infection with HIV</th>
<th>VDRL (TPHA) titres before and at intervals (years) after treatment</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>Before treatment</td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>Early latent (1975)</td>
<td>?</td>
<td>8 (N.A.)</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>Secondary (1981)</td>
<td>1982</td>
<td>16 (1280)</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>Secondary (1980)</td>
<td>?</td>
<td>8 (5120)</td>
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<tr>
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<td>28</td>
<td>Early latent (1983)</td>
<td>1981</td>
<td>16 (N.A.)</td>
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<td>42</td>
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<tr>
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<td>24</td>
<td>Primary (1983)</td>
<td>?</td>
<td>8 (80)</td>
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<tr>
<td>17</td>
<td>20</td>
<td>Secondary (1975)</td>
<td>1986</td>
<td>32 (1280)</td>
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</table>

* Dark field microscopy positive for Treponema pallidum.
† Fluorescent treponemal antibody absorbed (FTA-Abs) test positive.
‡ FTA-Abs weakly positive.
§ Patient had been treated for primary syphilis in 1979, but 1 year before he presented with secondary syphilis the serum VDRL and TPHA tests were negative.
N.A. = Data not available.
? = Unknown.
— = Negative.
± = Weakly positive.
(see above). Although symptomless, he requested HIV antibody testing in 1985 with positive results. In 1986 he developed Bowenoid papulosis on the shaft of his penis but the lesions resolved spontaneously within 6 months. He remained well but at routine attendance in February 1987 it was noted that his VDRL test had become positive at a titre of 16. At this time, he had no symptoms of syphilis or of HIV infection. There were no clinical abnormalities. He declined lumbar puncture. His haemoglobin was 15·6 g/dl, WBC count $9·2 \times 10^9/l$ with a differential of 73% polymorphs, 23% lymphocytes and 4% monocytes; the platelet count was $265 \times 10^9/l$. The CD4+ and CD8+ cell counts were 810 per mm$^3$ and 900 per mm$^3$ respectively. His plasma bilirubin was 8 $\mu$mol/l, alanine aminotransferase 32 units/l, alkaline phosphatase 68 units/l, γ-glutamyl transferase 16 units/l; total serum protein was 74 g/l, albumin 42 g/l, IgG 13·6 g/l, IgM 1·3 g/l, IgA 2·4 g/l. Serum antibodies against Epstein-Barr virus and cytomegalovirus were detected at titres of 160 and 320 respectively, but specific IgM against these viruses was not detected. Hepatitis B surface antigen was not present but antibodies against both surface and core antigens were found in the serum; a change in titre of antibodies to HBV antigens was not observed. T. gondii antibodies were not identified and antinuclear factor was not detected by latex agglutination. As he was unable to attend for injections of penicillin, the patient was treated with doxycycline and within 1 year titres in the VDRL and TPHA tests had declined to 4 and 640 respectively.

In the preceding year he had had two sexual partners. Both men were reviewed in the department; neither had clinical evidence of syphilis or HIV infection and treponemal and HIV antibodies were not found on repeated testing.


Discussion

Our clinical and serological data have failed to show recrudescence of syphilis in 19 of the 20 HIV infected men, some of whom had moderately severe immunodeficiency as judged by low numbers of CD:4 + cells in the peripheral blood. These results support the contention that procaine penicillin cures syphilis and prevents the occurrence of late manifestations in spite of infection with HIV. Similar conclusions were reported from London, U.K. by Terry et al.,7 who studied eight HIV infected men who had been treated previously for syphilis. Although case reports from the U.S.A. have described the development of neurosyphilis in HIV infected patients who had prior therapy for syphilis with benzathine penicillin,8,9 it has been shown that the treatment regimen used does not produce treponemicidal concentrations of penicillin in the CSF10 and indeed, in the pre-AIDS era, neurosyphilis had been reported in patients so treated.11

The significance of the serological findings in case no. 12 is uncertain. When increasing titres of cardiolipin antibodies were noted there were no clinical signs of syphilis and his two most recent sexual contacts showed no clinical or serological features of the disease. Reinfection, however, cannot be entirely discounted. Polyclonal B cell activation is a feature of HIV infection,12 and might explain the increasing cardiolipin antibody titres though this seems an unlikely explanation as serum immunoglobulin concentrations were normal (although his serum IgG was at the upper limit of normal), while there was a decline in titre after doxycycline therapy. It had been noted that almost all of these patients were, or had been infected with hepatitis B virus. In some of them a decline or loss of antibody to HBV surface antigen (HBsAg) was observed (unpublished work). The titre of antibody to HBsAg in case no. 12 remained constant throughout the surveillance period. Cardiolipin antibodies are found in conditions other than the treponematoses and are common in autoimmune diseases. Autoantibodies can be detected in the sera of HIV infected individuals13 and indeed may play a part in its pathogenesis. The possibility that the increasing titres in the VDRL test resulted from autoimmune phenomena is unlikely but cannot be entirely discounted. In diseases such as systemic lupus erythematosus cardiolipin antibodies persist for years14 and it is unlikely that doxycycline treatment would have resulted in the rapid decline in titres noted here. The TPHA titre declined after treatment which also supports the view that the cardiolipin antibody response was not related to an autoimmune phenomenon. There was no serological evidence of any other acute infection that might have led to the formation of these antibodies. Although specific IgM against T. pallidum was not detected in serum from this patient, the possibility of reactivation of this organism is not precluded. Defects of B-cell function that result in an impairment of specific IgM production during reactivation of latent infections such as T. gondii are a feature of HIV infection.5 Limited data indicate that a new treponemal infection in an HIV infected individual may not result in the development of cardiolipin or specific antibodies.15 We consider then that the most likely explanation for the rising cardiolipin antibody titres is reactivation of latent treponemal infection with production of IgG antibodies. Interestingly, Radolf
and Kaplan\textsuperscript{16} noted a deficient antibody response to \textit{T. pallidum} antigens in a patient with secondary syphilis. By immunoblotting, antibodies to fewer treponemal antigens were found than are usually present in secondary syphilis. The finding that the TPHA titre increased for between one and three years after treatment and persisted at a high level (\( \geq 5120 \)) thereafter may also be a pointed to inadequate control of the infection. Interestingly, the TPHA titre increased several years before that of the VDRL. The antibody response in treated and untreated infection may be due to a number of different treponemal antigens and this may explain the temporal difference in TPHA and VDRL titres.

In conclusion, we feel that although reactivation of the disease is uncommon, regular serological testing should be undertaken in HIV infected patients who have previously been treated for syphilis. As neurosyphilis can develop rapidly\textsuperscript{8} examination of the CSF should be undertaken in patients whose sera show rising titres of cardiolipin or treponemal antibodies, and if indicated, appropriate treatment with penicillin or other antimicrobial agents should be instituted without delay.

(We wish to thank Drs P. L. Yap and A. G. Bird, Department of Transfusion Medicine, Edinburgh Royal Infirmary, and Dr C. M. Steel, Cytogenetics Unit, Western General Hospital, Edinburgh for undertaking the serum immunoglobulin and lymphocyte subset analysis.)

\textbf{References}


Topley & Wilson's
PRINCIPLES OF BACTERIOLOGY, VIROLOGY AND IMMUNITY

Eighth edition in four volumes with cumulative Index Volume

Volume 3
BACTERIAL DISEASES

Edward Arnold
A division of Hodder & Stoughton
LONDON MELBOURNE AUCKLAND
3.31 Syphilis and related treponematoses
Hugh Young and Charles Penn

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follow the same course (Knox et al. 1976). Permanent recovery may occur at any stage. New observations on the natural course of untreated syphilis are now unlikely to be obtained, since treatment is cheap and effective, and cannot ethically be withheld.

Up to the beginning of the secondary stage, syphilis appears clinically as a localized infection, but dissemination of treponemes throughout the body occurs at a very early date. Bauer and colleagues (1952) examined cerebrospinal fluid (CSF) from patients with untreated early syphilis and found that 1.3% of seronegative primary, 2.9% of seropositive primary and 6.1% of secondary cases showed abnormalities. Treponemes have been isolated from the CSF of patients with secondary syphilis (Chesney and Kemp 1924). In rabbits, Collart and colleagues (1974) demonstrated them in the CSF and aqueous humour 18–24 h after infection.

Invasion by only a few organisms may be sufficient to establish infection. Magnuson et al. (1948) regularly infected rabbits intracutaneously or intratesticularly with less than 10 treponemes. During the primary stage of the disease in man, spirochaetes are numerous in the local chancre, and can sometimes be demonstrated in the blood by infection of rabbits (Uhlenhuth and Mulzer 1913). Spirochaetes are also numerous in all the secondary lesions, and may be excreted in the semen (Uhlenhuth and Mulzer 1913). In the tertiary lesions such as gummatas they are demonstrable, but only in small numbers; their virulence, however, appears to be maintained. Noguchi and Moore (1913) found them in the brain of patients dying of general paralysis. In congenital syphilis, spirochaetes are distributed in large numbers throughout the viscera. Both in congenital and acquired syphilis the organisms may remain latent for long periods of time without giving rise to any clinical manifestations of disease.

Immunity to syphilis

Even before the discovery of T. pallidum it was known that reinfection with syphilis was rare, suggesting the development of immunity. Extensive studies on the development and nature of immunity were carried out, mainly in the rabbit model; see reviews by Chesney (1927), Gastinel and Pulvenis (1934), Urbach and Beerman (1947) and Cannefax (1965). One theory was that immunity in syphilis was due to the persistence of the original infection, an ‘infection’ or ‘chancre’ immunity, but the work of Chesney and his colleagues led to the view that a true immunity to reinfection does develop slowly in syphilis, and is not necessarily due to the persistence of the original infection. When the immunizing infection was allowed to persist for more than 3 months before adequate treatment, subsequent challenge usually failed to produce overt infection. The early workers used arsenamines for treatment. Arnold and his colleagues (1947, 1950a, b) treated rabbits with early infections of only 6–8 weeks’ duration with penicillin. On rechallenge 10 days later, 10 of 37 contracted microscopically positive lesions and the remainder subclinical infections, as shown by subsequent positive lymph-node transplants. When the original infection was allowed to persist for 8 months before treatment, no lesions developed in 34 reinoculated animals, but 18 were infected subclinically. Magnuson and Rosenau (1948) used graded doses of treponemes to challenge rabbits treated with penicillin after infections lasting 3–24 weeks. They concluded that some immunity was demonstrable as early as 3 weeks after infection and that it increased progressively during the 24 weeks, as shown by the need to give increasing numbers of organisms to produce a given response.

The number of infecting organisms may influence the degree of immunity that develops. Turner and Nelson (1950) infected rabbits with graded doses of treponemes and challenged them after treatment; infection with small numbers produced less resistance than that resulting from massive inocula. Turner and his colleagues (1947) showed that within 6 months of intratesticular inoculation of rabbits with T. pallidum, the pertenue subspecies, or T. fallicus (p. 600) an appreciable degree of immunity had developed which protected against intracutaneous challenge with heterologous strains.

The observations of Magnuson and his colleagues (1956) on the experimental inoculation of human volunteers have given results confirming those from experimental animals. The 50% infective dose by the intradermal route in normal persons was found to be about 57 treponemes. Syphilitic patients were inoculated with 100,000 treponemes, about 2000 times the ID50. None of 5 patients with untreated latent syphilis showed lesions or a rise in serological titre; in 11 patients previously treated for early syphilis, lesions developed at the site of inoculation, and only showed a rise in titre; in 10 of 26 patients previously treated for late latent syphilis, lesions developed; 4 of 5 patients with treated congenital syphilis showed local lesions, one of which contained treponemes.

Infectivity by sexual transmission to others in untreated human syphilis is greatest during the first 2 years of the disease, spanning the primary and secondary stages and early latency. After 5 years the risk of transmission is thought to be minimal. The risk of the female transmitting congenital syphilis also lessens with time. The lesions of late syphilis are not infectious to others.

The mechanism of immunity to infection remains obscure, despite the vigorous antibody responses which are apparent from an early stage in the disease and are exploited in immunodiagnosis. The viable, intact organism appears to be refractory to the effects of combination with antibody, a situation fundamentally different from that exhibited by many other gram-negative bacterial pathogens.

Humoral immune responses

Antibody responses to T. pallidum have been recognized and measured by means of a variety of diagnostic tests (see p. 591). These responses have now been shown by the highly discriminating technique of immunoblotting to be induced by a number of polypeptide components of the organism (see Chapter 2.30), but their role in
immunity is undefined. In the absence of methods for the direct assessment and measurement of treponemal viability in vitro, the only test available for the study of humoral antitreponemal immunity was the treponemal immobilization test. The relevance of the results so obtained to protection in vivo remains unclear, but data are now emerging on antigens that do or do not appear to induce immobilizing antibody. For example, prominent polypeptides of mol. wt $47 \times 10^3$ (Jones et al. 1984) and $44 \times 10^3$ (Bailey et al. 1987) both induce the formation of either polyclonal or monoclonal antibodies that exhibit immobilizing activity. It might be inferred that these antigens are significant in induction of immunity, because they might be expected to immobilize (or kill) treponemes in vivo.

An alternative to the immobilization test is 'in vitro/in vivo neutralization' (T. B. Turner 1939, Turner et al. 1948, Bishop and Miller 1976b, Blanco et al. 1984). In this procedure, reliance on immobilization as an indicator of the bactericidal activity of antibody is avoided: treponemes are incubated in vitro with antibody and complement, then inoculated into rabbits intradermally to determine their survival and virulence. This can be done semiquantitatively by the inoculation of serial dilutions and monitoring for the development of dermal lesions.

Despite the demonstration of humoral antitreponemal activity in both immobilization and neutralization tests, the role of humoral factors in resistance to infection remains uncertain (Lukehart 1985). Passive administration to rabbits of antibodies against \textit{T. pallidum} appears to confer partial protection, detectable by delay in the induction and by the atypical appearance of lesions; often motile treponemes cannot be detected, and lesions fail to progress to ulceration (Perine et al. 1973, Turner et al. 1973, Bishop and Miller 1976a, Graves and Alden 1979, Titus and Weiser 1979). In some circumstances, the antitreponemal immunity conferred by passive transfer of antibody may be complete; Azadegan et al. (1983) were able to protect hamsters totally against the Bosnia A endemic syphilis strain. On the other hand, a state of immunity may exist in the absence of immobilizing antibody (Miller 1973); conversely, the high-titre antibodies against a broad spectrum of treponemal polypeptides that develop during primary syphilis do not prevent the development of secondary lesions (Lukehart 1986). The enigma that antibodies do not interact with the native treponemal surface (see Chapter 2.30) provides a powerful argument against a role for antibodies in protective immunity.

**Cellular immune responses**

Considerable evidence exists that cellular immune responses play a significant part in resistance to syphilis, but the experimental results are diverse and confused. There were several reports in the 1970s of cellular immune responses in both man and rabbits demonstrable by \textit{in vitro} methods such as lymphocyte transformation or macrophage migration-inhibition (reviewed by Folds 1983). In general, cellular responses - in rather small numbers of infected human subjects - to both mitogens and antigens of \textit{T. pallidum} appeared to be reduced early in infection, but after treatment they appeared normal (Levene et al. 1969, Mush er et al. 1974, From et al. 1976). There was also substantial histopathological evidence for depletion of cell populations in thymus-derived areas in lymphoid organs, both in man (D. R. Turner and Wright 1973) and the rabbit (Festenstein et al. 1967). The evidence from infected rabbits is both more abundant and more contradictory.

Pavia et al. (1976, 1977) reported evidence of suppression of cell-mediated immune responses - lymphocyte responsiveness to treponemal antigens - early in infection. On the other hand, Lukehart et al. (1980) and Baughn and Mush er (1982) showed no such apparent depression in responses of lymphocytes to treponemal antigens. Indeed, the work of Sell, Lukehart and colleagues has produced convincing evidence for (1) an abundant infiltration of T lymphocytes into healing primary lesions in infected rabbits (Baker-Zander and Sell 1980, Lukehart et al. 1980, Sell et al. 1980), and (2) appe riable ingestion by macrophages of treponemal antigen in such lesions (Sell et al. 1982). The inference from this work is that infiltrating lymphocytes, sensitized to treponemal antigen, are stimulated to activate macrophages which are then responsible for the elimination of treponemes from the infected site. It must be emphasized, however, that supporting evidence from \textit{in vitro} studies is weak, and the possibility remains that macrophages present in resolving lesions are merely phagocytosing treponemes which are already moribund from other causes. Certainly it is a common observation that treponemes obtained from rabbit testes several days after maximal orichiss, at the start of resolution of the lesion, are frequently sluggish in their motility and may appear damaged, for example, showing uneven waveforms, despite being extracellular.

Substantial evidence for a role of T lymphocytes in acquired resistance to infection has been obtained by studies in hamsters and guinea-pigs. Schell et al. (1980, 1981) demonstrated transfer of resistance to the Bosnia A endemic syphilis strain in hamsters, mediated by a suspension of cells enriched for T lymphocytes. Bagasra et al. (1985) demonstrated complex changes in T and B-lymphocyte populations during infections of hamsters with this strain. Lymphocyte transfer in guinea-pigs has also demonstrated a role for these cells in protective immunity: recipient inbred strains of guinea-pigs were partially protected against the development of cutaneous lesions by spleen and lymph-node cells from infected animals, and a delayed-type hypersensitivity response was also conferred by these cells (Pavia and Niederbuhl 1985, Wicher et al. 1987). Both B and T lymphocytes took part in this transfer, and antibody levels also rose substantially in recipients after cell transfer. In general, this work indicates that substantial cell-mediated immune responses do occur later in infection, and do play a part in protective immunity. The exact mechanisms and the potential for aberrant or harmful immunopathological consequences of such responses remain undefined.

**Diagnosis**

Serological tests are the mainstay for the diagnosis of syphilis at all stages, other than, perhaps, the very early
stages of the disease; they provide the sole means of
detecting latent infection. *T. pallidum* may be detected by
microscopy in the genital and rectal lesions of primary
syphilis. Microscopy is not suitable for examining the
dry skin rash of secondary syphilis, but treponemes are
plentiful in the moist lesions at mucocutaneous junctions,
such as mucous patches or condylomata lata.

**Demonstration of treponemes in exudate**

Dark-field microscopy is the standard method of dem¬
strating *T. pallidum* in exudate (Fig. 31.1). Lesions
should be cleansed by swabbing with saline and squeezed
gently until clear serum exudes. The material can be
collected directly on a coverslip which is then placed on
a glass slide. The morphology of *T. pallidum* has been
described in Chapter 2.30. Features that help to distin¬
guish it from the other treponemes that may be found
on mucosal surfaces are its slender structure and slightly
pointed ends; other spirochaetes are usually thicker and
have rounded ends. Movement is characteristically slow
with a to-and-fro ‘drilling’ motion and occasional flexion
of the body. The regularity of its spirals, about 7 of which
span the diameter of the red cells that are usually present
in the preparation, also help in identification. Dark-field
microscopy is unsuitable for examining oral lesions
because many of the non-pathogenic treponemes found
there may be confused with *T. pallidum*.

The specificity of dark-field microscopy is dependent
on the skill of the microscopist in distinguishing *T. pal¬
idum* from the numerous commensal spirochaetes that
are found in the normal flora of the genital and rectal
mucosal surfaces. Owing to the marked decline in the
incidence of early syphilis in recent years many clin¬
icians have inadequate experience in dark-field micro-
scopy. This, together with the potential hazard of
handling untreated exudate from patients at high risk of
HIV infection will hasten the more widespread use of
fluorescent-antibody staining methods.

The direct fluorescent-antibody staining for *T. pal¬
idum* (DFA-Tp) test has the advantage that a smear of
the exudate can be made on a slide, fixed in acetone, and
sent to the laboratory. In remote areas specimens can be
posted. Originally smears were stained with a conjugated
syphilitic serum made specific for *T. pallidum* by absorp¬
tion with cultivable treponemes (Daniels and Fer¬
neyhaugh 1977). Luger (1981) considered that the DFA-
Tp test gave non-specific results and was less reliable
than dark-field microscopy. The use of a pathogen-
specific monoclonal antibody has improved the reliabil¬
ity of the DFA-Tp test. Oral lesions, which cannot be evalu¬
atated by dark-field microscopy, can be accurately exam¬
ined by means of pathogen-specific monoclonal antibodies (Lukehart and Baker-Zander 1987b). Hook et al. (1985), using monoclonal antibody H9-1, which is
specific for the *T. pallidum* antigen of mol. wt 47000-
48000, found that the DFA-Tp test was 100% sensitive
and specific; dark-field microscopy was 97% sensitive
and 77% specific. Romanowski et al. (1987), using the
same antibody, reported a sensitivity of 73% for the
DFA-Tp test and 79% for dark-field microscopy; both
methods were 100% specific.

Because of variation between samples, if the initial test
is negative the procedure should be repeated daily for at
least 3 days; antibiotics should be withheld during this
period but cotrimoxazole in an oral dose of 960 mg/12 h
for 5 days and local saline lavage may be used to reduce
local sepsis.

The DFA-Tp test with monoclonal antibodies has
many advantages, including the ability to identify non-
motile *T. pallidum* and its applicability to the examination
of oral lesions. It may also be used to examine lymph¬
node aspirates and tissue specimens, making other
methods such as silver staining obsolete. For a more
detailed discussion on microscopy for the identification
of *T. pallidum* see Lukehart and Baker-Zander (1987b).

**Serological tests**

A number of different antibodies are produced in infec¬
tions with *T. pallidum* and the other pathogenic tre¬
onemes. These antibodies can be classified into non-
specific antitreponemal antibodies and antibodies specific
for pathogenic treponemes: the main tests that are or
have been available commercially for use in clinical prac¬
tice are shown along with their year of introduction in
Table 31.1.

**Antibodies to cardiolipin** Cardiolipin antibody
tests now use defined antigens comprising cardiolipin
mixed with lecithin and cholesterol. The development of
these antigens followed the identification of cardiolipin
as the active component and resulted from the sequential
use of saline extracts of fetal syphilitic tissues. For details
of these developments see the previous editions of this
book.
Table 31.1 Tests to detect antibodies produced during treponemal infection

<table>
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<th>Non-specific antitreponemal antibodies</th>
<th>Antibodies specific for pathogenic treponemes</th>
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<tr>
<td>Treponema pallidum immobilization (TPF) test (1949)</td>
<td>T. pallidum haemagglutination assay (TPHA) (1967)</td>
</tr>
<tr>
<td>Fluorescent treponemal antibody absorption (FTA-ABS) test (1964)</td>
<td>Bio-Enza Bead test (1985)</td>
</tr>
<tr>
<td>Venerreal Disease Research Laboratory (VDRL) test (1946)</td>
<td></td>
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<tr>
<td>Rapid plasma reagin test (1957)</td>
<td></td>
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<tr>
<td>Automated reagin test (1968)</td>
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<tr>
<td>Antibodies to group-specific treponemal antigen</td>
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<tr>
<td>Reiter-protein complement-fixation test (1953)</td>
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The Wassermann complement-fixation test introduced in 1906 (Wassermann et al. 1906) was the first serological test for the diagnosis of syphilis. Although of enormous value in the past it is now mainly of historical interest; the World Health Organization (1982) recommended that its use should be discontinued. Because it is easy to standardize simply and reproducibly, the Venerreal Diseases Research Laboratory (VDRL) test (Harris et al. 1946), or one of its modifications, is the preferred method of detecting antibodies against cardiolipin. All other tests, including complement fixation, Kline, Kolmer, Mazzini and Meimicke tests, give less reliable results and have been discontinued in most laboratories (Luger 1987).

Venereal Diseases Research Laboratory (VDRL) test and its modifications In its original form the VDRL test is performed as a slide test in which patient’s serum (3 volumes), previously heated to inactivate complement, is mixed with a freshly prepared suspension of cardiolipin–lecithin–cholesterol antigen (1 volume) on a glass slide. The mixture is rotated, usually mechanically, for 4 min after which time flocculation (aggregation of antigen–antibody complexes in suspension) is detected microscopically with a low-power objective. Quantitative tests with serial dilutions of patients’ serum are easily performed; the highest dilution that can be classified as reactive is reported as the titre. For details of the performance and reading of this test see Young (1989). The major disadvantages of the VDRL slide test are the need to prepare fresh antigen each day and to use a microscope to read the results. Heat inactivation of sera is necessary to destroy a heat-labile inhibitory factor of IgM class which may be present in both normal and syphilitic serum (Lantz and Falcone 1968, Butler and Brenner 1978). Although this was once considered a disadvantage, heat treatment is now favoured by many laboratories as it inactivates the HIV.

Unheated serum reagin (USR) test, rapid plasma reagin (RPR) test (VDRL carbon-antigen test) and automated reagin test (ART) USR antigen comprises VDRL antigen and choline chloride which blocks inhibitory factors in the serum, thus eliminating the need to heat the serum before testing. The antigen is also stabilized with EDTA, allowing it to be used for up to 6 months when stored at 4–10 °C. Results with the USR test are very similar to those of the VDRL test (Pedersen et al. 1980).

In the RPR test, finely divided carbon particles are added to USR test antigen (VDRL carbon antigen). The test can be performed with unheated serum or plasma and the results can be read with the naked eye instead of microscopically. It is simpler to perform than the VDRL test. Although the RPR test has been reported as both more sensitive (Walker 1971) and slightly less sensitive (Fischer et al. 1984) than the VDRL test, the performance of the 2 tests is probably comparable. In the ART, unheated serum or plasma is sampled automatically with an autoanalyzer and mixed with RPR antigen. The mixture is deposited on a moving strip of filter paper and the results are read photometrically. The ART may be the preferred cardiolipin test in laboratories serving blood-transfusion centres.

Reagin screen test (RST) and toluidine red unheated serum test (TRUST) The RST and TRUST are similar to the RPR test except that the antigen is stained with the lipid-soluble dye Sudan black B (RST) or toluidine red paint pigment (TRUST) instead of adding charcoal. The TRUST may have advantages in hot climates as the antigen is more stable than RPR antigen on storage at room temperatures of 26–31 °C. The sensitivity, specificity and reproducibility of the VDRL, RPR and TRUST tests are similar (Luger 1987).

Choice of test Factors such as the number of specimens to be examined, staffing (manpower and degree of expertise), reagent costs, available equipment, laboratory-based or on-site testing in developing countries, etc. must be taken into account in selecting one of these tests. The sensitivity and specificity of the various tests are comparable, so the most important factor in governing performance is careful attention to detail of test procedure combined with a suitable system of internal quality control (Wasley 1985). Tests based on VDRL carbon antigen are now preferred in many laboratories in the UK. However, interpretation of weak positive results may be difficult in tests that are read with the naked eye and there may be slight differences in the end-point reading in different types of quantitative test.

A recently developed ELISA test for determining IgG and IgM against VDRL antigen may be suited to large-scale testing for syphilis and may replace other non-treponemal tests (Pedersen et al. 1987). The sensitivity and specificity of the VDRL ELISA were similar to those of other non-treponemal tests; the test yielded quantitative results on a single serum dilution that correlated with the quantitative results of the traditional non-treponemal tests obtained by titration. The VDRL ELISA test has also supported previous observations on the nature of the cardiolipin-antibody response.

The nature of antibodies to cardiolipin Both treponemal and host antigens may be concerned in inducing antibodies to cardiolipin (Wicher and Wicher 1983). Car-
Antibodies to group-specific treponemal antigen The antigen used in tests to detect group-specific antibodies is derived from the Reiter treponeme, previously reported to be an adapted strain of *T. pallidum* but now classified as *T. phagedenis* (see Chapter 2.30). Before the advent of methods such as microhaemagglutination which require smaller amounts of antigen, the use of the cultivable Reiter treponeme overcame the problems of producing large amounts of antigen from non-cultivable virulent *T. pallidum*. The once popular Reiter-protein complement-fixation (RPCF) test (D’Allesandro and Dardanoni 1953) made use of a lipopolysaccharide–protein–complex antigen most probably derived from the axial filaments. The sensitivity and specificity of the RPCF test are lower than that of tests based on *T. pallidum* antigen. False-positive RPCF test reactions are distinct from non-specific cardiolipin reactions and may be due to antibody against lipopolysaccharide impurities in the antigen (Dupouey 1972). With the advent of newer *T. pallidum* antigen tests such as the *T. pallidum* haemagglutination assay (TPHA), the RPCF test is very rarely used nowadays. However, new ELISA tests based on purified axial filaments (AF-ELISA) of Reiter treponemes have been developed (Pedersen et al. 1982, van Eijk et al. 1986). The AF-ELISA, TPHA and fluorescent treponemal antibody absorption (FTA-ABS) tests (Table 31.1) showed similar specificity and sensitivity in untreated syphilis; the AF-ELISA was significantly less sensitive in treated syphilis. Because axial filament is more readily available than *T. pallidum* antigen, van Eijk et al. (1986) considered the AF-ELISA could be an alternative to the TPHA when large-scale screening is required such as in blood-bank or prenatal screening. However, they acknowledged that much more extensive testing was essential to confirm this view. The role of the AF-ELISA must now be considered in relation to the newly developed commercial ELISA tests based on *T. pallidum* antigen (p. 596).
Antibodies specific for pathogenic treponemes The antigen used to detect the antibodies is derived from the virulent Nichols strain of *T. pallidum*. Antibody produced as a result of endemic treponemal diseases such as yaws, bejel or pinta will also react in tests with this antigen.

*Treponema pallidum* immobilization (TPI) test This test, which determines the ability of patient’s serum to immobilize motile virulent *T. pallidum* is complicated, technically difficult to perform, and expensive in animals and reagents; details are given in the previous edition of this book. Once the only reliable serological test for distinguishing between syphilitic and non-treponemal cardiolipin-antibody responses, the test has now been superseded by the widespread use of the FTA-ABS test (see below). The TPI test may be negative in primary syphilis; this may result in discrepancies with the FTA-ABS test (Spratt et al. 1982). As the FTA-ABS test is more sensitive than the TPI test in all stages of untreated infection and remains positive longer after treatment than does the TPI test, Rein et al. (1980) considered that the TPI test failed to provide useful additional diagnostic information. False-positive TPI reactions have been reported (Rein et al. 1980). The main role of the TPI test nowadays is for research purposes, but a few reference laboratories still perform it on selected sera. In general, it is of more value to repeat the FTA-ABS test on a further serum than to perform a TPI test.

*Treponema pallidum* immune adherence (TPIA) test This test is based on the principle that a specific antigen-antibody and complement complex will adhere to primate erythrocytes or to non-primate platelets (Nelson 1953). In spite of an improved method for the TPIA test (Tanaka et al. 1978) the technique remains more time-consuming and technically demanding than other tests such as the *T. pallidum* haemagglutination assay. It is unlikely to be used for other than research purposes.

The fluorescent treponemal antibody absorption (FTA-ABS) test *T. pallidum* are acetone-fixed on slides and incubated with patient’s serum which has been absorbed to remove group-reactive antibody. Immunoglobulins that bind to the fixed organisms are detected by means of fluorescein-labelled anti-human immunoglobulin. The review of the FTA-ABS test by Hunter (1974) provides details of the evolution of the test through the early FTA 1.5 test (poor in specificity but high in sensitivity), the FTA-200 test (with improved specificity but decreased sensitivity) to the FTA-ABS test (high in specificity and sensitivity) in which group-specific antibody is removed by an absorption stage.

Various factors influence the performance of the test, including the type of absorption reagent, the purity of the treponemes, and the type of conjugate used. Efficient removal of group-reactive antibody, which may be present in non-syphilitic sera and usually predominates over specific antibody in treponemal infection (Wilkinson and Rayner 1966) is important. Initially ultrasonically disintegrated Reiter treponemes were used to neutralize the group-reactive antibody (Hunter et al. 1964). This reagent or ‘sonicate’ was later replaced by ‘sorbent’, the concentrated supernatant from an autoclaved culture of Reiter treponemes (Deacon et al. 1966). After the demonstration by Rathlev (1968) that un inoculated concentrated Reiter culture medium was also effective in blocking group-reactive antibodies, some ‘sorbents’ now lack treponemal antigen. The blocking action of these sorbents is largely due to their high osmolarity. Wilkinson and Johnston (1975) considered that the use of sonicate improved the performance of the FTA-ABS test without diminishing its sensitivity.

Treponemes for FTA antigen should be harvested early (7–9 days) from rabbits as more prolonged growth results in increased binding to the treponemes of rabbit immuno-globulin. Antiglobulin factors in patient’s serum may react with treponemes sensitized in this way and produce false-positive reactions. Purification of antigen is also important. Treponemes purified by Percoll density-gradient centrifugation were ultrastructurally intact, motile, virulent and free from detectable soluble or insoluble host-testicular material, and resulted in few equivocal reactions (Hanff et al. 1986). The use of monospecific anti-IgG conjugate is also considered to reduce the number of borderline reactions (Hunter et al. 1976). A monoclonal anti-IgM conjugate can also be used to detect specific anti-treponemal IgM (p. 597).

Although not suitable for routine screening, the FTA-ABS test is now the accepted reference test; when reagents of good quality are used and the test is properly controlled the sensitivity and specificity are high. Improved performance and greater standardization of commercial reagents is desirable. In an evaluation of 4 different kits, the ability to detect reactive samples varied from 82.5 to 95%, and the agreement with non-reactive and borderline samples ranged from 80.9 to 96.4% (Beebe and Nouri 1984).

The FTA-ABS test becomes reactive (i.e. gives a positive result) around the 3rd week of infection and in primary infection has a sensitivity ranging from 86 to 100%. It is positive in all secondary cases and 96–100% of late-stage infections (Lukehart and Baker-Zander 1987b). Reactivity persists after adequate therapy. Occasionally the test may become non-reactive (i.e. negative) if treatment is given early in the disease.

The total prevalence of false reactivity is around 1% in normal persons, but higher rates have been reported in hospital patients (Luger 1987). In recent evaluations of the test, specificity ranged from 92 to 99% (Hunter et al. 1986). False-positive reactions have been found in pregnancy (Drew and Sarandria 1975); they are no more common in pregnant women than in normal persons (Manikowska-Lesinska et al. 1978). False-positive reactivity is associated with rheumatoid arthritis (Tuffanelli et al. 1967), lupus erythematosus (McKenna et al. 1973), cirrhosis (Mackey et al. 1969) and hypergammaglobulinaemia (Bradford et al. 1967). Transitory false-positive results have been reported in some cases of genital herpes infection (Wright et al. 1975) although this was not confirmed by others (Chapel et al. 1978). Excessive growth of commensal treponemes in oral or genital lesions may give rise to false-positive reactions if
the group-reactive antibody is not completely blocked. In false-positive reactions associated with lupus erythematosus and collagen disease the treponemes have a typical beaded fluorescence pattern (McKenna et al. 1973) due to anti-DNA antibody in the serum reacting with treponemal nucleic acid. These false-positive results can be distinguished from positive FTA-ABS tests due to treponemal infection in that the sera from patients with collagen disorders also give fluorescence with Treponosoma cruzi and Toxoplasma gondii (Wright 1973). Fluorescence is absent when organisms are preheated with deoxyribonuclease. A high rate of false reactivity (22.5%) was reported in patients with Lyme disease (Hunter et al. 1986). Simultaneous titration of sera against T. pallidum and Borrelia burgdorferi antigens can be used to distinguish between the 2 infections.

Modifications of the FTA-ABS test These include a double-staining procedure with a rhodamine-labelled class-specific anti-human-IgG primary stain and fluorescein-labelled anti-treponemal globulin as counterstain (Farshy et al. 1983). This method, which makes it easier to see treponemes on non-reactive slides, is more suitable for newer fluorescence microscopes equipped with incident illumination. Increased sensitivity resulting from the use of biotinylated antibody and avidin–fluorescein has also been described (Bertsson 1985) but the system has not been evaluated in clinical practice. A solid-phase fluorimmunoassay makes use of a lyse of virulent T. pallidum absorbed on to a cellulose acetate disc; it has been investigated as a potential automated alternative to the FTA-ABS test (Stevens and Schell 1982).

The Treponema pallidum haemagglutination assay (TPHA) The TPHA (Tomizawa and Kasamatsu 1966, Rathlev 1967) is simple to perform and became the first of the specific tests suitable for routine screening. Because of greater convenience and lower cost, most laboratories perform the TPHA in microtitre plates; this is often referred to as the MHA-TP but the 2 terms are used synonymously.

Sera are absorbed with diluent containing components of Reiter treponemes, rabbit tests, and erythrocyte membranes before being incubated with sheep erythrocytes coated with sonically treated T. pallidum. Antibody in the serum of patients with syphilis results in agglutination of the sensitized erythrocytes. Sera are usually screened at a final serum dilution of 1 in 80. Quantitative tests can be performed with serial doubling dilutions ranging from 1 in 80 to >1 in 5120. All sera showing agglutination of sensitized cells should also be tested against control erythrocytes (not coated with antigen). About 0.1% of specimens agglutinate erythrocytes in the absence of antigen: this non-specific agglutination makes the individual test result invalid. The use of fowl erythrocytes may decrease the number of non-specific agglutination reactions (Sequeria and Eldridge 1973).

Luger (1981) reported 0.07% false-positive and 0.008% false-negative results; the TPHA was considered to be the most reliable and practical test for mass screening for syphilis. Doubts on the high specificity of the TPHA were expressed by Garner et al. (1973) who investigated patients from areas with a high incidence of tropical diseases and found that 11.3% showed reactivity in the TPHA but not the TPI or FTA-ABS tests. Other workers considered these results to be due to the high sensitivity of the TPHA in detecting the residue of previous infection by endemic treponematosis (Fischman et al. 1974), Manikowska-Lesinska et al. (1978).

TPHA reactivity may be detectable around the 4th week of infection. The sensitivity in untreated primary infection ranges from 64% to 87% (Lukehart and Baker-Zander 1987b). Reactivity depends on the exact duration of the infection and the IgM-binding capacity of the reagents. When the TPHA is positive in primary infection the titre is low (80–320). Most authors (Young et al. 1974, Larsen et al. 1981, Huber et al. 1983, Sequeira 1983) found that antibody detected by the TPHA appeared later or was of relatively lower titre than that detected by cardiolipin-antigen tests. In contrast, Dyckman et al. (1980) reported that the TPHA generally became reactive earlier than the VDRL test. In infections beyond the primary stage, the TPHA is almost always positive and is more sensitive than the TPI or FTA-ABS tests (Luger 1987). Titres rise sharply during the secondary stage and usually reach 5120 or greater. Although the titre decreases during the latent stage it invariably remains positive at low titre (80–640). High titres (>5120) can be expected in active late syphilis but persistently high titres do not always reflect active disease. After treatment the TPHA invariably remains positive for life although a rapid fall in titre may occur in some patients treated for secondary infection.

Kobayashi et al. (1983) described an agglutination test in which erythrocytes were replaced by polyurea microcapsules as the carrier for treponemal antigen. Microcapsules are chemically stable and antigenically inert and should not deteriorate or give rise to non-specific agglutination due to heterophile antibody. The microcapsule agglutination test was superior to the TPHA for detecting primary syphilis.

Enzyme immunoassay (EIA) Veldkamp and Visser (1975) used an ultrasonicate of T. pallidum antigen coated on to tubes and concluded that EIA was simple, reliable and relatively quick; its sensitivity in all stages of syphilis was equivalent to that of the FTA-ABS test. The total sensitivity and specificity were 99% and 98% respectively. A lower sensitivity (89%) but comparable specificity (99%) was found by Pope et al. (1982) in a similar assay performed in microtitre plates. Sodium-deoxycholate-extracted antigen resulted in improved sensitivity (96%) and stability of antigen without affecting specificity (Hunter et al. 1982). Another modification (Farshy et al. 1985) incorporated an antigen-capture step in which rabbit antiserum against T. pallidum was used; specificity was poorer (95%) without any corresponding increase in sensitivity (96%). In a preliminary study, Pospisil (1983) reported a sensitivity and specificity of 100%, using white polystyrene carriers which were dipped into wells in polystyrene plates to coat them with antigen. Antigen-coated carriers were then placed sequentially in patients’ serum, conjugated immunoglobulin and substrate. A handle on the carrier simplified the transfer and
washing stages. Small plastic discs containing iso-thiocyanate groups were coated with sodium-deoxycholate-extracted antigen in the EIA described by Chen et al. (1986). The sensitivity (96%) and specificity (99%) were similar to those of the microtitre plate test.

EIAs with *T. pallidum* antigen are now available commercially. The Bio-Enza Bead test (Organon Teknika Corporation) makes use of ferrous metal beads as a solid-phase carrier for antigen. Magnetic forces are used to transfer the beads from one reaction mixture to another. The sensitivity of the Bio-Enza Bead test has been reported as 75% in a population where 72% of the treponemal sera tested were VDRL reactive (Borobio et al. 1985), 99.5% when all sera were VDRL reactive (Stevens and Schmidt 1985) and 95% when 94% of the sera were VDRL reactive (Moyer et al. 1987). The specificity of the test was 98%. In the Captia Syphilis-G test (Merica Diagnostics) antibody bound to *T. pallidum* antigen coated on to a plastic well is detected by a tracer complex comprising biotinylated monoclonal antibody against human IgG and streptavidin-horseradish peroxidase conjugate. The sensitivity and specificity of the assay were 98.4% and 99.3% respectively in an evaluation in which only 41% of the treponemal sera were VDRL reactive (Young et al. 1989). This test would seem to be more sensitive than the Bio-Enza Bead test in detecting late, including latent, infection irrespective of treatment status.

Although all of the above EIAs use anti-human IgG conjugate, they are able to detect primary infection. The EIA of Veldkamp and Visser (1975) detected all 32 cases of untreated primary infection. The Bio-Enza Bead test was reactive in 5 of 6 cases (Borobio et al. 1985) and all 3 cases (Stevens and Schmidt 1985) of untreated primary infection. Farsky et al. (1984) described a double-conjugate assay for IgG and IgM and found that in infected patients a positive IgG result always accompanied a positive IgM result. The potential for automation makes EIA attractive as a first-line screening procedure for treponemal infection.

### Use and interpretation of serological tests

#### Screening schedules

In the United Kingdom, the combination of a cardiolipin-antigen test and the TPHA is the most widely used screening procedure. When used together these tests provide a highly efficient screen for the detection or exclusion of all stages of treponemal infection. The only stage of syphilis likely to escape detection is early primary syphilis, although repeated tests over a 3-month period will detect such an infection. Most such cases of seronegative primary syphilis should be detected by a careful clinical examination and dark-ground investigation. Diggory (1983) suggested that the VDRL test should be withdrawn from initial testing for syphilis except where primary disease is suspected. However, as the VDRL is both cheap and simple to perform and may be positive in primary syphilis when the TPHA is negative, it is probably worth retaining. However, if the prevalence of primary syphilis decreases further and is maintained at a low level for several years there would be less objection to relying on the TPHA alone.

In contrast, screening in the USA often relies solely on the VDRL (or RPR) test. This gives limited 'cover', because the VDRL test may fail to detect about 1% of patients with secondary syphilis owing to the prozone phenomenon (Luger 1987). It will also fail to detect about 30% of patients with late syphilis. Detection of latent infection is very important because if untreated, a portion of the affected patients will develop the clinical manifestations of late infection. However, because of the sensitivity of the TPHA its use as a screening test presents the clinician with the problem of distinguishing between treated and untreated or partially treated infection.

Traditionally, in situations such as blood transfusion, cardiolipin-antigen tests have been used to detect donations from patients with early syphilis. Puckett and Pratt (1982) described a modified miniaturized version of the TPHA for use in blood-transfusion centres but considered that neither the TPHA nor cardiolipin tests should be relied upon alone in screening. Routine antenatal screening early in pregnancy is also important. Although the risk to the fetus is highest in early infection, the TPHA is valuable in detecting women with infections of uncertain treatment status. Because congenital infection is preventable by appropriate treatment during pregnancy and the costs of congenital syphilis are so high, screening may be cost-beneficial when the prevalence of maternal infection is as low as 0.005% (S'r-ray-Pedersen 1983).

EIA is a more sensitive system than agglutination; in spite of using anti-human IgG conjugate it is more sensitive than the TPHA in primary syphilis (Pope et al. 1982). Results of screening with the Captia Syphilis-G EIA were comparable with screening with a combination of VDRL and TPHA tests (Young et al. 1989). The potential for automation and computerized data analysis and reporting makes such tests attractive for rapid screening of large numbers of specimens for current or past infection. Theoretically, however, there is a small 'window' of 2–3 weeks during which very early primary infection may escape serological detection by screening with an EIA method that makes use of an anti-human IgG conjugate. Because the diagnosis of early primary infection relies on a high index of clinical suspicion, an FTA-ABS test should be requested in such cases.

Whichever screening system is used, human and technical error may occur, and a diagnosis of syphilis should never be made on the results of a single blood specimen. Caution is required in excluding treponemal infection in persons with HIV infection, because limited data indicates that a new treponemal infection may not result in the development of cardiolipin or specific antibody (Hicks et al. 1987).

#### Confirmatory tests

Because of its high sensitivity at all stages of syphilis the FTA-ABS test is the accepted reference test for confirming the treponemal nature of reactivity in one of the screening tests. It is essential for distinguishing between non-specific cardiolipin reactions and early syphilis in the case of VDRL-positive TPHA-negative sera. Repeated serological tests are also helpful in this situation. In the absence of a suitable history, the FTA-ABS test is useful for confirming the treponemal
nature of TPHA-positive VDRL-negative sera. However, it should be borne in mind that the specificity of the FTA-ABS is no greater than that of other tests and that its value as a specific confirmatory test depends on the greater probability of treponemal infection in cases reactive in a screening test. Very rarely more than one treponemal test may be falsely reactive. Russell-Jones et al. (1983) described a patient with essential mixed cryoglobulinaemia whose serum gave false-positive reactions in the FTA-ABS and TPHA tests and an anti-complementary TPI test. The Bio-Enza Bead test (Borobio et al. 1985, Stevens and Schmidt 1985, Moyer et al. 1987) has been advocated as an alternative to the FTA-ABS test. Because it lacks sensitivity in late and latent disease, the Bio-Enza Bead test could be used only as a confirmatory test in cases in which cardioplin-antigen tests are employed as the sole means of screening.

Quantitative tests
Quantitative tests are used in follow-up to show the effectiveness of treatment and to allow prompt retreatment when this has failed. They may also give some guidance as to the stage of infection as well as detecting reinfection.

All cardioplin-antigen tests tend to become negative after treatment, particularly in early syphilis. The titre declines approximately 4-fold at 3 months and 8-fold at 6 months (Braun et al. 1985). In interpreting a fall or rise in titre in early-stage syphilis a 4-fold change is considered significant but a 2-fold change is not. The mean period of reactivity after treatment is 4 months for primary syphilis, 17 months for secondary, 13 months for early latent disease and 60 months for latent infection of indefinite duration (Luger 1987). As a general guide, the VDRL test should become negative within 1–2 years of effective treatment of early syphilis. After adequate treatment of late infection the VDRL test will show a slow decline in titre, some cases eventually becoming negative while others may remain reactive (titre <8) for many years. VDRL titres of >16 rarely persist in adequately treated infections. In patients who have been reinfected the rate of seroconversion is slower (Fiumara 1980).

Although a rapid fall in TPHA titre may occur after treatment for secondary syphilis, the TPHA is of little help in monitoring the efficacy of treatment. Quantitative tests, when interpreted in the light of clinical signs and symptoms, and with a knowledge of the patient's history, will support a diagnosis of untreated early syphilis. A significant increase in titre may indicate reinfection and provides a safeguard against missing reinfection when the VDRL is falsely negative on screening as a result of the prozone phenomenon. However, the TPHA titre is of little help in assessing treatment status in cases of late syphilis. Because the TPHA remains positive for life even in those who have been adequately treated, its use as a screening test has increased the importance of specific antitreponemal IgM as an indicator of treatment status.

Detection of specific antitreponemal IgM
After infection the first humoral response is the production of antibodies of the IgM type; these are usually detectable during the 2nd week of infection. As IgM synthesis through memory cells does not occur, specific IgM production ends soon after elimination of the antigen. A reactive IgM test in untreated patients suggests active disease and the need for treatment. In general, the titres of specific IgM decline rapidly after adequate treatment of early syphilis and reactivity ceases within 3–9 months; reactivity may be found from 1 to 1.5 years after treatment of late disease (Luger 1987). Persistence of IgM reactivity after therapy suggests treatment failure whereas the reappearance of IgM reactivity after repeated non-reactivity suggests reinfection. As IgM cannot cross the intact placenta the detection of antitreponemal IgM in neonatal sera indicates congenital infection. Similarly, IgM does not cross the normal blood–brain barrier and its detection in CSF is indicative of neurosyphilis.

Many different methods are available for detecting antitreponemal IgM (Table 31.2). For a more detailed discussion of the individual tests see Luger (1987) and Robertson et al. (1989). The IgM-FTA-ABS test was the first method used for the detection of specific antitreponemal IgM. In common with other methods that use unfractionated serum, the test is subject to false-positive results caused by rheumatoid factors and false-negative results caused by competitive inhibition of IgM binding by IgG antibodies of the same specificity for the binding site on the antigen. At present the 19S (IgM) FTA-ABS is the most sensitive and specific test for the detection of specific IgM in all stages of syphilis. Unfortunately the need for fractionation of sera makes the test technically complicated and unsuitable for routine purposes. The IgM SPHA was one of the first specific antitreponemal IgM tests that was simple and cheap enough to be applied on a large scale. Merlin et al. (1985) found that the SPHA had a sensitivity of 96% and a specificity of 97.4% when compared with the 19S (IgM) FTA-ABS. However, false-negative results may occur, particularly in early infection (Müller and Lindenschmidt 1982). If high VDRL titres suggest active disease a non-reactive SPHA result should be checked by the 19S (IgM) FTA-ABS test (Luger 1987). The use of erythrocytes as the solid phase in the Tp-IgM-HA method improved IgM detection in early syphilis while maintaining high specificity (Sato et al. 1984). Unfortunately reagents for these tests are not available commercially. ELISA may be more sensitive than the IgM-SPHA (Luger 1987) but in using whole serum it is subject to interference from rheumatoid factor. Müller et al. (1987) described a modified ELISA test in which immunoprecipitation of total IgG was used to overcome the problem of false-positive results from rheumatoid factor and false-negative results from competitive inhibition by IgG. The modified Tp-IgM ELISA gave a sensitivity of 98% and a specificity of 97.6%, and results accorded well with those of the 19S (IgM) FTA-ABS (Müller et al. 1987). The Captia Syphilis-M test was the first specific antitreponemal IgM test available commercially. Conventional capture ELISAs are subject to interference from rheumatoid factor. Because rheumatoid factor binds to the anti-IgM antibodies coated on the solid phase it can in turn bind the Fc portion of the IgG conjugate used to detect bound
treponemal antigen. This type of false-positive result should not occur in the Captia Syphilis-M test because a tracer complex is used instead of an IgG-class conjugate (Ijsselmuiden et al. 1989). The Captia Syphilis-M test was positive in all 5 cases of congenital infection; the sensitivity was 82% (18/22) in primary syphilis, 60% (12/20) in secondary, 53% (16/30) in latent and 34% (11/32) in neurosyphilis (Ijsselmuiden et al. 1989). Reactivity of the 19S (IgM) FTA-ABS test in the various stages was 82% (primary), 70% (secondary), 60% (latent) and 72% (neurosyphilis).

In patients with positive serological tests who lack a history of recent adequate treatment, a reactive antitreponemal IgM test supports the need for treatment. However, occasionally the sera of patients who have been adequately treated will contain 8S IgM which will react in μ-chain capture assays (Müller and Lindenschmidt 1982). Untreated or inadequately treated infections, particularly beyond the early stages, as well as reinfection, cannot be excluded reliably on the basis of a negative antitreponemal IgM test, and due consideration must be given to clinical findings, the history of the patient, and quantitative VDRL and TPHA tests. Until more experience is gained with the simpler IgM tests, quantitative VDRL testing remains the method of choice in assessing the efficacy of treatment and detecting reinfection.

**Table 31.2 Tests for specific antitreponemal IgM**

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Immunoglobulin M fluorescent treponemal antibody absorption test (IgM-FTA-ABS)</td>
<td>Immunofluorescence with unfractinated serum and specific anti-human IgM conjugate</td>
<td>Atwood and Miller (1970)</td>
</tr>
<tr>
<td>19S immunoglobulin M fluorescent treponemal antibody absorption test (19S (IgM) FTA-ABS)</td>
<td>Immunofluorescence with IgM fraction obtained by gel filtration or ultracentrifugation</td>
<td>Müller and Loa (1974)</td>
</tr>
<tr>
<td>Immunoglobulin M solid phase haemadsorption test (IgM-SPHA)</td>
<td>Microtitre plate wells act as solid phase for μ-chain capture. Specific IgM detected by TPHA reagents</td>
<td>Schmidt (1980)</td>
</tr>
<tr>
<td>19S IgM Treponema pallidum haemagglutination assay (19S (IgM) TPHA)</td>
<td>IgM fraction obtained by gel filtration titrated with standard TPHA reagents</td>
<td>Müller and Lindenschmidt (1982)</td>
</tr>
<tr>
<td>T. pallidum specific IgM haemagglutination test (Tp-IgM-HA)</td>
<td>Erythrocytes act as solid phase for μ-chain capture. Specific IgM detected by erythrocytes sensitized with sonicate of T. pallidum</td>
<td>Sato et al. (1984)</td>
</tr>
<tr>
<td>Immunoglobulin M enzyme linked immunosorbent assay (IgM ELISA)</td>
<td>Unfractionated serum reacts with T. pallidum antigen on microtitre plate. Specific IgM detected by enzyme-labelled anti-human IgM</td>
<td>Müller and Moskophidis (1994)</td>
</tr>
<tr>
<td>Captia syphilis-M monoclonal antibody enzyme linked assay (Captia Syphilis-M)</td>
<td>Microtitre plate wells act as solid phase for μ-chain capture. Specific IgM detected by tracer complex comprising T. pallidum antigen-biotinylated monoclonal antibody against T. pallidum-streptavidin conjugated to horseradish peroxidase</td>
<td>Ijsselmuiden et al. (1989)</td>
</tr>
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</table>

**Congenital syphilis** Early-stage congenital syphilis is rare in the United Kingdom; only 12 of the 126 cases of congenital syphilis reported in 1982 were in children aged <2 years (Report 1985). Antenatal screening is an important and effective preventive measure because treatment given during pregnancy will prevent congenital infection (Stray-Pedersen 1983). The diagnosis of congenital syphilis may present difficulty because most syphilitic neonates are symptomless at birth and the standard serological tests for syphilis depend on IgG and IgM antibody responses: the IgG found in the serum of neonates is largely passively acquired through the placenta. The demonstration of specific antitreponemal IgM by the 19S (IgM) FTA-ABS test confirms a diagnosis of congenital infection. Fractionation of serum prevents false-negative reactions resulting from competition between IgG and IgM for treponemal binding sites. IgM capture may also overcome this problem, and Ijsselmuiden et al. (1989) considered the Captia Syphilis-M test might prove useful for easy and sensitive detection of antitreponemal IgM antibodies in congenital infection. Serial examinations are recommended because specific IgM may not be detectable until several weeks after birth. This phenomenon is thought to result from suppression of IgM synthesis in the neonate due to high levels of circulating maternal antitreponemal IgG (Johnston 1972). However, according to Borobio et al. (1980), there was no suppression of IgM synthesis. As well as specific IgM, infants had raised total IgM, usually in the range...
of 1–4 g/l. After treatment, total IgM returned to normal (<0.7 g/l), specific antitreponemal IgM and other antibodies decreased in amount, and tests tended to become negative.

It is helpful to test maternal and neonatal sera in parallel, particularly when specific IgM tests are not available. A significantly higher antibody titre in the neonate than in the mother suggests infection, whereas a lower titre in the neonate than the mother suggests passively transferred antibody. Serial testing is important: in the absence of infection, passively transferred antibody detected by the VDRL will decrease and the tests will become negative in approximately 3 months. In the case of the TPHA, the titre will become low and the test negative in 6–12 months.

In late-stage infection, either treated or untreated, test results tend to fluctuate over a period of months or years. The VDRL test often remains positive at low titre as does the TPHA. Data on specific antitreponemal IgM in late stage congenital infection is inadequate.

Neurosyphilis Because invasion of the central nervous system can be detected before symptoms develop, and because the early effects of syphilis on the system can often be reversed by penicillin treatment, examinations of the CSF are important in the assessment of patients with syphilis. Diagnosis may be made more difficult if atypical forms of neurosyphilis become more prevalent (Simon 1985), but Talbot and Morton (1985) consider that there has been no major deviation from the classical symptoms. Unfortunately there are no acceptable figures, past or present, to indicate the relative proportions of 'atypical' and 'classical' cases, but recent publications reflect an increased awareness of atypical forms (Luger 1987). Although experience is limited, it may be that, when there is concomitant infection with HIV, neurosyphilis can usually develop rapidly (Johns et al. 1987). CSF examination is necessary when there is evidence of clinical relapse or a 4-fold rise is noted in the titre of follow-up serological tests on serum (Catterall 1977). It is generally agreed that the CSF should be examined in all patients with clinical neurosyphilis, and in all infections of more than 2 years' duration in order to exclude symptomless neurosyphilis.

It is unnecessary to perform routine screening tests for syphilis on the CSF of patients with symptoms referable to the central nervous system in whom there is no suspicion of syphilis. A negative T. pallidum antigen test, such as the TPHA, on serum will virtually exclude active neurosyphilis and is a better screen for the detection of all forms of late syphilis than examination of the CSF (Leading Article 1977). The latter should be reserved for cases selected on clinical grounds and backed by a positive TPHA test on serum. A negative TPHA test on CSF excludes neurosyphilis. A negative VDRL test on CSF does not exclude neurosyphilis as a third to a half of patients with clinically active neurosyphilis will give a negative VDRL result on the CSF (World Health Organization 1982).

Although active neurosyphilis can be excluded reliably as indicated above, obtaining unequivocal serological evidence of CNS involvement is essentially a procedure for a specialized laboratory. For a detailed discussion of criteria for making a diagnosis of neurosyphilis see Catterall (1977), World Health Organization (1982), Johnson and Thompson (1986), Luger (1987), Robertson et al. (1989). The major problem is that measurement of the amount of any antibody in CSF is of limited value without simultaneous knowledge of the serum content and the degree of permeability of the serum–CSF barrier. A cell count of > 5 x 10⁶/l and total protein concentration above 0.45 g/l indicates inflammation without indicating its cause. In the presence of other evidence of syphilis, however, they are reliable in defining the activity and type of neurosyphilis, and their rapid reversal to normal after treatment is a measure of resolution. TPHA titres of > 2560 in the CSF are highly suggestive of active neurosyphilis (Luger 1981). To exclude errors that may arise from disturbed function of the blood–brain barrier several new variables have been proposed. These include evidence of the local production of IgG, IgM and treponemal IgG by estimating the ratio of the CSF to serum IgG concentration/the ratio of CSF to serum albumin concentration (the IgG index), the ratio of the CSF to serum IgM concentration/the ratio of the CSF to serum albumin concentration (the IgM index), or the ratio of CSF TPHA titre to the CSF IgG/the ratio of serum TPHA titre to serum IgG; or by detecting oligoclonal IgG, IgM or treponemal IgG in the CSF (van Eijk et al. 1987). Hook et al. (1986), using Western-blot analysis, revealed the presence in serum of IgG antibodies to at least 17 treponemal antigens and in CSF of antibodies to at least 10 treponemal antigens. After ceftriaxone therapy, serum and CSF IgG reactivity to all antigens decreased in intensity. It was concluded that ceftriaxone may provide useful alternative treatment for penicillin-allergic patients with neurosyphilis. Most penicillin-allergic patients with neurosyphilis have been treated with tetracycline or erythromycin, which give poor penetration into the CSF. Because confidence and experience with these antibiotics in the treatment of neurosyphilis is less than with penicillin, very careful CSF follow-up is required (Robertson et al. 1989).

Conclusions
Although there have been many improvements in diagnosis over the past few years there are further significant changes on the horizon. Our better understanding of the immune response to treponemal antigens, the development of monoclonal antibodies, and the production of treponemal antigens by gene cloning are likely to lead to a new generation of serological tests. Indeed, several groups of workers have already started to characterize the immune response to cloned antigens (Coates et al. 1986, Hindersson et al. 1986, Radolf et al. 1986).

Prophylaxis and treatment
At present there is no effective means of immunization against syphilis in man. Current research into the roles of a number of individual antigens, possible now that monoclonal antibody and gene cloning technology is
being applied, may lead to identification of potentially protective antigens (Chapter 2.30). The ethical, practical and logistic obstacles to a vaccination programme are, however, substantial (Lukehart 1985): for example, the effect of any vaccine on serological status would have to be defined and shown not to interfere with diagnostic procedures.

Penicillin is by far the most widely used treatment for syphilis. The curative effect of this antibiotic in man was first demonstrated by Mahoney et al. (1943). Incubating syphilis can be aborted by very small doses of penicillin, and the fall in incidence of early syphilis since 1946 is probably due in part to the widespread use of penicillin for gonorrhoea and other illnesses, unsuspected early infections being aborted at the same time. In early infections a course of 10–12 daily injections of $0.6 \times 10^6$ units of aqueous procaine penicillin will produce cure in more than 95% of cases. In patients who are thought likely to default a single dose of $2.4 \times 10^6$ units of benzathine penicillin can be given. In late syphilis the aim of treatment is to prevent progression of the disease. Longer courses, such as $0.6 \times 10^6$ units of aqueous procaine penicillin daily for 20 days or 3 injections of $3 \times 10^6$ units of benzathine penicillin at weekly intervals, are recommended. Penicillin penetrates poorly into the cerebrospinal fluid after such courses of treatment (Dunlop et al. 1979, Polinikorn et al. 1980). In neurosyphilis the injection of $0.5 \times 10^6$ units of crystalline penicillin with $0.3$ g probenecid orally every $6$ h for 15–20 days has been advocated.

If penicillin cannot be given because of sensitization, 500 mg of tetracycline or erythromycin 4 times daily for 15 days in early syphilis and 30 days in later infections are alternatives. Experience of the long-term results of these last 2 methods is still inadequate. Tetracycline should not be used in pregnant women or in young children. There have been reports of the failure of erythromycin to prevent fetal infection when it was given during pregnancy. The newer derivative roxithromycin, proven to be effective in rabbit infection (Lukehart and Baker-Zander 1987a), may be a better alternative. There have been no reports of significant, proven resistance to penicillin in T. pallidum, although treatment failures of unknown cause have been described. There is, however, evidence of resistance to erythromycin in one isolate (Stamm et al. 1988), and the possibility that the organism may ultimately acquire resistance to penicillin cannot be ruled out. Alternatives to penicillin are not numerous (M. F. Rein 1976), but the newer cephalosporins appear effective in vitro (Korting et al. 1986) and should have potential clinically.

Whatever form of treatment is given, it is essential that the patient is kept under clinical observation and progress monitored by regular serological tests. The initial treatment may be accompanied by a Jarisch–Herxheimer reaction. This is common in early syphilis in which it comprises fever, headache and an exacerbation of the early lesions or rash. It is transient and unimportant at this stage of the disease. In some forms of late syphilis with lesions in vital structures, such as gumma of the larynx or involvement of the coronary ostia, serious results have been attributed to it, and preliminary treatment with steroids has been advocated. The reaction may be a hypersensitivity due to a sudden release of treponemal products (Heyman et al. 1952). It appears unlikely, however, that release of a classical endotoxin-like substance is responsible for the phenomenon (E. J. Young et al. 1982).

### Related treponematoses

#### Rabbit syphilis

Rabbit syphilis is a naturally occurring venereal disease of rabbits due to the spirochaete Treponema paraluisani. In many respects it resembles syphilis infection in man and experimental T. pallidum infection in the rabbit (Small and Newman 1972, Beamer-Cunliffe and Fox 1981, DiGiacomo et al. 1983, 1985). The lesions consist of small scaly patches, often slightly eroded and covered with a brownish crust, situated on the genitals or in the perineal region. Sometimes the nostrils or eyelids are affected. The spirochaetes are found in large numbers in scrapings from the lesions and in sections; they are confined apparently to the superficial layers. The disease can be transferred to normal rabbits by inoculation of a tissue suspension on to the scarified skin of the genital region; transmission can also be effected by mating, but with less constancy (Graves et al. 1980), or by the inoculation of lymph-node material intratessicularly (DiGiacomo et al. 1985). The incubation period is from 2 to 8 weeks; once established, infection persists for months. In males, spontaneous cure usually occurs, but in females the disease is very chronic. Rabbits suffering from rabbit syphilis can be infected with human syphilitic material, indicating that the 2 diseases are not identical (Noguchi 1922). T. paraluisani is morphologically indistinguishable from T. pallidum, and infection is accompanied by the production of similar antipoidal and antitreponemal antibodies (DiGiacomo et al. 1985).

Baker-Zander and Lukehart (1984) showed that sera from rabbits infected with T. paraluisani reacted in Western blots with a wide range of antigens from T. pallidum, indicating a close antigenic and structural relationship between them. Such experiments have not, however, been done with antigens from T. paraluisani. Penicillin is curative. Infection of rabbits with T. paraluisani gives some protection against both syphilis and yaws strains (Turner and Hollander 1957).

Graves (1981) found there was an initial depression of immunity followed after 5 months by limited protection against low doses of T. pallidum. Inoculation of a human volunteer intradermally with $2 \times 10^7$ virulent T. paraluisani produced a transient skin lesion and the Wassermann and TPHA tests became weakly positive for a short time (Graves and Downes 1981).

#### Yaws, pinta and endemic syphilis

**Yaws** is a contagious inoculable disease characterized classically by the appearance of papules, which generally
develop into a fungating, encrusted granulomatous eruption (Manson 1914). In recent years, particularly in areas of low or declining incidence, atypical attenuated or receding forms of yaws with less abundant, less florid lesions have become common (Vorst 1985). Infection occurs in childhood. Its spread is favoured by a warm humid atmosphere, scanty clothing, poor personal cleanliness and a low standard of living. Latent cases are 2-5 times as common as clinically manifest disease, and the patients are apt to suffer from infectious relapses in the early years after infection (Report 1960). The disease is not of venereal origin. It is spread by contact and possibly by insects. Congenital infection is unknown. The commonest site for the primary lesion is the lower extremities. The secondary eruption usually appears in another 2-4 weeks. In the late stages of yaws, bone lesions and ulcerative skin lesions develop. The causative organism, *T. pertenue*, or *T. pallidum* subsp. *pertenue*, was discovered by Castellani in 1905. It is indistinguishable from *T. pallidum*. Serologically, yaws cannot be distinguished from syphilis, even by the TP1 test, thus indicating the extremely close relation of the 2 organisms (see Chapter 2.30 for details of molecular comparisons).

Extensive campaigns were waged in the past against yaws in many tropical countries under the auspices of the World Health Organization (see Report 1960, Guthe 1969, Guthe et al. 1972). The need for further control programmes has recently been reviewed by Hopkins (1985). Infection with yaws provides considerable immunity against syphilis, although this is not absolute and may wane with time. In areas where treatment campaigns have been conducted young adults may no longer have the protection given by childhood yaws; there is evidence that venerally acquired syphilis is invading some of these regions. Resurgences in the incidence of yaws in some areas where it was formerly virtually eradicated are also causing considerable concern (Agazdi et al. 1983, 1985). For information on the nomenclature, pathology, epidemiology, diagnosis and treatment of yaws, see Symposium (1953), Hackett (1957), L. H. Turner (1959) and Symposium (1985).

**Pinta**, like yaws, is a contagious inoculable disease. It is restricted to Central and South America. Infection occurs by contact under conditions similar to those favouring spread of yaws. Children 10-15 years old are most commonly affected. The skin bears the brunt of the disease. The lesions, which are dry and scaly and variously coloured, progress slowly and run a prolonged course. Other tissues of the body are seldom attacked. The causative organism, *T. carateum*, was first demonstrated by Saenz and his colleagues (1938). It is morphologically indistinguishable from *T. pallidum*, and antibodies from pinta cases recognize a full spectrum of antigens from *T. pallidum* by immunoblot, suggesting a very close relationship between these organisms (Fohn et al. 1988). The disease has been transmitted to chimpanzees (Kuhn et al. 1970). Antipoidal antibodies appear in a low proportion of patients in the primary stage, and in most in the late stages (C. R. Rein et al. 1952). Penicillin is curative (Rein et al. 1952).

Numerous forms of endemic syphilis are known, frequently called by different names in different countries, such as bejel of the Middle East, and skeri)jevo of Bosnia (see Grin 1953, Hudson 1961). Transmission is usually by contact. Venereal spread is uncommon. Primary chancres are rare. The outstanding features are the frequency of mucous patches in the mouth, and the occurrence of skin and bone lesions in both the early and late stages.

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Syphilis and related treponematoses

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Immunoblots of PBC serum (1 in 100) against:

(1) rat liver mitochondria, (2) rat brain mitochondria, (3) rat brain myelin, (4) bovine heart mitochondria, (5) bovine brain mitochondria, (6) bovine brain myelin. Myelin prepared according to procedure of Dodd et al. Same amount of protein was applied to each track.

Escherichia coli, Proteus mirabilis, and Klebsiella pneumoniae. Myelin is a target for autoimmune attack in multiple sclerosis, and infections, including those of the urinary tract, may be associated with exacerbation of that disease. Might an immunological cross-reaction between E3 antigens of infecting bacteria and homologous autoantigens present in myelin be involved in such exacerbation—or indeed in the pathogenesis of the disease?


Non-typhoidal quinolone-resistant gonococci

Sir—Dr Jephcott and Dr Turner (Jan 20, p 165) describe nine isolates of an unusual type of penicillinase-producing Neisseria gonorrhoeae (PPNG) with diminished sensitivity to ciprofloxacin; these strains were contracted in the UK, Spain, or the Canary Isles, they carried 3.2 and 245 MD plasmids, were of non-requiring auxotype, and were non-typhoidal with the standard panel of six protein 1A and six 1B specific monoclonal antibodies (Genetic systems). We have isolated similar strains from three patients in Glasgow. None was typable by the above panel but all reacted with monoclonal antibody Bj, and they typed as serovar AvBx in the Pharmacia panel.

The first isolate (October, 1989) was from a man infected after intercourse in Glasgow. One week after a single dose of 400 mg ciprofloxacin he was still culture positive. He denied intercourse during the intervening period. Pretreatment and post-treatment isolates were identical. He was treated successfully with spectinomycin and erythromycin. His partner was traced and an apparently identical strain was isolated from her urethra, cervix, and rectum. She was treated with spectinomycin and erythromycin, which proved successful on follow-up cultures. She had been infected in Benidorm, Spain, in the summer of 1989. The third patient was seen elsewhere in mid-October when she was found to be infected with a penicillin-resistant gonococcus. This isolate was not available for serotyping. She attended the department of genitourinary medicine 2 weeks later, when urethral culture yielded a scanty growth of PPNG Bj/AvBx; cervical and rectal cultures were negative. She was treated successfully with erythromycin. Despite the apparently identical strain she was not connected with the other two patients, but she had been infected after being raped by a Spanish man in Benidorm at the end of September.

Our cases confirm the link of unusual PPNG isolates with Spain and support the view that quinolone therapy developed for the normal range of susceptibilities may fail in patients infected with strains of diminished sensitivity. Although the minimum inhibitory concentration (MIC) of ciprofloxacin was not determined for our isolates the MIC of ciprofloxacin was 0·04 mg/l. It is likely that the MIC of ciprofloxacin was greater than this since in one study, involving both PPNG and non-PPNG, the MIC of ciprofloxacin was 0·04 mg/l compared with 0·015 mg/l for ciprofloxacin. These isolates also show that penicillinase plasmids can become established in rare strains of gonococcus. Strains of serotype AvBx contain epitopes of both protein 1A and 1B, which are generally considered to be mutually exclusive forms of protein 1. PIA-PIB hybrids are rare in nature. The rarity of these strains may indicate a diminished capacity of hybrid strains to enter and maintain themselves in the gonococcal pool. If this is the case then the spread of this particular quinolone-resistant clone may be limited.

W. J. Young
A. Coyne
I. B. Tait
A. C. McClinton
G. Gallagher

Portable infuser for victims of catastrophes

Sir—Intravenous therapy is often needed for casualties of disasters, whether military or civilian. However, brackets for bottles (or packs) are not always available and it is often necessary for someone to keep the bottle in a raised position. We have devised a portable infuser that can be handled easily and attached without the use of a stand to a patient’s arm.

This infuser consists of a sealed box containing a plastic bag, filled with 500 ml of the infusion solution (other sizes of plastic bags are available). Pressure is exerted on the bag by means of liquid gas which is fed into the bag through a relief valve. A small gas cartridge is positioned on the outside of the box. The pressure is regulated to allow a mean rate of 500 ml in 60 min. This rate can be adjusted. A small ball flowmeter is incorporated in the tube (figure).
GONOCOCCAL INFECTION WITHIN SCOTLAND: ANTIGENIC HETEROGEPNEITY AND ANTIBIOTIC SUSCEPTIBILITY OF INFECTING STRAINS


*Sexually Transmitted Diseases Diagnostic Laboratory, Department of Bacteriology, and **Genitourinary Medicine Unit, Department of Medicine, Edinburgh University Medical School, Edinburgh EH8 9AG, Scotland.
***Departments of Bacteriology, Glasgow Royal Infirmary and ****Southern General Hospital, and *****Department of Microbiology, Fife Area Laboratory, and *******Sexually Transmitted Diseases Clinic, Victoria Hospital, Kirkcaldy and Dunfermline and West Fife Hospital, Fife, Scotland.
********Department of Genitourinary Medicine, Glasgow Royal Infirmary, Southern General Hospital, Glasgow, Scotland.

Key words: N. gonorrhoeae - Serotyping - Geographical variation - Antibiotic susceptibility - PPNG

Two panels of monoclonal antibody reagents were used to serotype all strains of Neisseria gonorrhoeae isolated from four separate geographical areas serving two million of the five million Scottish population. Serotype IB isolates accounted for 60% of the 869 strains examined and were more prevalent than 1A isolates in each geographical area. A total of 11 1A serovars and 47 1B serovars were recognised. Only two of the 11 1A serovars (Aedgk/Arost and Aedih/Arst) were found in every centre but these accounted for over 90% of the 1A isolates. Although there was a total of 47 different 1B serovars over 80% of the isolates were accounted for by the ten most commonly encountered serovars. There were, however, marked geographical differences within both major and minor serovars. There was a highly significant difference (P < 0.001) between protein 1A and 1B serovars with respect to their susceptibility to penicillin. Within each protein 1 type there were also differences in antibiotic susceptibility. Penicillinase-producing N. gonorrhoeae (PPNG) were found in all centres and accounted for 24 (2.8%) of the 869 isolates. The majority of the PPNG (71%) were serotype 1A and with one exception were serovar Aedih/Arst. PPNG strains accounted for 37% (16) of the 43 Aedih/Arst isolates. Epidemiological, diagnostic and therapeutic implications arising from the distinct geographical differences in the pool of circulating gonococci are discussed.

INTRODUCTION

Typing of individual isolates of a given bacterial species provides information of value in the diagnosis, control and prevention of many bacterial infections. Until recently there was no recognized serotyping system for differentiating isolates of Neisseria gonorrhoeae, but now the advent of monoclonal antibodies against epitopes on the gonococcal major outer membrane protein (Protein I), has produced a
powerful epidemiological tool for the study of gonococcal infection (3). Protein 1 is a stable surface protein that is identical to the gonococcal porin. It is stable to heat and subsequent storage as an antigen and undergoes little change when the gonococcus is grown in vivo and in vitro. It does, however, exhibit enough natural variation to serve as good marker (12).

By providing detailed macro- and micro-epidemiological information, monoclonal antibody serotyping (serovar analysis), in conjunction with contact tracing should prove useful in the control of infection, e.g. in the recognition and control of micro-epidemics by detecting the occurrence of new serotypes or increased prevalence of existing ones; in monitoring the spread of plasmid and chromosomally mediated antibiotic resistant isolates; and by recognizing re-infections and double infections. To achieve the full potential from serovar analysis, however, it is necessary to have accurate data on the serotype of all of the strains circulating within a given population. Whilst our initial studies (5) defined the serovar distribution of gonococci isolated in Edinburgh and identified particular serovars associated with male rectal (4) and female rectal (6) infections, serotyping of a small number of isolates from Glasgow (9) suggested that there may be marked geographical differences in the prevalence of certain serovars. This study was undertaken to examine the distribution of gonococcal serovars from centres within Greater Glasgow, Tayside, Fife and Lothian Health Board Areas, serving a total population of approximately 2 million (Fig. 1).

MATERIALS AND METHODS

The participating centres outwith Edinburgh included:
Department of Bacteriology, Glasgow Royal Infirmary; Department of Bacteriology, the Southern General Hospital, Glasgow; Department of Medical Microbiology, Fife Area Laboratory; Medical Microbiology Department, University of Dundee Medical School (Stracathro Hospital and Perth Royal Infirmary were included with Dundee). During 1988 all isolates of N. gonorrhoeae from the above centres were sent to the Sexually Transmitted Diseases Diagnostic Laboratory, Department of Bacteriology,

Figure 1. - Health Board Areas in Scotland with estimated population at 30th June 1987: participating Health Areas are shown in enclosed box.
(Source: Communicable Diseases Scotland Unit, Ruchill Hospital, Glasgow - reproduced by permission of Dr. E. Walker, Editor CDS Weekly Report).
Edinburgh University Medical School. Cultures were sent by First Class Mail either on chocolate agar slopes or on charged swabs in Amies’ transport medium with charcoal.

After recovery, isolates were incubated for 18-24 hours on clear GC medium prior to serotyping as described previously (5) by means of two different sets of monoclonal antibody coagglutination reagents developed by Pharmacia (Ph) and Genetic Systems (GS). The Minimum Inhibitory Concentrations (MICs) of penicillin were determined by the agar plate dilution method. Concentrations of penicillin of 0.015, 0.06, 0.12, 0.5 and 1.0 mg/L were prepared in modified New York City (MNYC) medium lacking selective antibiotics. Strains of N. gonorrhoeae were grown overnight on MNYC medium and a light suspension, approximately 10^7 to 10^8 colony forming units/ml, was made in nutrient broth. By use of a multipoint inoculator, a drop (approximately 1 μl) of each suspension was inoculated on a series of antibiotic-containing plates of medium and a control plate. The plates were allowed to dry before being incubated overnight at 37°C in a moist atmosphere containing 10% carbon dioxide.

The MIC was defined as the lowest concentration of penicillin which completely inhibited growth of the inoculum. Occasionally, a spot was observed which had two or three colonies or a very thin film of growth and this was scored as negative. Susceptibility to other antimicrobials was determined in parallel on agar plates containing cefuroxime (0.02, 0.1 and 0.5 mg/L); tetracycline (0.1 and 0.5 mg/L); erythromycin (0.125 and 0.5 mg/L); ciprofloxacin (0.002, 0.004 and 0.008 mg/L) and spectinomycin (16 mg/L). Isolates growing on 0.5 mg/L tetracycline were re-tested on a series of plates containing two-fold increasing concentrations of tetracycline up to 32 mg/L.

Whenever possible gonococci isolated from the rectum and/or pharynx were tested as well as any isolate from a genital site. For analysis, however, isolates from multiple sites in the same patient which belonged to the same serovar were considered as a single episode of infection. Isolation of the same

**TABLE 2**.

and which had Serovar Edinburgh in parallel Susceptibility to growth and this of penicillin which completely inhibited growth overnight carbon 10% plates were containing plates was suspension in inoculator, approximately 10^7 overnight on York New Carolina. Cultures made in nutrient broth. Antibiotics. Strains of N. gonorrhoeae were grown overnight on MNYC medium and a light suspension, approximately 10^7 to 10^8 colony forming units/ml, was made in nutrient broth. By use of a multipoint inoculator, a drop (approximately 1 μl) of each suspension was inoculated on a series of antibiotic-containing plates of medium and a control plate. The plates allowed to dry before being incubated overnight at 37°C in a moist atmosphere containing 10% carbon dioxide.

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**TABLE 1.** - Prevalence and geographical distribution of protein IA and IB serotypes (1988).

<table>
<thead>
<tr>
<th>Area</th>
<th>Number (and percentage) of isolates belonging to serotype:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IA</td>
</tr>
<tr>
<td>Edinburgh</td>
<td>123 (43)</td>
</tr>
<tr>
<td>Glasgow - Royal Infirmary</td>
<td>81 (44.5)</td>
</tr>
<tr>
<td>Glasgow - Southern General</td>
<td>59 (36.4)</td>
</tr>
<tr>
<td>Dundee</td>
<td>51 (37.7)</td>
</tr>
<tr>
<td>Fife</td>
<td>26 (32.1)</td>
</tr>
<tr>
<td>Total</td>
<td>350 (40.3)</td>
</tr>
</tbody>
</table>

**TABLE 2.** - Prevalence and geographical distribution of protein IA serovars (1988).

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Edinburgh</th>
<th>Glasgow (Royal Infirmary)</th>
<th>Glasgow (Southern General)</th>
<th>Dundee</th>
<th>Fife</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae/Av</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Aedgk/Aro</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Aedgk/Arost</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Aedgk/Arot</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aedgki/Arost</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aedgkih/Arost</td>
<td>107</td>
<td>65</td>
<td>49</td>
<td>46</td>
<td>22</td>
<td>289</td>
</tr>
<tr>
<td>Aedih/Arost</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Aedih/Arst</td>
<td>20</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>Aedikh/Arost</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Aegk/Aros</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Aegk/Arot</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>133</td>
<td>81</td>
<td>59</td>
<td>51</td>
<td>26</td>
<td>350</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Edinburgh (Royal Infirmary)</th>
<th>Glasgow (Southern General)</th>
<th>Dundee</th>
<th>Fife</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacejk/Brpyust</td>
<td>49 (27.8)</td>
<td>28 (27.2)</td>
<td>19 (22.6)</td>
<td>24 (43.6)</td>
<td>124</td>
</tr>
<tr>
<td>Back/Brogyt</td>
<td>22 (12.5)</td>
<td>3 (2.9)</td>
<td>4 (4.8)</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Bajk/Bropt</td>
<td>22 (12.5)</td>
<td>41 (40.6)</td>
<td>21 (20.4)</td>
<td>50 (59.5)</td>
<td>144</td>
</tr>
<tr>
<td>Bacjk/Bropt</td>
<td>14 (7.9)</td>
<td>12 (11.9)</td>
<td>11 (10.7)</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>12 (6.8)</td>
<td>1 (1)</td>
<td>4 (3.9)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Bacjk/Bropt</td>
<td>10 (5.7)</td>
<td>0</td>
<td>2 (1.9)</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Back/Brogyt</td>
<td>10 (5.7)</td>
<td>0</td>
<td>2 (1.9)</td>
<td>1 (1.2)</td>
<td>14</td>
</tr>
<tr>
<td>Baejk/Brpyust</td>
<td>6 (3.4)</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Bcegjk/Brpyust</td>
<td>0</td>
<td>9 (8.9)</td>
<td>10 (9.7)</td>
<td>4 (7.3)</td>
<td>23</td>
</tr>
<tr>
<td>Bj/Bro</td>
<td>0</td>
<td>10 (9.9)</td>
<td>10 (9.7)</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Others*</td>
<td>31 (17.7)</td>
<td>19 (18.8)</td>
<td>11 (10.7)</td>
<td>12 (21.8)</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>176 (100)</td>
<td>101 (100)</td>
<td>103 (100)</td>
<td>84 (100)</td>
<td>55 (100)</td>
</tr>
</tbody>
</table>

*Other serovars not represented: Edinburgh (15); Glasgow Royal Infirmary (12); Glasgow Southern General (5); Dundee (7); Fife (7).

serovar from a patient treated within the preceding two to four weeks was also considered as a single episode of infection.
Statistical analysis was by the Chi square test with Yates’ correction.

RESULTS

Distribution of serogroups and serovars

Isolates from a total of 869 episodes of infection were examined. The distribution of these isolates between the various centres is given in Table 1 which also shows the proportion of isolates belonging to protein IA (serogroup W1) and protein IB (serogroup WII/III) serovars.

Overall 40% of isolates were of the protein IA type. In each centre protein IA strains were less prevalent than IB strains: the difference between the lowest proportion of IA isolates (32.1% in Fife) and the highest (44.5% in Glasgow Royal Infirmary) just fails to reach significance at the 5% level (x² = 3.08; P > 0.05).

The number and distribution of protein IA serovars found in the various centres is given in Table 2.

Although eleven different serovars were isolated only two (Aedgkh/Arost and Aedih/Arost) were found in every centre; these two serovars accounted for between 92 and 97% of the protein IA serovars.

Serovar Aedgk/Arost was isolated only in Glasgow while serovar Ae/Av was found only in Edinburgh and was associated with homosexually acquired infection.


<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates with following MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae/Av</td>
<td>0.015 0.06 0.12 0.5 1.0 &gt;1.0 Total</td>
</tr>
<tr>
<td>Aedgk/Aro</td>
<td>1 0 2 3 0 0 0 1</td>
</tr>
<tr>
<td>Aedgk/Arost</td>
<td>3 2 0 0 0 0 0 5</td>
</tr>
<tr>
<td>Aedgk/Arot</td>
<td>1 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>Aedgk/Arost</td>
<td>0 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>Aedgkh/Arost*</td>
<td>233 54 1 0 0 0 0 288</td>
</tr>
<tr>
<td>Aedih/Arost</td>
<td>2 0 0 0 0 0 0 2</td>
</tr>
<tr>
<td>Aedih/Arost</td>
<td>19 4 4 0 0 0 0 43</td>
</tr>
<tr>
<td>Aedgk/Arost</td>
<td>1 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>Aegk/Arost</td>
<td>1 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>Aegk/Arost</td>
<td>1 0 0 0 0 0 0 1</td>
</tr>
</tbody>
</table>

(a) = PPNG isolates.
* = One isolate not available for testing.
There is a much greater diversity of protein IB serovars compared to the limited number of protein IA serovars. Although there was a total of 47 different IB serovars over 80% of the isolates were accounted for by the ten most commonly encountered serovars shown in Table 3.

Unlike protein IA strains no single serovar accounted for the majority of isolates or was prevalent among the various centres. Serovar Bacejk/Brpyust was the most prevalent serovar in Edinburgh, Fife and the Southern General Hospital, Glasgow but accounted for only 4% of IB strains isolated at Glasgow Royal Infirmary. Serovar Bajk/Bropt was most common in Glasgow Royal Infirmary and in Dundee. Serovar Back/Bropyt isolated more often in Edinburgh than in order areas has been associated with homosexually acquired infection (4). Other serovars such as Bj/Bro were almost exclusively associated with Glasgow (both centres) while serovars such as Bacejk/Brpyust, Bajk/Bropt, Back/Bropyt and Bacejk/Brpyust were more common in Edinburgh than in other centres.

### Antibiotic Susceptibility

There was a highly significant difference (P < 0.001) between protein 1A and IB serovars with respect to their susceptibility to penicillin at MIC values < 0.5 mg/L (Tables 4 and 5).

Approximately 5% of both protein 1A and IB serovars had MIC values ≥ 1.0 mg/L; protein 1A isolates were accounted for entirely by PPNG strains whereas only 29% (7) of the 24 IB serovars with MIC values ≥ 1.0 mg/L were PPNG.

Within each protein 1 type there were also differences in antibiotic susceptibility. Of the protein 1A serovars, Ae/Av which was associated with homosexually acquired infection tended to be less susceptible. Serovar Aedih/Arst which accounts for most of the PPNG strains also tended to be less susceptible even in the absence of the Beta-lactamase encoding plasmid.

Within the two common protein 1B serovars Bacejk/Brpyust was significantly less susceptible (P < 0.001) than serovar Bajk/Bropt with 76% and 14% of

### Table 5. - Penicillin susceptibility of protein IB serovars (1988)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates with following MIC (mg/L)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 0.015 0.06 0.12 0.5 1.0 &gt; 1.0</td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>3 8 19 91 2 0 123</td>
<td></td>
</tr>
<tr>
<td>Back/Bropyt</td>
<td>1 7 16 4 0 5(a) 33</td>
<td></td>
</tr>
<tr>
<td>Bajk/Bropt</td>
<td>1 50 73 20 0 0 144</td>
<td></td>
</tr>
<tr>
<td>Bacejk/Bropyt</td>
<td>7 12 11 9 0 0 39</td>
<td></td>
</tr>
<tr>
<td>Bajk/Brpyut</td>
<td>0 7 8 4 0 0 19</td>
<td></td>
</tr>
<tr>
<td>Back/Brbropt</td>
<td>2 1 4 5 0 0 12</td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyut</td>
<td>0 0 2 1 3 8(b) 14</td>
<td></td>
</tr>
<tr>
<td>Bajk/Brpyut</td>
<td>0 2 6 0 0 0 8</td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>1 3 19 0 0 23</td>
<td></td>
</tr>
<tr>
<td>Bj/Bro</td>
<td>0 5 13 2 0 0 20</td>
<td></td>
</tr>
<tr>
<td>Others*</td>
<td>12 17 16 31 1 5(c) 82</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26 110 171 186 6 18 517**</td>
<td></td>
</tr>
</tbody>
</table>

(a) = 1 PPNG isolate.
(b) = 3 PPNG isolates.
(c) = 3 PPNG isolates.
* = 37 other serovars not represented individually.
** = Two isolates not available for testing.

### Table 6. - Serovar and geographical distribution of PPNG isolates (1988).

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Edinburgh (Royal Infirmary)</th>
<th>Glasgow (Southern General)</th>
<th>Dundee</th>
<th>Fife</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedgki/Arost</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Aedih/Arst</td>
<td>5/20</td>
<td>7/10</td>
<td>4/8</td>
<td>0/3</td>
<td>16/43</td>
</tr>
<tr>
<td>Back/Bropyt</td>
<td>0/22</td>
<td>1/4</td>
<td>0/3</td>
<td>0/4</td>
<td>0/33</td>
</tr>
<tr>
<td>Back/Bropyst</td>
<td>1/10</td>
<td>0</td>
<td>2/2</td>
<td>0/1</td>
<td>3/14</td>
</tr>
<tr>
<td>Bacejk/Bos</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
<td>2/2</td>
<td>0/23</td>
</tr>
<tr>
<td>Bacejk/Byus</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/1</td>
</tr>
<tr>
<td>Others</td>
<td>0/255</td>
<td>0/168</td>
<td>0/149</td>
<td>0/125</td>
<td>0/774</td>
</tr>
<tr>
<td>Total</td>
<td>7/309</td>
<td>8/182</td>
<td>6/162</td>
<td>2/135</td>
<td>24/869</td>
</tr>
<tr>
<td>(%)</td>
<td>(2.3)</td>
<td>(4.4)</td>
<td>(3.7)</td>
<td>(1.5)</td>
<td>(1.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype</th>
<th>Cumulative percentage MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td></td>
<td>≤ 0.015 0.06 0.12 0.5 1.0</td>
</tr>
<tr>
<td></td>
<td>IA</td>
<td>75.0 92.2 94.2 95.1 95.1(b)</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>5.0 26.3 59.1 95.1 95.6(b)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td></td>
<td>≤ 0.02 0.1 0.5 1.0</td>
</tr>
<tr>
<td></td>
<td>IA</td>
<td>87.9 98.8 100 100</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>21.0 84.9 98.6 99.6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td>≤ 0.1 0.5</td>
</tr>
<tr>
<td></td>
<td>IA</td>
<td>23.7 96.8</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>15.2 91.3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td>≤ 0.125 0.5</td>
</tr>
<tr>
<td></td>
<td>IA</td>
<td>49.6 99.1</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>60.2 95.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>≤ 0.002 0.004 0.008</td>
</tr>
<tr>
<td></td>
<td>IA</td>
<td>12.3 88.5 100</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>13.9 85.3 98.8</td>
</tr>
</tbody>
</table>

(a) = 17 isolates MIC > 1.0 mg/L; all PPNG.
(b) = 18 isolates MIC > 1.0 mg/L; 7 PPNG.


<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates</th>
<th>Cefuroxime ≤ 0.02</th>
<th>0.1</th>
<th>0.5</th>
<th>Tetracycline ≤ 0.1</th>
<th>0.5</th>
<th>&gt; 0.5 ≤ 0.125</th>
<th>0.5</th>
<th>&gt; 0.5 ≤ 0.002</th>
<th>0.004</th>
<th>0.008 &gt; 0.008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedgki/Arost</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aedih/Arst</td>
<td>16</td>
<td>3</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Back/Bropyt</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Back/Bropyst</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Begjk/Bos</td>
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<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Begjk/Byus</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Total</td>
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<td>16</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td>15</td>
<td>15</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

* = All with: penicillin MIC > 1 mg/L; spectinomycin MIC < 16 mg/L.

DISCUSSION

Over the past few years there has been a marked decrease in the number of cases of gonorrhoea reported in Scotland. Between 1986 and 1987 there was a decrease of 35% from 3921 to 2536 cases with the 1987 figure decreasing a further 40% to give the 1988 level of 1532 cases (Information and Statistics Division, Scottish Health Service Common Services Agency).
The centres covered by this survey serve a population of approximately 2 million of the 5 million population served by Health Board Areas in Scotland. The 869 gonococcal isolates examined account for almost 60% of the 1532 new cases of gonorrhoea reported at clinics in Scotland in 1988. Therefore in contrast to other epidemiological serotyping studies which are restricted to a very small geographical area (or areas), or to selected isolates, the routine serotyping of unselected isolates from specific areas of Scotland has enabled us to gain an accurate representation of circulating gonococcal strains. Surveillance studies based on such discontinuous sampling methods may lead to deficiencies in the data concerning the prevalence of certain strains within the total N. gonorrhoeae strain population (11).

Using the combination of GS and Ph monoclonal antibody reagents a total of 11 different protein 1A serovars and 47 different protein 1B serovars were recognised. In a worldwide epidemiological survey 27 GS/Ph 1A serovars and 93 GS/Ph 1B serovars were recognised (13). Clearly therefore there is ample opportunity for new strains of gonococci to gain entry to, and be disseminated within, the local population. By having accurately defined baselines it should be possible to recognise the entry of such strains. More extended temporal studies however are needed to determine the dynamic nature of the circulating population of gonococci throughout Scotland.

Although there were overall similarities in the serovars isolated from the various centres there were also marked differences not only between centres but with areas outwith Scotland. For example, serovars Aedgkih, Aedih and Ae were the most common serovars from different geographical areas and accounted for 92% of the 1A isolates (3). Whilst Aedgkih and Aedih were common in all centres in Scotland accounting for 95% of isolates Ae is extremely rare and was found only in Edinburgh. The main foci of Ac strains is thought to be Africa and parts of the Western Pacific (3). Of the 1B serovars, four (Bajk, Bacejk, Bacejk and Back) are included amongst the ten most common 1B serovars isolated in other geographical areas (3). Serovar Bajk was more common than Bacejk in the following locations: Stockholm, Copenhagen, Oslo, Helsinki, Sydney, Perth (Australia), Adelaide, Auckland, Singapore and Korea. A similar situation has been reported in Athens (15) where serovar Bropt (corresponding to Bajk) was more common (27.7%) than Bropyst (10.7%) which corresponds to Bacejk. In our study Bajk was more common than Bacejk in both the locations, Glasgow Royal Infirmary and Dundee. The finding that the proportion of these serovars can vary within two centres from the same city (Glasgow Royal and Glasgow Southern General) combined with the observation that in an earlier Edinburgh study (5) serovar Bajk accounted for 35% of 1B isolates and was more common than Bacejk which accounted for only 12% of isolates suggests that routine sampling of isolates detects the true dynamic situation which is masked by more limited numerical and temporal sampling studies. The reasons for these changes remain speculative at present although it is interesting to observe that there is a significant difference (P < 0.001) in penicillin susceptibility between serovars Bacejk and Bajk. A study of the dynamics of circulating gonococcal strains is important in studying basic aspects of transmissibility as well as problems such as immunity, re-infection and vaccine development. Serovar analysis is of immediate and direct practical benefit however in monitoring strains associated with therapeutic and diagnostic problems.

Although penicillinase-producing *Neisseria gonorrhoeae* (PPNG) are now endemic in the UK they are being reasonably well controlled through surveillance, accurate diagnosis and selection of an appropriate antibiotic (1). Isolates of gonococci exhibiting chromosomally mediated resistance (CMRNG) are now more common than PPNG in some areas and may be considered a greater danger because they are more likely to be resistant to a range of antibiotics (7). Plasmid-mediated high level tetracycline resistant *Neisseria gonorrhoeae* (TRNG) first occurred in the USA in 1985. During 1987, TRNG spread from the USA to Europe and the first cases in England were reported in Leeds and London (8).

According to Bygdeman (3) the main IA serovars associated with PPNG were Aedih, Aedgkih, Ae and Aed. With regard to PPNG (Table 6) it is clear that the majority (67%) of our PPNG belong to serovar Aedih/Arst. Serovar Arst also predominated amongst PPNG strains in Athens (15) while in Amsterdam Aedih/Arst was the only serovar common in strains isolated in 1981-82 as well as in 1985 (2). Ansink-Schipper et al. (2) considered that Aedih/Arst strains may have a specific capacity to survive and be transmitted compared with strains of the 1B serovars or may have resulted from repeated importation of PPNG strains of this type from places such as South Asia where this serovar is dominant in IA strains. Clearly we have a limited population of IA PPNG within Scotland and as well as controlling these isolates we should be vigilant to prevent the establishment of additional PPNG serovars. In this context it is interesting that in contrast to the single isolate of PPNG serovar Aedghk/Arost isolated in Fife in October 1988 there were three such isolates in Glasgow in the first quarter of 1989.

The 1B PPNG isolates were spread over four different serovar combinations. The most likely geographical origin of these isolates is Asia (3). Of the two 1B PPNG isolates in Edinburgh one was imported from Bangkok and the other from Switzerland. Serovar analysis is of particular value when contact tracing a PPNG micro-epidemic. A PPNG strain may lose the beta-lactamase encoding plasmid and this must be taken into account.

Certain serovars may also be linked with the problem of chromosomally mediated penicillin resistance. Serovars Back/Bropt and Back/Bropt have a significant proportion of isolates with a penicillin MIC ≥ 1.0 mg/L, a level at which the overall
treatment failure rate has been reported as 21% (14). It is reassuring to note that there were no isolates of TRNG.

Information provided by serovar analysis is also valuable in monitoring the efficacy of immunological reagents used for confirming the identity of N. gonorrhoeae. The serovar Bj/Bro which accounted for approximately 10% of IB isolates in Glasgow is the only serovar encountered which is non-reactive in the widely used Phadebact Monoclonal GC test (16). The serovar Beh/BX which is non-reactive in a monoclonal antibody based micro-immuno fluorescence test (10) was represented by a single isolate from Dundee. In a previous study (5) there was a small outbreak of infection with this serovar which accounted for around 9% of isolates in Edinburgh.

In addition to the advantages described above serovar analysis is also of value in detecting double infections. Apart from detecting “high-risk” individuals and the need to trace more than one contact such infections are important in that they provide the means of genetic exchange, including spread of antibiotic resistance, between different strains of gonococci. Provided that accurate, comprehensive and up-date information is maintained serovar analysis also has potentially valuable medico-legal applications.

Acknowledgements

We are grateful for the help and interest of the many members of staff within the various Departments of Bacteriology and Microbiology who contributed to this study. In particular, Dr. I.A. Harper, Perth Royal Infirmary and the staff at Strachathro Hospital who sent isolates which were included with those from Dundee for analysis. The help of Dr. Cyril Lafong and Mrs. Jeanette Malone of the Department of Medical Microbiology, Fife Area Laboratory is also acknowledged.

REFERENCES

ARTICLE

Gonococcal Infection within Scotland: Antigenic heterogeneity and antibiotic susceptibility of infecting strains (1989)

(Contributed by H Young and A Moyes, STD Diagnostic Laboratory, Department of Bacteriology, Edinburgh University Medical School and others*)

Introduction

Epidemiological data provided by gonococcal antibody serotyping (serovar analysis) is useful in the control of gonococcal infection, e.g. in the recognition and control of micro-epidemics by detecting the occurrence of new serotypes or increased prevalence of existing ones; in monitoring the spread of plasmid and chromosomally mediated antibiotic resistant isolates; and by recognising re-infection and double infections. The distribution and antibiotic susceptibility of gonococcal serovars circulating within various areas of Scotland during 1988 was reported in CDS 89/34. This paper reports comparable data for gonococci isolated during 1989.

Materials and Methods

During 1989 all isolates of N. gonorrhoeae from the centres shown in Table 1 were sent to the Sexually Transmitted Diseases Diagnostic Laboratory, Edinburgh University Medical School. All isolates were serotyped with two different panels of monoclonal antibody coagglutination reagents developed by Pharmacia and Genetic Systems and the minimum inhibitory concentration (MIC) of several antibiotics determined by an agar dilution method (1). Whenever possible gonococci isolated from the rectum and/or pharynx were tested as well as any isolate from a genital site. For analysis, however, isolates from multiple sites in the same patient which belonged to the same serovar were considered as a single episode of infection. Isolation of the same serovar from a patient treated within the preceding two to four weeks was also considered as a single episode of infection.

Statistical analysis was by the Chi square test with Yates' correction.

Result

Distribution of serogroups and serovars

Isolates from a total of 574 episodes of infection were examined compared with a total of 869 episodes of infection in 1988 (Table 1). Although 1A serovars were less prevalent than 1B when the overall gonococcal population is taken into account, 1A serovars predominated among isolates from Perth Royal Infirmary and the Clinical Bacteriology Laboratory within Lothian.

The number and distribution of protein 1A serovars found in the various centres is given in Table 2. For this analysis the four laboratories within Lothian and the three centres from Tayside are grouped together.

Nine different serovars were found among the 263 isolates compared with eleven serovars among the 350 1A isolates examined in 1988. Again serovars Aedgk/Arest and Aedh/Arst were the most prevalent and between them accounted for 90% of isolates. There was, however, a significant decrease ($X^2 = 5.8, P < 0.05$) in the prevalence of Aedh/Arst between 1988 and 89. This is due in part to a decrease in penicillinase-producing Neisseria gonorrhoeae (Table 7).

Again there is much greater diversity among protein 1B isolates with 37 different serovars represented within the 311 isolates (Table 3): in 1988 there were 47 serovars among 519 isolates.

* see Acknowledgements
a considerable overlap with 1988 when eight of these also appeared within the top ten. Serovars Bacejk/Brpyust (2.9%) and Bcegjk/Brpyu (3.2%) were newcomers in 1989 while Back/Bropt and Baejk/Brpyust dropped out from the top ten. Of the two most common serovars Bajk/Bropt increased in prevalence between '88 and '89 while Bacejk/Brpyust decreased: these changes were mainly due to a change within the gonococcal population at Glasgow Southern General where Bajk/Bropt increased from 20.4% to 38.7% and Bacejk/Brpyust decreased from 27.2% to 14.1%. Certain geographical variations were similar to those found in 1988 with serovars Bj/Bro and Bcegjk/Brpyu1(t) showing a strong correlation with Glasgow and serovars Back/Bropt and Bacejk/Brpyust occurring much more frequently in Lothian.

**Antibiotic susceptibility:**

There was a highly significant difference (P<0.001) between 1A and 1B serovars with respect to their susceptibility to penicillin at MIC values ≥ 0.5 mg/L (Tables 4 and 5). Comparing 1A isolates between 1988 and 1989 there has been a significant increase (P<0.001) in highly susceptible isolates: excluding PPNG 235 (90.7%) of isolates had an MIC≥ 0.015 mg/L during 1989 compared with 262 (78.9%) of isolates in 1988.

There has also been a significant decrease (P<0.001) in the proportion of less susceptible 1B isolates between 1988 and 1989: excluding PPNG 80 (26%) of isolates had an MIC≥ 0.5 mg/L compared with 203 (40%) of isolates in 1988.

The susceptibility of isolates to cefuroxime, tetracycline, erythromycin and ciprofloxacin is summarised in Table 6: all isolates were susceptible to 16 mg/L spectinomycin. Of 35 isolates (4 1A and 31 1B) which grew on 0.5 mg/L tetracycline 34 were available for re-testing at higher concentrations: the MICs for these isolates were 0.5 mg/L (11 isolates), 1.0 mg/L (18 isolates) and 2.0 mg/L (5 isolates).

The serovar and geographical distribution of PPNG strains is given in Table 6. Although there is a marked decrease of PPNG/total isolates (1.4% for 1989 compared with 2.8% for 1988) this is not statistically significant (X²=2.4; P>0.1). The three serovar Aedgki/Arost isolates in 1989 were proline requiring and contained the 3.2 Mdal resistance plasmid and the 2.6 Mdal cryptic plasmid. The serovar Aedgki/Arost strain isolated in 1988 had the same plasmid profile but was arginine requiring. The three Bj/BxAv isolates were all non-requiring and contained the 3.2 Mdal resistance plasmid as well as the 2.6 Mdal cryptic plasmid and 24.5 Mdal transfer plasmid.

**Discussion**

The 574 episodes of gonorrhoea analysed in this report represent a decrease of 34% on the 869 episodes of gonorrhoea analysed in 1988. This decrease is, however, less than the 53% fall in the 464 cases of gonorrhoea reported for the first 6 months of 1989 compared with 858 for the same period in 1988 (2).

The decrease in the overall prevalence of gonorrhoea is reflected in a decrease in the number and diversity of different serovars circulating within the gonococcal population. The decrease in the number of different serovars is largely due to a decrease in unusual serovars which each account for only a few isolates. This may reflect a lower level of importation and subsequent spread of strains from outwith Scotland.

Decreased importation of strains may account to some extent for changes in antibiotic susceptibility observed during 1989 when there was an increase in the proportion of more susceptible isolates (Table 4 and 5). The increase in the prevalence of serovar Bajk/Bropt (more sensitive) and decrease in Bacejk/Brpyust (less sensitive) accounts to some extent for this change. There was only one non-PPNG isolate with a penicillin MIC≥1.0 mg/L during 1989.
compared to 17 such isolates in 1988. This finding is significant in terms of therapy as the overall failure rate is approximately 20% for isolates with a penicillin MIC of 1.0 mg/L [3].

It is again re-assuring to note that there were no plasmid-mediated high level tetracycline resistant Neisseria gonorrhoeae (TRNG). Overall, isolates remained highly sensitive to tetracycline with an MIC of 0.004 mg/L (Table 6). Decreased susceptibility to ciprofloxacin was associated with PPNG strains belonging to the very unusual serovar BJ/BxAv; these strains have been linked epidemiologically with Spain (4,5). Such strains may necessitate re-appraisal of the widely practised regimen of a single 250 mg/L dose of ciprofloxacin to treat infections suspected of penicillin resistance on clinical or epidemiological grounds. The overall decrease in PPNG strains between 1988 and '89 is in contrast to a significant increase in PPNG in several areas of the United States during the same period (6).

References


Acknowledgements

We acknowledge the help and support of numerous bacteriological and clinical colleagues throughout Scotland. We are however particularly indebted to: Dr. D.H.H. Robertson, Genitourinary Medicine Unit, Edinburgh Royal Infirmary: Dr. A.C. McCartney, Department of Microbiology, Glasgow Royal Infirmary: Dr. G. Gallacher and Dr. G. Lindsay, Department of Bacteriology, Southern General Hospital: Dr. I.B. Tait, Department of Genitourinary Medicine, Glasgow Royal Infirmary and Southern General Hospital, Glasgow: Dr. O. Brogan, Dr. D. Lafong, C. Fox, and J. Malone. Department of Microbiology, Fife Area Laboratory: Dr. G. Kohiyar, Sexually Transmitted Diseases Clinic, Victoria Hospital, Kirkcaldy and Dunfermline and West Fife Hospital: Dr. D. Parratt, Department of Medical Microbiology, Ninewells Hospital, Dundee: Dr. I.A. Harper, Perth Royal Infirmary: Dr. I. Alexander, Department of Genitourinary Medicine, Dundee Royal Infirmary: and Dr. R. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Glasgow.

Special thanks are extended to Mrs Joan McElhinney for careful record keeping and preparation of the manuscript.
# TABLE 1
Source of specimens, prevalence and geographical distribution of protein 1A and 1B serotypes (1989)

<table>
<thead>
<tr>
<th>Area/Laboratory</th>
<th>Number (and percentage) of isolates belonging to serotype:</th>
<th>Total</th>
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</thead>
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<td>1A</td>
<td>1B</td>
</tr>
<tr>
<td>LO/STD</td>
<td>63</td>
<td>18</td>
</tr>
<tr>
<td>LO/CB</td>
<td>12</td>
<td>18</td>
</tr>
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<td>LO/CML</td>
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<td>10</td>
</tr>
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</tr>
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<td>GG/SGH</td>
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<td>75</td>
</tr>
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<td>8</td>
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<tr>
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<td>31</td>
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<td>FF/FAL</td>
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<td>38</td>
</tr>
<tr>
<td>TOTAL</td>
<td>263</td>
<td>311</td>
</tr>
<tr>
<td>1988</td>
<td>350</td>
<td>519</td>
</tr>
</tbody>
</table>

- **LO** = Lothian
- **STD** = STD Diagnostic Laboratory serving Genito-urinary Medicine Unit.
- **CB** = Clinical Bacteriology, University Medical School.
- **CML** = Central Microbiology Laboratory, Western General Hospital.
- **CITY** = Bacteriology Laboratory, City Hospital.
- **GG** = Greater Glasgow
- **GRI** = Glasgow Royal Infirmary.
- **SGH** = Southern General Hospital.
- **RUC** = Ruchill.
- **TY** = Tayside
- **NWH** = Ninewells Hospital, Dundee.
- **PRI** = Perth Royal Infirmary.
- **SH** = Stracathro Hospital.
- **FF/FAL** = Fife Area Laboratory.

# TABLE 2
Prevalence and geographical distribution of protein 1A serovars (1989)

<table>
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<tr>
<th>Serovar</th>
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<th>GG/GRI</th>
<th>GG/SGH</th>
<th>GG/RUC</th>
<th>TY</th>
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<th>TOTAL</th>
<th>1988</th>
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<td>39(88.6)</td>
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</tr>
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</table>

**TOTAL:** 82(100) | 45(100) | 44(100) | 3 | 60(100) | 29(100) | 263(100) | 350(100)
**TABLE 3**


Number (and percentage) of isolates of each serovar in:

<table>
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<tr>
<th>Serovar</th>
<th>LO</th>
<th>GG/GRI</th>
<th>GG/SGH</th>
<th>GG/RUC</th>
<th>TY</th>
<th>FF</th>
<th>Total</th>
<th>1988</th>
</tr>
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<tr>
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</table>

*Ten most prevalent serovars during 1988; Baejk/Brpyut accounted for 8(1.5%) isolates during 1988 but was not isolated during 1989.*
## TABLE 4
Penicillin susceptibility of Protein 1A Serovars (1989)

<table>
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| Total         | 235              | 14   | 7    | 2   | 1   | 4    | 263   |

1988

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TOTAL 349

(a) PPNG isolates

## TABLE 5
Penicillin susceptibility of Protein 1B Serovars (1989)

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<th>1.0</th>
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TOTAL 311

1988

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TOTAL 517

(a) PPNG isolates (b) 7PPNG isolates
### TABLE 6

**Antibiotic susceptibility of serotype 1A and 1B isolates (1989)**

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<td>73</td>
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<td>31.2</td>
<td>88.4</td>
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<td>19.3</td>
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<td>14.8</td>
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(a) 3 isolates MIC >1.0 mg/L; all PPNG
(b) 4 isolates MIC >1.0 mg/L; all PPNG
(c) 4 isolates MIC >0.008 mg/L; 3 PPNG
MIC 0.06 mg/L and one non-PPNG MIC 0.016 mg/L.

### TABLE 7

**Serovar and geographical distribution of PPNG isolates (1989)**

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<th>Serovar</th>
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<th>GG-GRI</th>
<th>GG-SGH</th>
<th>GG-RUC</th>
<th>TY</th>
<th>FF</th>
<th>Total</th>
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<td>Aedgki/Arost</td>
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<td>0/63</td>
<td>0/548</td>
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<td><strong>Total</strong></td>
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<td>0/98</td>
<td>1/67</td>
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(a) Strains with decreased susceptibility to ciprofloxacin (MIC 0.06 mg/L).
EPIDEMIOLOGICAL TYPING OF NEISSERIA GONORRHOEAE: A COMPARATIVE ANALYSIS OF THREE MONOCLONAL ANTIBODY SEROTYPING PANELS

A. MOYES and H. YOUNG

STD Diagnostic Laboratory, Department of Medical Microbiology, Edinburgh University Medical School - Edinburgh EH8 9AG - Scotland.

Key words: N. gonorrhoeae - Serotyping - Monoclonal antibodies

Sixteen hundred and thirty seven isolates of Neisseria gonorrhoeae isolated over a two year period were serotyped using three panels of monoclonal antibodies. The isolates comprised 687 serogroup W1 strains and 950 serogroup W11/III strains. The antibodies used in the panels were those developed by Pharmacia (Ph) and Genetic Systems (GS). The GS antibodies were used as two separate panels; the American (GS-A) panel which designates serovars by a simple numerical nomenclature and the Swedish (GS-S) panel which designates serovars by a more detailed descriptive nomenclature. Using only a single panel the Ph panel gave the greatest discrimination yielding 42 serovars compared with 28 serovars with the GS-S panel and 23 serovars with the GS-A panel. Using two panels in combination, the GS-A/Ph panel combination gave the greatest discrimination with 81 serovar combinations while a combination of the GS-S and Ph panels yielded 77 serovar combinations. A compilation of antibodies from all three panels yielded 90 serovar combinations. As the combination of GS-A and Ph panels gave a greater degree of discrimination than the combination of the GS-S and Ph panels we advocate that first-stage serotyping should be performed with the more widely used GS-A panel while second-stage serotyping should be performed with the Ph panel. We propose that serovar combinations should be reported using a dual nomenclature e.g. IA-1/Arost, IB-1/Brop, IB-1/Bropyt etc. The use of such a dual system would allow "core comparison" between all centres while maintaining a degree of flexibility regarding the extent of discrimination required.

INTRODUCTION

Serological classification of Neisseria gonorrhoeae using monoclonal antibodies raised to epitopes on the gonococcal outer membrane Protein I is a powerful tool in studying gonococcal epidemiology (5) and resistance patterns. Local monitoring of serovar patterns from geographical areas around the world should allow the origin of outbreaks to be identified and the world-wide spread of gonorrhoea to be studied. To compare results from around the world a standardised panel of monoclonal antibodies is desirable. Currently, however, serotyping studies may employ one of two panels of reagents derived from antibodies produced by Genetic Systems (GS) Corporation, Seattle, USA either alone or in combination with an additional reagent panel prepared from antibodies produced by Pharmacia (Ph), Uppsala, Sweden: these panels are shown in Tables IA and IB and include the GS-S (Swedish) panel comprising seven IA and seven IB GS monoclonal antibodies (5); the GS-A (American) panel comprising six IA and six IB GS monoclonal antibodies (14); and the Ph panel comprising five IA and nine IB Ph monoclonal antibodies (5).

Not only do different workers use different serotyping panels they may also use one of two nomenclatures to report the serotypes found (12).
Both nomenclatures distinguish between two species of protein I with the designation A or B where A-reactivity corresponds to serogroup WI and B-reactivity to serogroup WII or WIII (17). The purpose of one nomenclature in which reaction patterns with a fixed set of antibodies are numbered according to the prevalence of serovars in a large worldwide survey is to present a system that is easy to use; thus the most common serovar of strains with protein IA was designated IA-I etc. The other nomenclature is descriptive and all reactions are indicated. Each monoclonal antibody reaction is represented by a small letter corresponding to that antibody; for example, a strain of the serovar Aedgkih reacts with six different anti-protein IA antibodies designated e, d, g, k, i and h (6). The flexibility of this system allows the recognition of new serovars and caters for the possibility of adding additional antibodies to the panel. Ph serovars are always reported by this descriptive nomenclature.

Depending on the GS antibody panel used the different nomenclatures may, or may not, be interchangeable. For example certain studies report the use of the GS-S panel and a descriptive nomenclature (3, 9) whereas others use the GS-A panel and a numerical system of nomenclature (14, 16). Yet other studies have used the GS-S panel in combination with the Ph panel and the descriptive nomenclature (1, 2, 6, 8). Occasionally the Ph panel has been used alone (20).

It has been suggested that the GS-A panel could be referred to as the standard serotyping panel as the descriptive and numerical nomenclatures are interchangeable with this panel (15). To our knowledge, however, there have been no studies comparing the GS-A panel with both the GS-S and Ph panels to support this recommendation. By serotyping unselected gonococcal isolates from an area covering a population of approximately two million with all three panels we have assessed the degree of discrimination achieved by typing with each panel individually and in combination.

MATERIALS AND METHODS

A total of 1637 unselected strains of gonococci isolated in various parts of Scotland during 1988 (24) and 1989 were included in the study. All isolates were identified as Neisseria gonorrhoeae by the rapid carbohydrate utilisation test and the Phadebact Monoclonal GC test (23). Prior to serotyping isolates were incubated for 18-24 hours on clear gonococcal (GC) agar. Using a cotton swab, growth was harvested into 1 ml phosphate buffered saline pH 7.2 to give a smooth milky suspension. The suspension was boiled for 10 min and allowed to cool before testing. Monoclonal coagglutination (CoA) reagents and antigen preparations were each mixed well before testing. The tests were carried out by adding 20 μl of each coagglutination reagent on premarked areas of a glass tile. The antigen/CoA reagent mixtures were rocked gently for two minutes before reading the CoA reactions.

The Ph serotyping panels were supplied by Dr. Solgun Bygdeman (Karolinska Institute, Sweden) as ready to use CoA reagents. Monoclonal antibodies for the standard GS-A panel were supplied by Dr. Catherine Ison (St. Mary's Hospital, London) and the CoA reagents were prepared in our own laboratory by a standard method. The GS-S panel was prepared by adapting the standard GS-A panel. Monoclonal antibodies 6G9 and 5G2 required to supplement the WI typing reagents (Table 1A) and 2H7 and 3B10

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+ = Present in panel
- = Absent from panel

312
TABLE IB. - Monoclonal antibodies used for the serological classification of serogroup WII/III Neisseria gonorrhoeae.

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<td>GS-A panel</td>
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<th>Pharmacia Monoclonal Antibodies</th>
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<tr>
<td>Ph panel</td>
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<td>+ + + + + + + +</td>
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* = Present in panel
- = Absent from panel

required to supplement the WII/III typing reagents (Table IB) were kindly supplied by Dr. Richard Rodgers (Syva, California).

The serovar of each isolate was defined as the pattern of reactivity with a given set of monoclonal antibodies specific for either the WI or WII/III serogroup. Any new pattern of reactivity was re-tested as were isolates showing discrepancies in their reaction with the same monoclonal antibody reagent contained in the GS-S and GS-A panels. On re-testing, when a different pattern of reactivity occurred in the monoclonal antibodies not shared by the GS-A and GS-S panels the isolate was re-tested for a third time. The serovar was recorded as the pattern giving agreement on two out of three occasions.

RESULTS

The 1637 isolates comprised 687 (42%) serogroup WI and 950 (58%) serogroup WII/III strains.

As shown in Table 2 the 687 serogroup WI isolates were divided into eight different serovars with the GS-A panel and seven serovars with the GS-S and Ph panels. For the majority of isolates (95%) each serovar with one panel corresponded to one specific serovar with the other panel.

A combination of the Ph and GS-S panels yielded 12 serovar combinations while the combination of the GS-A and Ph panels yielded thirteen serovar combinations. A compilation of antibodies from all three panels yielded fourteen serovar combinations. The increased discrimination provided by the combination of panels accounts for only 5% of isolates.

TABLE 2. - Correlation between serovars for 687 serogroup WI isolates serotyped with standard GS-A panel, GS-S panel and Ph panel.

<table>
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<tr>
<th>Serovar designation (and number of isolates) obtained with each panel:</th>
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<td>IA5 (1)</td>
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<td>IA9 (1)</td>
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<td>IA21 (22)</td>
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</table>

* Nomenclature of Knapp et al. (14)
** Nomenclature of Bygdeman et al. (6)
The 950 serogroup WII/III isolates were divided into 35 serovars with the Ph panel; 21 with the GS-S panel and 15 with the GS-A panel (Tables 3 and 4). A combination of the Ph panel with the GS-S panel yielded 65 serovar combinations (Table 3) whilst a combination with the GS-A panel yielded 68 serovar combinations (Table 4). The compilation of antibodies from all three panels yielded 76 serovar combinations.

For the 687 serogroup WI isolates there was a total of 7 strains (0.1%) each of which showed one epitope discrepancy between the common epitopes of the GS-A and GS-S panels (Table 5). A further 5 epitope discrepancies occurred in these strains to epitopes not shared by the GS-S and GS-A panels: the four isolates which failed to react with “g” and one of the isolates giving false reactivity with “h” also failed to react with “k” initially.

Sixty four (6.7%) of the 950 serogroup WII/III isolates showed one epitope discrepancy between the common epitopes in the GS-A and GS-S panels (Table 6). Eleven isolates failed to react with the “k” reagent and six isolates failed to react with the “h” reagent. Thirty two isolates failed to react with the “c” reagent while another 14 isolates gave an unconfirmed positive reaction. One isolate gave an unconfirmed positive reaction to the “a” reagent. Two of these isolates also showed discrepancies in the epitopes not shared by the GS-A and GS-S panels: the isolate that gave an unconfirmed reaction with the “a” reagent also failed to react to the “e” reagent while one of the isolates that gave an unconfirmed reaction with the “c” reagent also failed to react to the “b” reagent.

**DISCUSSION**

Factors to take into account when considering which serotyping panel to use include the level of discrimination given, the reproducibility of reactions with individual monoclonal antibodies, the occurrence of non-typable strains and the extent to which individual panels of antibodies are already used.

We find very little difference in the degree of discrimination with any of the individual panels for serogroup WI strains. Our findings are similar to the level of discrimination found by other studies using the same panels (5, 9, 10, 14, 16, 21, 24).

We find the predominant WI serovar Aedgkih/Arost corresponding to IA-2 represents 83.6% of the serogroup WI strains. Although also the predominant IA serovar reported by Bygdeman (S) and Dillon et al. (10) it accounts for only 33% and 46% of the serogroup WI strains in these studies. No IA-1 serovars were found in this study. Grouping together serovars IA-1 and IA-2 as suggested by Knapp et al. (13) accounts for; 93% of serogroup WI strains from the Federal Republic of Germany (16), 43% of serogroup WI strains from Southern Florida (21) and 41% of serogroup WI strains in the worldwide study of Knapp et al. (14). The frequency of isolation of this serovar reflects the differences in geographical distribution of gonococcal strains.

A greater diversity of serovars is found among serogroup WII/III strains than serogroup WI strains. The degree of discrimination obtained corresponds to the number of monoclonal antibodies in each panel or combinations of panels. The use of “e” and “j” in the GS-S panel as opposed to “b” in the GS-A panel accounts for the extra discrimination obtained with the GS-S panel; “b” distinguishes between IB-1 and IB-2, IB-3 and IB-6, and between IB-5 and IB-7. Without “b” these 6 major groupings would be condensed into three groups (IB-1/IB-2; IB-3/IB-6; IB5/IB-7) each of which could be divided into three further groups by “e” and “j” i.e. reactive with both “e” and “j”, reactive with “j” but not “e” and reactive with neither “e” nor “j”. When a single panel is used alone we find the Ph panel gives the greatest discrimination. The greatest discrimination using a combination of panels is given by the GS-A and Ph panels. The greatest overall discrimination is given by a compilation of antibodies from all three panels.

As with serogroup WI isolates the level of discrimination obtained with serogroup -WII/III isolates is similar to that obtained in other studies using the same panels (5, 9, 10, 14, 16, 21, 24). The number of antibodies employed in the use of combined panels greatly exceeds what is practical for routine testing. Panels of five or six antibodies as suggested by Sandstrom et al. (18) appear to be more appropriate as a high percentage of routine isolates are covered by a few common serovars. In this study the Ph panel gives the greatest discrimination but the 10 major Ph serovars account for 91.6% (1500/1637) of all isolates.

The seven discrepancies between the GS-A and GS-S panels for the 687 serogroup WI isolates comprised three unconfirmed positive and four unconfirmed negative reactions involving three separate monoclonal antibodies but no single antibody predominated. However, as there were five antibodies in common between the two panels there were ten agglutination reactions per strain, therefore only 0.1% (7/6870) of the total common agglutination reactions were discrepant, a level which is highly acceptable for a biological test subject to subjective interpretation.

The 17 serogroup WII/III unconfirmed negative reactions between the GS-A and GS-S panels occurred in 11 “k” and 6 “h” monoclonal antibody reactions. These took place towards the end of a set of reagents and may have been caused by monoclonal antibody disassociating from the staphylococcal cells. However, 46 (72%) of the 64 discrepancies occurred with the “c” reagent which resulted in 32 unconfirmed negative and 14 unconfirmed positive reactions. As shown in Table 6 these discrepancies may result in
TABLE 3 - Correlation between serovars for 950 serogroup WII/III isolates serotyped with GS-S panel and Ph panel.

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*NT = non typable.
confusion between serovars 1B-1 and 1B-3 and serovars 1B-2 and 1B-6. However, as “c” is in both the GS-A and GS-S panels this does not influence our choice of panels. Again taking the five common antibodies into account the overall level of discrepant agglutination reactions was only 0.7% (64/9,500).

According to Sarafian and Knapp (19) two criticisms of serotyping have been voiced; one is that too much

TABLE 5. – Discrepancies between GS-A panel and GS-S panel serovars for 687 serogroup WI isolates.

<table>
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<th>Initial GS-S serovar</th>
<th>Initial GS-A serovar</th>
<th>Retest both panels</th>
<th>Comment on initial reactivity for shared epitopes</th>
<th>Corresponding Ph serovar</th>
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TABLE 6. – Discrepancies between GS-A panel and GS-S panel serovars for 950 serogroup WII/III isolates.

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</tr>
<tr>
<td>4</td>
<td>Behj</td>
<td>NT</td>
<td>Behj (IB24)</td>
<td>non-reactive with “h”</td>
<td>Av/Bx</td>
</tr>
</tbody>
</table>

* = No corresponding numerical code.
NT = non-typable.
discrimination results; the other is that more discrimination is required within the major serovars such as IB-1 and IB-3. The level of discrimination required will of course depend on the nature of a particular epidemiological study. In our view the criticism that serotyping results in too much discrimination carries little support. If required individual serovars with similar biological properties, for example IA-1 and IA-2, IB-5 and IB-7, IB-2 and IB-16, can be grouped together as suggested by Kohl et al. (15). The need for more discrimination is supported by the use of a dual classification system based on auxotyping and serotyping. The so called auxotype/serovar (A/S) classification system (14). The A/S classification system is widely used particularly in North America to perform a detailed analysis of gonococcal strain populations (4, 13). Unfortunately auxotyping is a complex and tedious procedure and many centres do not wish to employ this method. Our studies show that discrimination can be greatly increased by using a second panel (Ph) of monoclonal antibodies. In using the same simple methodology this system has certain advantages over the A/S classification system. As the combination of GS-A and Ph panels gave a greater degree of discrimination than the combination of the GS-S and Ph panels we advocate that first-stage serotyping should be performed with the GS-A panel while second-stage serotyping should be performed by the Ph panel. Depending on the degree of discrimination required not all laboratories would require second-stage serotyping. This proposal has several advantages: (i) the GS-A panel is becoming increasingly recognised as the standard panel and workshops have been established to instigate quality control procedures and to ensure standardisation of the technique (15); (ii) nomenclature is interchangeable when the GS-A panel is used. The numerical nomenclature is considered by many workers to be easier to use, particularly with the dual A/S classification system; (iii) increased discrimination can be achieved by either using the dual A/S system or by sub-dividing GS-A serovars with the Ph panel. Individual laboratories can select the system best suited to their needs on the basis of methodology and degree of discrimination required while maintaining GS-A serovar data for “core” comparison in global studies. For very detailed studies A/S classification and Ph serotyping could be performed; (iv) second-stage serotyping with the Ph panel allows the classification of isolates that do not react with any of the antibodies in the GS-A panel (5, 22); non-typable GS-A strains may be of considerable epidemiological significance as in the case of quinolone resistant isolates (11, 25); (v) because of the extensive antigenic heterogeneity of IB isolates and the many single epitope differences the use of the Ph panel acts as an in-built quality control and increases confidence in the results. This is because serovars found with one system usually correspond to one of a known group of serovars with the other system (5, 8).

We propose that serovar combinations should be reported using a dual nomenclature, e.g. IA-1/Arost, IB-1/Bropt, IB-1/Bropt etc. The use of such a dual system would allow “core comparison” between all centres while maintaining a degree of flexibility regarding the extent of discrimination desired. The dual system not only meets the need for greater discrimination within the common serovars (IB-1, IB-2 and IB-3) it also allows the recognition of certain epidemiological associations that may be hidden within these broad groups. As shown in table 4 serovars IB-1 and IB-2 can each be divided into II and IB-3 into 9 serovar combinations. Although strains of serovar IB-2 were associated with homosexually acquired infection (22) we have shown in our locality this association is specific for a sub-group of IB-2 isolates viz. IB-2/Bropt (7). Similarly sub-populations within serovar IB-1 isolates may account for the bimodal pattern of susceptibility to penicillin observed with serovar IB-1 (22).

For serotyping IA serovars the combination of GS-A and Ph panels also yielded the greatest degree of discrimination. Although the case is less strong for sub-dividing the protein IA GS-A serovars with the Ph panel many of the advantages outlined above for IB isolates apply and we advocate the dual classification system for detailed epidemiological studies.

Acknowledgements

We are grateful to our many colleagues in the Departments of Microbiology and Genitourinary Medicine for the great interest they have shown in this work and for sending us gonococcal isolates.

REFERENCES


An analysis of false positive reactions occurring with the Captia Syph G EIA

J Ross, A Moyes, H Young, A McMillan
An analysis of false positive reactions occurring with the Captia Syph G EIA

J Ross, A Moyes, H Young, A McMillan

Abstract

Aim—The Captia Syph G enzyme immunoassay (EIA) offers the potential for the rapid automated detection of syphilis antibodies. This study was designed to assess the role of other sexually transmitted diseases (STDs) in producing false positive reactions in the Captia Syph G EIA. The role of rheumatoid factor (RF) as a potential source of false positives was also analysed.

Methods—Patients who attended a genitourinary medicine (GUM) department and gave a false positive reaction with the EIA between 1988 and 1990 were compared with women undergoing antenatal testing and with the control clinic population (EIA negative) over the same time period. The incidence of sexually transmitted disease (STD) in the clinic population and the false positive reactors was measured in relation to gonorrhoea, chlamydia, genital warts, candidiasis, “other conditions not requiring treatment” and “other conditions requiring treatment.” Male:female sex ratios were also compared. Ninety two RF positive sera were analysed with the EIA.

Results—The rate of false positive reactions did not differ with respect to the diagnosis within the GUM clinic population. The antenatal group of women, however, had a lower incidence of false positive reactions than the GUM clinic group. No RF positive sera were positive on Captia Syph G EIA testing.

Conclusions—There is no cross reaction between Captia Syph G EIA and any specific STD or with RF positive sera. The lower incidence of false positive reactions in antenatal women is unexplained but may be related to physiological changes associated with pregnancy.

Introduction

The recent introduction of the Captia Enzyme Immunoassay (Captia Syph G) to detect immunoglobulin G (IgG) antibodies against Treponema pallidum presents an opportunity for automated processing and reporting for large numbers of specimens.1-3 This EIA test with an overall sensitivity of over 98% and specificity of over 99%4,5 is comparable in performance with the traditional combination of VDRL (Venereal Disease Research Laboratory) and TPHA (Treponema pallidum Haemagglutination Assay) tests even in primary syphilis.1 False negatives in the EIA were associated specifically with primary infection where the sensitivity was 82%.6 False positive reactions were not associated with conventional Biological False Positive Reactors.1

The incidence of syphilis in general, and the primary stage in particular, is low in the United Kingdom, however, and the occurrence of false positive reactions, albeit at a low level, decreases the positive predictive value of a positive test. The factors responsible for these erroneous reactions with the EIA are not known.

The antigen in the Captia Syph G EIA is a sonicate of T. pallidum (Nichols strain) coated onto microtitration wells. Unlike other T. pallidum antigen tests such as the Fluorescent Treponemal Antibody Absorbed (FTA-ABS) test4 and the TPHA5 that comprise an absorption stage to neutralise group treponemal antibody, the EIA involves no such absorption and relies on serum dilution (1 in 20) to overcome interference from group treponemal antibody.

Because of the lack of an absorption stage it is possible that more false positive reactions might occur in patients attending an STD clinic; this could result from damage to the mucosal epithelium encouraging the growth of commensal treponemes. Excessive growth of such treponemes in genital lesions may give rise to false positive FTA-ABS reactions if the group reactive antibody is not completely neutralised.6 In view of this we compared the rate of false positive EIA reactions in patients attending an STD clinic with that for women attending an antenatal clinic. A number of sera positive for rheumatoid factor (RF) were also tested in order to exclude this as a cause of false positive reactions.
Methods

All patients who attended the Department of Genitourinary Medicine, Edinburgh Royal Infirmary between January 1988 and December 1990 and were screened for syphilis by the Captia Syph G (Mercia Diagnostics UK) EIA were included in the study. All sera were heat inactivated at 56°C for 30 minutes prior to serological testing. Sera giving an antibody index greater than or equal to 0.9 were repeat tested once by the EIA and were examined by the VDRL, TPHA and FTA-ABS and an EIA for specific antitreponemal IgM (Captia Syph M). Whenever requested, sera from patients at high risk for syphilis, particularly early primary syphilis were examined by all of the above tests. A false positive EIA was taken as an antibody index of greater than or equal to 0.9 which persisted on one repeat test but was not supported by confirmatory treponemal tests and signs or symptoms of treponemal infection.

Comparison was made between the patients with false positive reactions and the overall clinic population with regard to the prevalence of gonorrhoea, chlamydial infection, genital warts, candidiasis, “other conditions not requiring treatment” and “other conditions which required treatment.” The last group included patients who had infected partners and received therapy on an epidemiological basis. Patients who had more than one diagnosis were counted in each diagnostic group separately. The male to female sex ratio (M:F) was calculated for each group.

The overall false positive rate in the clinic population was also compared with that of pregnant women screened for syphilis at the Simpson Memorial Maternity Pavilion, Edinburgh between 1988 and 1990.

Sera found positive on screening with the Serascan Rheumatoid Arthritis Latex test (Hycor) were also tested with the Captia Syph G EIA.

Statistical analysis was performed using the chi square test on the Minitab PC statistics package.

Results

Over the 3 year period 12,842 new patients were seen in the Department of Genitourinary Medicine. False positive reactions occurred in 197 (1.5%) compared with 117 confirmed positive tests for syphilis, giving a positive predictive value of 37%. The M:F ratio was 124:73 (1.7:1) in the false positive group compared with 7593:5249 (1.4:1) in the STD clinic population, which is not statistically significant. The prevalence of sexually transmitted diseases in the clinic population and in those giving false positive reactions are shown in Table 1. The only statistically significant difference between the groups is a lower incidence of genital warts in those with false positive EIA (p = 0.02).

Of patients who had positive EIA results 182 (1.4%) became negative on repeat testing.

Antenatal patients (10,314) were tested with 82 false positives (0.8%). This level is significantly lower than the STD clinic population (p < 0.01). Only three (1.5%) of the 197 GUM patients and 1 (1.2%) of the antenatal patients with false positive IgG reactions also gave a false positive IgM test.

The RF titres of the RF positive sera are shown in Table 2. All 92 of the RF positive sera were negative on testing with the Captia Syph G EIA.

Discussion

Despite a specificity of greater than 99% a positive
IgG EIA, in the population studied, has a positive predictive value of only 37%. This is due to the low prevalence of syphilis and the large number of screening tests performed. Clearly, however, a positive EIA reaction must be confirmed by other treponemal tests. It is essential that laboratories that do not have confirmatory tests on site send EIA reactive sera to a reference laboratory for confirmation.

The incidence of positive tests with the EIA that are unconfirmed (1.4%) is comparable to that with using VDRL and TPHA as screening tests (1.6%).

Our results indicate that false positive EIA reactions are commoner in an STD population when compared with antenatal women but are not associated with any individual STD. The lower incidence of genital warts in patients with false positive tests compared with the control clinic population is difficult to explain. Warts are controlled mainly via cell mediated immunity and humoral factors are thought to be relatively unimportant.

There were fewer women in the false positive reactor group than the clinic population as a whole but this was not statistically significant. In this context it is interesting that the incidence of false positive IgG EIA results was lower in the group of pregnant women studied compared with the GUM population (p < 0.01). Since pregnant women are generally in more stable relationships than the GUM clinic population and are therefore less likely to have genital infections this may indicate that STDs do contribute to false positive reactions. Many other physiological changes in immunity and hormones also occur in pregnancy however and it is unclear as to the relative contribution of these different factors.

Women have a higher incidence of autoimmune diseases in general but this may not be an important factor in determining a serological false positive reaction in this assay in view of the sex ratio observed in the false positive patients.

Rheumatoid factor, usually in the form of immunoglobulin M (IgM) directed against host IgG, is well recognised in syphilis and increases in incidence in the latter stages of the disease. The IgM-IgG complex may produce a false positive reaction by mimicking IgM specific antibody in EIA reactions. The absence of any false positive reactions would indicate that rheumatoid factor is not important in producing false positive reactions in the Capita Syph G EIA system, however. RF would also not appear to be a problem in the IgM EIA as evidenced by the very low number of false positive IgM reactions.

It is also possible that false positive reactions may occur owing to the presence of heat shock proteins and endoflagella present in the sonicate of Treponema pallidum.

In conclusion, no individual sexually transmitted disease is associated with false positive reactions in the Capita Syph G EIA although false reactivity is commoner in an STD population than in pregnant women. This may suggest that the cause of these reactions is multifactorial or due to a different mechanism. Despite a low positive predictive value the test is comparable to traditional screening methods with regard to sensitivity and specificity but is potentially cheaper when large numbers of samples are processed, particularly by automated systems.

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References:

Accepted for publication 5 August 1991.
Changing trends of gonococcal infection in homosexual men in Edinburgh

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(Accepted 18 July 1991)

SUMMARY

In an attempt to explain the recent resurgence of homosexually-acquired gonorrhoea in the Lothian region of Scotland the number of infections and pattern of infection (urethral, rectal and pharyngeal) of all gonococcal isolates from homosexual men attending the Department of Genitourinary Medicine at Edinburgh Royal Infirmary between 1985 and 1990 were analysed. Serovar typing data were available from infections acquired between January 1986 and December 1990. A correlation between one serovar, Bacejk/Brpyust, and the overall pattern of gonorrhoea was observed. The number of infections caused by minor serovars also correlated with rates of gonococcal infection. The number of minor serovars isolated, which may represent strains from other geographical locations, is related to the total incidence of gonorrhoea. It is possible that the incidence of Bacejk/Brpyust may be determined by the size of the infected pool of gonorrhoea. The most likely explanation for the recent increase in gonorrhoea is a change in sexual behaviour and/or an influx of strains from other geographical areas.

INTRODUCTION

Gonorrhoea was well recognized as a common sexually transmitted disease in homosexual men but following the health campaigns in the mid 1980s encouraging safer sexual practices with respect to the transmission of the human immunodeficiency virus (HIV) the incidence of gonococcal infection dropped sharply in this population [1]. More recently a number of centres have reported an increase in gonococcal infection [2–4].

In order to increase our understanding of the mechanisms underlying these recent trends we analysed the sites of gonococcal infection and variety of gonococcal serovars in homosexual men in an attempt to correlate individual serovars with the changing patterns of infection.

METHODS

All male patients admitting to homosexual contact who attended the Department of Genitourinary Medicine at Edinburgh Royal Infirmary, Edinburgh
with a diagnosis of gonococcal infection between January 1985 and December 1990 were included in the study. The age of each patient was noted.

The diagnosis of infection with Neisseria gonorrhoeae was made by culture of material from the urethra, rectum or pharynx on modified New York culture medium and identification of isolates by sugar utilization and immunological methods as described elsewhere [5]. Most patients giving a history of homosexual contact have cultures taken routinely from all three sites regardless of symptoms.

The number of individual serovars was calculated for each quarter of the years 1986–90. The geographical origin of each serovar isolated between 1986 and 1990 was also noted.

Statistical analyses were performed using Fishers exact probability test and Spearman's Rank Correlation test.

RESULTS

The number of episodes of gonococcal infection in homosexual men is seen to drop significantly in 1987 compared to 1986 (Table 1). There is a marked increase in the number of gonococcal infections in homosexual men in 1990 which have risen from 14 (4% of the clinic population) in 1989 to 50 (13%) in 1990 (P < 0.001). The sites of gonococcal infection are shown in Table 2. The occurrence of rectal gonorrhoea had been falling since 1985 reaching statistical significance in 1988 (28 cf. 8, P < 0.01). There was a marked increase in rectal gonorrhoea from 3 cases in 1989 to 26 cases in 1990 (P = 0.04). The number of cases of pharyngeal gonorrhoea varied over the 5-year period but with no consistent trends apparent.

Over the 5-year period 5 serovars were isolated on at least 3 occasions in 2 or more years; Ae/Av, Back/Brpyt, Bacejk/Brpyut, Bacejk/Brpyust, Baejk/Brpyut (Table 3). The dominant gonococcal serovar varies from year to year but the only direct association between any individual serovar and the total number of infections was for Bacejk/Brpyust (Table 3, Fig. 1: r = 0.85). There is also a correlation between the number of infections due to minor serovars (taken as those not isolated at least three times per year in two or more years) and the total number of infections (r = 0.82).

The geographical origin of the infections are shown in Table 4.

In 1990 six serovars which had not previously been isolated in this area were detected: Baghjk/Bpyvut, Bajk/Broput, Behjk/Byvut, Bbck/Bys, Baejk/Bpyu and Beegjk/Bpyust. These accounted for 12 (28%) of the 50 infections seen in 1990.
Changing trends in gonorrhoea

Table 2. Sites of gonococcal infection in homosexual men in Edinburgh 1985–90

<table>
<thead>
<tr>
<th>Year</th>
<th>U only</th>
<th>R only</th>
<th>T only</th>
<th>U Total</th>
<th>R Total</th>
<th>T Total</th>
<th>No. of infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>20</td>
<td>17</td>
<td>1</td>
<td>26</td>
<td>28</td>
<td>16</td>
<td>60</td>
</tr>
<tr>
<td>1986</td>
<td>20</td>
<td>12</td>
<td>7</td>
<td>31</td>
<td>19</td>
<td>17</td>
<td>62</td>
</tr>
<tr>
<td>1987</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>16</td>
<td>7</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>1988</td>
<td>14</td>
<td>6</td>
<td>5</td>
<td>24</td>
<td>8</td>
<td>12</td>
<td>38</td>
</tr>
<tr>
<td>1989</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>11</td>
<td>3</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>1990</td>
<td>14</td>
<td>11</td>
<td>5</td>
<td>26</td>
<td>26</td>
<td>15</td>
<td>50</td>
</tr>
</tbody>
</table>

U, urethra; R, rectum; T, throat

Table 3. Quarterly distribution of gonococcal serovars 1986–90

<table>
<thead>
<tr>
<th>Year</th>
<th>1986</th>
<th>1987</th>
<th>1988</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1989</td>
<td>1990</td>
<td></td>
</tr>
<tr>
<td>Back/Bro pyt</td>
<td>9 4 11 6</td>
<td>4 0 2 2</td>
<td>1 1 1 0</td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>1 4 7 1</td>
<td>1 0 0 1</td>
<td>2 3 0 0</td>
</tr>
<tr>
<td>Ae/Av</td>
<td>0 0 0 0</td>
<td>3 0 0 0</td>
<td>3 0 0 0</td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>0 0 0 0</td>
<td>0 0 0 1</td>
<td>0 0 2 3</td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 3 6 1</td>
</tr>
<tr>
<td>Minor serovars</td>
<td>4 4 6 2</td>
<td>1 1 2 2</td>
<td>6 2 3 1</td>
</tr>
<tr>
<td>Total</td>
<td>14 12 24 12</td>
<td>6 4 4 6</td>
<td>12 9 12 5</td>
</tr>
</tbody>
</table>

Table 4. Geographical origin of serovars

<table>
<thead>
<tr>
<th></th>
<th>Scotland</th>
<th>Outside</th>
<th>Not known</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(not Lothian)</td>
<td>Scotland (%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Back/Bro pyt</td>
<td>38 (76%)</td>
<td>2 (4%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>19 (58%)</td>
<td>2 (6%)</td>
<td>5 (15%)</td>
</tr>
<tr>
<td>Ae/Av</td>
<td>7 (78%)</td>
<td>3 (17%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td>9 (50%)</td>
<td>0</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Minor serovars</td>
<td>34 (57%)</td>
<td>4 (7%)</td>
<td>12 (20%)</td>
</tr>
</tbody>
</table>

The mean age of homosexual men with gonorrhoea was 26 (range 18–38) over the study period.

DISCUSSION

In the population studied there was a sharp increase in the number of cases of homosexually acquired gonorrhoea in 1990. The observed increase is probably not due to one or two promiscuous infected index cases in that there were 14 different serovars represented amongst the 50 homosexually acquired infections in 1990 but
only a single infection was due to serovar Back/Bropyt. In previous years serovar Back/Bropyt has correlated strongly with homosexually acquired infection and from November 1985 to June 1986 accounted for 69% of homosexually acquired infection [6]. The drop in the number of cases in 1987 was presumably as a result of safer sexual practices following widespread health education messages encouraging ‘safe sex’ as a response to the threat of HIV infection. In particular this encouraged the use of condoms and discouraged promiscuity and could therefore be expected to reduce the incidence of gonorrhoea.

The mean age of homosexual men with gonorrhoea has not changed over the past decade [1].

The distribution of sites that were positive for gonococcal culture showed little relative change compared with the past decade [7] despite the overall increase in infections in 1990. In particular the increase cannot be attributed to more pharyngeal infections. This has important implications for HIV infection in this risk group. The number of cases of rectal gonorrhoea had been falling up to 1989 but increased markedly in 1990. ‘Oral sex’ is relatively low risk for the transmission of HIV but anal intercourse is high risk and the increase in gonococcal infection rate possibly parallels an increase in high risk behaviour amongst homosexuals.

It has been suggested that using gonorrhoea as a surrogate marker for unsafe sexual behaviour is inaccurate and that the history obtained from the patient is more reliable [8]. Although no single measurement can be used to assess changes in sexual behaviour the risk activity involved in contracting gonorrhoea is very similar to that for HIV.

Apart from changes in sexual behaviour another possible explanation for the recent increase in gonorrhoea is that there has been a change in the virulence or infectivity of the organism leading to more effective transmission and producing
an increase in the total number of infections. It is possible that the increased isolation rate of Bacekj/Brpyut and Bacekj/Brpyust in 1990 may reflect such changes. The natural history of isolation rates for individual serovars is to vary from year to year however [6] and the factors responsible for these variations are not known. A large influx of new strains may explain a localized rise in the incidence of gonorrhoea and the proportion of infections due to minor serovars did increase in 1990. It is therefore likely that the importation of new serovars contributed to the observed increase.

The incidence of only one serovar, Bacekj/Brpyust, correlates with the total number of infections. It is unlikely that this serovar should determine the overall total as it occurs relatively infrequently (33 times over 5 years). It is more likely that the overall incidence of gonorrhoea is a determinant for the incidence of Bacekj/Brpyust. A sufficiently large pool of sexually active individuals at risk of infection may be required before this serovar can become established in any one year.

There is also an association between the total number of infections and the number of less commonly isolated strains. These might represent new serovars being imported from different geographical locations giving support to the theory that the pattern of dominant serovars is determined by a continual influx of new strains from different areas some of which go on to become established. Although there is a trend towards more minor serovars originating outside Scotland this fails to reach statistical significance. It remains to be seen whether any of the serovars isolated for the first time in 1990 become dominant in future years.

In addition to changes in sexual behaviour and serovar pattern over the time period studied there has also been intense media attention directed at homosexual men with regard to HIV and AIDS. This may have resulted in the decline in attendance rates that has occurred (Table 1) and the recent increases may represent a 'rebound' effect of previously undiagnosed infection. It is unlikely however that the observed change in incidence in gonorrhoea is directly related to clinic attendance rates since the proportion of total attendances at the clinic with gonorrhoea has varied considerably and independently from the total number of attendances.

In conclusion the recent increase in the incidence of gonorrhoea in homosexual men is most likely a result of a change in sexual behaviour although other factors, in particular an influx of new gonococcal serovars from other geographical areas, may be contributing. A change in sexual behaviour producing an increase in the number of gonococcal infections may in itself produce a change in the serovar pattern.

REFERENCES


Increasing Incidence of Gonorrhoea and Syphilis in Homosexual Men in Edinburgh

Jonathan D C Ross1, Alexander McMillan1 and Hugh Young2

Gonorrhoea was well recognised as a common sexually transmitted disease in homosexual men but following the health campaigns encouraging safer sexual practices with respect to the transmission of the human immunodeficiency virus (HIV) the incidence of gonococcal infection has dropped sharply in this population. As it was felt that the incidence was increasing in our region this year, a retrospective survey of the number of cases of gonorrhoea and syphilis was undertaken.

Methods

All patients attending the Department of Genito-Urinary Medicine at Edinburgh Royal Infirmary with a diagnosis of gonococcal infection or early syphilis (primary, secondary or early latent disease) between January 1985 and September 1990 were included in the study.

Results

Results are tabulated in Tables 1 and 2. The incidence of gonorrhoea in homosexual men fell significantly in 1987 compared to 1986. There was a marked increase in the number of gonococcal infections in homosexual men for the first nine months of this year which have risen from 4% to 10.4% of those attending within this group (p=0.0012). The prevalence of rectal gonorrhoea has generally been falling since 1985 but has increased markedly this year with 15 cases in the first 3 quarters of this year compared with 3 cases throughout 1989. The prevalence of pharyngeal gonorrhoea has fluctuated over the past 5 years but there has been no significant rise this year.

The number of cases of early syphilis also declined in 1986 and remained low until this year. In the first nine months of this year there have been more cases of early syphilis in homosexual men than in the past four years combined and a similar increase is seen in heterosexual men.

Discussion

These results show a sharp increase in the number of cases of homosexually acquired gonorrhoea and syphilis this year. The decline in the number of cases in 1986 and 1987 presumably resulted from the use of safer sexual practices following widespread health education messages encouraging "safe sex" as a response to the threat of HIV infection. In particular this encouraged the use of condoms and discouraged multiple changes of partner.

The distribution of sites that were infected by gonococci showed little relative change compared with the past decade despite the overall increase in total infections for the first 9 months of 1990. In particular this increase cannot be attributed to more pharyngeal infections. This has important implications for HIV infection in this risk group. The number of cases of rectal gonorrhoea had been falling up to 1989 but increased markedly in 1990. "Oral sex" is low risk for HIV transmission but anal intercourse is high risk and the increase in gonococcal infection rate possibly parallels an increase in high risk behaviour amongst homosexuals. Similar findings have been noted in Amsterdam and King County, Washington.

The increase in the number of homosexually acquired cases of syphilis is paralleled by an increase in the number of heterosexualy acquired infections indicating that heterosexuals may also be failing to adhere to safe sexual practices.

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2Department of Medical Microbiology, University of Edinburgh
TABLE 1

Number of cases of gonorrhoea and syphilis in homosexual and heterosexual men from January 1985 to September 1990

<table>
<thead>
<tr>
<th>Year</th>
<th>Syphilis</th>
<th>Gonorrhoea</th>
<th>Total Attendances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homosexual</td>
<td>Heterosexual</td>
<td>Homosexual</td>
</tr>
<tr>
<td>1985</td>
<td>8 (1.3%)</td>
<td>4</td>
<td>54 (8.9%)</td>
</tr>
<tr>
<td>1986</td>
<td>0 (0.0%)</td>
<td>1</td>
<td>50 (9.9%)</td>
</tr>
<tr>
<td>1987</td>
<td>0 (0.0%)</td>
<td>2</td>
<td>17 (4.6%)</td>
</tr>
<tr>
<td>1988</td>
<td>1 (0.3%)</td>
<td>2</td>
<td>31 (8.3%)</td>
</tr>
<tr>
<td>1989</td>
<td>1 (0.3%)</td>
<td>0</td>
<td>14 (4.0%)</td>
</tr>
<tr>
<td>1990</td>
<td><strong>5 (1.6%)</strong></td>
<td>3</td>
<td>*32 (10.4%)</td>
</tr>
</tbody>
</table>

(up to Sept)

percentage of total number of new patients attending in parenthesis for homosexual men.

* significant change from previous year p=0.0012 (Fishers Exact Test)

** p=0.08 compared to previous year (Fishers Exact Test)

TABLE 2

Sites of Gonococcal Infection in Homosexual Men in Edinburgh

<table>
<thead>
<tr>
<th>Year</th>
<th>U only</th>
<th>R only</th>
<th>T only</th>
<th>R Total</th>
<th>T Total</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td>20</td>
<td>17</td>
<td>1</td>
<td>28</td>
<td>16</td>
<td>54</td>
</tr>
<tr>
<td>1986</td>
<td>20</td>
<td>12</td>
<td>7</td>
<td>19</td>
<td>17</td>
<td>50</td>
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<tr>
<td>1987</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>1988</td>
<td>14</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>12</td>
<td>31</td>
</tr>
<tr>
<td>1989</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>1990</td>
<td>5</td>
<td>2</td>
<td>8</td>
<td>15</td>
<td>18</td>
<td>31</td>
</tr>
</tbody>
</table>

(up to Sept)

U = urethra  R = rectum  T = throat

References


2. McMillan A; Young H. Gonorrhoea in the Homosexual Man: Frequency of Infection by Culture Site Sexually Transmitted Diseases 1978; 5: 146-150


Patterns of homosexually acquired gonococcal serovars in Edinburgh 1986–90

H Young, A Moyes, J D C Ross, A McMillan
Patterns of homosexually acquired gonococcal serovars in Edinburgh 1986–90

H Young, A Moyes, J D C Ross, A McMillan

Abstract
Aim—The aim of this study was to observe the changes in gonococcal serovar pattern in homosexual men over a 5 year period.
Methods—All men who presented to the Genitourinary Medicine clinic at Edinburgh Royal Infirmary between 1986 and 1990 with homosexually acquired gonococcal infection were included in the study. Gonococcal isolates were serotyped and the temporal change in isolated serovars noted.
Results—Over the 5 year period 32 different serovars were associated with 75 homosexually acquired infections. There was a dynamic temporal change in the dominant serovars with a continual influx of new strains of which become established in the community but most of which appeared only transiently. Rapid variation in incidence over time was observed for certain serovars while others remained at more constant levels. There was a marked association between certain serovars (Ae/Av; Back/Brophy; Bacejk/Brpyut; Bacejk/Brpyust; Bacejk/Brpyut) and homosexually acquired infection.
Conclusions—Possible determinants for the patterns observed are discussed but the underlying mechanism is probably multifactorial.

Introduction
In the early 1980s monoclonal antibodies specific to Protein I on the outer membrane of Neisseria gonorrhoeae were developed. The subsequent ability to subdivide gonococcal isolates into serovars was soon applied to study the epidemiology of gonococcal infection. This work has demonstrated wide geographical variation in the prevalence of different gonococcal serovars in addition to changes in the dominant serovars of any one region with time. The distribution of serovars has also been noted to vary with sexual orientation with certain serovars, such as Back/Brophy' or 1B-2, being associated with homosexually acquired infection. Data relating to temporal changes in serovar prevalence are, in general, less extensive and to our knowledge no information on the temporal variation in serovars associated with homosexually acquired gonorrhoea in this country has been published.

The aim of the study was to observe the prevalence of gonococcal serovars producing homosexually acquired infection over a period of 5 years and to see if any pattern could be discerned.

Methods
All isolates of Neisseria gonorrhoeae from men giving a history of homosexual contact who attended the Department of Genitourinary Medicine at Edinburgh Royal Infirmary between January 1986 and December 1990 were included in the study. Comparison was also made with the serovars isolated in the heterosexual population over the same time period.

Serovar identification
Monoclonal coagglutination reagents The serovar of each isolate was determined using two panels of monoclonal reagents. The Pharmacia (Ph-) panel consisted of five Protein IA specific reagents (Ar, Ao, As, At, Av) and nine Protein IB specific reagents (Br, Bo, Bp, Bv, By, Bu, Bs, Bt, Bx) and the Genetic Systems (GS-) panel consisted of seven Protein IA specific reagents (Af, Ac, Ad, Ag, Ak, Ai, Ah) and seven Protein IB specific reagents (Ba, Bc, Be, Bg, Bh, Bj, Bk).

Antigen preparation Eighteen to 24 hour cultures on GC medium were harvested into 1 ml phosphate buffered saline pH 7.2 to give a smooth milky suspension. This suspension was boiled for 10 minutes and allowed to cool before testing. Prepared antigen was stored at 4°C for up to two weeks if not tested immediately.

Test procedure Monoclonal coagglutination reagents and antigen preparations were each mixed well before testing. The tests were carried out by adding 20 μl of prepared antigen suspension to 20 μl
of each coagglutination reagent on defined areas of large glass slides. Slides were rocked gently for two minutes, and coagglutination reactions were read using an oblique light against a dark background.

**Scoring results** Reactions were scored according to the strength of the coagglutination observed. They were graded negative (−) if they showed a smooth milky background; borderline (+) if they showed a slightly granular milky background; weakly reactive (1+) if they showed a granular background with some clearing; or moderately reactive (2+), strongly reactive (3+), or very strongly reactive (4+) if they showed increasing degrees of clumping and background clearing.

**Designation of serovars** A serovar is defined as the pattern of reactivity of a test strain with a given set of monoclonal antibodies specific for either the protein I-A or I-B subtype. Each serovar is therefore depicted by an upper case letter A or B followed by lower case letters representing positive reactions with the corresponding coagglutination reagents. Reactions of 1+ or more were scored positive in designating a strain to a serovar.

**Statistical analysis** Statistical analysis was by the chi square test with Yates' correction.

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**Results**

The numbers (and percentages) of different serovars isolated each year and total number of homosexually acquired infections are shown in the table. It can be seen that new serovars were continually appearing over the study period with a small number beginning to be established and the rest being only transient. Over the 5 year period the total of 175 infections were caused by 32 different serovars: 29 protein IB serovars accounted for 88% (154) of the 175 isolates and three protein IA serovars for 12% (21).

Five serovars were isolated at least three times per year in 2 or more years—Ae/Av, Bacjek/Brypyst, Back/Bropt, Bacjk/Brypyst and Bacjk/Brypyst. The changing prevalence of these serovars by year is illustrated in fig 1. The prevalence of three serovars changed significantly over the 5 year period—Back/Bropt, Bacjek/Brypyst and Bacjek/Brypyst. Comparison with the serovars from heterosexually acquired gonorrhoea (fig 2) confirmed that all five of the above serovars were significantly associated with homosexual transmission over the 5 year period. Although these five serovars were strongly associated with homosexual infection they also occurred in the heterosexual community. However, within the
Serovar by Year

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae/Av</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back/Brypt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baejk/Brpyut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1  Temporal change in gonococcal serovars 1986–90. Significant year to year changes marked *(p < 0.05) and ***(p < 0.01).

heterosexual group the percentage prevalence of certain serovars had a high male:female ratio (M:F), for example, Back/Brypt (M:F ratio 1:6:1), Ae/Av (M:F ratio 2:4:1).

Discussion

In common with previous studies there was a pre¬dominance of serogroup W11/III (protein IB) infections in the homosexual group.37 From fig 1 it is evident that there is a marked variation in serovar prevalence with time in this patient group. Certain serovars remain at relatively constant low levels (Ae/Av, Baejk/Brpyust) while others have wide and rapidly varying levels (Back/ Brypt, Bacejk/Brpyut, Bacejk/Brpyust). These wide variations in homosexually acquired infection have not been previously reported in this country.

The reasons for this diversity in serovars and the greater success of some serovars in becoming established are not clear. Previous studies have shown that Ae/Av and Baejk/Brpyut have a relatively high level of chromosomally mediated resistance to penicillin with more isolates having a MIC ≥ 0.5 mg/l than < 0.5 mg/l when compared with most other strains.6 The ability of these two serovars to persist in the homosexual population may be related to this anti¬biotic resistance. However, Back/Brypt has persisted over a 5 year period despite being significantly more susceptible to penicillin.

The cause of the year to year variation in the levels of serovars may also be mediated via individual strain infectivity or virulence. In addition there has been a change in sexual behaviour over the study period following a health education campaign directed against human immunodeficiency virus infection. This has led to safer sexual practices and a fall in the number of cases of gonorrhoea.11 This reduction in high risk sexual activity presumably makes it more difficult for new strains to survive in a population that was previously more promiscuous.

Morse et al12 reported an association between homosexually acquired infection and a mutation (mtr) that encodes for increased resistance to both hydrophilic and hydrophobic molecules such as penicillin and erythromycin. It was postulated12 that possession of the mtr mutation may enhance survival of gonococci in the hydrophobic environment of the rectum, most likely as a result of decreased cell envelope permeability; there is, however, little direct
Patterns of homosexually acquired gonococcal serovars in Edinburgh 1986–90

Serovar

<table>
<thead>
<tr>
<th></th>
<th>Male Homosexual</th>
<th>Male Heterosexual</th>
<th>Female Heterosexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ae/Av</td>
<td>✠</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back/BroPyt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyut</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacejk/Brpyust</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baejk/Brpyut</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 Gonococcal serovars grouped by sexual orientation of patient.

evidence for this. Whereas a decrease in permeability may constitute a selective advantage in the hydrophobic environment of the rectum, in other situations decreased permeability may be a disadvantage in that it might limit the flow of nutrients into the cell and restrict growth. Presumably complex host and bacterial factors will determine the relative benefits of a particular strain characteristic in a given environment and influence the ability of a strain to compete in the circulating pool of isolates causing infection within a particular community.

The presence of transient populations of particular serovars has been previously described but not for homosexually acquired infections over such a prolonged time period. It has been postulated that all strains are intrinsically transient and strains only persist by frequent reintroduction from neighbouring areas. Another theory proposes that eventually a population will develop protective immunity to a particular strain which results in the strain dying out.

Three new serovars were isolated exclusively from homosexual men on two or more occasions in 1990—Bajk/Brpyut, Bajk/Brpyut and Behjk/Byvut. It remains to be seen whether any or all of these will become established over subsequent years.

The association between gonococcal serovar and sexual activity suggests a use for serovar determination as a marker of homosexually acquired infection. For example in 1986 19 out of the 21 heterosexual isolates of Back/BroPyt were male, strongly suggesting that some of these men acquired their infection from homosexual contact. Most “homosexual” serovars also appear in the heterosexual community, however, albeit at low levels. Because of the dynamic nature of the serovars continuous surveillance within any one area is required to identify strains associated with homosexually acquired infection.

The use of two panels of monoclonal antibodies is more suited to this detailed epidemiological surveillance as certain serovars can be further subdivided. For example the three major serovars, Bacejk/Brpyust, Back/BroPyt, Bacejk/Brpyut and five minor serovars (table) would all be classified as IB-1 or IB-2 with the standard GS panel.

In conclusion, there are large variations in the prevalence of different gonococcal strains over a 5 year period with some strains persisting at low levels while others appear and disappear. The reasons for these observations are unclear but are almost certainly multifactorial. If strains are continually monitored in any one area then serovar analysis may
be used as a marker for homosexually acquired infection.

We thank Dr Solgun Bygdeman, Karolinska Institute Sweden, Dr Catherine Ison, St Mary's Hospital, London and Dr Richard Rodgers, Syva, California for supplying the monoclonal antibodies used in this study.

Address correspondence to Dr H Young.


Accepted for publication 22 March 1991
Rectal gonorrhoea and unsafe sex

Sir,—Dr Tomlinson and colleagues (Feb 23, p 501) challenge the tenet that male rectal gonorrhoea reflects unsafe sexual behaviour and assert that a detailed history of sexual practices is both informative and reliable. In our view it is difficult to generalise and a more complete picture can be obtained by the combination of microbiological findings and patient history. Tomlinson et al suggest that the partner with rectal gonorrhoea may be monogamous and his only partner could have been infected through receptive oral sex with a casual contact; some 20% of men with rectal gonorrhoea had had oral-anal or digital-anal contact, neither route of infection being important in the transmission of HIV. These routes of acquisition of rectal gonorrhoea are dependent on high levels of pharyngeal gonorrhoea as well as the ready transmission of gonococci to the rectum. We report data on colonisation with pathogenic neisseriae that support the hypothesis that oral-anal contact is likely to spread gonorrhoea.

Over the five years from 1986 to 1990, 141 men with homosexually acquired gonorrhoea attending the genitourinary medicine unit, Edinburgh Royal Infirmary, had urethral, rectal, and throat cultures. Neisseria gonorrhoeae and N meningitidis (confirmed by biochemical and immunological tests) were isolated from the throats of 41 (29%) and 42 (30%) patients, respectively. Rectal gonorrhoea was seen in 58 (41%) patients and rectal carriage of N meningitidis in 13 (9%). This has important implications for diagnostic laboratories because 18% of rectal isolates of neisseriae from men were meningococci. Clearly, if N meningitidis, whose primary habitat is the nasopharynx, can become established in the rectum of 9% of patients, then the gonococcus with a predilection for the oromucosal epithelial cells of the ano-genital tract may be transferred with even greater frequency. However, as Tomlinson et al point out, the relation between sexual behaviour and sexually transmitted diseases is complex and the presence of one infection cannot be used to infer the presence or absence of another. Equally, the acquisition of infection by one route does not preclude acquisition by other routes, and until such time as it can be clearly shown that oral transfer is the exclusive route of infection in most cases of rectal gonorrhoea it would seem prudent to maintain the view that rectal gonorrhoea is an indicator of unsafe sexual behaviour.

Sir,—Our report on pharyngeal gonorrhoea (March 16, p 620) was meant to be illustrative, not comprehensive. We agree that other possible high-risk populations need to be assessed. The contraceptive pill was not evaluated. We ourselves have been surprised at the prevalence of gonococcal carriage in young women who showed no symptoms, who claimed to have had single heterosexual intercourse, and who had used a condom regularly.

Françoise Gray
Christian Gény
Gilles Fenelon
François Lionnet
Romain Gherardi

Departments of Medical Neuroscience and Clinical Immunology, Hôpital Henri Mondor, 94010 Créteil, France; Tropical and Infectious Pathology Unit, Hôpital Raymond Poincaré, Garches; and Neuroradiology Service, Hôpital Tenon, Paris.


Clomiphene and neural-tube defects

Sir,—Our report that women who used ovulation-inducing drugs to conceive were not at increased risk of producing children with neural tube defects has drawn many inquiries about the effect of clomiphene use in particular. Clomiphene was not used more frequently by mothers of offspring with neural tube defects (5/571, 0.88%) than mothers with abnormal control children (5/546, 0.92%) or normal control children (9/573, 1.57%). Thus, we find no evidence for an association between maternal clomiphene use and neural tube defects.

Pediatric Epidemiology Section, Epidemiology Branch, NICHD, National Institutes of Health, JAMES L. MILLS, Bethesda, Maryland 20892, USA for the NICHD-Neural Tube Defect Study

For the NICHD-Neural Tube Defect Study

Preliminary evaluation of "Clearview Chlamydia" for the rapid detection of chlamydial antigen in cervical secretions

H Young, A Moyes, H Lough, I W Smith, J G McKenna, C Thompson
Preliminary evaluation of “Clearview Chlamydia” for the rapid detection of chlamydial antigen in cervical secretions

H Young, A Moyes, H Lough, I W Smith, J G McKenna,* C Thompson*

Abstract
Clearview Chlamydia (Unipath) is a rapid monoclonal antibody based latex immuno-diffusion test for detecting chlamydial antigen in endocervical specimens. The assay does not require specialised equipment or extensive training and takes less than 30 minutes from sample to results. The clinical performance of Clearview Chlamydia was evaluated with 478 paired endocervical swabs from patients attending a genitourinary medicine clinic. In the first part of the study, 221 non-randomised specimens were tested by cell culture (1st swab) and Clearview (2nd swab) whereas in the second part of the study 257 randomised swabs were examined by Clearview, cell culture and immunofluorescence. The overall prevalence of chlamydial infection was 8.8% and the sensitivity, specificity, positive and negative predictive values for Clearview were 85.7%, 99.1%, 90% and 98.6%. The test requires further evaluation to establish its role in the management and control of chlamydial infection.

Introduction
Infection with Chlamydia trachomatis is probably the most common sexually transmitted disease in the western world. In the case of men, presentation with a non-gonococcal urethritis (NGU) with urethral discharge will lead to the prescription of an appropriate effective antibiotic, whereas diagnosis in women on the grounds of clinical signs is quite unreliable and appropriate therapy will often not be given. Whilst the majority of infections in women may be asymptomatic, infection with C trachomatis is capable of inducing a plasma cell endometritis, salpingitis and peritonitis with associated perihepatitis (Curtis Fitz-Hugh syndrome) and periappendicitis. Resolution of upper genital tract infection may result in chronic pelvic pain, tubal infertility or ectopic pregnancy.

The wide clinical spectrum of C trachomatis infection combined with the lack of simple and rapid diagnostic methods is a major limitation in the control of chlamydial disease. Current diagnostic protocols include tissue culture and antigen detection by enzyme immunoassay and immunofluorescence (IF) staining which have time scales of days, 3–4 hours and less than an hour respectively. Although IF staining is rapid it requires highly skilled personnel, expensive equipment and can only be applied to relatively small numbers of specimens.

A simple, rapid and reliable test for chlamydial antigen that could be performed at the time of the patient’s visit would be of enormous value in the management and control of infection. Clearview Chlamydia (Unipath) is a new simple and rapid (less than 30 minutes from sample to result) test that does not require specialised equipment or extensive training. Our aim was to evaluate the performance of this new test in female patients attending a genitourinary medicine clinic.

Materials and methods
Patients
A total of 478 new and returned-new female patients attending the Genitourinary Medicine Unit, Edinburgh Royal Infirmary were included in the study. In the first part of the study involving 221 patients a comparison was made between Clearview Chlamydia (antigen detection) and cell culture. The cervical swab for culture was always taken before the swab for antigen detection. The second part of the study compared the performance of Clearview Chlamydia, cell culture and IF in 257 patients. In this case the order of taking swabs was randomised: one swab was used for culture and the other used to prepare a smear for IF before extracting antigen for the Clearview test.

A retrospective analysis of the case notes was made to determine if the women were known contacts of
men with non-gonococcal urethritis (NGU) and if they had received antibiotics within the preceding 10–14 days.

**Cell culture**

Swabs to be cultured for chlamydiae were expressed in sucrose-phosphate transport medium (2SP) which was then placed in a freezer at −70°C. Specimens were thawed and vortexed prior to culture in cycloheximide treated McCoy cells. Incubation of the infected cells was continued for three days at 35°C. Cells were then stained with iodine and *C. trachomatis* was considered to be present if characteristic intracytoplasmic inclusions were seen.

**Immunofluorescence (IF)**

Smears for IF were allowed to air dry and fixed in methanol. The Syva MicroTrak test was performed according to the manufacturer's instructions.

**Clearview Chlamydia**

Immediately after the smear had been prepared the swab was replaced in its sleeve (no transport medium) and transported to the laboratory within three hours. If the Clearview test was not performed the same day, swabs were stored at 4°C and tested within three days according to the manufacturer's instructions. The swab was placed in a small flexible plastic extraction tube containing 0.6 ml extraction buffer and agitated. The extraction tube containing the swab was then placed in a heating block at 80°C for ten minutes. On removing the extraction tube from the heating block the swab was rotated in the buffer and removed from the tube: liquid was thoroughly removed from the swab by pinching the rim of the extraction tube between thumb and forefinger and squeezing the swab as it was removed from the tube. After cooling for five minutes at room temperature a drop of antibody coated latex suspension (blue latex particles coated with genus specific monoclonal antibody against chlamydial lipopolysaccharide) was added and mixed with the extract. The extraction tube was then capped with an integral cap which has an in-built filter and acts as a dropper for applying sample to the test device. Five drops of this mixture were then added to an absorbent pad in the sample window of a small (8.5 cm × 3.0 cm) immunochromatographic device. The mixture migrates from the sample window along a strip to the "Result Window": the formation of a blue line in the result window (within 5 minutes) indicates the presence of chlamydial antigen in the extract. The line is formed due to the binding of chlamydial antigen to the blue latex and its immobilisation by a zone of antibody located beneath the result window. If no antigen is present the result window remains clear. Formation of a blue line in a "Control Window" shows that the test has been performed correctly. The control line results from binding of some of the antibody sensitised latex by an immobilised zone of antibodies to mouse immunoglobulin. Test performance was validated by appropriate positive and negative controls.

**Results**

In the first part of the study a total of 20 patients gave a positive result by culture and/or Clearview. The correlation between the two methods is shown in table 1.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Clearview Pos</th>
<th>Clearview Neg</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Positive</td>
<td>16</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>201</td>
<td>203</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>203</td>
<td>221</td>
</tr>
</tbody>
</table>

Based on culture the sensitivity of Clearview was 88.9% (16/18) and the specificity was 99% (201/203). Neither of the patients with an unconfirmed Clearview result was a known contact of NGU although one of the women had gonorrhoea.

The result pattern for the 257 patients examined by all three tests in the second part of the study is shown in table 2.

All three tests were negative in 230 patients and positive in 18 giving an overall correlation of 96.5% (248/257). The nine discrepancies comprised two patients with a positive Clearview test confirmed by IF, two with a positive IF test confirmed by culture, two with a positive culture only, two with an unconfirmed Clearview test, and one with an unconfirmed IF test. This last patient had received antibiotic at a family planning clinic prior to the chlamydial culture being taken and was excluded from further analysis. Of the 24 positive chlamydia cases only 15 (62.5%) were known contacts of men with NGU. Eight of the nine women not known to be contacts of men with NGU were Clearview positive. Of the 232 chlamydia negative patients 24 (10.3%) were known contacts of men with NGU.

The overall correlation between Clearview and

**Table 1** Correlation between Clearview and culture for 221 female patients

<table>
<thead>
<tr>
<th>Culture</th>
<th>Clearview Pos</th>
<th>Clearview Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>16</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>201</td>
<td>203</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>203</td>
<td>221</td>
</tr>
</tbody>
</table>

**Table 2** Test result pattern for 257 female patients

<table>
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<tr>
<th>Clearview</th>
<th>IF</th>
<th>Culture</th>
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<td>Neg</td>
<td>Neg</td>
<td>230</td>
</tr>
<tr>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>18</td>
</tr>
<tr>
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<td>Neg</td>
<td>1</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>2</td>
</tr>
</tbody>
</table>

IF = Immunofluorescence

Correlation for three tests: 96.5% (248/257)
Table 3 Overall correlation between Clearview and chlamydial infection detected by culture and/or IF for 477 female patients

<table>
<thead>
<tr>
<th></th>
<th>Clearview Pos</th>
<th>Clearview Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture/IF Positive</td>
<td>36</td>
<td>6</td>
<td>42</td>
</tr>
<tr>
<td>Culture/IF Negative</td>
<td>4</td>
<td>431</td>
<td>435</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>437</td>
<td>477</td>
</tr>
</tbody>
</table>

detection of chlamydia by IF and/or cell culture is shown in Table 3.

The overall prevalence of chlamydial infection was 8.8% (42/477) and the sensitivity and specificity of Clearview were 85.7% (36/42) and 99.1% (431/435) respectively. The corresponding positive and negative predictive values were 90% (36/40) and 98.6% (431/437).

Discussion

Non-cultural methods for the diagnosis of chlamydial infection are widely used but none is wholly reliable and discrepancies may occur. It is therefore necessary to ensure that new tests are evaluated in such a way as to determine as accurately as possible whether the results agree with those of the best techniques available. Although cell culture is often taken as the gold standard for detection of C. trachomatis infection it is now generally accepted that the sensitivity of culture is less than 100%. Because the sensitivity of culture may be less than 100% and as non-cultural methods detect non-viable organisms a third test (IF) was used to help resolve any discrepancies.

The sensitivity and specificity of Clearview as determined against the combination of cell culture and IF were 85.7% (36/42) and 99.1% (431/435) with a corresponding positive predictive value of 90% and a negative predictive value of 98.6%. The overall agreement between all three tests was 96.5% which is almost identical to the overall agreement of 97% between the Pharmacia EIA, IF and cell culture. The sensitivity, specificity, positive and negative predictive values for the Pharmacia EIA in detecting endocervical chlamydial infection were 86%, 97.2%, 87.8% and 96.8% respectively.

Chlamydiazyme (Abbott Diagnostics) is the most widely evaluated EIA and reported sensitivities in detecting endocervical chlamydial infection generally range from 70–100% with most studies in the 85–95% range. Taylor-Robinson et al. reported a poorer performance for Chlamydiazyme with a sensitivity of 67% and a specificity of 89% for endocervical specimens. False positive Chlamydiazyme tests may be due to cross-reaction with other organisms: various bacteria including Achromobacter spp., Klebsiella spp., Streptococcus spp., and Gardnerella spp. at concentrations of >10^7/l react in the Chlamydiazyme assay to yield false positive results. Similar performance parameters have been reported between Chlamydiazyme and other EIAs such as the amplified EIA, IDEIA (Novo Bio Labs, formerly Celltech Diagnostics). However, the sensitivity of IDEIA (96.3%) was higher than that of Chlamydiazyme (85.2%). The IDEIA III version of the assay which used Fab fragments rather than whole monoclonal immunoglobulin G as the capture antibody yielded sensitivity, specificity, positive and negative predictive values of 93.8%, 99%, 92.9% and 99.1% respectively.

Processing of samples by the above non-cultural assays takes several hours and although they are ideally suited to batch testing in the laboratory they cannot be used as an "on-the-spot" test when the patient is at the clinic. The new rapid tests which are available yield similar results to the above EIAs but can be performed at the time of the patient's visit. TestPack Chlamydia (Abbott Laboratories) is a direct EIA for the detection of chlamydial antigen in endocervical specimens. The assay requires no specialised equipment and yields results in less than 30 minutes. When the assay was evaluated against cell culture and/or chlamydial antigen positive (confirmed Chlamydiazyme or IF staining) in a population with a prevalence of chlamydial infection of 13.8% the assay yielded a sensitivity, specificity, positive and negative predictive values of 76.5%, 99.5%, 96.2% and 96.5% respectively.

In our study Clearview Chlamydia gave a slightly higher sensitivity (85.7%) in a population with a lower prevalence (8.8%) of chlamydial infection. The various factors that must be taken into account when selecting tests for chlamydial infection are discussed in detail by Barnes. The development of these new tests, however, means that the advantages of rapid antigen detection thus enabling the patient to receive immediate effective treatment require greater consideration. The use of these immediate tests must be deliberated against the setting where epidemiological treatment is widely practised. Within Genitourinary medicine clinics epidemiological treatment is usually given to women who are known contacts of men with NGU. In this context it is interesting that 40% (8/20) of the Clearview positive patients were not known contacts of men with NGU and would not have been treated on epidemiological grounds. In contrast, 10.3% (24/232) of the chlamydia negative patients were contacts of men with NGU and would have received epidemiological treatment. Despite epidemiological treatment the knowledge of whether a patient is chlamydia positive or negative may be useful, particularly when there is a risk to other contacts.

Because culture following storage at −70°C may result in a slightly decreased sensitivity it is possible
that we have over-estimated the sensitivity of Clearview. Nevertheless the test obviously merits further evaluation against stricter diagnostic methodology. Assuming that our favourable results are confirmed the test could be of value in several situations where rapid detection of chlamydial infection is important. For example, there is a need to screen women before vaginal termination of pregnancy. Greater access to chlamydial diagnosis would also be helpful in managing women with pelvic inflammatory disease who present to gynaecology departments, women who attend family planning clinics and who attend inner city general practices.

We thank our clinical and laboratory colleagues in Genitourinary Medicine and Medical Microbiology, particularly Alistair McCulloch, for their help and cooperation with this study.

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Accepted for publication 21 December 1990
GONOCOCCAL INFECTION WITHIN SCOTLAND: ANTIGENIC HETEROGENEITY AND ANTIBIOTIC SUSCEPTIBILITY OF INFECTING STRAINS (1990)

H Young and A Moyes

Gonococci were serotyped and tested for antibiotic susceptibility as described previously (CDS 90/29). Isolates from a total of 544 episodes of infection were examined compared with a total of 574 in 1989 (Table 1). Again the distribution of serotypes varied geographically: IB isolates were more common in the larger centres of population (Greater Glasgow and Lothian) whereas IA isolates were more common in Fife and Tayside.

The number and distribution of protein IA serovars found in the main centres are given in Table 2: for convenience serovars are designated by a simple numerical nomenclature based on typing with a standard panel of monoclonal antibody typing reagents1. Global studies have defined a total of 23 IA serovars but only six were found amongst Scottish isolates with serovar IA-2 predominating in all locations (Table 2).

There is a greater antigenic diversity within serotype IB isolates with 32 different serovars defined in global studies. Sixteen different serovars were found amongst the Scottish isolates (Table 3) although three (IB-1, IB-2 and IB-3) accounted for 75% of the total. Again there were geographical differences in the relative distribution of these strains with IB-2 being more common than IB-3 in Lothian and Greater Glasgow, whereas IB-3 was more common than IB-2 in Fife and Tayside. Serovar IB-6 isolates were more common in Lothian than in other areas. Within Lothian these isolates have been associated with homosexually acquired infection2. Localised pockets of infection also occurred with other serovars such as IB-15 in Lothian and IB-17 in Greater Glasgow. The eight isolates of serovar IB-17 in Glasgow represents a considerable decrease from the 20 isolates reported in 1989: serovar IB-17 is of interest in that it is negative in the Phadebact monoclonal GC test2.

There is a highly significant difference in the penicillin susceptibility of IA and IB serotypes (Tables 4 and 5): excluding penicillinase-producing strains of Neisseria gonorrhoeae 86% of IA serovars had a minimum inhibitory concentration (MIC) to penicillin of ≤0.015 mg/L compared with 15% of IB serovars (P <0.001). Comparing IA isolates between 1989 and 1990 there was no significant difference in the proportion of highly susceptible isolates. Likewise there was no significant difference in the proportion of less susceptible (MIC ≥0.5 mg/L) isolates between 1989 (26%) and 1990 (31%). A total of four non-PPNG isolates had an MIC of 1 mg/L compared with one such isolate in 1989 and 17 in 1988. These results suggest that the significantly lower level of less susceptible strains reported in 1989 as compared to 1988 (CDS 90/29) has been maintained in 1990.

The susceptibility of isolates to cefuroxime, tetracycline, erythromycin and ciprofloxacin is summarised in Table 6. Spectinomycin susceptibility is not shown in this table but all isolates had an MIC <16 mg/L. Significantly more isolates grew on 0.5 mg/L tetracycline in 1990 than in 1989 (P <0.001). Of 97 isolates (18 IA and 79 IB) that grew on 0.5 mg/L, 81 were available for re-testing: the MICs for these isolates were ≤ 1.0 mg/L (33 isolates), 2 mg/L (41 isolates), 4 mg/L (6 isolates) and 8 mg/L (1 isolate). Plasmid-mediated, high-level-tetracycline-resistant isolates of N. gonorrhoeae (TRNG) were not encountered.

The serovar and geographical distribution of PPNNG strains is given in Table 7. Overall 2.6% of isolates were PPNNG compared with 1.4% for 1989 and 2.8% for 1988: these differences are not significant. Plasmid analysis was performed on 13 of the 14 PPNNG isolates: ten contained the 3.2 Mdal resistance plasmid, two the 4.5 Mdal plasmid and one the 2.9 Mdal plasmid. All four serovar IA-2 isolates from Glasgow Royal Infirmary were plqline-requiring strains and contained the 3.2 Mdal resistance plasmid and the 2.6 Mdal cryptic plasmid. Only one of the IA-6 isolates was available for plasmid analysis: although this isolate had the above plasmid profile it was prototrophic. The two IB-1 strains isolated in Lothian both contained the 24.5 Mdal transfer plasmid but one was plqline-requiring and contained the 3.2 Mdal plasmid whereas the other was prototrophic and contained a 2.9 Mdal plasmid. The IB-2 isolate was plqline-requiring and contained the 4.5 Mdal plasmid, the IB-4 strain was prototrophic and contained the 3.2 Mdal plasmid, the IB-5 strain plqline-requiring and contained the 4.5 Mdal plasmid while the IB-7 strain was prototrophic and contained the 3.2 Mdal plasmid. The diverse pattern

1STD Diagnostic Laboratory, Department of Medical Microbiology, Edinburgh University Medical School
of PPNG isolates suggests occasional importation and subsequent spread rather than major indigenous acquisition and spread of PPNG.

References


Acknowledgements

We thank our numerous bacteriological and clinical colleagues for their help and support in making this surveillance possible. We are aware, however, that there are geographical gaps in the coverage of this report and in our co-ordinating role as the National Reference Laboratory we would welcome isolates from areas not covered at present - anyone wishing to send isolates should contact Dr H Young (Tel 031-650-3143). We are also indebted to Dr Eva Tzelepi of the Institute Pasteur Hellenique for performing auxotyping and plasmid analysis on the PPNG isolates.

Special thanks are extended to Mrs Joan McElhinney for careful record keeping and preparation of the manuscript.
TABLE 1

Source of specimens, prevalence and geographical distribution of IA and IB serotypes (1990)

<table>
<thead>
<tr>
<th>AREA/LABORATORY</th>
<th>NUMBER (%)</th>
<th>NUMBER (%)</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IA</td>
<td>IB</td>
<td></td>
</tr>
<tr>
<td>LO/STD</td>
<td>45 (28.8)</td>
<td>111 (71.2)</td>
<td>156</td>
</tr>
<tr>
<td>LO/CB</td>
<td>11 (55)</td>
<td>9 (45)</td>
<td>20</td>
</tr>
<tr>
<td>LO/CML</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>LO/CITY</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>GG/GRI</td>
<td>49 (38.3)</td>
<td>79 (61.7)</td>
<td>128</td>
</tr>
<tr>
<td>GG/SGH</td>
<td>24 (30)</td>
<td>56 (70)</td>
<td>80</td>
</tr>
<tr>
<td>GG/RUC</td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>TY/NWH</td>
<td>31 (60.8)</td>
<td>20 (39.2)</td>
<td>51</td>
</tr>
<tr>
<td>TY/PRI</td>
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<td>2</td>
<td>2</td>
</tr>
<tr>
<td>TY/SH</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>FF/FAL</td>
<td>41 (59.4)</td>
<td>37 (40.6)</td>
<td>78</td>
</tr>
<tr>
<td>AC/VOL</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>216 (39.7)</td>
<td>328 (60.3)</td>
<td>544</td>
</tr>
</tbody>
</table>

1989  263 (45.8)  311 (54.2)  574

LO = Lothian: STD = Diagnostic Laboratory serving Genito-urinary Medicine Unit.
CB = Clinical Bacteriology, University Medical School.
CML = Central Microbiology Laboratory, Western General Hospital.
CITY = Bacteriology Laboratory, City Hospital.

GG = Greater Glasgow: GRI = Glasgow Royal Infirmary.
SGH = Southern General Hospital.
RUC = Ruchill.

TY = Tayside: NWH = Ninewells Hospital, Dundee.
PRI = Perth Royal Infirmary.
SH = Stracathro Hospital.

FF/FAL = Fife Area Laboratory.

AC/VOL = Argyll and Clyde, Vale of Leven Hospital, Alexandria.
### TABLE 2

Prevalence and geographical distribution of protein IA serovars (1990)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>LO</th>
<th>GG/GRI</th>
<th>GG/GSH</th>
<th>GG/RUC</th>
<th>TY</th>
<th>FF</th>
<th>AC/VOL</th>
<th>TOTAL</th>
</tr>
</thead>
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<tr>
<td>IA-1</td>
<td>5(8.3)</td>
<td>1(2)</td>
<td>6(25)</td>
<td>0</td>
<td>6(17.1)</td>
<td>8(19.5)</td>
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<td>27(12.5)</td>
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<tr>
<td>IA-2</td>
<td>35(58.3)</td>
<td>40(81.6)</td>
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<td>28(68.3)</td>
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<td>148(68.5)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
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<td>0</td>
<td>1(2.4)</td>
<td>1</td>
<td>6(2.8)</td>
</tr>
<tr>
<td>IA-21</td>
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<td>0</td>
<td>1(2.9)</td>
<td>0</td>
<td>0</td>
<td>10(4.6)</td>
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<td><strong>TOTAL</strong></td>
<td>60(100)</td>
<td>49(100)</td>
<td>24(100)</td>
<td>4</td>
<td>35(100)</td>
<td>41(100)</td>
<td>3</td>
<td>216(100)</td>
</tr>
</tbody>
</table>

### TABLE 3

Prevalence and geographical distribution of protein IB serovars (1990)

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<tr>
<th>Serovar</th>
<th>LO</th>
<th>GG/GRI</th>
<th>GG/GSH</th>
<th>GG/RUC</th>
<th>TY</th>
<th>FF</th>
<th>AC/VOL</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB-1</td>
<td>14(11.2)</td>
<td>6(7.6)</td>
<td>4(7.1)</td>
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<td>2(8.2)</td>
<td>6(16.2)</td>
<td>1</td>
<td>33(10.1)</td>
</tr>
<tr>
<td>IB-2</td>
<td>54(42.2)</td>
<td>32(40.5)</td>
<td>23(41.1)</td>
<td>1</td>
<td>7(29.2)</td>
<td>9(24.3)</td>
<td>2</td>
<td>128(39.0)</td>
</tr>
<tr>
<td>IB-3</td>
<td>15(12)</td>
<td>24(30.4)</td>
<td>12(21.4)</td>
<td>1</td>
<td>11(45.8)</td>
<td>20(54.1)</td>
<td>1</td>
<td>84(25.6)</td>
</tr>
<tr>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>5(1.5)</td>
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<td>0</td>
<td>1(0.3)</td>
</tr>
<tr>
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<td>3(3.8)</td>
<td>3(5.4)</td>
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<td>0</td>
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<td>0</td>
<td>26(7.9)</td>
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<tr>
<td>IB-7</td>
<td>3(2.4)</td>
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<td>5(8.9)</td>
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<td>1(4.2)</td>
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<td>0</td>
<td>14(4.3)</td>
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<td>4(1.27)</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>IB-17</td>
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<td>2(3.6)</td>
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<td>0</td>
<td>8(2.4)</td>
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<td>0</td>
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<td>1(0.3)</td>
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<td>0</td>
<td>6(1.8)</td>
</tr>
<tr>
<td>Non-</td>
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<td>3(5.4)</td>
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<td>0</td>
<td>8(2.4)</td>
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<td>typable</td>
<td><strong>TOTAL</strong></td>
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<td>56(100)</td>
<td>3</td>
<td>24(100)</td>
<td>37(100)</td>
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</table>
TABLE 4

Penicillin susceptibility of IA serovars (1990)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>≤ 0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1.0</th>
<th>&gt;1.0</th>
<th>TOTAL</th>
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</thead>
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<td>IA-1</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>27</td>
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<tr>
<td>IA-2</td>
<td>133</td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4(a)</td>
<td>148</td>
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<tr>
<td>IA-4</td>
<td>0</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td>IA-6</td>
<td>9</td>
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<td>0</td>
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<td>10</td>
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<tr>
<td>TOTAL</td>
<td>181</td>
<td>12</td>
<td>10</td>
<td>6</td>
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<td>1989</td>
<td>235</td>
<td>14</td>
<td>7</td>
<td>2</td>
<td>1</td>
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<td>263</td>
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</table>

(a) = PPNG isolates.

TABLE 5

Penicillin susceptibility of IB serovars (1990)

<table>
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<tr>
<th>Serovar</th>
<th>≤ 0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1.0</th>
<th>&gt;1.0</th>
<th>TOTAL</th>
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<td>27</td>
<td>52</td>
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<td>47</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>84</td>
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<td>1</td>
<td>0</td>
<td>1(a)</td>
<td>5</td>
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<td>4</td>
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<td>0</td>
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<td>14</td>
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<tr>
<td>IB-8</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<td>0</td>
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</tr>
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<td>IB-14</td>
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<td>0</td>
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<td>1</td>
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<td>0</td>
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<td>NON-TYPABLE</td>
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<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>2(a)</td>
<td>8</td>
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<tr>
<td>TOTAL</td>
<td>48</td>
<td>56</td>
<td>116</td>
<td>97</td>
<td>2</td>
<td>8(a)</td>
<td>327</td>
</tr>
<tr>
<td>1989</td>
<td>31</td>
<td>79</td>
<td>117</td>
<td>80</td>
<td>0</td>
<td>4(a)</td>
<td>311</td>
</tr>
</tbody>
</table>

(a) = PPNG isolates.
### TABLE 6

**Antibiotic susceptibility of serotype IA and IB isolates (1990)**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype</th>
<th>Cumulative percentage</th>
<th>MIC (mg/L)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤0.015</td>
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<tr>
<td>Penicillin</td>
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<tr>
<td>IA</td>
<td>83.8</td>
<td>89.4</td>
<td>94</td>
</tr>
<tr>
<td>IB</td>
<td>14.7</td>
<td>31.8</td>
<td>67.3</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td></td>
<td></td>
<td>≤0.02</td>
</tr>
<tr>
<td>IA</td>
<td>93.1</td>
<td>98.6</td>
<td>100</td>
</tr>
<tr>
<td>IB</td>
<td>31.5</td>
<td>82.9</td>
<td>100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td></td>
<td></td>
<td>≤0.1</td>
</tr>
<tr>
<td>IA</td>
<td>9.3</td>
<td>91.7</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>1.2</td>
<td>75.8</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td></td>
<td>≤0.125</td>
</tr>
<tr>
<td>IA</td>
<td>64.4</td>
<td>97.2</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>56.3</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td>&lt;0.002</td>
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<tr>
<td>IA</td>
<td>22.7</td>
<td>96.3</td>
<td>100</td>
</tr>
<tr>
<td>IB</td>
<td>20.8</td>
<td>94.5</td>
<td>100</td>
</tr>
</tbody>
</table>

(a) 6 isolates MIC > 1.0 mg/L; all PPNG
(b) 8 isolates MIC > 1.0 mg/L; all PPNG

### TABLE 7

**Serovar and Geographical Distribution of PPNG Isolates (1990)**

<table>
<thead>
<tr>
<th>Serovar</th>
<th>LO</th>
<th>GG/GRI</th>
<th>GG/SGH</th>
<th>GG/RUC</th>
<th>TY</th>
<th>FF</th>
<th>AC/VOL</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>0/35</td>
<td>4/40</td>
<td>0/16</td>
<td>0/4</td>
<td>0/24</td>
<td>0/28</td>
<td>0/1</td>
<td>4/148</td>
</tr>
<tr>
<td>IA-6</td>
<td>1/11</td>
<td>1/4</td>
<td>0</td>
<td>0</td>
<td>0/4</td>
<td>0/4</td>
<td>0</td>
<td>2/23</td>
</tr>
<tr>
<td>IB-1</td>
<td>2/14</td>
<td>0/6</td>
<td>0/4</td>
<td>0</td>
<td>0/2</td>
<td>0/6</td>
<td>0/1</td>
<td>2/33</td>
</tr>
<tr>
<td>IB-2</td>
<td>0/54</td>
<td>0/32</td>
<td>1/23</td>
<td>0/1</td>
<td>0/7</td>
<td>0/9</td>
<td>0/2</td>
<td>1/128</td>
</tr>
<tr>
<td>IB-4</td>
<td>1/4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
<td>1/5</td>
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<tr>
<td>IB-5</td>
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<td>0</td>
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<td>1/1</td>
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<td>1/1</td>
</tr>
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<td>1/3</td>
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<td>0/5</td>
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<td>0/1</td>
<td>0/2</td>
<td>0</td>
<td>1/14</td>
</tr>
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<td>Non-typable</td>
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<td>2/3</td>
<td>0/3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2/8</td>
</tr>
<tr>
<td>Others</td>
<td>0/62</td>
<td>0/40</td>
<td>0/29</td>
<td>0/2</td>
<td>0/19</td>
<td>0/29</td>
<td>0/3</td>
<td>0/184</td>
</tr>
<tr>
<td>Total</td>
<td>5/185</td>
<td>7/128</td>
<td>1/80</td>
<td>0/7</td>
<td>1/59</td>
<td>0/78</td>
<td>0/7</td>
<td>14/544</td>
</tr>
<tr>
<td>1989</td>
<td>1/162</td>
<td>4/120</td>
<td>2/119</td>
<td>0/8</td>
<td>0/98</td>
<td>1/67</td>
<td>-</td>
<td>8/574</td>
</tr>
</tbody>
</table>

Syphilis diagnosis: screening by enzyme immunoassay and variation in fluorescent treponemal antibody absorbed (FTA-ABS) confirmatory test performance

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STD Diagnostic Laboratory, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland, UK

(Accepted 19 November 1991)

Abstract: Four fluorescent treponemal antibody absorbed (FTA-ABS) test kits were evaluated with 86 treponemal and 84 non-treponemal sera selected with enzyme immunoassay (EIA). Agreement between all four kits was 63% for treponemal, and 50%, for non-treponemal, sera. Discrepancies with treponemal sera were associated with low levels of antibody characterised by T. pallidum haemagglutination assay (TPHA) titres ≤160 and a negative Venereal Diseases Research Laboratory (VDRL) test. Discrepancies with non-treponemal sera were significantly associated with false reactivity on screening with EIA. Possible reasons for the differences obtained between kits, the importance of screening policies for pre-selecting sera, and the significance of equivocal (borderline) reactions on positive and negative predictive values are discussed.

Key words: Enzyme-linked immunosorbent assay. Fluorescent antibody technic. Reagent kits, diagnostic. Syphilis.

Introduction

The fluorescent treponemal antibody absorbed (FTA-ABS) test is the accepted reference test for confirming the treponemal nature of sera screened by other methods. The specificity of the FTA-ABS test is not absolute, however, and varies from 92% to 99%, and the purity and quality of the T. pallidum antigen preparation may have a direct effect on the occurrence of equivocal and borderline reactions. Borderline reactions are common, however, and attempts have been made to diminish their significance by altering reporting systems. Elimination of the borderline report increased the specificity of the test from 82.5% to 88.7%, but decreased the sensitivity from 100% to 99.5%. Apart from sensitivity and specificity, various other factors influence the reliability of the FTA-ABS test including the prevalence of syphilis and the pre-selection of sera by screening with cardiolipin tests and/or the T. pallidum haemagglutination assay (TPHA).

It has been estimated that at a prevalence of syphilis of 1.4%, around 30% of reactive FTA-ABS tests would be 'false-positives' if the FTA-ABS test was used for screening. The particular test used to pre-select sera may also have an important influence on FTA-ABS test utility. Early evaluations of enzyme immunoassay (EIA) showed a link between reactivity in EIA and the FTA-ABS test in non-treponemal sera. The importance of this early observation has been increased recently with the introduction of EIA as a screening test for syphilis. Most laboratories now use commercial kit systems to perform FTA-ABS tests. It has been reported, however, that there may be considerable variation in the quality—and hence performance—of such kits. In view of these factors we considered it important to evaluate test perfor-
mance (sensitivity and specificity) of four of the most widely used commercial FTA-ABS kits in Europe.

Materials and methods

The four FTA-ABS tests used were: Treplite;7 FTA-ABS Fluoro-Kit;8 Mastallour FTA-ABS;9 and Trepo-Spot IF.10 Reagents for the Trepo-Spot IF were supplied individually whereas all other reagents were supplied as packaged kits.

Procedures were performed according to the recommendation of the manufacturer of the corresponding kit. Slides were examined independently by two readers using a Zeiss Fluorescence Microscope. Fluorescence was scored as follows:

**Negative (−)** No fluorescence, treponemes visible or completely invisible;

**Equivocal (+)** Weak but definite fluorescence, less than weakly reactive control;

**Weak positive (1 +)** Weak but definite uniform green fluorescence, equivalent to weakly reactive control;

**Positive (2 +)** Moderate to bright green fluorescence;

**(3 +)** Brilliant to green fluorescence.

The above classification of fluorescence is essentially the same as that used by the Centers for Disease Control (CDC), Atlanta, Georgia, USA, the only difference being that positive reactions were scored 2+ or 3+ rather than 2+, 3+ and 4+.

**Sera**

One hundred and seventy sera that had been sent to the Sexually Transmitted Diseases (STD) Diagnostic Laboratory for serological tests for syphilis were included in our evaluation. Sera were stored at \(-20^\circ C\) without preservative. Before testing they were thawed and heat-inactivated at 56\(^\circ\)C for 10 min. All sera were clear and showed no evidence of haemolysis or lipaemia. All sera had been screened by the Captia Syph G anti-treponemal EIA.11 and if reactive on screening had also been examined by the Venereal Diseases Research Laboratory (VDRL) test, TPHA and a specific anti-treponemal IgM EIA as described previously.5 Fourteen sera that were negative on EIA screening were also subjected to the full range of tests prior to inclusion in the evaluation.

Sera were classified as treponemal or non-treponemal on the basis of specific treponemal tests, and clinical examination or history. Sera from 86 patients were classified as treponemal. The stage of infection and treatment status was known for 54 patients and included: primary infection 18 (untreated 4, treated 14); secondary 10 (all treated); early latent 14 (untreated 2, treated 12); neurological 2 (both treated) and late latent 10 (all treated). Sera from 84 patients were classified as non-treponemal. Although 70 of these patients gave a reactive result on EIA screening this finding was not corroborated by the TPHA test or by clinical findings and history. Statistical analysis was by the \(\chi^2\) method with Yates’ correction. Predictive values were calculated as follows:

**Positive Predictive Value**
\[
\frac{\text{True positives}}{\text{True positives} + \text{False positives}} \times 100\%
\]

**Negative Predictive Value**
\[
\frac{\text{True negatives}}{\text{True negatives} + \text{False negatives}} \times 100\%
\]

**Results**

Each kit performed according to the control criteria laid down in the manufacturer’s kit insert. However, there were marked differences in the number of treponemes present on the slides between the four kits. The BioMerieux and Mast slides had an even distribution of large and moderate numbers of treponemes respectively. Although the Mercia slides contained abundant treponemes, the distribution was uneven, with some areas showing clumping of treponemes while other areas were almost devoid of organisms. There was an even distribution of organisms on the CSI slides, but the treponemes were sparse.

**Treponemal sera:** Differences in scoring of results between reagent kit and reader for 86 treponemal sera are shown in Table 1.

Sensitivity of the four kits based on the results for Reader 1 (and Reader 2) when equivocal reactivity was scored negative was: Mast 74.4% (73.3%); CSI 82.6% (82.6%); Mercia 76.7% (72.1%) and BioMerieux 89.5% (89.5%). In spite of the similar sensitivity
values reported by the two readers there were differences in the scoring of individual sera. The percentage (and category) of discrepancies between the two readers was Mast 8.1% (five sera equivocal/1+ and two sera 1+/2+); CSI 17.4% (two sera equivocal/1+, 11 sera 1+/2+ and two sera 2+/3+); Mercia 11.6% (four sera equivocal/1+, four sera 1+/2+ and two sera 2+/3+) and BioMerieux 20.9% (four sera equivocal/1+, six sera 1+/2+ and eight sera 2+/3+). When results were analysed according to the CDC recommendations3 on the basis of a reactive result (1+, 2+ or 3+) that would denote treponemal infection, and an equivocal or negative result that would denote absence of treponemal infection, a much higher agreement between readers was obtained. Using these criteria to analyse meaningful differences the concordance between readers was: Mast 94.2%; CSI 97.7%; Mercia 95.3%; and BioMerieux 95.3%. As indicated by the almost identical specificity values obtained for the same kit by the two readers, differences were not due to one reader scoring consistently under- or over-sensitive. The differences in sensitivity of the four kits therefore represent true differences that are not related to reader variation.

Based on meaningful differences, 54 (62.8%) of the sera gave the same category of result with all four kits. Differences were significantly associated with low levels of antibody as determined by TPHA titre: discrepancies occurred in 60.5% (23/38) of sera with TPHA titres <160 compared with 18.8% (9/48) of sera with TPHA titres >320 (P<0.02). There was also a significant correlation between discrepancies in kits and VDRL reactivity: discrepancies occurred in 52.9% (27/51) of the VDRL negative sera compared with 14.3% (5/35) of the VDRL positive sera (P<0.05).

Non-treponemal sera: Differences in scoring of results between reagent kit and reader for 84 non-treponemal sera are given in Table 2.

Specificity of the four kits based on the results for Reader 1 (and Reader 2) when equivocal reactivity was scored negative was: Mast 96.4% (96.4%); CSI 77.4% (75%); Mercia 61.9% (63.1%); and BioMerieux 71.4% (70.2%). In spite of the similar specificity values reported by the two readers, there were differences in the scoring of individual sera. The percentage (and category) of discrepancies between the two readers was: Mast 2.4% (two sera negative/equivocal); CSI 6% (one serum negative/equivocal, two sera equivocal/1+ and two sera 1+/2+); Mercia 6% (one serum negative/equivocal, three sera equivocal/1+ and one serum 1+/2+); and BioMerieux 6% (one serum negative/equivocal, three sera equivocal/1+ and one serum 1+/2+). When results were analysed according to the CDC recommendations3 (as described under treponemal sera) a much higher agreement between readers was obtained: Mast 100%; CSI 97.6%; Mercia 96.4%; and BioMerieux 96.4%. Again, as indicated by the almost identical specificity values obtained for the same kit by the two readers, differences were not due to one reader scoring consistently under- or over-sensitive. The differences in specificity of the four kits therefore represent true differences that are not related to reader variation.

Based on meaningful differences, 42 (50%) of the sera gave the same category of result with all four kits. Differences were significantly associated with false

---

**Table 1. Category of reactivity given by 86 treponemal sera according to reagent kit and reader**

<table>
<thead>
<tr>
<th></th>
<th>Mast R1</th>
<th>CSI R1</th>
<th>Mercia R1</th>
<th>BioMerieux R1</th>
<th>Mast R2</th>
<th>CSI R2</th>
<th>Mercia R2</th>
<th>BioMerieux R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>4</td>
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<tr>
<td>Equivocal</td>
<td>16</td>
<td>17</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>16</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>1+</td>
<td>34</td>
<td>32</td>
<td>38</td>
<td>26</td>
<td>31</td>
<td>27</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>2+</td>
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<td>1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

R1 = Reader 1  
R2 = Reader 2

---

**Table 2. Category of reactivity given by 84 non-treponemal sera according to reagent kit and reader**

<table>
<thead>
<tr>
<th></th>
<th>Mast R1</th>
<th>CSI R1</th>
<th>Mercia R1</th>
<th>BioMerieux R1</th>
<th>Mast R2</th>
<th>CSI R2</th>
<th>Mercia R2</th>
<th>BioMerieux R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>70</td>
<td>70</td>
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<td>48</td>
<td>43</td>
<td>44</td>
<td>47</td>
<td>46</td>
</tr>
<tr>
<td>Equivocal</td>
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<td>11</td>
<td>16</td>
<td>15</td>
<td>9</td>
<td>9</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>1+</td>
<td>3</td>
<td>3</td>
<td>18</td>
<td>18</td>
<td>22</td>
<td>19</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>2+</td>
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<td>0</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>3+</td>
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<td>0</td>
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<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

R1 = Reader 1  
R2 = Reader 2

---

reactivity on screening with EIA: discrepancies occurred in 58.6% (41/70) of sera tested on the basis of false reactivity on EIA screening, compared with 7.1% (1/14) of sera negative on EIA screening ($P<0.01$).

**Discussion**

Our results demonstrate that differences occurring in test performance between different FTA-ABS kits are more important than the differences obtained between two readers. Combining the results for treponemal and non-treponemal sera, the overall agreement between the four kits was 56.5% (96/170). Beebe and Nouri\(^6\) reported that the reproducibility of reactive and non-reactive results measured by between-assay and within-assay studies averaged 42%. They concluded that such substantial variation in performance characteristics between assay kits had important implications for the diagnosis of syphilis.

The performance of the various kits examined in our study is summarised in Table 3.

In general, sensitivity and specificity are inversely correlated with a corresponding effect on the negative and positive predictive value. Clearly the Mast kit gave the highest positive predictive value. This value (95.5% when equivocal reactions are taken as positive) approximates to the 97.9% positive predictive value for the FTA-ABS as a confirmatory test reported by Larsen et al.\(^3\) The poor specificity and positive predictive value of the other kits results from our sera being selected as equivocal or reactive on screening with EIA. It should be stressed that the FTA-ABS is a highly specific test when used to confirm the treponemal nature of sera that are reactive in the TPHA, and when used in this way the differences that we have observed in the four FTA kits would be extremely unlikely to occur. This is because the TPHA is the most specific method for the detection of antibody to *T. pallidum*, with false reactivity as low as 0.07%\(^1\). In keeping with this finding it is not surprising that none of the non-treponemal sera evaluated in our study were reactive in the TPHA, further supporting our view that the differences observed are related to pre-selection on the basis of EIA reactivity. It should also be emphasised that our non-treponemal sera reactive in the EIA were selected from a large number of sera tested. The EIA is a highly specific screening test, with a reported specificity of 99.3%\(^5\).

A correlation between unexplained reactivity in the FTA-ABS and EIA was noted by Veldkamp and Visser\(^4\), who tested sera from ten people with a weakly reactive FTA-ABS (1+) test with no clinical evidence or history of syphilis: the EIA was reactive in five of the ten patients. The reasons for this correlation are not known, but it would seem that the Mast kit differs from the others with respect to dual false reactivity with the EIA. Differences are unlikely to be due to differences in the efficacy of the sorbent in neutralising group reactive anti-treponemal antibodies; apart from the Mercia kit which contains a chemically defined sorbent the other three sorbents were prepared from an extract of 'Reiter' treponemes. In a recent study\(^13\) six different sorbent preparations were examined, but there was no difference in their performance in the FTA-ABS test; it was suggested that the effect of sorbent is based on a non-specific inhibition of the reaction between anti-treponemal antibodies and *T. pallidum*.

Differences in the conjugate may be important as the use of mono-specific anti-IgG conjugate reduces the number of borderline reactions. In this context it is interesting that the Mast kit uses anti-human IgG, whereas the other three kits use anti-human immunoglobulins. The nature of the treponemal antigen is also likely to contribute to differences between kits. Hanff and colleagues\(^2\) found that percoll-purified *T. pallidum* antigen essentially eliminated borderline and false positive reactions in the FTA-ABS test. There are obviously difficulties associated with obtaining large quantities of purified antigen that are free from contaminating host testicular tissue, and yet retain

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**Table 3. Effect of equivocal reactions on sensitivity, specificity, positive and negative predictive values**

<table>
<thead>
<tr>
<th></th>
<th>Mast</th>
<th>CSI</th>
<th>Mercia</th>
<th>BioMerieux</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Equivocal -ve</td>
<td>73.9</td>
<td>82.6</td>
<td>74.4</td>
<td>89.5</td>
</tr>
<tr>
<td>Equivocal +ve</td>
<td>93</td>
<td>95.3</td>
<td>90.7</td>
<td>95.3</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equivocal -ve</td>
<td>96.4</td>
<td>76.2</td>
<td>62.5</td>
<td>70.8</td>
</tr>
<tr>
<td>Equivocal +ve</td>
<td>83.3</td>
<td>57.7</td>
<td>51.8</td>
<td>55.4</td>
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<tr>
<td><strong>Positive Predictive Value</strong></td>
<td></td>
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<tr>
<td>Equivocal -ve</td>
<td>95.5</td>
<td>78</td>
<td>67</td>
<td>77</td>
</tr>
<tr>
<td>Equivocal +ve</td>
<td>85.1</td>
<td>69.8</td>
<td>66.4</td>
<td>68.6</td>
</tr>
<tr>
<td><strong>Negative Predictive Value</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Equivocal -ve</td>
<td>78.3</td>
<td>81</td>
<td>70.5</td>
<td>86.9</td>
</tr>
<tr>
<td>Equivocal +ve</td>
<td>92.1</td>
<td>92.3</td>
<td>84.5</td>
<td>92.1</td>
</tr>
</tbody>
</table>

\(^*\)Values averaged from results of Reader 1 and Reader 2
their morphological characteristics and immunological reactivity: manufacturers do not give details of antigen preparation in kit inserts.

Apart from a high positive predictive value, a confirmatory test should also have a high negative predictive value in order that sera are not falsely categorised as non-treponemal. The CDC changes in reporting criteria, aimed at improving specificity and hence positive predictive value, reduced the sensitivity of the FTA-ABS as a confirmatory test from 100% to 99.5%; no details were given regarding the influence of this change on negative predictive value, but presumably it remained high. The minimal reduction in sensitivity brought about by the reporting change is due to the way that screening is generally performed in the United States: it is recommended that treponemal tests should not be used as screening tests, but as tests to confirm the positive results of a non-treponemal test or specific diagnostic tests for patients with signs and/or symptoms that suggest late syphilis. Our results illustrate how this difference in screening policy can affect the reliability of confirmatory FTA-ABS tests. When equivocal reactions were scored negative, and the four tests are analysed with respect to the 35 VDRL positive treponemal sera, sensitivity varied from 91.5% (Mercia) and 94.2% (Mast and CSI) to 100% (BioMerieux). When equivocal reactions were scored positive, sensitivity was 100% in each case, apart from CSI, which was 97.1%.

These results show that if the CDC recommendations are applied to sera selected on the basis of screening by a treponemal test such as the TPHA (or EIA), then a significant number of treponemal sera fail to be confirmed. This is because the TPHA is more sensitive than the FTA-ABS (except during the third to fourth week of infection). The advent of HIV infection has increased the importance of screening with highly sensitive treponemal tests. As noted by Haus and colleagues in the United States and Johnson and colleagues in Australia, there is a tendency for serological markers of syphilis to disappear in HIV infected patients, particularly as immune dysfunction progresses. Another important reason for not relying on screening with the VDRL test is the possibility of false negative results occurring as a result of the prozone phenomenon. False negative prozone reactions have been reported recently in antenatal patients and in HIV infected individuals such reactions have occurred on testing cerebrospinal fluid.

In conclusion we have observed significant differences in the performance of four different FTA-ABS test kits, with respect to confirming the treponemal nature of sera pre-selected on the basis of EIA. The highest positive and negative predictive values were given by the Mast kit when equivocal reactions were scored positive. The differences in specificity that we observed were related to screening with EIA, and would not be found when sera were selected on the basis of the TPHA. With all four kits it was necessary to interpret equivocal reactions as positive in order to obtain a high negative predictive value and avoid categorising sera falsely as non-treponemal. This means that the recommendation of CDC to abolish the borderline report is not suitable for laboratories testing sera that have been screened by treponemal tests.

References

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Syphilis diagnosis


An analysis of lectin agglutination as a means of sub-dividing gonococcal serovars

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STD Diagnostic Laboratory, Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG

Summary. Sixteen lectins were examined for their ability to agglutinate 298 strains of Neisseria gonorrhoeae. Seven lectins failed to agglutinate any of the strains; the remaining nine lectins gave 22 different agglutination patterns. The 298 strains were divided into 14 serovars with a single panel of monoclonal antibody typing reagents; lectin agglutination subdivided these into 57 serovar/lectin patterns. A combination of two monoclonal antibody serotyping panels divided the strains into 32 serovar combinations; lectin agglutination further subdivided these into 79 serovar/lectin patterns. There was no correlation between lectin pattern and serovar. Lectin agglutination is a simple supplementary typing method and could be particularly useful in micro-epidemiological studies.

Introduction

Lectins are natural proteins that are not part of immune mechanisms and that react with sugar residues. They have been used in the study of cell-surface carbohydrates of bacteria and can detect inter-strain variations in cell-wall carbohydrate composition. The selectivity of lectins for microbial surfaces may be advantageous in epidemiological studies of bacterial disease.

The ability of animal and plant lectins to react with microbial substances has been known for some time. Summer and Howell first reported bacterial reactions with lectins by demonstrating the ability of Canavalin A to agglutinate species of Mycobacterium and Actinomyces. In the late 1970s, agglutination of Neisseria gonorrhoeae by wheat germ lectin was advocated as a confirmatory test for identifying this organism. The interaction between lectins and members of the genus Neisseria has been well documented in several other studies and has been evaluated for identification and epidemiological typing.

Vázquez and Berron examined lectin agglutination in relation to serogroup and auxotype. However, there have been no detailed studies correlating lectin agglutination with serotyping based on monoclonal antibodies (MAbs). In this study we examined the reactivity of 298 clinical isolates of N. gonorrhoeae with a panel of 16 lectins and assessed the increased epidemiological discrimination achieved by the combination of lectin agglutination and serotyping.

Materials and methods

Bacterial strains

A series of 298 clinical isolates of N. gonorrhoeae from patients attending the Genitourinary Medicine Unit, Edinburgh Royal Infirmary during 1988 and 1989 was studied. The isolates were identified as N. gonorrhoeae by the rapid carbohydrate utilisation test and the Phadebact Monoclonal GC test. 149 isolates were serogroup 1A and 149 were serogroup IB. Serotyping of the isolates was performed as described previously with both the Genetic Systems (GS) panel of MAbs supplied by Dr C. Ison, St Mary's Hospital, London, and the Pharmacia (PH) panel of MAbs supplied by Dr S. Bygdeman, Karolinska Institute, Sweden. All of these MAbs are directed against epitopes on gonococcal outer-membrane protein I.

Lectin agglutination

Bacterial suspensions. Isolates were inoculated on to clear gonococcal (GC) agar and incubated at 37°C in an atmosphere of CO₂ 5% in air for 18–24 h. Growth was harvested with a cotton swab into 1 ml of phosphate-buffered saline, pH 7.2 (PBS), to give a smooth suspension. The suspension was boiled for 10 min, allowed to cool and adjusted to an optical density of 0.5 at 450 nm with a Titertek Multiplan MCC 340 spectrophotometer.

Lectins. Sixteen lectins were obtained from Sigma, reconstituted in distilled water to a concentration of 1 mg/ml and stored in small divided volumes at −20°C. For use, lectin solution was thawed and diluted with distilled water to the concentrations...
Table I. Panel of lectins and concentrations used

<table>
<thead>
<tr>
<th>Code</th>
<th>Lectin</th>
<th>Common name</th>
<th>Concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNA</td>
<td>Arachis hypogea</td>
<td>Peanut</td>
<td>62</td>
</tr>
<tr>
<td>BSI</td>
<td>Bandeiraea simplicifolia I</td>
<td>Griffonia seeds</td>
<td>62</td>
</tr>
<tr>
<td>Cona</td>
<td>Concanavalin A</td>
<td>Jack bean</td>
<td>250</td>
</tr>
<tr>
<td>DBA</td>
<td>Dolichos biflorus</td>
<td>Horse gram</td>
<td>62</td>
</tr>
<tr>
<td>SBA</td>
<td>Glycine max</td>
<td>Soybean</td>
<td>62</td>
</tr>
<tr>
<td>HPA</td>
<td>Helix pomatia</td>
<td>Snail</td>
<td>62</td>
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<tr>
<td>SJA</td>
<td>Sophora japonica</td>
<td>Pagoda tree</td>
<td>62</td>
</tr>
<tr>
<td>LCH</td>
<td>Lens culinaris</td>
<td>Lentil</td>
<td>62</td>
</tr>
<tr>
<td>MPA</td>
<td>Maclura pomifera</td>
<td>Osage orange</td>
<td>62</td>
</tr>
<tr>
<td>PSA</td>
<td>Pisum sativum</td>
<td>Pea</td>
<td>62</td>
</tr>
<tr>
<td>STA</td>
<td>Solanum tuberosum</td>
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</tr>
<tr>
<td>WGA</td>
<td>Triticum vulgare</td>
<td>Wheat germ</td>
<td>62</td>
</tr>
<tr>
<td>UEA</td>
<td>Ulex europeaus</td>
<td>Gorse</td>
<td>62</td>
</tr>
<tr>
<td>PHA</td>
<td>Phaseolus vulgaris</td>
<td>Red kidney bean</td>
<td>62</td>
</tr>
<tr>
<td>BSII</td>
<td>Bandeiraea simplicifolia II</td>
<td>Griffonia seeds</td>
<td>62</td>
</tr>
<tr>
<td>LPA</td>
<td>Limeus polyphemus</td>
<td>Horseshoe crab</td>
<td>62</td>
</tr>
</tbody>
</table>

Table II. Lectin agglutination patterns of 298 strains of N. gonorrhoeae

<table>
<thead>
<tr>
<th>Agglutination with</th>
<th>Number of strains</th>
<th>Lectin patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PNA BSI SBA HPA SJA MPA STA WGA BSI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 2 LP12</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 184 LP1</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 17 LP3</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 7 LP6</td>
</tr>
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<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 3 LP9</td>
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</tr>
<tr>
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<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 1 LP16</td>
</tr>
<tr>
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<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 4 LP8</td>
</tr>
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<td>- + 3 LP10</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 2 LP13</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 1 LP17</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 3 LP11</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 40 LP2</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 10 LP4</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 5 LP7</td>
</tr>
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<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 2 LP14</td>
</tr>
<tr>
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<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 1 LP18</td>
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<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 8 LP5</td>
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<td>- + 1 LP19</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 1 LP20</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 1 LP21</td>
</tr>
<tr>
<td></td>
<td>+ + + + + + + + + + + + + + + + + +</td>
<td>- + 1 LP22</td>
</tr>
</tbody>
</table>

described in previous studies.3-8 The lectins and concentrations used are shown in table I.

Agglutination tests. Standardised test suspension (20 μl) was pipetted into two wells on a glass tile; 20 μl of the appropriate lectin solution was added to one well and 20 μl of PBS was added to the other well. The suspensions were mixed and placed on a rotary shaker for 5 min. Reactions were scored as positive when agglutination occurred in the lectin suspension but not in the control suspension. Strains showing agglutination in the control suspension were re-tested.

Results

Seven lectins did not agglutinate any of the 298 gonococcal isolates (ConA, DBA, LCH, PSA, UEA, PHA and LPA). The agglutination patterns with the remaining nine lectins are shown in table II. Twenty-two lectin patterns or groups were recognised: these are designated LP1–LP22 based on their frequency of occurrence. All isolates were agglutinated by soybean lectin (BSA). Only two isolates (0.7%) were agglutinated by maclura pomifera lectin (MPA); four strains were agglutinated by PNA and SBA and three strains by BSI, SBA, HPA and SJA; and seven isolates (2.3%) failed to agglutinate wheat germ lectin (WGA).

Combining lectin agglutination with serotyping with MAbs (table III) markedly increased the discrimination between isolates. The 298 isolates were divided into 14 different serovars with the GS panel of MAbs.
A combination of the GS panel with the PH MAb panel yielded 32 serovar/lectin patterns. A combination of GS panel serotype with lectin agglutination yielded 57 serovar/lectin patterns and a compilation of GS panel serotype with PH panel serotype and lectin agglutination yielded 79 serovar/lectin combinations.

The combination of serotyping and lectin agglutination enabled subdivision of serogroup IA and IB strains. The two most common lectin patterns (LP1 and LP2) accounted for 184 (61.7%) and 40 (13.4%) of strains, respectively (tables IV and V). The most common IA serovar was IA-2, representing 85.2% of the IA serotypes; this could be subdivided into 12 lectin patterns. Less discrimination was obtained with the remaining IA serovars: IA-4, IA-6 and IA-21 yielded two, five and three lectin patterns, respectively. The most common IB serovar was IB-2 (36.9% of IB serotypes); unfortunately, the degree of further discrimination was less with IB-2 than with IA-2 serovars—only seven lectin patterns were recognised. Serovars IB-1 and IB-3 could be subdivided into 10 and six lectin patterns, respectively; the remaining seven IB serovars yielded only 12 serovar/lectin patterns.

### Discussion

Typing of *N. gonorrhoeae* is useful in the control of gonococcal infection, e.g., in the recognition and control of micro-epidemics by detecting the occurrence of new strains or increased prevalence of existing ones. It is also useful in monitoring the spread of isolates with plasmid and chromosomally mediated antibiotic resistance and in recognising re-infections and double infections.

In this study, we have shown that agglutination with a panel of 16 animal and plant lectins subdivided 149 serogroup IA isolates into 13 types and 149 serogroup IB isolates into 19 types and merits consideration as a supplementary means for epidemiological typing of *N. gonorrhoeae*. A similar study with 101 strains of *N. gonorrhoeae* from outbreaks in Georgia, California and Hawaii, and a panel of 14 lectins, yielded 24 lectin patterns. Korting and Abeck distinguished 29 lectin patterns amongst 102 gonococcal isolates from Munich with a panel of 14 lectins.

The four major lectin patterns reported here (LP1-LP4) accounted for 84% (251 out of 298) of isolates, which is similar to the results of serotyping studies in which a few major serovars account for the majority of strains.

As seven of the lectins tested did not react with any of the strains, the panel could be reduced to nine lectins without any decrease in discrimination. Inclusion of ConA in any typing panel is debatable because it failed to agglutinate any of 54 strains, 193 strains, and 40 strains tested in other studies. However, all 101 strains were agglutinated by ConA in the study by Korting and Abeck, and two of 101 penicillinase-producing and non-penicillinase-producing strains examined in a

### Table III. Discrimination given by lectin agglutination and serotyping among 298 gonococcal isolates

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Number of groups given by typing method(s) when results are considered alone or in combination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lectin</td>
</tr>
<tr>
<td>IA isolates (149)</td>
<td>13</td>
</tr>
<tr>
<td>IB isolates (149)</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>22*</td>
</tr>
</tbody>
</table>

GS, Genetic Systems MAb. PH, Pharmacia MAb.* The same lectin pattern often occurred with isolates of serogroups IA and IB.

### Table IV. Distribution of lectin patterns within serogroup IA isolates

<table>
<thead>
<tr>
<th>Lectin pattern</th>
<th>Number of isolates of serotype</th>
<th>Total number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IA-2</td>
<td>IA-4</td>
</tr>
<tr>
<td>LP1</td>
<td>92</td>
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</tr>
<tr>
<td>LP2</td>
<td>13</td>
<td>4</td>
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</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>5</td>
</tr>
</tbody>
</table>
further study by Schalla et al. The reasons for the variations in results in these studies is unclear, but it is unlikely to be due to the inclusion of minor serovars or types because of the large number of strains tested. Similarly, MPA might also be omitted from the panel because only two of the 298 isolates were reactive and none were reactive in two further studies with a total of 247 strains. 4,6

All the strains in this study gave a positive reaction with SBA, which is one of the criteria in the five tests comprising lectins and chromogenic substrates for the rapid identification of strains of *N. gonorrhoeae*. SBA agglutination in this case was useful in the identification of strains that were not agglutinated by WGA.

We found that seven (2.3%) strains failed to agglutinate WGA compared with 0.5–9.9% of strains in several other studies. 5,6,8,10,18 WGA agglutination cannot, therefore, be relied upon as a single criterion for the confirmatory identification of *N. gonorrhoeae*. All seven WGA-negative strains belonged to serovar IB-1/Bropt: a further 16 isolates of serovar IB-1 Bropt gave a positive reaction with WGA and could be differentiated into six lectin patterns.

A combination of lectin pattern with serotype markedly increased the discrimination over that obtained with either method alone. The greatest overall discrimination was given by lectin agglutination in combination with both the GS and PH serotyping panels of MAb; this resulted in 79 serovar/lectin combinations. We have shown that the major serovars, such as IA-2 and IB-2, can be subdivided into 12 and seven lectin patterns, respectively. This is relevant in the micro-epidemiological approach to gonorrhoea control, in which auxotyping has been the primary means of further subdivision of serovars, i.e., auxotype/serovar classification. 10 In this study we have been unable to show any correlation between lectin pattern and serovar when either a single panel or a combination of panels of MAb was used. Others have been unable to show any correlation between lectin pattern and auxotype 11 or between lectin pattern and serovar. 8

We have shown previously that the use of two panels of MAb (GS and PH panels) increases discrimination in typing *N. gonorrhoeae*. 13 Provided that lectin agglutination is shown to be reproducible, the combination of GS/PH serovar with lectin pattern could provide sufficient discrimination to be a simple alternative to auxotype/serovar classification. There is, however, a need for an agreed reference panel of lectins to be used in such an epidemiological typing system.

### References

Classification of oral pigmented anaerobic bacilli by pyrolysis mass spectrometry and biochemical tests

J. T. MAGEE*, J. M. HINDMARCH, B. I. DUERDEN* and L. GOODWIN

Department of Experimental and Clinical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX

Summary. Clinical (66) and reference (5) strains of pigmented gram-negative anaerobic bacilli, identified as Prevotella intermedia (47), Pr. melaninogenica (1), Pr. corpora (8), Porphyromonas asaccharolyticus (12), P. endodontalis (1) and P. gingivalis (2), were examined by pyrolysis mass spectrometry (PMS) and in conventional tests. Numerical classification based on conventional test reaction patterns (CTRPs) resolved five clusters, four comprising strains identified as Pr. intermedia, Pr. corpora, Pr. melaninogenica, and P. gingivalis respectively, and one comprising strains identified as P. asaccharolyticus and P. endodontalis. Numerical classification based on PMS showed a similar division, with decreasing homogeneity of chemical composition in the order Pr. intermedia, Pr. corpora, P. asaccharolyticus, which agreed with the order of homogeneity in CTRPs. PMS clusters corresponding to the genus Porphyromonas were clearly distinct from those of the genus Prevotella. PMS and CTRP classification disagreed on cluster membership for six strains. PMS identification from blind challenge sets was in agreement with conventional identification for 64 of 67 strains.

Introduction

Gram-negative anaerobic bacilli that produced black or brown colonies on blood-containing media were first described by Oliver and Wherry and called Bacterium (later Bacteroides) melaninogenicus. This species encompassed all pigmented bacteroides but was subsequently divided into three subspecies. Subsequently the asaccharolytic strains were removed, first to a distinct species, B. asaccharolyticus, later as a subgroup of species within the Bacteroides and then to a separate genus, Porphyromonas, comprising three species, P. asaccharolyticus, P. endodontalis and P. gingivalis. The saccharolytic pigmented strains remained members of the melaninogenicus-oralis group of Bacteroides which included pigmented and nonpigmented strains; but these too were later separated from the genus Bacteroides sensu stricto (formerly the fragilis group of Bacteroides) as a new genus, Prevotella. The pigmented Prevotella spp. comprise Pr. intermedia, Pr. melaninogenica, Pr. corpora, Pr. loescheii and Pr. dentiicola. These changes were largely the result of DNA homology studies.

Pigmented bacteroides are members of the normal flora of the mouth, vagina and colon, and are also implicated in a range of purulent and necrotising infections at these sites. In the mouth they are found in the gingival crevice in both health and disease. Pr. melaninogenica is part of the normal commensal gingival flora and appears to be of little pathological significance, whereas Pr. intermedia is more commonly associated with acute necrotising gingivitis and chronic periodontal disease. P. gingivalis is linked with severe, rapidly progressive periodontitis and generalised juvenile periodontal disease. P. asaccharolyticus has not been associated with oral colonisation or disease, but in a study of the gingival flora in patients with adult periodontal disease we isolated large numbers of pigmented strains, about half of which appeared asaccharolytic in rapid tests, produced small amounts of n-butyric acid, and were identified as P. asaccharolyticus. Most of the saccharolytic strains were identified as Pr. intermedia. Similar results were obtained in a subsequent longitudinal study of adult periodontal disease. The work described here was done partly because of doubts as to the validity of these identifications as P. asaccharolyticus, based solely on rapid tests.

Pyrolysis mass spectrometry (PMS) is a rapid characterisation method that has been used in classification, identification and typing of micro-organisms. The spectra produced reflect cell chemical composition as 'fingerprint' or pattern data with a complex statistical structure. Analysis of these data requires multivariate statistical strategies. However, modern statistical program suites available for micro- and mini-computers can be used to reduce raw...
Forfar and Arneil's
Textbook of Paediatrics

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FOURTH EDITION

CHURCHILL LIVINGSTONE
EDINBURGH LONDON MADRID MELBOURNE NEW YORK AND TOKYO 1992
22. Infections

Chapter editor: A. G. M. Campbell

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therapy, as few centres have large enough numbers of infected children to conduct meaningful trials.

PROGNOSIS

Despite the wealth of information that has accumulated on HIV disease in children, data on the natural history of the disease are lacking. It is known that the incubation period is shorter in children compared to adults, and that the length of survival following diagnosis is decreased. Children who develop an opportunistic infection in the first year of life have the poorest prognosis, with few surviving beyond 2 years of age. In developing countries, common causes of death include protracted diarrhoea with wasting, pneumonia and measles.

Children who present with LIP have a very low incidence of opportunistic infections although morbidity is high with recurrent respiratory infections and chronic hypoxaemia. Some children with LIP respond favourably to steroid therapy and survive to later childhood.

Encephalopathy is reported to affect about 50% of children infected with HIV, and persistence of HIV antigen in the cerebrospinal fluid has been reported to be pathognomonic for progressive encephalopathy. Neurological signs also tend to worsen coincident with progression of immune deficiency.

There is a need to define early clinical and laboratory parameters to delineate those children with mild disease from those with rapid progression to AIDS. In one study (Robert-Guroff et al 1987), children who survived for more than 2 years following the onset of symptoms had LIP, candidiasis, recurrent bacterial infections, failure to thrive and lymphadenopathy. All the stable children possessed some neutralizing antibody. The clinical spectrum of those children who did not have neutralizing antibody included progressive encephalopathy, P. carinii pneumonia and thymic depletion.

Disappearance of antibodies against HIV core proteins (e.g. p24) as well as the persistence of HIV antigenaemia correlate with poor outcome and predict the occurrence of full blown AIDS. (Goudsmit et al 1987). The possible prognostic value of other variables such as T4 lymphopenia, impaired mitogen-induced lymphocyte proliferation, neopterin and B2 microglobulin levels remains to be assessed in children.

PREVENTION

With the routine screening of blood and blood products and the self-referral of donors, the number of children infected via this route will diminish. This decrease will however be compensated by the increase in the number of children infected by vertical transmission. The rise of HIV infection amongst women of childbearing age represent those who acquire infection through heterosexual transmission as well as those who are intravenous drug users. The present challenge is therefore to limit the current spread of HIV by sexual and parenteral routes. While no effective vaccine or cure exists, an intensive educational approach is important. This should include information on the modes of transmission of HIV. For the paediatrician, it means educating schoolchildren and teenagers, as well as women of childbearing age, about good hygiene standards, avoidance of intravenous drug use and safer sexual practices.

TREPONEMATOSES AND SEXUALLY TRANSMISSIBLE DISEASES

In sexually transmissible disease (STD) the paediatric interest arises for a number of reasons. The need for prevention and protection in children necessitates an unambiguous commitment to education in the difficult and controversial field of sexual behaviour. Sexual abuse of children is being increasingly revealed as a serious medicosocial problem (Lancet 1987b); this will need understanding of the approach and procedures necessary to detect or exclude sexually transmitted infection. In STD infection of one parent is likely to lead to infection of the other and in the case of pregnancy vertical transmission to the fetus or neonate may take place. Specific treatment will have to take into account the sensitivity of the organism to antibiotic or chemotherapy prevailing in the locality and in the control of STD the tracing of contacts is essential. In endemic treponematosis, however, it is not sexual transmission which is the method of spread but close contact among children and others with skin lesions in an infectious stage.

PREVENTION

The need for primary prevention, always important, has been sharpened in recent times and becomes more pressing for everyone responsible for the education of the young as human immunodeficiency virus (HIV) infection and the acquired immune deficiency syndrome (AIDS) spread inexorably since first recognized in 1981. In STD secondary prevention has an important role and this will require insight by the young person of the possible risks of sexual behaviour and a willingness to seek help when needed. It will need readily available medical facilities for diagnosis and specific treatment as well as those for counselling and the tracing and treatment of contacts.

World-wide, wherever AIDS is found the causative agent, the human immunodeficiency virus (HIV) is spread only in the same ways — through penetrative sexual intercourse, blood transfer and from infected mother to infant. There is now increasing evidence that other STDs, particularly those like syphilis, chancroid and genital herpes, that cause genital ulceration, may increase the likelihood of the spread of HIV. For the reasons stated, because effective vaccines have not yet been created and because there is not yet a fully effective drug for treatment, sex education and information programmes are critical to AIDS prevention and STD control. The short-term actions, often strident in their urgency, have to be replaced by education programmes which will last and which will be brought into the schools (Director of the World Health Organization Global Programme on AIDS, 1988).

With the highest total prevalence rate in Europe — 34.9 cases per million population on 31 March 1987 — Switzerland's response, for example, may be quoted as a model. AIDS care and prevention policies are based on the need for new attitudes towards sexual relationships, different from those of young people 10–15 years ago. Some 15% of the male homosexuals and more than half of the drug misusers using intravenous injections have been found to be seropositive. New ways are being devised to reach the young from the age of 13–14 years onwards and serious endeavours are being made to generate dialogue, personal interest
and commitment. Contacts are maintained both formally and informally with homosexual groups and those who misuse intravenous drugs. Approaches to heterosexuals are being developed. The values and safety of long-term monogamous relationships (Martin & Michaud 1988) are being explained and promoted and safer forms of sexual expression and the use of condoms discussed.

In relation to the transmission of STD it is appropriate here to refer to the evidence that psychosocial development in both sexes is determined by nutrition. In developed countries sexual maturity in girls, for example, occurs at an earlier age than in the developing countries as a result of good nutrition. Intellectual maturity, upon which her capacity to cope with her sexuality depends, is however more dependent upon chronological age and comes later. The social consequences of lowering the age of puberty are profound: earlier sexual activity requires access to sex education. Rights to health education, including sex education, can be claimed from the European Convention of Human Rights. Judgements of the European Court of Human Rights have made these obligations clear as well as the bounds that may be set in this subject. The principles characterizing a ‘democratic society’, which are relevant to discussion on education and have been outlined in the European Court of Human Rights (ECHR) at Strasbourg, emphasize the value of ‘freedom of expression’. The importance of pluralism, tolerance and broadmindedness is again and again emphasized as well as the balance which must be achieved to secure the fair and proper treatment of minorities. None the less, teaching materials should not contain sentences or paragraphs that young people, at a critical stage of their development, could interpret as encouragement to indulge in precocious activities harmful for them, or even to commit criminal offences for further discussion see Robertson et al 1989, pp. 56–58).

SUMMARY OF CLINICAL AND LABORATORY PROCEDURES FOR THE DETECTION OR EXCLUSION OF STD

The clinician, when considering the possibility of a sexually transmitted disease in a patient, will find it useful to follow the summary of the clinical procedures (detailed more fully in the text) necessary to detect or exclude such infection and to consider a number of other important issues as listed below.

EARLY STAGE SYPHILIS

Detect or exclude syphilis. If detected give specific treatment for patient and trace contacts.

1. Clinical inspection: surface lesions of early syphilis, e.g. chancre, condylomata lata.
3. Serological tests: e.g. venereal disease research laboratory (VDRL) test and Treponema pallidum haemaggulination assay (TPHA) (confirmation if necessary by the fluorescent treponemal antibody-absorbed (FTA-ABS) test at 0, 30 and 90 days); trace particularly contacts of primary and secondary syphilis.

GONORRHOEA

Detect or exclude: if infected give specific treatment and trace contacts.

Bacteriological diagnosis:

2. Selective medium, direct plating, when immediate incubation available, or use transport (e.g. Amies) (serogroup/serovar analysis in medicolegal cases).
3. N. gonorrhoeae: test all for beta-lactamase production, chromosomally mediated resistance.
4. Sites examined (number of occasions): heterosexual male — urethra (×1); throat (×2) if indicated; homosexual male — urethra (×1); anorectum (×2); throat always (×2); female — urethra (×2); cervix (×2); throat (×2) if indicated.

(Indications for throat culture: history, if Neisseria gonorrhoeae is detected in any site or if patient is a contact of N. gonorrhoea case.)

OTHER STD

Detect or exclude: specific treatment for affected patient; trace contacts. Some other issues to consider at this stage:

1. By inspection: Phthirius pubis, Sarcoptes scabiei (or characteristic lesions), molluscum contagiosum, human papillomavirus (HPV): or herpes simplex virus (HSV): lesions.
2. Heterosexual male: non-gonococcal urethritis (NGU) and recurrences (common).
3. Homosexual male: positive HBsAg, HIV, enteric infection (possibly common).
4. Female: cervical cytology (annual smears/colposcopy when indicated); pelvic inflammatory disease (PID); Trichomonas vaginalis: (diagnosis by microscopy of wet vaginal film); Candida: (diagnosis by microscopy of Gram-stained film); Chlamydia: 0.2 M sucrose phosphate (2SP) transport medium (see later), smear fixed in acetone for immunofluorescence test; ‘anaerobic vaginal infection’ (Amies) (pH ≥ 4.5; amines released by potassium hydroxide solution (10%).

OTHER MEDICAL CONDITIONS

History:

Hepatitis B (see p. 549)
Allergy to drugs
Recent or present antibiotic or chemotherapy (if any)
Drug misuse/addiction (especially i.v. and sharing of needles)
Contraception/pregnancy
Glycosuria (diabetes important cause of balanitis).

SOCIAL ASPECTS

Non-judgemental attitude in staff
Conciliation important
Avoid making situation worse
Right of patient to truth
Confidentiality strict, information within STD department
Medicolegal (sexual abuse, marital issues)
Trace contacts: persuasion: no threats, no coercion, legal or otherwise.
HIV INFECTION AND AIDS

HIV antibody test requires the following:
- Pretest counselling
- Consent
- Informing of results
- Learning to live
- Partners, consideration of prevention; unambiguous counselling
- Infection control (HIV, HBV, M. tuberculosis)
- Safer sex, information on STD, exclusion or detection of other
- Link with other support services
- Follow-up
- Tests predictive of AIDS

SUMMARY OF AIMS

- Organisational diagnosis first; specific treatment afterwards
- Contact tracing (source and secondary contact) after diagnosis; priority related to seriousness
- Epidemiological treatment
- Prevention: counselling and risk reduction

OTHER GROUPS (SPECIAL CONSIDERATIONS)

PREGNANT FEMALE

Treponema pallidum can be transmitted transplacentally long after a woman has ceased to be infectious sexually. Early diagnosis and treatment with penicillin is essential to prevent spread to or to cure fetus (crystalline benzyl penicillin, procaine penicillin).

Neisseria gonorrhoeae — cervical infection, with spread to the fetus during passage down birth canal to cause conjunctivitis of newborn (benzylpenicillin, or ampicillin for mother).

Chlamydia trachomatis — cervical infection, treat with erythromycin.

Herpes simplex virus — see text for discussion on prevention in newborn.

Hepatitis B virus — babies of HBeAg positive mothers: give to newborn 0.5 ml HBIG at delivery within 48 h, and vaccinate at 0, 1 and 6 months.

Human papillomavirus: genital warts — commonly increase in size and spread during pregnancy, recede during puerperium. Laryngeal papillomas rare in neonate/children.

Human immunodeficiency viruses: intra-uterine and postnatal transmission.

NEONATE

Congenital syphilis is rare in areas where antibiotics are easy to obtain: ‘early’ form and ‘late’ form

Conjunctivitis of newborn:

- N. gonorrhoeae (see p. 293)
- Chlamydia trachomatis spread to fetus during delivery to cause conjunctivitis and sometimes pneumonia (erythromycin ethyl-succinate 50 mg/kg/day orally and topical tetracycline for 3 weeks for affected infant).

Hepatitis B: HBIG/vaccine

Herpes simplex virus — acyclovir treatment if any lesions seen.

Human immunodeficiency viruses: acquired immune deficiency syndrome.

THE PREPUBERTAL CHILD

When sexual abuse of prepubertal children is suspected, screening for sexually transmissible disease forms an important part of medical investigation. Firstly it allows treatment of what otherwise might be an undetected and damaging infection. Secondly, the presence of an STD, particularly early stage syphilis and gonorrhoea, may be supportive evidence that sexual abuse has occurred and in the case of gonorrhoea serotyping of the organism may allow it to be matched with a supposed assailant. Furthermore STD including HIV infection may be an important factor to consider when compensation for criminal injury is being assessed (Criminal Justice Act 1988, UK legislation).

The circumstances of the examination of young children are important. At all times a sympathetic and understanding approach is required in the management of these children and repeated examinations by different doctors are to be avoided. When possible a single detailed examination should be performed in the presence of both a police surgeon and paediatrician, with the child sedated if necessary. A general physical examination which will include throat, eyes, ears, chest and abdomen should precede the examination of genitalia and anus. Time should be given to set the child at ease, so that examination of the genitalia and anus is seen as a routine part of the procedure (DHSS 1988b). Evidence of trauma can be sought and satisfactory specimens obtained for the detection of STD; follow-up examinations for the exclusion of STD are not avoided.

In the case of children suspected of having been sexually abused or proved to have been so abused the clinician should be aware of the importance of the multidisciplinary process and ensure that medical confidentiality does not work against the protection of the child (DHSS 1988b).

Diagnosis of STD (for further detail see summary of clinical laboratory procedures for the detection or exclusion of STD above) in prepubertal children.

1. Direct plating on selective media for Neisseria gonorrhoeae: pharynx, anorectum, urethra and vagina. Obtaining specimens may be facilitated by gentle insertion of an anucleate or a suitable nasal swab (e.g. Killian's Nasal Swab, 2-3", Downs Surgical plc, Church Path, Mitcham, Surrey CR4 3UE, England); a swab is then passed along the speculum to sample the vaginal contents. Taking the anorectal specimen may be facilitated by the gentle use of a child's proctoscope.

2. Collection in 2SP (0.2 M sucrose phosphate transport medium) for isolation of Chlamydia trachomatis:* pharynx, rectum, urethra and vagina.


4. Note any anogenital discharge and exclude presence of foreign body in vagina or rectum.

5. In the presence of genital/oral vesicular lesions collect vesicular fluid or scrapings from roof of mouth in virus transport medium for Herpes simplex virus.*

6. Serological tests for syphilis (e.g. VDRL and TPHA) and hepatitis B (e.g. HBsAg and antibody).

7. Store serum (for retrospective anti-HIV testing if necessary).

Specific consent from person with legal responsibility for the child for testing is necessary.

*If delay more than 3 h store at -70°C.
THE TREPONEMATOSES

AETIOLOGY

Treponema pallidum, the causative organism of syphilis and other treponematoses, is a delicate tightly coiled spiralled filament 6–10 μm by 0.1–0.18 μm in diameter; its ‘subspecies’ or ‘variants’ causative of other treponematoses are similar in appearance. It is feebly refractile and too narrow to be seen well by ordinary light microscopy. Dark ground illumination has been the technique normally used to detect this organism in the lesions of early syphilis since its demonstration as the cause of syphilis by Schaudinn and Hoffman in 1905. Immunofluorescent techniques can now be used to demonstrate the organism in tissue and body fluids. Pathogenic T. pallidum can grow in vivo in the testicular tissue of rabbits but although there has been some success in cultivation in vitro serial in vivo cultivation has not been achieved. DNA/DNA hybridization methods, however, are now available and these have shown that there is, for example, 100% homology between T. pallidum and T. pertenue (Miao & Fieldsteel 1980). The non cultivable pathogenic treponemes can now be classified as follows:

1. Sexually transmitted: Treponema pallidum subspecies pallidum (causative organism of syphilis, world-wide in distribution)
2. Not sexually transmitted, spread mostly by skin lesion to skin contact in childhood: Treponema pallidum subspecies pertenue (causative of yaws, endemic in some warmer countries)
3. Treponema pallidum subspecies endemium (causative organism of endemic syphilis, e.g. bejel of semi-nomads of Arabian peninsula and sub-Saharan Africa)
4. Treponema carateum (causative organism of pinta, found in scattered foci in northern South America or Mexico).

SYPHILIS

Transmission

Syphilis is spread principally by sexual intercourse but it may be transmitted congenitally, that is to say transplacentally to the fetus in utero by the infected mother (see p. 1469).

Transfusion acquired syphilis is now rare. When infected blood is stored at 4°C in citrate anticoagulant, infectivity is lost between 96 and 120 h. The actual survival times may depend on the number of treponemes present in donor blood (van der Sluis et al 1982). The risk of accidental syphilis is highest where fresh heparinized blood is used as in exchange transfusion in neonates (Risseeuw-Appel & Kothe 1983).

Pathogenesis and immunology

Attachment to host tissues is an important initial step in T. pallidum infection (Fitzgerald 1981), which can be considered as a generalized infection of vascular tissues since, within lesions in each stage, treponemes localize primarily in vascular areas. Capillary destruction inhibits blood supply leading to necrosis and ulceration, characteristics of syphils pathoslogy (Quist et al 1983).

Human immunodeficiency virus infection may have a profound influence upon the natural history of syphilis and be associated with unusual presentations of this disease (Shulkin et al 1988).

Acquired syphilis

General description

Syphilis is systemic from the onset and produces short symptomatic and prolonged asymptomatic stages. In the early infectious stage of the disease lesions, containing many treponemes, occur on the moist mucocutaneous parts of the body, particularly the genitalia, and enable transmission to occur by sexual intercourse. Even if untreated these lesions tend to heal but may recur during the first 2–4 years, after which the disease becomes latent or hidden. The latent form or the non-infectious late stage of the disease may persist for decades without producing obvious clinical changes, but a proportion of patients will unpredictably develop active involvement of the cardiovascular system (about 10%), the central nervous system (about 10%), or localized gummatous destructive lesions which can affect the musculoskeletal system (about 10%), the viscera and mucous membranes (about 15%) (Fig. 22.20).

If treated in the early stage clinical cure can be achieved by penicillin treatment and with certain other antibiotics. In the late stages curative effects are often spectacular in some forms of neurosyphilis and in gummatous syphilis. In cardiovascular forms of the disease the effects of antibiotic therapy are not easy to define.

![Fig. 22.20 Simplified diagrammatic representation of the course of untreated syphilis from the time of infection. The early stages, whether primary, secondary or early relapse of latent syphilis, are indicated together with the subsequent development of late stage effects, whether late benign or gummatous, cardiovascular, central nervous system or late stage latent syphilis. The percentage of effects may vary in different populations. (Modified from Kampmeier 1964, Robertson et al 1989.)](image)

Congenital infection

See page 1469.

Laboratory diagnosis of syphilis

In syphilis a clinical diagnosis must be confirmed in the laboratory either by:

1. Demonstrating T. pallidum in the serous exudate obtained from the depth of early lesions
2. Demonstrating specific antibodies in the serum.
Demonstration of T. pallidum in primary and secondary lesions

Dark ground microscopy. After cleansing the surfaces of the primary or secondary lesions with a swab soaked in physiological saline, serum is obtained from the depth of the lesion by gentle squeezing and examined by dark ground microscopy using the oil immersion objective. T. pallidum is recognized by its slender structure, characteristic slow movements and angulation. If the initial test is negative the procedure should be repeated daily for at least 3 days: antibiotics should be withheld during this period although cotrimoxazole orally may be used to reduce local sepsis. Many commensal treponemes occur in the mouth and therefore dark ground illumination is not suitable for examining oral lesions. Organisms are not easily found in skin lesions of secondary syphilis except those in moist skin areas.

Fluorescence microscopy. A smear of the material to be tested is made on a glass slide, dried and fixed (5 mm immersion in acetone). Staining with fluorescein-labelled antibody specific for T. pallidum may give rise to non-specific results and is less reliable than dark ground microscopy (Luger 1981) but the value of the method should be improved with the advent of monoclonal antibodies. Hook et al (1985) found a pathogen-specific fluorescein conjugated monoclonal antibody to be reliable.

Demonstration of antibodies in the serum

During treponemal infection, whether in syphilis or in the endemic treponemal diseases such as yaws or pinta, a variety of antibodies are produced. These can be classified into non-specific anti-treponemal antibodies and antibodies specific for pathogenic treponemes.

After infection, the first humoral immune response is the production of antibodies of the IgM class. Specific anti-T. pallidum IgM is detectable during the second week of infection but disappears usually within 3 months of the beginning of treatment in cases of early syphilis or within 1 year of the beginning of therapy in late disease.

Production of IgG begins around the fourth week after infection and usually reaches much higher titres than those for IgM. IgG secretion may be continued by memory cell clones long after elimination of the antigen thus accounting for the persistence of reactivity in sensitive tests such as the Treponema pallidum haemagglutination assay (TPHA) and the fluorescent treponemal antibody-absorbed (FTA-ABS) test.

Tests to detect non-specific treponemal antibodies

Cardiolipin antigen tests

These tests are suitable for routine screening and usually become positive 10–14 days after the appearance of the chancre, the titre gradually increasing. After treatment the titre may diminish and the test becomes negative. In late or latent syphilis the cardiolipin antigen tests are often negative.

The serum of patients with an acute febrile infectious disease may give a positive cardiolipin antigen test but negative specific treponemal antigen tests in the absence of past or present treponemal infection, so called ‘biological false positive reaction’ (BFP). Other tests are required to distinguish between positive cardiolipin test results due to BFP reactions and those resulting from treponemal infection (Robertson et al 1989).

Venereal disease research laboratory (VDRL) test

The VDRL test (Harris et al 1946) is the cardiolipin antigen test recommended. The test can be performed as a slide test in which the patient’s serum, previously heated to inactivate complement, is mixed with a freshly prepared suspension of cardiolipin-lecithin-cholesterol antigen on a glass slide. The mixture is rotated, usually mechanically, and after a few minutes flocculation (aggregation of antigen–antibody complexes in suspension) is detected microscopically using a low power objective. Quantitative tests with serial dilutions of patient’s serum are easily carried out.

The VDRL test is versatile and can be modified for testing unheated serum or plasma either by automated or manual techniques (Robertson et al 1989). These modifications include the addition of carbon particles to the antigen enabling the test results to be read by the naked eye instead of microscopically. The VDRL cardiolipin antigen test is now the preferred cardiolipin antigen test in most laboratories.

The antibody is now generally considered to be directed against cardiolipin present in treponemes, i.e. it is antibody against a non-specific antigen shared by treponemes and mammalian tissues.

Tests to detect antibodies specific for pathogenic treponemes

The antigen used in these tests is derived from Nichols strain of T. pallidum which is maintained in rabbits by intratesticular inoculation and weekly passage. Tests using pathogenic T. pallidum as antigen have been used as verification tests to confirm the treponemal nature of a positive cardiolipin antigen test; in such tests results tend to remain positive for a very long time after treatment.

Newer tests have superseded the T. pallidum immobilization (TPP) test in which live treponemes are incubated with heat-inactivated serum in the presence of complement and the number of organisms immobilized is determined by dark ground microscopy.

The fluorescent treponemal antibody absorbed (FTA-ABS) test

In the FTA-ABS test (Hunter et al 1964) the patient’s serum is absorbed with a sonicate of Reiter’s treponemes in order to remove group-specific antibody. Binding to T. pallidum of antibody specific for pathogenic treponemes is then demonstrated by the indirect immunofluorescence technique. The FTA-ABS test is an accepted reference test and is highly specific and sensitive at all stages of syphilitic infection although a small percentage of false positive reactions occur.

False positive results have been reported in patients with systemic lupus erythematosus and other connective tissue diseases (McKenna et al 1973) and these sometimes give problems in diagnosis (Robertson et al 1989).

The FTA-ABS test is not suitable for screening large numbers of sera and is used as a confirmatory test when one of the screening tests is positive. It is also useful in suspected cases of primary syphilis. A modified procedure has been developed for the FTA-ABS test for new microscopes equipped with incident illumination (Farshy et al 1983).

The T. pallidum haemagglutination assay (TPHA)

In the TPHA (Rathlev 1967, Tomizawa & Kasamatsu 1966)
sheep erythrocytes coated with an extract of *T. pallidum* are agglutinated by antibody from serum of patients with syphilis. Components of Reiter treponemes, rabbit tests and erythrocyte membranes are used to absorb test sera in order to eliminate haemagglutination due to antibody against any of these agents. Any serum giving a positive reaction is tested against control erythrocytes (i.e. not coated with *T. pallidum* antigen) to check the specificity of the agglutination. In spite of the absorption procedure about 0.1% of specimens agglutinate erythrocytes in the absence of antigen: this non-specific agglutination makes the individual test result invalid. The use of fowl erythrocytes in the TPHA test (Sequeira & Eldridge 1973) may decrease the number of non-specific agglutination reactions. The TPHA is simple to perform and as reagents based on sheep and fowl erythrocytes are available commercially, it has become the first of the specific tests suitable for routine screening. Because of the expense of reagents the TPHA is almost always performed in microtitre plates.

The TPHA is often negative in untreated primary syphilis but otherwise has a sensitivity and specificity comparable with the FTA-ABS test.

**Enzyme-linked immunosorbent assay (ELISA)**

ELISA is a relatively simple and fairly new serological technique. Patients' serum is allowed to react with antigen coated on the surface of small plastic beads or on the inside surface of plastic tubes or wells in a microhaemagglutination plate. Specific antibodies binding to the antigen are then quantitated by means of an-anti-immunoglobulin conjugated to an enzyme such as alkaline phosphatase or horseradish peroxidase. By use of appropriate enzyme substrates colour changes can be measured spectrophotometrically allowing objective interpretation of the results. Another advantage of ELISA is the potential for automation; it merits further study as a front-line screening procedure.

**Serological tests in clinical practice**

Since syphilis can be acquired concomitantly with any other STD those at risk should be screened to exclude syphilis. As congenital infection is preventable by appropriate treatment during pregnancy and the costs of congenital syphilis are so high screening may be cost beneficial when the prevalence of maternal infection is as low as 0.005% (Stray-Pedersen 1983).

**Screening with a combination of VDRL and TPHA tests**

When used together, the VDRL and TPHA tests provide a highly efficient screen for the detection or exclusion of treponemal infection. The VDRL test is more sensitive than the TPHA in the detection of very early syphilis while the TPHA is more sensitive in the detection of latent and late stage infection (Young et al 1974).

Although the VDRL carbon antigen and TPHA tests can be performed without inactivation, heating patient's serum at 56°C for 30 min is recommended: this not only decreases the incidence of non-specific agglutination but also destroys the acquired immune deficiency syndrome (AIDS) associated virus HIV.

The VDRL test usually becomes positive 10–14 days after the appearance of the chancre or approximately 3–5 weeks after acquiring the infection. It is positive in approximately 75% of cases of primary syphilis. After the secondary stage the VDRL titre declines and eventually becomes negative in approximately 30% of untreated latent and late cases. The VDRL test also tends to become negative after treatment, particularly in early syphilis.

**The TPHA test**

Patient's serum is screened at a final dilution of 1 in 80: if any haemagglutination is noted the test is repeated with a series of twofold dilutions from 1 in 80 to 1. The reciprocal of the final serum dilution resulting in marked haemagglutination is termed the titre. TPHA titres are reported as positive-80, positive-160, etc. Each specimen is also tested against control cells (no antigen) at a serum dilution of 1 in 80: if the control cells agglutinate the serum is reported as 'non-specific agglutination — test invalid'.

The TPHA is usually negative in early primary syphilis but may become positive at low titre (80–320) towards the end of the primary stage. The TPHA titre may give some indication of the duration of the infection. Titres rise sharply during the secondary stage and commonly reach 5120 or greater. The TPHA titre declines during the latent stage but invariably remains positive at low titre (80–640).

The only stage of syphilis likely to escape detection by screening with a combination of VDRL and TPHA tests is early primary syphilis, although repeated tests over a 3-month period will detect such an infection.

**Activity of infection and response to treatment**

All cardiolipin antigen tests tend to become negative after treatment particularly in early syphilis. Serial quantitative tests should be carried out for up to 2 years following treatment for early acquired syphilis and for up to 5 years in late stage infection.

The VDRL titre should become negative within 1–2 years of effective treatment for early syphilis. After adequate treatment of late infection the VDRL test may remain reactive (titre ≤ 8) for many years.

The TPHA test remains positive for life even in those who have been fully treated with adequate doses of penicillin: in such cases further treatment and investigation is not necessary unless the patient has again been at risk.

**Screening with an anti-T. pallidum IgG ELISA**

ELISA is a more sensitive system than agglutination and in spite of using an anti-human IgG conjugate is more sensitive than the TPHA in primary syphilis. Results of screening with an antitreponemal IgG ELISA were comparable to screening with a combination of VDRL and TPHA tests (Young et al 1989). The potential for automation makes ELISA attractive for screening large numbers of specimens.

**Detection of anti-treponemal IgM**

The presence of anti-treponemal IgM in the sera of neonates and adults is an indication of the persistence of treponemal antigen within the body. Very occasionally false negative reactions may result from suppression of the specific IgM antibody response by high levels of circulating IgG (Araujo & Remington 1975, Uhr & Moller 1968, Müller & Moskophidis 1984). Of the various methods of detecting anti-treponemal IgM reactivity, the 19S
The diagnosis of congenital syphilis can present a considerable problem since it depends mainly on the results of serological tests and also because most so infected neonates are asymptomatic at birth. Early stage congenital syphilis is a rarity in the United Kingdom and there are few up-to-date serological data available. Recently, there has been an increase in the number of cases of congenital syphilis occurring in certain areas of the United States. There remains a considerable risk for congenital infection in many developing countries.

As the standard serological tests for syphilis depend on responses involving IgG and IgM antibodies their interpretation is extremely difficult; the IgG found in the serum of neonates is largely passively acquired through the placenta and does not represent the infant’s own response. Whereas a rising or higher titre in the neonate than in the mother is suggestive of infection, the 19S (IgM)-FTA-ABS test is currently the most reliable test for confirming a diagnosis of congenital infection. Whilst fractionation of serum will overcome the problem of false negative reactions resulting from competition between IgG and IgM for treponemal binding sites during the test, it can not overcome the theoretical possibility of suppression of IgM synthesis in the neonate due to high levels of circulating maternal anti-treponemal IgG (Johnston 1972).

There was no suppression of IgM synthesis in the nine cases of congenital syphilis reported from Seville, Spain, where the mean number of cases of congenital syphilis over a 3-year period was 0.81 per 1000 live births (Borobio et al 1980). In addition to the demonstration of specific treponemal IgM, Borobio et al found that all infected infants had raised total IgM, usually in the range of 1-4 g/l. After treatment, total IgM returned to normal, specific anti-treponemal IgM and other antibody test titres decreased and tended to become negative. In three cases considered to have passively transferred antibody anti-treponemal IgM was absent and total IgM levels were near normal (≤0.7 g/l). Lower titres in neonates when compared to their mothers are also suggestive of passively transferred antibody. In the absence of infection passively transferred antibody detected by the VDRL will decrease and the tests will become negative in approximately 3 months. In the case of the TPHA, the titre will become low and the test negative in 6-12 months.

In late stage infection, either treated or untreated, test results tend to fluctuate over a period of months or years. The VDRL test often remains positive at low titre in association with a low TPHA titre and positive FTA-ABS test. Data on specific antitreponemal IgM in late stage congenital infection are inadequate.

Diagnosis of neurosyphilis

The use of cerebrospinal fluid (CSF) for routine screening tests in patients in whom there is no clinical suspicion of syphilis is unjustified; a negative TPHA test on the blood will virtually exclude active neurosyphilis and is a better screen for the detection of all forms of late syphilis (Lancet 1977a).

In the case of early syphilis it is traditional policy to carry out a lumbar puncture 12 months after treatment as part of the test of cure. In the case of patients with syphilis of uncertain duration or in the late symptomatic or late latent stage, CSF examination is an essential part of the investigation necessary before treatment. When there is evidence of clinical relapse or a fourfold rise is noted in the titre of the serological tests in the follow-up of a patient, CSF examination is necessary (Catterall 1977).

Examination of the cerebrospinal fluid

Investigation of the CSF should include:

1. Serological tests (TPHA or FTA-ABS)
2. A total white cell (normally less than 3 leucocytes/mm³, 3 × 10⁹/l) and differential count

Serological tests and estimation of IgG and albumin should be performed in parallel on serum.

Blood-CSF barrier function and the TPHA index

Conventional criteria such as raised CSF cell count and increased total protein may give evidence of an inflammatory response in the CNS but these examinations are not specific for neurosyphilis. In addition normal cell counts and total protein values do not exclude absolutely involvement of the CNS. Lugger et al (1981) have investigated the other parameters as aids to the accurate diagnosis of neurosyphilis. These include:

1. Albumin quotient
2. TPHA index
3. IgG index, about which details are provided in Robertson et al (1989).

New technological approaches to diagnosis and control

Monoclonal antibodies

Monoclonal antibodies should facilitate the development of diagnostic tests designed to detect low numbers of pathogenic treponemes in lesion exudates or other body fluids such as CSF (Lukehart & Baker-Zander 1987). Therapeutic use of monoclonal antibodies remains another possibility.

Gene cloning

The combined technologies of gene cloning and monoclonal antibodies may aid the selection and testing of candidate antigens for diagnosis and vaccine production but as a proportion of patients may develop syphilis in a latent form only, the assessment of the effectiveness of any vaccine would be very difficult.

Acquired syphilis: clinical features

Early stage

Primary syphilis. Following a period of about 3 weeks (range 10-90 days), the primary lesion or chancre develops at the site of
inoculation of *T. pallidum*. The initial lesion noted in primary syphilis is a single dull red papule which becomes an ulcer, well demarcated from the surrounding tissue, with a smooth, flat, dull red surface, which may be covered by a thin yellow or brown crust. Characteristically the ulcer is painless, not tender and on pressure serous fluid, but no blood, exudes from the lesion. Induration of the ulcer is often marked giving it a cartilaginous consistency. Occasionally there may be considerable oedema of the adjacent tissues.

In the male the chancre may be found on any part of the external genitalia, especially in the coronal sulcus, on the inner surface of the prepuce, on the glans or on the shaft of the penis. Rarely an intra-urethral chancre may occur. In male passive homosexuals the chancre may be found at the anal margin or less frequently in the rectum. Chancre may also occur in the lips, buccal cavity, tongue, tonsil and pharynx, particularly in homosexual patients. Lesions of the tonsil and pharynx may be painful.

Chancre may occur on the labium majus (Fig. 22.21), labium minus, fourchette, clitoris or cervix. Lesions of the cervix usually produce no symptoms and as the lymph drains to the iliac nodes, these may be found to be enlarged on abdominal examination. Extragenital chancres are uncommon.

Many lesions of primary syphilis are atypical (Chapel 1978) and in the anal region, primary lesions may resemble slightly indurated anal fissures (Fig. 22.22).

Within a few days of the appearance of the chancre there is usually regional lymph node enlargement; the enlarged lymph nodes are typically discrete, rubbery and painless. Without treatment, the primary lesion heals within 3–8 weeks, leaving a thin atrophic scar.

**Secondary syphilis.** Signs of secondary syphilis usually appear 7–10 weeks after infection or 6–8 weeks after the appearance of the primary lesion, which may still be present and unnoticed by the patient, in about a third of patients with early secondary syphilis.

**Symptoms.** The patient often feels generally unwell, with mild fever, malaise, headache and anorexia. He may complain of a non-itchy skin rash, patchy loss of hair, hoarseness, swollen lymph nodes, bone pain and, rarely, deafness or other evidence of neural damage.

**Signs.** (a) Skin lesions (syphilides). Skin lesions are seen in over 80% of patients with secondary syphilis. Mucocutaneous lesions, particularly, contain many treponemes and are infectious. Skin eruptions are often polymorphic — several types of eruption appear simultaneously — during the course of secondary syphilis and, although early skin lesions are usually symmetrically distributed, later lesions are not always in this pattern. Just over 40% of patients with secondary syphilis complain of pruritus (Chapel 1980). Histological appearances are not always classical but more variable (Jeerapaet & Ackerman 1973, Abell et al 1975) and in at least 25% of biopsies plasma cells are either inconspicuous or absent.

**Macular syphilide** (*roseola*): these lesions are usually the earliest to appear, but are often overlooked, being faintly coloured. The individual macules are rose pink in colour, about 1 cm in diameter, discrete and with indistinct margins.

**Papular and papulosquamous syphilide:** these are the commonest lesions to be detected in secondary syphilis. Papules are dull red
lesions, variable in size, distributed symmetrically — during the early stages of secondary syphilis — over the body, and especially prominent on flexor aspects. They are firm to touch and initially have a shiny surface. Later, as the papule ages, scaling is noted on the surface. When scaling papules predominate in the eruption, the term papulosquamous syphilis is applied.

Although papules may be found anywhere on the body, the following sites require special mention:

1. Face: this is often affected, papules being especially prominent in the nasolabial folds and on the chin. Occasionally a group of papules may be noted on the forehead just below, and parallel to, the hairline, sometimes described as 'corona ceneris'.

2. Scalp: when a hair follicle is involved in the inflammatory changes in the skin, hair growth is arrested and shedding of the contained hair occurs. Hair loss in secondary syphilis is characteristically irregular, the scalp having a 'moth-eaten' appearance (syphilitic alopecia). Occasionally, as a non-specific reaction to a systemic disease, there may be a more diffuse hair loss (telogen effluvium) after recovery from the secondary stage.

3. Palms and soles (palmar and plantar syphilis): papular lesions on these sites do not project much above the surface of the skin, but appear as firm lesions. Dull red in colour, associated with thickening and scaling (Fig. 22.23). A collar of scales may often surround individual lesions.

4. External genitalia: on moist areas such as the vulva and perianal region, papules may become hypertrophied, forming broad-based, flat-topped, moist, wart-like lesions called condylomata lata (Fig. 22.24). The surface is often eroded and the exudate from the erosion contains large numbers of T. pallidum. Commonly papules encircle the free margin of the prepuce, and, as a result of moisture, trauma and secondary infection, deep painful fissures develop. Papulosquamous lesions are often found on the shaft of the penis and on the scrotum.

In the later stages of secondary syphilis, papules become fewer in number and asymmetrical in distribution. Occasionally, a large papule may be found, surrounded by smaller satellite lesions — corymbose syphilis. Nail growth may be affected, particularly in the late secondary stage. The nail loses its lustre, becomes brittle and may be shed.

Pustular syphilis: rarely papules become pustular due to necrosis of the upper dermis and epidermis as a result of occlusion of the lumen of blood vessels. Multiple pustular lesions are very seldom found in western countries (Miller 1974).

With the exception of pustular syphilis, the skin lesions of secondary syphilis heal without leaving scars. Areas of faint pigmentation may persist for months. Occasionally, depigmentation of the skin of the neck may be noted, particularly in dark-haired women — leucoderma coll. This residual depigmentation lasts for life.

(b) Lesions of the mucous membranes. These are found in about 30% of patients with secondary syphilis. The characteristic lesion is the so-called 'mucous patch' which appears at the same time as the skin rash. The mucosal lesions appear as round or oval grey areas surrounded by a narrow zone of erythema. Shedding of the grey necrotic membrane reveals superficial ulceration and if several patches coalesce, a 'snail-track ulcer' (Fig. 22.25) may result. Mucosal lesions are generally painless and resolve.

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Fig. 22.23 Papulosquamous syphilis of the palms (early stage syphilis).

Fig. 22.24 Perianal condylomata lata of early stage syphilis in a homosexual male.

Fig. 22.25 Mucous patch of the uvula in early stage syphilis in a heterosexual male.
within a few weeks, or, less commonly, within a few days. Various sites may be involved in secondary syphilis, viz. tonsils, cheeks, palate and lips, tongue, larynx (produces hoarseness), nasal mucosa and mucous membranes of the genitalia (patches may be found on the glans penis, subpreputial surface of the prepuce, vulva, fourchette and cervix). (c) Lymph node enlargement. Generalized lymph node enlargement is found in at least 60% of cases of secondary syphilis. Cervical, suboccipital, axillary, epitrochlear and inguinal nodes are often palpably enlarged. The lymph nodes are discrete with a rubbery consistency and are not tender. Not uncommonly, the spleen is enlarged in secondary syphilis. Histologically there is follicular hyperplasia with fibrosing endarteritis and periarteritis. Treponemes are rarely found in affected lymph nodes. (d) Periostitis. Bone pain may occasionally be the presenting feature of the disease (Waugh 1976). Periostitis is usually a localized process most commonly affecting the anterior tibia. Localized bone pain, especially at night, relieved by movement and exacerbated by immobilization is the chief symptom and localized tenderness may be noted on examination. Radiological examination usually reveals no abnormalities although osteolytic foci and periostitis may be seen. Bone scanning using technetium-99 may show areas of increased bone uptake (Hansen et al 1984); the superficial bones, including the skull, are chiefly affected. These changes resolve within about 9 months of completion of therapy. (e) Arthritis and bursitis. Painless effusion into joints and bursae occurs rarely during the course of secondary syphilis. Arthralgia, however, either localized or generalized, is more common, affecting at least 6% of patients. (f) Hepatitis. Rarely, jaundice may be associated with secondary syphilis. The serum alkaline phosphatase — of hepatic origin is often disproportionately elevated in comparison with only a moderate elevation in the case of alanine aminotransferase. Usually within 6 weeks of treatment the plasma enzyme activities revert to normal (McMillan et al 1977). (g) Glomerulonephritis and the nephrotic syndrome. Patients with secondary syphilis frequently have mild albuminuria, possibly as the result of immune complexes trapped by the glomeruli and setting up an inflammatory reaction there. Such changes are usually mild and transient, but rarely a membranous glomerulonephritis results, being manifest as the nephrotic syndrome (Gamble & Reardon 1975). If untreated, the nephrotic syndrome appears to resolve spontaneously. (h) Iridocyclitis and choroidoretinitis. Iritis, usually discovered late in secondary syphilis, is now a rare complication in western countries (less than 1% of cases) (Tait 1983). Uveitis and choroidoretinitis may be precipitated by the use of corticosteroid preparations for some other complication (e.g. glomerulonephritis) or for some intercurrent illness. (i) Neurological abnormalities in secondary syphilis. Headache, especially noticeable in the morning, is a common complaint and probably reflects meningeal inflammation. Although transient abnormalities of the white cell count and protein content in the cerebrospinal fluid occur in only about 5% of patients with secondary syphilis, frank meningocerebralitis may rarely be encountered (Parker 1972). Peripheral neuritis may be a rare complication. Perceptive nerve deafness with or without vestibular dysfunction is another uncommon complication. It is usually associated with tinnitus and the CSF tends to show some abnormality. Improvement, both subjective and objective, occurs following antibiotic treatment (Vercoe 1976). Although pure tone, speech and impedance audiometry are usually normal in patients with early syphilis, brain stem electrical response audiometry often indicates subclinical brain stem disease (Rosenhall & Roupe 1981). (j) Parotitis. Unilateral parotitis has been described as a complication of secondary syphilis (Hira & Hira 1984). Differential diagnosis of secondary syphilis. The appearance of the rash of secondary syphilis is variable and as a result many dermatological conditions have to be considered in the differential diagnosis; the problem is generally resolved by the serological tests for syphilis and by the generally rapid response to antibiotic treatment.

Early latent syphilis. The lesions of early syphilis may heal and the disease may become latent. During this stage, known as early stage latent syphilis, recurrence of infectious mucocutaneous lesions may be seen. Latency may, however, persist, and early stage latent syphilis is arbitrarily taken to last for 2 years. Late stage

In the early years of syphilis the lesions already described (chancre, mucous patch, condyloma latum) are infectious and there is evidence of a recurrent spirochaetaemia and recurring mucocutaneous lesions. In pregnant women infection of the fetus in utero is inevitable in early untreated syphilis. Syphilis then enters a subclinical stage of latency, in which the only readily detectable evidence of infection is serological and this latency may persist for years or even for life. Transmission of the disease by sexual intercourse does not occur although in the case of pregnancy the woman can infect her fetus long after she has ceased to be infectious sexually. Further activity of the disease may, at any time during latency, cause profound effects (Kampmeier 1964) and lead to death as long as three decades or more after infection. The main forms of late stage acquired syphilis are more appropriately discussed in a general text (Robertson et al 1989).

Treatment of syphilis: general considerations

Among the kaleidoscopic changes in medical practice since 1946 when penicillin first became easy to obtain, the effect of this antibiotic in microbial disease (Hare 1970) has nowhere been more spectacular than in syphilis where it continues to be the antibiotic of first choice for the treatment of all stages of the disease (Idsoe et al 1972, Willcox 1979). The persisting susceptibility of Treponema pallidum to penicillin over an extensive period of time, as is also found in the case of Streptococcus pyogenes, suggests that these organisms, in distinction from many other pathogens, do not have the genetic capacity to develop resistance to this antibiotic (Lamanna et al 1973).

Penicillin is only effective against actively growing bacteria, the optimum effect being achieved when there is unhindered and rapid multiplication. It follows that penicillin will be most effective against the treponeme during early syphilis where there is rapid multiplication of the organism. Treponemes, like other bacteria, can exist in a resting phase when there is minimal cell wall synthesis and when penicillin effects are minimal (Kern 1971).

It is proper to express dosage of penicillin in terms of mass or weight. To accommodate these, however, used to referring to international units, all dosages of benzylpenicillin and long-acting forms of benzylpenicillin are given in weights bracketed with
weight equivalents and the obsolete 'international unit' equivalents of benzylpenicillin (Table 22.28).

In the case of rapidly growing bacteria, such as gonococci, the organism will be particularly sensitive to the action of penicillin many times over a 24-h period. In organisms with a longer generation time these phases of optimal sensitivity are correspondingly less frequent, for example, as in the case of Treponema pallidum (Turner & Holland 1957, Kern 1971). It is, therefore, an important determinant for therapeutic success to ensure that effective plasma concentrations are maintained over an adequate time.

T. pallidum is one of the most penicillin-sensitive microorganisms known. For penicillin to be effective in the therapy of syphilis, however, two requirements are believed to be essential: a minimal benzylpenicillin concentration of 0.018 mg (30 IU)/1 of serum, which gives several times the serum and tissue levels needed to kill T. pallidum, should be maintained for at least 7–10 days in early syphilis; penicillin-free or subtreponemical intervals during treatment should not exceed 24 h.

Healing of lesions occurs rapidly and treponemes disappear from early stage lesions; biological cure, that is total eradication of treponemes, is difficult to prove, however, as T. pallidum cannot be cultured in vitro. In patients, however, the passage of time has given confidence that individuals treated for early syphilis will not suffer ill effects due to late syphilis provided that they have had a course of penicillin which gives adequate blood levels over a sufficient length of time.

Clinical and serological follow-up after treatment has always been maintained as important in clinical practice and there are occasional reports of failure after a generally accepted course of penicillin (Giles & Lawrence 1979).

After treatment of some early, but especially of latent or late stage syphilis with penicillin or indeed after treatment with any anti-treponemal agent, the T. pallidum immobilization test, positive before treatment, remains positive afterwards and remains thus often for life (Moore & Mohr 1952), although cardiolipin antigen tests, such as the VDRL, become negative.

The finding of treponemes, apparently avirulent and incapable of causing further clinical disease, persisting after treatment of late syphilis (Collart et al 1962a, b) does not alter the fact that the treatment of early syphilis produces a clinical cure and prevents the emergence of late effects and that it is only in early syphilis with moist lesions that transmission can occur by sexual contact.

There are a multiplicity of empirically developed treatment plans but in spite of these variations in the case of long-acting penicillins results have been good (Kern 1971). Imperfections in the understanding of penicillin effects in late and latent syphilis and more particularly in the long-term value of alternative antibiotics leave some questions unanswered.

Long-acting forms of penicillin diminish the need for repeated injections and consist of procaine penicillin, an equimolecular compound of penicillin and procaine; benzathine penicillin; and benzathine penicillin; with these relatively low concentrations are produced for periods respectively of 24 h, 5 days or for some weeks.

Guidelines in treatment

Guidelines established by a group of experts and the staff of the Centers for Disease Control (CDC), US Public Health Service (1985) form a valuable source of guidance when considering therapy. In keeping with practice in the United Kingdom, the authors tend to place less reliance on single dose schemes than is given in current recommendations in the USA. In all cases regular clinical and serological follow-up is advised following treatment.

Treatment is given in dosages for adults or young persons over 50 kg weight or past puberty. In children the dosage suggested should follow those outlined for the treatment of congenital syphilis. Doxycycline or tetracycline should not be used in children under the age of 12 years.

Doses for young persons over 50 kg body weight or past puberty for treatment of early syphilis (primary, secondary, latent syphilis of less than 1 year's duration) are given in Table 22.28. In early latent syphilis it is difficult to obtain direct information regarding the duration of the disease and it is advisable in cases of doubt to examine the CSF, because when it is abnormal a diagnosis of asymptomatic neurosyphilis can be made and adequate treatment ensured. In patients with secondary syphilis admission to hospital is advisable for the first 1 or 2 days of treatment at
least. The patient can then receive care during the Jarisch-Herxheimer reaction (Lancet 1977b) and the opportunity can be taken to give explanations, to help with social problems, and to interview closely regarding possible sexual contacts.

Doses are given in Table 22.29 for treatment of early syphilis (young persons over 50 kg body weight or past puberty) who are hypersensitive to penicillin. These antibiotics appear to be effective but results have been evaluated less fully than in the case of penicillin therapy. Clinical and serological follow-up are therefore very important.

Treatment of syphilis of more than 1 year's duration (latent syphilis of indeterminate or more than 1 year's duration, cardiovascular or late benign syphilis) except neurosyphilis is given in Table 22.30. Optimal treatment schedules for syphilis of greater than 1 year's duration are less well established than those for early syphilis. Cerebrospinal fluid (CSF) examinations are mandatory in suspected symptomatic neurosyphilis and desirable in other patients with syphilis of greater than 1 year's duration.

Alternative antibiotics and the doses given for those who are hypersensitive to penicillin are given in Table 22.31. Cerebrospinal fluid examinations and follow-up are important in patients being treated with these regimens as their efficacy in the long term is not yet clear.

**Neurosyphilis.** Early diagnosis and the rapid institution of treatment are of prime importance in neurosyphilis. Results of treatment depend to a very great extent upon how much irreversible damage to the central nervous system has occurred before treatment has begun and it is clear that any patient should be seen as a matter of urgency. For a long time it seemed that in neurosyphilis there was little evidence that more clinical benefit could be obtained by using doses higher than those recommended, or by giving benzylpenicillin rather than long-acting forms such as benzathine penicillin or procaine penicillin, but now the use of intravenous benzylpenicillin in high doses is being reconsidered (Ducas & Robson 1981, Ritter et al 1975, Robertson et al 1989).

**Table 22.30** Treatment of syphilis of more than 1 year's duration (latent syphilis of indeterminate or more than 1 year's duration, cardiovascular or late benign syphilis) except neurosyphilis. Doses are given for young persons over 50 kg body weight or past puberty.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Route</th>
<th>Daily dose</th>
<th>Approximate benzylpenicillin equivalent</th>
<th>Number of doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzathine penicillin</td>
<td>i.m.</td>
<td>1.8 g</td>
<td>1.4 g</td>
<td>2.4 x 10⁶ IU</td>
</tr>
<tr>
<td>Procaine Penicillin</td>
<td>i.m.</td>
<td>900 mg</td>
<td>550 mg</td>
<td>0.9 x 10⁶ IU</td>
</tr>
<tr>
<td>Procaine penicillin</td>
<td>i.m.</td>
<td>1.2 g*</td>
<td>730 mg</td>
<td>1.2 x 10⁶ IU</td>
</tr>
</tbody>
</table>

* Dose for much heavier patients.

**Syphilis in pregnancy and the care of the fetus.** In localities where the number of reported cases of transplacental (congenital) syphilis are more than very rare the antenatal care and the regular serological testing of pregnant women is a matter of major importance and will enable the eradication of congenital syphilis. Penicillin is recommended for pregnant women in dosage appropriate for the stage of syphilis.

**Alternative antibiotic therapy in pregnant women with a history of hypersensitivity to penicillin.** In the case of syphilis in the pregnant woman who gives a history of hypersensitivity to penicillin, treatment is problematic since tetracyclines are not used. Treatment with erythromycin may control the infection in the gravid patient but it may fail to prevent or cure the infection in her fetus. The infection in the fetus might or might not be seriously damaging and would be treatable effectively with penicillin at birth although damage already sustained would not necessarily be reversed.

**Follow-up after treatment**

The CDC (1985) guidelines advise that quantitative non-treponemal tests (e.g. VDRL) should be taken 3, 6 and 12 months after treatment. If disease is of more than 1 year's duration serological tests for syphilis should be repeated 24 months afterwards. Follow-up is especially important in patients treated with antibiotics other than penicillin. CSF is examined at the last follow-up after treatment with alternative antibiotics. In patients with neurosyphilis a follow-up after treatment is advised with periodic quantitative cardiolipin tests (e.g. VDRL), clinical evaluation and repeat CSF examinations, for at least 3 years (CDC 1985). A life-time follow-up is, however, generally recommended.

In the case of early syphilis a 2-3 year follow-up is maintained where possible.

In the case of cardiovascular syphilis follow-up by a cardiologist is advisable and in neurosyphilis a life-time follow-up is best.

**Retreatment**

Reinfection with syphilis is a possibility particularly in the promiscuous who have casual relationships and where contacts may fear or neglect to surface for medical help. A CSF examination should be carried out before retreatment unless reinfection and a diagnosis of early syphilis is clearly established.

Retreatment should be considered under the following circumstances (CDC 1985):

1. If clinical signs or symptoms persist or recur
2. If there is a fourfold increase in the titre of a non-treponemal test
3. If an initially high titre non-treponemal test fails to show a fourfold decrease in titre within a year.

**Table 22.31** Treatment of syphilis for more than 1 year's duration (latent syphilis of indeterminate or more than 1 year's duration, cardiovascular or late benign syphilis) except neurosyphilis, in those hypersensitive to penicillin. Doses are given for young persons over 50 kg body weight or past puberty. Results have been evaluated less fully than in the case of penicillin.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Route</th>
<th>Daily dose</th>
<th>Number of divided doses</th>
<th>Number of days treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline hydrochloride</td>
<td>Oral</td>
<td>2 g</td>
<td>4</td>
<td>30 days</td>
</tr>
<tr>
<td>Oxacytetracycline</td>
<td>Oral</td>
<td>2 g</td>
<td>4</td>
<td>30 days</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Oral</td>
<td>2 g</td>
<td>4</td>
<td>30 days</td>
</tr>
</tbody>
</table>
When a patient is retreated one retreatment course is indicated and the course used will be that recommended for syphilis of more than 1 year's duration.

Treatment on epidemiological grounds

Patients who have clearly been exposed to infectious syphilis and who are not likely to cease sexual intercourse for 3 months, or to attend for surveillance, should be considered on epidemiological grounds for treatment and followed up; the regimen advised is that for early syphilis. It is our practice to treat couples such as husband and wife simultaneously if possible, if one partner develops early syphilis. Every effort is made, however, to establish a diagnosis beforehand in such cases.

In the case of early stage syphilis immediate treatment for the infected person or 'epidemiologic' treatment for potentially infected contacts will interrupt a possible chain of infection (Kaufman et al 1974). The CDC (1985) guidelines recommend that those exposed to infectious syphilis within the preceding 3 months and those who, on epidemiological grounds, are at high risk for early syphilis should be treated as for early.

Response to treatment and assessment of cure

Early stage syphilis. Penicillin in sufficient dosage causes disappearance of T. pallidum from early surface lesions within 6–26 h (Idsoe et al 1972). Infectivity appears to be lost very soon and lesions heal often within a few days. Obvious induration, depth, size and number of lesions will vary in relation to the duration of the disease and healing may be correspondingly longer. The infection can no longer be transmitted by sexual contact and relapse after adequate treatment is exceedingly rare, recurrences being usually due to reinfection.

In addition to careful clinical assessment of the patient, serological tests (cardiolipin tests, e.g. VDRL) must be performed serially over a period of time to ensure that treatment has been satisfactory. These tests should be carried out immediately after completion of treatment, monthly for the first 3 months and thereafter 3-monthly in the first year and twice in the second year. Following satisfactory treatment, quantitative tests for cardiolipin antibody (e.g. VDRL) gradually become negative, usually within 3–6 months.

The TPHA, which detects mainly IgG antibody, remains positive for years after treatment, unless this was given very early in the course of the disease when the titre in the serum was low (e.g. 1 in 80). Unlike the cardiolipin antibody tests, the TPHA is therefore of very little value in assessing efficacy of treatment because it remains positive often for years, or even for life. The FTA-ABS test and probably the ELISA also remain positive for many years after treatment. More experience is required in the use of the FTA-IgM test before it can be used routinely.

To ensure that treatment of secondary syphilis has been adequate it is generally considered good practice, even after penicillin therapy, to examine the CSF fluid (cell count, total protein content, IgG content, VDRL, TPHA and FTA-ABS test) about a year after treatment. Such examinations are essential after treatments with antibiotics other than penicillin.

Abstinence from sexual intercourse is advised in a patient being treated for early syphilis until the disease cannot be transmitted. This is usually achieved within a few days but it is wise for the patient to abstain until after the course of antibiotic when all lesions will be healed and possibly for 1 month after treatment if resumption of intercourse with an uninfected partner is to take place. If one partner is infected and has put the other at risk simultaneous treatment of both is advisable as reinfection of the treated by the untreated can occur. Such treatment on epidemiological grounds in a person at very high risk will call for the same follow-up and assessment of cure as in the patient in whom a diagnosis is established.

Late stage syphilis. Titres of cardiolipin antigen tests tend to diminish over the years in some patients but persistence of a high titre does not necessarily indicate a need for further treatment. If penicillin has been given in an adequate dosage further courses are not necessarily indicated. In cases of neurosyphilis the advisability of higher penicillin dosage requires consideration as levels in the cerebrospinal fluid after doses of long-acting penicillin preparations may be very low.

Jarisch–Herxheimer reaction (JHR)

This reaction is a complication that usually follows within a day of the initial treatment of a number of organismal diseases, including syphilis, and is characterized by fever and an aggravation of existing lesions together with attendant symptoms and signs (Warrell et al 1971; Bryceon 1976, Lancet 1977b). The salient features are as follows:

1. Prodromal phase with aches and pains.
2. A rise and then a fall in body temperature: the rise is accompanied by a chill and the fall by sweating.
3. Defervescence lasts up to 12 h.
4. Aggravation of pre-existing lesions and their attendant symptoms and signs, e.g. flaring of the rash in syphilis or more seriously exacerbation in deafness in laryngitis.
5. Characteristic physiological changes, which include early vasoconstriction, hyperventilation and a rise in blood pressure and cardiac output, and later a fall in blood pressure associated with a low peripheral resistance.

CONGENITAL SYphilis

Congenital syphilis is an uncommon disease in the United Kingdom. In England during the 12 months ending in December 1982 there were 126 cases reported and a case rate of 0.27 per 100 000 population for that year (Department of Health and Social Security 1983). Antenatal examination routinely includes serological tests for syphilis and this screening, together with adequate treatment of infected mothers, accounts for the low incidence of the disease in this country. In Africa and South America, congenital syphilis is still common although where yaws is endemic an immune effect may modify the consequences of syphilis in the adult females and its transmissibility to the fetus. It can be prevented in utero by treatment of the infected woman with penicillin during early pregnancy or cured later in pregnancy; its occurrence in a community is an indicator of defective antenatal care and the result of insufficient primary medical care.

Transmission of infection

Although it had long been considered that involvement of the fetus did not occur before the fourth month of gestation, recent studies have clearly demonstrated that infection may occur with:
10 weeks of conception (Harter & Benirschke 1976). Any infection before 20 weeks' gestation will not stimulate immune mechanisms because the fetal immune system is not as yet well developed and thus no histological evidence of fetal reaction to infection will be seen (Silverstein 1962). The theory that the cytotrophoblastic layer (Langhan's layer) of the early placenta protects, until its disappearance at 18–20 weeks' gestation, against transmission of the organism from the maternal to the fetal circulation (Curtis & Philpott 1964) has now been discounted. Electron microscopic studies have shown that this layer of cells does not completely atrophy (Benirschke 1974).

Infection of the fetus is more likely to occur when the mother's infection is in the early stage, as at this time considerable numbers of organisms are present in the circulation. During the first year of infection in an untreated woman there is an 80–90% chance that the infection will be transmitted to the fetus. The probability of fetal infection declines rapidly after the second year of infection in the mother and becomes rare after the fourth year. In general, the greater the duration of syphilis in the mother, the less chance there is of the fetus being affected.

In preantibiotic days it was common for a mother of a child with congenital syphilis to give a history of previous miscarriages succeeded by a premature stillbirth, then a stillbirth at term, and later an apparently healthy child at birth. The widespread use of antibiotics for concomitant infection has completely altered this pattern of events, and such an obstetrical record is now virtually unknown.

Although uncommon, a woman with late syphilis, however, may give birth to a child with syphilis, although the child of a previous pregnancy had been apparently healthy. This may be explained by the speculation that there is an intermittent release of treponemes from lymphoid tissue into the circulation in late syphilis. Should such an event occur the fetus may become infected.

If a mother with early-stage syphilis is not treated, 25–30% of fetuses die in utero, 25–30% die after birth, and of the infected survivors 40% develop late symptomatic syphilis (Thomas 1949).

**Clinical features**

The manifestations of congenital syphilis may conveniently be divided into two stages, early and late; the end of the second year of life is the arbitrary point of division between the two stages. Fuller details of the clinical features of congenital syphilis may be found in Nabarro's book (Nabarro 1954) and in a review by Robinson (1969) as well as by reference to individual papers quoted.

**Early congenital syphilis**

When congenital syphilis was common it was rare to find acute signs of syphilis in the newborn and in cases that occurred, death usually followed within a few days. Infants were often born prematurely or, if full term, were often of low birthweight. The skin was wrinkled and there was a bullous skin rash (syphilitic pemphigus), particularly on the soles and palms. The clear or purulent fluid from the bullae contained large numbers of *T. pallidum* and was highly infectious. Other skin lesions, most often maculopapules, were usually present and were found around the body orifices. Rhinitis produced a mucoid or mucopurulent nasal discharge, and a hoarse cry resulted from laryngitis.

Abdominal distension was common, and hepatic and splenic enlargement almost invariably found. Haemorrhagic manifestations occasionally occurred. This has been shown to have been due to thrombocytopenia and macroglobulinaemia (Marchi et al 1966). The majority of infants infected with syphilis appear healthy at birth, as the characteristic clinical features do not develop until between 2 and 12 weeks. After a period of normal development, the child fails to thrive and the clinical picture of congenital syphilis becomes apparent.

It is convenient to describe the manifestations according to the particular part of the body affected.

**Cutaneous manifestations.** Skin rashes of varied character are found in 70–90% of infants with congenital syphilis. The rash is symmetrical in distribution and erythematous macular, papular, and papulosquamous lesions may exist together in different parts of the body. On the face the eruption is particularly prominent around the mouth. Where the skin is moist, for example on the buttocks and external genitalia, the rash appears eczematous. In these sites hypertrophic lesions resembling condylomata lata may appear, usually as a manifestation of a recurrence following resolution of the initial rash. Deep fissures develop round the body orifices, and healing of these lesions leaves characteristic scars (rhagades).

The skin of the palms and soles may show peeling. In severe cases, the hair becomes scanty and brittle and involvement of the nails leads to shedding, and replacement by narrow, atrophic nails.

In addition to the eruptions described, the skin may show wrinkling from weight loss and there is café-au-lait pigmentation.

If an infant is not treated, or is inadequately treated, the skin lesions usually heal within a year, but there may be recurrences during the second year. Recurrent lesions usually differ from those seen in the original rash and include condylomata lata.

**Mucosal lesions.** Clinical evidence of rhinitis is found in 70–80% of infected infants. There is nasal obstruction and a mucoid nasal discharge which becomes mucopurulent and occasionally blood stained (syphilitic snuffles). Numerous treponemes may be demonstrated in the discharge which is highly infectious. Arrested development of nasal structures, and continued pressure changes within the nose as a result of obstruction, lead to deformities of the nose (saddle nose).

Mucous patches resembling those seen in secondary acquired syphilis may occur in the mouth and pharynx. Laryngitis produces a hoarse or aphony cry.

**Lymphadenitis and splenic enlargement.** Although not a constant accompaniment of early congenital syphilis, moderate generalized enlargement of the lymph nodes is common. The spleen is enlarged in at least 60% of infected infants.

**Bone and joint manifestations.** Bone disease, diagnosed by clinical or radiological examination, or both, occurs in at least 85% of infected infants under the age of 1 year (Nabarro 1954). In only about 40% of cases is there clinical evidence of bone involvement. Bones are usually affected symmetrically, but one side may be more involved than the other. The child cries when adjacent joints are passively moved and he rarely moves affected limbs (Parrot's pseudo-paralysis).

Radiological examination of infected infants under the age of 12 months, who have no clinical evidence of bone involvement, demonstrates abnormalities in at least 75% of cases. Multiple long bone involvement is most commonly found, the metaphyses being
particularly affected. Variable degrees of calcification at the growing ends of the bone result in a variety of radiological changes (Hira et al 1985). Most commonly there is an irregular (saw tooth) dense zone of calcification overlying an osteoporotic area at the metaphysis. Peripheral osteoporosis of the metaphysis is less often observed, as is the appearance of dense bands sandwiching such zones.

Irregular patchy areas of loss of bone density are commonly found in both metaphyses and diaphyses. A characteristic sign is loss of density of the upper medial aspect of the tibiae (Wimberger’s sign). In severe cases there may be a fracture at the site of bone destruction in the metaphysis, with impaction or displacement of the epiphysis.

Periostitis appears radiologically either as a single layer or as multiple layers of new bone formation along the cortex of the shaft of the bone; it is common in early congenital syphilis particularly amongst children aged 4 weeks and over (Hira et al 1985). Although any long bone may be affected, the distal femur and radius and the proximal tibia and humerus are the most often involved. The changes described are not specific for syphilis, similar radiological findings being encountered in rubella, cytomegalovirus infection, rickets and haemolytic disease of the newborn (Cremin & Fisher 1970). Occasionally in early congenital syphilis lens-shaped areas known as Parrot’s nodes appear around the anterior fontanelle on the frontal and parietal bones. These nodes are probably due to periostitis. Usually the changes described resolve within the second 6 months of life, but periostitis persists and may become more pronounced.

During the later stages of early congenital syphilis, dactylitis, manifest clinically as painless, spindle-shaped swelling of the fingers, may occur in a small number of cases (less than 5%). Radiographic examination shows that up to 25% of all infected children under the age of 2 years have dactylitis.

**Hepatic and pancreatic involvement.** The liver is almost invariably enlarged, usually in association with the spleen, in congenital syphilis appearing in the neonatal period and in at least 60% of older infants. Jaundice is an uncommon feature, but its presence in the neonate should alert the physician practising in areas where syphilis is common to the possibility that syphilis may be the cause of the jaundice.

Although not clinically apparent, pancreatitis is a common finding at autopsy of infants dying of congenital syphilis in the neonatal period (Oppenheimer & Hardy 1971).

**Renal involvement.** The nephrotic syndrome may rarely be associated with early congenital syphilis, and is thought to be the result of deposition of soluble complexes of treponemal antigen and anti-treponemal antibody in the glomeruli (Yuceoglu et al 1974). Acute nephritis is a rarity (Taitz et al 1961).

**Bronchopulmonary involvement.** In the aborted fetus and stillborn infant the lungs are always affected, as bronchi and lung parenchyma have developed abnormally.

**Neurological involvement.** Although meningitis is common in early congenital syphilis, particularly during the exanthem stage, clinical signs relating to the nervous system are uncommon. Epileptiform seizures, irritability and bulging of the anterior fontanelle may occur. There may be focal changes in the cerebral tissue due to thrombotic occlusion of blood vessels affected by a panarteritis. These cerebral lesions may produce hemiplegia, monoplegia and cranial nerve palsies.

In about a third of infants under the age of 12 months, the cerebrospinal fluid is abnormal with respect to cell content and protein levels, and gives positive results when examined by the serological tests for syphilis.

**Ocular manifestations.** Iritis is rare in early congenital syphilis. Chorioretinitis is considerably more common during the first few years of life. Examination with ophthalmoscope shows small spots of pigment surrounded by yellow areas (salt and pepper fundus). If untreated the inflammatory process progresses and if the macular or optic disc regions are involved, blindness may result.

**Haematological abnormalities.** Anaemia of varying severity occurs in at least 20% of infants with congenital syphilis. Normocytic, normochromic anaemia reflects depression of haematopoiesis in the bone marrow as a result of the chronic infection. Increased haemolysis probably plays a small part in the development of the anaemia. Secondary iron deficiency produces a macrocytic, hypochromic anaemia. Occasionally a leucoerythroblastic anaemia occurs.

Thrombocytopenia, associated with a bleeding disorder during the first few weeks of life, has been described (Freiman & Super 1966). Macroglobulinaemia may be associated with the bleeding diathesis (Marchi et al 1966).

In early congenital syphilis, the white cell count is usually elevated, with lymphocytosis.

**Late congenital syphilis**

Infected and untreated children are said to have entered the late stage of syphilis after their second birthday. In at least 60% of affected children there are no clinical signs of the disease, the only abnormal finding being positive serological tests, that is latent congenital syphilis.

**Intestinal keratitis.** This is the most common clinical manifestation of late congenital syphilis, occurring in about 40% of affected children. Intestinal keratitis appears to be the result of immunological reaction in the cornea to the treponeme, penicillin treatment having no influence on the course of this manifestation. In most cases this develops between the ages of 6 and 14 years. It may occur earlier or very much later (even over the age of 30 years). Although commencing in one eye, both become involved in more than 90% of cases; the second eye shows features of the condition a few days to several months after the first. The patient complains of pain in the affected eye, photophobia with excessive lacrimation and dimness of vision. A diffuse haze near the centre of the cornea of one eye is the earliest clinical sign, but within a few weeks the whole cornea becomes opaque. This is usually associated with circumcorneal scleral congestion.

Examination by slit lamp microscopy shows that these corneal changes are attributable to blood vessels extending into the cornea from the sclera, and to exudation of cells from these vessels.

The condition gradually improves over a period of 12–18 months, leaving a variable degree of corneal damage which may lead to blindness or be only detectable by slit lamp examination. This latter investigation may show empty blood vessels (ghost vessels) within the cornea of patients who have had intestinal keratitis earlier in life, but have had no apparent residual scarring (Dunlop & Zwink 1954).

After resolution of the initial episode of intestinal keratitis, 20–30% of patients suffer a relapse of this condition.

**Bone lesions.** The essential bone lesion in late congenital syphilis is hyperplastic osteopetrosis, a process which may be diffuse, resulting in sclerosis of bone, or localized (periosteal node
or gumma). Gumma formation may lead to necrosis of underlying cortex with softening of the bone. The tibia is most commonly affected by these changes.

Usually bone lesions develop between the fifth and twentieth year of life, when the patient complains of pain in the affected bone. Palpation may reveal nodules on the anterior surface of the bone, and rarely ulceration may be observed where a gumma has involved skin and bone. In older children, thickening of the anterior surface of the tibia may result in forward bowing of that bone (sabre tibia).

Painless gummatous lesions may be found on the hard and soft palates or in the pharynx. These are often extensive, with considerable necrosis of tissue. Perforation of the palate, absence of the uvula and scarring about the oropharynx may be the result.

Destructive gummatous lesions of the nasal septum may cause perforation of the septum with or without deformity of the lower part of the nose.

**Joint lesions.** The commonest type of joint lesion (Clutton's joints), seen in about 20% of untreated children, is bilateral effusion into the knee joints (Scott Gray & Philip 1963). This condition, like interstitial keratitis, is unaffected by antibiotic treatment, and appears to be an immunological reaction to *T. pallidum.* Less commonly, other joints are similarly affected. Although most frequently occurring in children between the ages of 5 and 10 years, joint involvement may be seen at any age from 3 years to the mid-twenties.

The onset of the arthritis starts acutely often with a history of antecedent trauma. Although most commonly painless, the affected joints may be acutely painful, particularly at the onset. Radiological examination reveals no specific changes in the joint.

There is gradual resolution of the arthritis over many months, with recovery of full function.

**Neurosyphilis.** In about 20% of infected children over the age of 1 year neurosyphilis is latent or hidden and diagnosis depends upon the detection of abnormalities in the cerebrospinal fluid. As a late result of the mumpsitis of early stage congenital syphilis, epileptiform seizures, mental deficiency and cranial nerve palsies may be found in children over the age of 2 years. Parenchymatous involvement produces two main clinical conditions, juvenile general paralysis of the insane and tabes dorsalis.

**Juvenile general paralysis of the insane (juvenile GPI).** This occurs in about 1% of affected children appearing about the age of 10 years, but occasionally much earlier, or much later as in middle age. The sexes are affected equally (in contrast to the GPI of acquired syphilis in which males are more often affected than females). There is usually a gradual onset of symptoms, the child becoming dull, irritable, apathetic and forgetful. Later, delusions, usually paranoid in type, occur and speech becomes disturbed. The voice is monotonous, articulation becomes stumbling and tremulous and speech is eventually lost. There is generally tremor of the lips, hands and legs. Handwriting becomes indistinct. Epileptiform seizures are common at a late stage of the disease.

Pupillary abnormalities are seen in over 90% of cases; the pupils are of the Argyll Robertson type or immobile and dilated. Optic atrophy occurs in between 10% and 35% of cases.

Other physical findings resemble those found in general paralysis of acquired syphilis.

**Juvenile tabes.** This is much rarer than general paralysis. The onset of the condition is generally between the ages of 10 and 17 years. Failing vision and paraesthesiae are the most common symptoms; lightning pains and ataxia are rare. Later in the course of the disease headaches, photophobia and diplopia occur frequently. Sphincter disturbances are uncommon although enuresis may be found. Clinical examination may detect nystagmus, pupillary abnormalities, optic atrophy, and absent or diminished tendon reflexes. Tropic disturbances are rare and it is unusual to find evidence of loss of cutaneous sensation.

**Ear disease.** The middle ear may be affected by a painless otolabyrinthitis, showing as a slight purulent aural discharge. Conduction deafness may result without treatment. The deafness of congenital syphilis, however, is predominantly sensory.

In sections of the temporal bone of individuals with long-standing sensorineural deafness, which has resulted from congenital syphilis, there is patchy otitis with inflammation of all three layers of the otic capsule. Hydrops of the cochlear duct, saccule and utricle occurs and there is degeneration of the organ of Corti with loss of cochlear neurons. Similar changes may affect the sensory epithelium or neurones of the vestibular system (Kar¬mody & Schuknecht 1966).

Even after what has been considered adequate penicillin treatment, treponemes have been demonstrated in endochondral bone, a dense structure into which antibiotics do not readily diffuse.

Subjective hearing impairment is commonly a late manifestation often not occurring until adult life, although it may occur in childhood. In addition the patient may not be seen first till middle age, when the diagnosis of congenital syphilis may not come readily to mind unless there are other stigmata of the disease.

Vestibular disease is frequent in patients with congenital syphilis, the symptoms, which include dizziness, unsteadiness of gait and paroxysmal vertigo, usually beginning with the onset of deafness (Morrison 1975).

Audiograms show a variety of patterns. The most common (35%) is high tone loss, followed by a flat audiogram (25% of cases) and low tone loss (15% of cases) (Morrison 1975). There is progressive deterioration of deafness although spontaneous fluctuation may occur. The most severe difficulty is in discrimination of speech. It is usually an isolated finding, bilateral, although one side is often more severely affected than the other. There are usually no abnormalities in the cerebrospinal fluid (Hahn et al 1961).

**Skin lesions.** Gummata similar to those occurring in late acquired syphilis may be found.

**Cardiovascular lesions.** Myocarditis may be found in children dying of congenital syphilis, but aortitis is exceedingly rare.

**Liver disease.** Gummatas of the liver are rarely found.

**Paroxysmal cold haemoglobinuria.** This rare condition occurring in less than 1% of patients with late congenital syphilis, may be seen also in acquired syphilis. Large quantities of haemoglobin are excreted in the urine after exposure to cold. Shivering or a rigor heralds the attack and this is rapidly followed by fever, headache and pains in the back or limbs. A generalized urticarial rash may also develop. Within the next few hours the urine becomes dark brown in colour and contains haemoglobin and methaemoglobin but few red blood cells. In most cases, the clinical features described resolve within several hours, but occasionally mild jaundice may develop and persist for some days. This condition is liable to recur periodically when the patient is exposed to cold of varying severity.

Cold haemolysins are found in the blood, and demonstrated by the Donath Landsteiner test. The basis of this test is the ability of the haemolysin to unite with red cells when the blood is
chilled; when the blood is then warmed to 37°C, these sensitized cells are lysed in the presence of complement.

**Stigmata of congenital syphilis**

Lesions of early and late congenital syphilis may heal leaving scars and deformities characteristic of the disease. Such scarring and deformities constitute the stigmata of congenital syphilis, but only in some 40% of patients do they occur.

**Stigmata of early lesions**

**Facial appearance.** The 'saddle nose' deformity may result from rhinitis. The palate may appear high arched as a result of underdevelopment of the maxilla.

**Teeth.** The tooth germs of deciduous teeth are fully differentiated by the 10th week of gestation before tissue reaction to treponemes appears to occur; hence these teeth are usually unaffected. Teeth which develop later may, however, be affected. Two groups of teeth bear the brunt, the upper central incisors and the first molars.

Typically the affected upper incisor is smaller than normal, and darker in colour and peg-like, instead of being flat, with the sides converging to the cutting edge which classically has a notched centre (Fig. 22.26), the so-called Hutchinson's incisor (Hutchinson 1858). Affected incisors do not always show this typical appearance but may often be thickened anteroposteriorly, with rounding of the incisal angles; they may have a shallow depression on the incisal edge rather than a notch.

The typically affected molar, Moon's molar, shows a constricted occlusal surface and rounded angles. The cusps of the molar are poorly developed and appear crowded together. Such teeth are prone to dental caries and as a result are lost early.

In one series (Putkonen 1962), in 45% of patients with congenital syphilis the upper central incisors were affected, and in about 20% the first molars were involved. The incidence of dental changes is high in patients who also develop interstitial keratitis.

**Ragades.** The deep cutaneous lesions around the orifices of
d the body heal producing scars radiating from the orifice known as ragades.

**Nails.** Atrophy and deformity of the nails may be seen in adult life as a result of nail bed inflammation in infancy.

**Choroidal scarring.** Healing of choroidoretinitis produces white scarred areas surrounded by pigmentation on the retina.

**Stigmata of late lesions**

**Corneal lesions.** Opacities of the cornea and ghost vessels observed on slit lamp examination are the result of interstitial keratitis (Dunlop & Zwink 1954).

**Bone lesions.** Sabre tibia resulting from osteoporosis may be observed, as may the scars of destructive lesions of the oropharyngeal and nasal regions. Broadening of the skull may result from osteoporosis of the frontal and parietal bones.

**Optic atrophy.** This may occur as a single entity without iridoplegia (e.g. Argyll Robertson pupils) (Robinson 1969).

**Nerve deafness.**

**Diagnosis of congenital syphilis**

It is important to ascertain that a child born to a mother who has been apparently adequately treated for syphilis is not infected. An infected infant may appear healthy at birth. Blood from the neonate should be examined at birth, using VDRL and TPHA tests. As a result of transplacental passage of maternal antibody, those tests which are positive in the mother are also likely to be positive in the infant and at a similar titre. Within 6 months, however, these maternal antibodies will have disappeared from the infant's serum and, if the child is not infected, the tests will have become negative. Persistently positive serological tests, or a rising titre, suggests congenital infection and the need for treatment.

The use of the FTA-ABS test, using monospecific antisera to detect specific IgM antibodies in the infant's blood, has been described. It is important to remember that this test may be negative at birth in a child with active infection and may not become positive until the age of 3 months. Serial serological tests up to 6 months are therefore required in apparently healthy babies born to mothers with positive serological tests for syphilis particularly if untreated or suspected to be so.

In western industrialized countries, the discovery of positive serological tests in an otherwise healthy person often raises the question as to whether syphilis has been acquired before birth or later. This problem is difficult as stigmata appear to be rare now. A family history may be misleading. Patients should, however, be carefully examined for the presence of obvious stigmata and slit lamp microscopy of the cornea should be included in the investigation to search for ghost vessels, as a trace of previous interstitial keratitis (Dunlop & Zwink 1954). Nerve deafness may be obvious or, if mild, demonstrable by audiography. In doubtful cases, serological examination of parents or brothers and sisters may be helpful, and, to avoid serious social upset, consultation and collaboration with the general practitioner is advised.

**Treatment of congenital syphilis**

**Early congenital syphilis**

Although infants with massive infection may still die in the neonatal period, the majority will be cured by adequate penicillin

![Fig. 22.26](image-url) Hutchinson's incisors, one of the classical stigmata of congenital syphilis. Note the peg-like appearance with convergence towards the cutting edge, which classically has a notched centre. Affected incisors do not always have this typical appearance (see text).
treatment. Stigmata, particularly dental, will, however, be detectable.

Prior to instituting treatment, the CSF should be examined to
detect neurogliosis involvement. Benzylpenicillin in an intramuscular
dose of 30 mg (50 000 IU)/kg should be given daily in two
divided doses. Alternatively, procaine penicillin 50 mg (approximat¬
ely equivalent to 30 mg or 50 000 IU benzylpenicillin)/kg/day
may be used (Hager 1978). Treatment should be continued for
at least 10 days, and preferably longer if the CSF is abnormal.
Further details on prevention, treatment and the problem when
erythromycin has been used for treatment of the pregnant woman
with syphilis are discussed elsewhere (p. 1468).

After the neonatal period the dose should be the same dosage
used for neonatal congenital syphilis. For larger children the total
dose need not exceed the dosage advised in adult syphilis for more
than 1 year's duration. If hypersensitive to penicillin,
erythromycin may be used. Tetracycline or oxytetracycline should
not be used in children under 12 years of age.

Late congenital syphilis

The dosage of procaine penicillin required for the treatment of
late congenital syphilis is similar to that used in the therapy of
late acquired syphilis. Treatment, however, does not prevent the
development or course of interstitial keratitis, hydrarthrosis and
neral deafness.

Management of interstitial keratitis. Patients with interstitial
keratitis should be managed in hospital, in consultation with an
ophthalmologist. Topically applied corticosteroids rapidly sup¬
press the inflammatory reaction in the cornea and anterior uveal
tract, and their use, until spontaneous cure occurs, has
revolutionized the management of this condition. Although the
infiltration of the cornea by inflammatory cells resolves, scarring
from previous episodes of keratitis is not affected.

Betamethasone eye drops, BPC 0.1%, instilled into the affected
eye(s) every 1 to 2 h is a useful preparation. Treatment should
be continued until the corneal inflammatory infiltrate has cleared,
and visual acuity restored to the patient's normal level. Slit lamp
examination is essential before steroid treatment is discontinued.

As mild degrees of keratitis may not be apparent otherwise.

Regular examination is required after cessation of treatment, as
corneal scarring may result from continuing mild inflammation.

During steroid treatment, mydriatics such as atropine eye drops
BPC 1% may be useful adjuvants by reducing ciliary muscle tension.

Corneal grafting may be required in patients with corneal scarring
acquired during attacks of interstitial keratitis.

Hydrarthrosis (Clutton's joints). This is a self-limiting disor-
der, and does not require any specific therapy.

Nerve deafness. Despite previous treatment of congenital
syphilis with what has been regarded as adequate doses of penicil¬
in, progressive neural deafness may develop at any age, but most
commonly in the adult of middle age. This may be the result of
failure of the drug to reach adequate concentrations in the
perilymph or endolymph.

Morrison (1975) advocated the use of benzylpenicillin in a
dosage of 300 mg (500 000 IU) given by intramuscular injection
every 6 h for 17 days, together with probenecid in a dose of 1 g
6-hourly by mouth. During the first week of treatment, 30 mg
of prednisone is given orally, the dose thereafter being reduced

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Fig. 22.27 Geographical distribution of the endemic treponematoses in the early 1950s — World Map, Arno Peters projection; data from WHO Chronicle. 1982.
to 25 mg daily for 3 weeks. As a response to treatment is seen within the first month, treatment may be discontinued if there has been no improvement at that time. When the response has been satisfactory, steroids may be continued for 3-6 months in gradually diminishing doses. A further course of penicillin is then given. Occasionally patients have required maintenance treatment with low doses of steroids to abolish vertigo and maintain hearing. In advanced cases where there has been considerable tissue damage, however, no response to medical treatment occurs.

Ampicillin in a dosage of 1.5 g 6-hourly for 4 weeks, together with prednisolone 30 mg daily for 10 days, tailing off over the succeeding 10 days, has been used in the management of this condition (Kerr et al 1973).

Audiometry may give useful information regarding response to treatment. The value of treatment has not been fully assessed. In some cases, the disease process may be arrested, but in others improvement in hearing may only be temporary.

ENDEMIC TREPONEMATOSES

The treponematoses of man have developed in differing geographical and epidemiological situations as parasite and host and have evolved a modus vivendi. The non-venereal diseases tend to exist among primitive peoples in rural communities, where transmission occurs by skin contact in childhood with other younger or older children who, themselves, have relapsing crops of infective skin lesions. If infected in childhood susceptibility to venereal syphilis later as adults is diminished.

In the world maps provided the geographical distribution of the endemic treponematoses in the early 1950s (Fig. 22.27) is contrasted with that of the 1980s (Fig. 22.28).

Syphilis, on the other hand, probably evolved as a venereal disease as a result of social and climatic change as in those who began to wear clothes and, apart from sexual contact, tended to live more separate existences. The survival and transmission of the treponeme under these circumstances became possible only when susceptible adults, escaping yaws in childhood, became infected by contact with genital lesions at sexual intercourse.

Pinta (synonyms: in Mexico, mal de pinto; in Colombia and Venezuela, carate; and in Chile and Peru, azul)

This is a disease of remote rural communities, affecting the skin (blue-stain disease) and it is the least damaging of the human treponematoses. The causative organism is Treponema carateum, the most attenuated of the pathogenic treponemes. Pinta used to be prevalent in semiarid regions of Brazil, Colombia, Cuba, southern Mexico and Venezuela with scattered foci in Central and South American countries and the Caribbean islands; today only scattered foci remain in northern South America and Mexico (Perine et al 1984). It differs from yaws and endemic syphilis in that it affects children and adults of all ages. Throughout its course the disease is confined to the skin, where pigmented and achronic lesions may remain infective for years, permitting spread by direct skin to skin contact.
Clinical features

The primary or initial lesion develops usually after 2–3 weeks, often on an uncovered part of the body as a lenticular and slightly scaly papule which enlarges to form a plaque; mostly the initial lesion is to be found on the legs, the dorsum of the foot, the forearm or the back of the hands. At first pink in colour in fair skins it becomes pigmented or hypochromic to a variable degree as it enlarges; lymph nodes draining the area enlarge. After 2 months or up to a year later, secondary lesions develop, some on occasions appearing on the same site as the initial lesion. At first erythematous and afterwards copper coloured these ‘pintos’ become pigmented to a varying degree, changing slowly from a copper colour to lead grey and slate blue as a result of photosensitization and areas of erythema, hypopigmentation and leucoderma develop. The polychromic lesions become keratotic.

In late stage pinta residual areas of hyperchromia and achromia develop in isolated patches to form multicoloured lesions; the de-pigmentation process occurs at different rates even within the same lesion. No disability or complication other than leucoderma occurs (Perine et al. 1984).

The causative organism, *T. carateum*, is detected by dark ground illumination microscopy in serum obtained from the base of a lesion after abrading the surface; although numerous in early stage lesions treponemes persist through to the late dyschromic stage (Marquez 1975).

**Yaws (synonyms: pia in French; framboesia in German, Dutch; bouba in Portuguese; buba in Kiswahili)**

Yaws has shown the greatest changes in regional prevalence since the mass treatment campaigns of the 1950s. In South America only scattered foci of active yaws persist; Brazil and Surinam are almost yaws free and in Colombia, Ecuador, French Guiana and Guyana only a few dozen or a hundred cases are reported annually. In south-east Asia yaws still exist in Indonesia and Papua New Guinea.

Africa remains the part of the world mostly affected although where there is improved rural medical care and improved standards of living, as in the Ivory Coast and Nigeria, the numbers of clinical cases are declining (WHO Scientific Group 1982). In Ghana, however, resurgence of yaws occurred following cessation of active yaws surveillance; the numbers of reported cases of infectious yaws increased 21-fold between 1969 and 1976 (Agadzi et al. 1983).

In a WHO survey in the Central African Republic, Congo and Gabon, clinical yaws was detected in over 20% of the pygmy population and positive serological tests in 80% (WHO Scientific Group 1982). Out of a total pygmy population of between 100 000 and 200 000 two major groups have undergone less assimilation than the others — the Bina of the tropical rain forests to the west of the Ubangi River and the Mburi, several hundred miles to the east in the Ituri Forest (Anonymous 1978). In a survey (458 examined) of the former group in 1978–1979, in the dry season, a time when these nomadic forest people were accessible, it was found that there were clinical signs of yaws in 50% and serological tests (VDRL and TPHA) were positive in 86% of the children and 95% of the adults. In the case of neighbouring non-pygmy peasant cultivators clinical evidence of yaws was also common (30%) and 78% of the children and 98% of the adults had positive serological tests (Widy-Wirski et al 1980). Eradication campaigns had clearly not reached such isolated popula-

<table>
<thead>
<tr>
<th>Yaws lesions</th>
<th>Examples</th>
<th>‘Infectiousness’ (+ to ++++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage cutaneous (often pruritic, tendency to cropping, often polymorphous, modified by climate, if lesions moist then infectious)</td>
<td>Papule, papilloma</td>
<td>+++</td>
</tr>
<tr>
<td>Initial lesion</td>
<td>Papilloma, some serpiginous, some ulcerated</td>
<td>+++</td>
</tr>
<tr>
<td>Macules</td>
<td></td>
<td></td>
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<tr>
<td>Micropapules, papules</td>
<td></td>
<td></td>
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<tr>
<td>Squamous macules</td>
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<td></td>
</tr>
<tr>
<td>Squamous micropapules, papules</td>
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<td></td>
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<tr>
<td>Polymorphous or mixed</td>
<td></td>
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</tr>
<tr>
<td>Plaques</td>
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<td></td>
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<tr>
<td>Nodules</td>
<td>Front of knees</td>
<td></td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>Palmar, plantar (as in crab yaws — painful)</td>
<td></td>
</tr>
<tr>
<td>Early stage bone</td>
<td>Polydactylitis tibia</td>
<td></td>
</tr>
<tr>
<td>Osteoperiostitis</td>
<td>Goundou (ostetis of nasal processes of maxilla)</td>
<td></td>
</tr>
<tr>
<td>Early stage joint</td>
<td>Ganglion, hydrarthrosis</td>
<td></td>
</tr>
<tr>
<td>Late stage cutaneous</td>
<td>Palmar, plantar</td>
<td></td>
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<tr>
<td>Hyperkeratosis</td>
<td>Ulcer with characteristic tissue destruction (gummatus)</td>
<td></td>
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<tr>
<td>Late stage bone</td>
<td>Sabre tibia, monodactylitis</td>
<td></td>
</tr>
<tr>
<td>Gummatus osteoperiostitis</td>
<td>Gangosa</td>
<td></td>
</tr>
<tr>
<td>Late stage joint</td>
<td>Ganglion</td>
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<tr>
<td>Hydrarthrosis</td>
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<tr>
<td>Bursitis</td>
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<td></td>
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<tr>
<td>Juxta-articular nodes</td>
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</tbody>
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the natural course of the infection. The classification and nomenclature for the lesions of yaws were established in an illustrated monograph of the World Health Organization (Hackett 1957) and the bone lesions were discussed more fully by Hackett (1951). A classification of lesions and the degrees of infectiousness of such lesions, based on Perine et al (1984), are summarized in Table 22.32.

The most characteristic lesion in early yaws is the papilloma (Fig. 22.29) and in the exudate of all early lesions, which may be macular, maculopapular or papular, treponemes are numerous. The early papule enlarges to form a papillomatous lesion bearing some resemblance to a raspberry (a synonym is framboesia). There may also be adenitis. After 2–6 months the initial lesion heals, often without scarring. Further papillomata often in crops develop most often around the body orifices, near the nose, mouth, anus and vulva. A change in climate may influence the number and morphology of yaws lesions: in the dry season lesions tend to be fewer in number and macular in form and papillomata tend to be more concentrated in moist areas of the skin such as the axilla (Fig. 22.30) and anal cleft. Hyperkeratotic lesions occur on the soles of the feet and palms of the hands. On the feet plaques develop which are painful and walking becomes difficult (crab yaws).

A periostitis may affect long bones or cause a polydactylitis affecting the phalanges and metacarpals (Fig. 22.31). An osteitis of the nasal processes of the maxilla produces paramanal swellings (goundou) and is common in Africa. The tibia may become sabre shaped. Nocturnal bone pain and tenderness of the tibial shaft are common in early yaws.

Ganglions, particularly at the wrist, and hydarthrosis can also occur in early yaws. There is also an early latent stage which may be interrupted by relapses of active early lesions.

Late lesions (Table 22.32) develop five or more years after the infection and the characteristic late lesion is a destructive ulcer, which may involve skin, subcutaneous tissue, the mucosa and the bones and joints. Deep destructive lesions are typified by the hideous mutilation of the central part of the face (rhinopharyngitis mutilans) called gangosa in which there is destruction of cartilage and bone structures of the septum, palate and posterior part of the pharynx (Vegas 1979). It is probable, however, that there is no bone lesion that occurs in yaws that does not also occur in syphilis (Hackett 1951).

There is no certain evidence of transplacental or congenital infection in yaws. Serologically it cannot be distinguished from syphilis.

**Endemic syphilis** (synonyms: *bejel* in Arabic; *ajovera*, *dichuchwa* in Zimbabwe; endemic syphilis in Bosnia; extinct forms of disease: *sibbes* in Scotland, *radesyege* in Norway and *skerlfiev*, of the Croatian coast, Yugoslavia)

Endemic syphilis is prevalent today primarily among the semi-nomads in the Arabian peninsula and along the southern border of the Sahara desert. A recent survey found thousands of cases of early endemic syphilis in Mali, Mauritania, Niger and Upper Volta; in sub-Saharan Africa the disease may be a greater problem today than it was formerly (WHO Scientific Group 1982). It used to be found also in scattered foci in central Asia, Australia and India but these have been eliminated by the mass penicillin treatment campaigns of the 1950s (Perine et al 1984).

The infection may be spread in the early stage through the use of recently contaminated drinking vessels (Grin 1953), by direct skin to skin contact, or by the fingers contaminated with saliva.
and mucus from infective lesions containing treponemes (Perine et al 1984).

In Arabia endemic syphilis, known as bejel, presents in its early stage and generally in children aged 2–15 years with a mucocutaneous eruption and exuberant papules predominantly around the genitalia and anus. Mucous papules or shallow ulcerations (mucous patches) appear on the lips, in the mouth and in the fauces. Symptoms such as hoarseness, dysphagia or dyspnoea have been attributed to extension of the mucous patches to the larynx. Condylomata may be seen in the moist areas of the skin. Periostitis also occurs (Hudson 1958).

Late lesions are granulomatous and destructive and the nose and its bony structure, the oral cavity and the hard palate and larynx are favourite sites. Hudson (1958) remarks that in his time ‘left palate voices were common in the market place’. Destructive skin ulcers, plantar keratosis, juxta-articular nodes and depigmented lesions are common late manifestations.

Control of endemic treponematoses

Although the treatment of whole communities with long-acting penicillin preparations for the control of endemic treponematoses of childhood was followed initially by a remarkable regression of the community disease, early clinical yaws has not been eliminated in large endemic areas where transmission continues and periodic focal outbreaks tend to occur (Guthe et al 1972, WHO Scientific Group 1982). Without renewed control programmes gains made by mass treatment campaigns of the 1950s and afterwards may be lost, particularly for yaws in some African countries (Perine et al 1984).

In the 1950s, on the basis of pilot studies in yaws in Haiti, endemic syphilis in Yugoslavia and pinta in Mexico, mass treatment campaigns with penicillin were undertaken in 46 countries in the context of the World Health Organization Treponematoses Campaign. Up till 1970 some 160 million people had been examined and in 50 million clinical cases, latent cases and contacts treatment had been given. In western Samoa, for example the prevalence of clinically active yaws was about 11% in 1955 with about 3% with infectious lesions. On resurvey of the population after mass treatment a year later clinically active yaws was found in only 0.06% and infectious cases in 0.02%. In Yugoslavia the careful campaign, follow-up and progressive environmental changes reduced the rate of endemic syphilis to nil and results of surveys in 1968–1970 confirmed that eradication had been achieved (Arslanagic et al 1989).

Clinical surveys for active yaws may be conducted without any sophisticated laboratory test but surveys for latent disease require serological tests (WHO Scientific Group 1970). The original indices were clinical and the detection of cases of active yaws is likely to continue to be the mainstay of surveillance and most appropriate for economic and logistic reasons. Age-specific seroreactor rates are, however, useful to define areas as hyperendemic, mesoendemic and hypoendemic and in surveys after mass treatment such profiles demonstrate the age at which infections are occurring.

The socioeconomic status of a large segment of the populations in the rural areas of West Africa has either not improved or has actually regressed in the last decade. In these areas, patients with reported yaws infection now number tens of thousands, but epidemiological estimates place the true incidence as four times higher. In areas of increasing prevalence, atypical early yaws lesions may be underdiagnosed owing to the inexperience of clinicians unfamiliar with the manifestations of the disease (WHO Scientific Group 1982).

Antibiotics in treatment and control in endemic treponematoses

Benzathine benzylpenicillin (available as Extencilline, Specia,
Paris) has been recommended by a recent WHO Expert Committee on Venerable Diseases and is preferred to other forms of penicillin for the treatment of treponemal diseases. Since a single deep intramuscular injection of benzathine benzylpenicillin 1.8 g (approximately equivalent to 1.4 g or 2.4 × 10^8 IU benzylpenicillin) in a healthy ambulant adult produces a penicillinemia above the treponemical level for more than 3 weeks, this dose is effective not only for curing treponemal diseases but also for providing protection against reinfection during this period.

Currently schedules for the endemic treponematoses (not including venereal syphilis) are: a single intramuscular injection of 460 mg benzathine benzylpenicillin (approximately equivalent to 360 mg or 600 000 IU of benzylpenicillin) for patients and contacts aged under 10 years; and 918 mg benzathine benzylpenicillin (approximately equivalent to 720 mg or 1.2 × 10^6 IU benzylpenicillin) for those over 10 years (Perine et al 1984).

The extent of treatment given to a community, village or other group living close to one another is based on the prevalence of clinically active yaws in the community. World Health Organization treatment policies recommend for hyperendemic areas (approximate prevalence of clinically active yaws in the community of over 10%) benzathine benzylpenicillin treatment to the entire population, viz. total mass treatment (TMT); for mesoendemic areas (approximate prevalence of clinically active yaws in the community of 5–10%) treatment with benzathine benzylpenicillin in all active cases, all children under 15 years of age, and obvious contacts of infectious patients, viz. juvenile mass treatment (JMT); and for hypendemic areas (approximate prevalence of clinically active yaws in the community under 5%) treatment with benzathine benzylpenicillin in all active cases and all household or other obvious contacts. In isolated and remote villages TMT may be appropriate even if the prevalence of active yaws is less than 10% (Perine et al 1984).

Penicillin treatment always carries the risk of serious side effects including fatal anaphylaxis. During the initial mass treatment campaigns when almost all are receiving penicillin for the first time risks are very low but those undertaking such campaigns should be prepared to treat penicillin reactions. Alternatives to penicillin have been suggested; children between 8 and 15 years may be given erythromycin 250 mg orally four times per day for 15 days. Although tetracycline is generally not advised in children under the age of 12, Perine et al (1984) have suggested that in those between 8 and 15 years of age who are allergic to penicillin a dose of 250 mg four times per day for 15 days is acceptable in mass campaigns. In children under the age of 8 years only erythromycin in doses appropriate to their body weight (8–12 mg/kg) may be used and tetracycline must not be given. Tetracycline is not recommended in pregnancy.

Adults who have acquired yaws in childhood may be seen in the clinics of western countries, when the results of serological tests will not differentiate from syphilis: in such cases, treatment appropriate for syphilis is advised.

GONORRHOEA

Gonorrhoea, an infection of the mucosal surfaces of the genitourinary tract with the bacterium Neisseria gonorrhoeae, is mainly transmitted by sexual intercourse. In men the infection is associated with an acute purulent urethritis in approximately 90% of cases, but the organisms may spread also to the epididymis and the prostate. In women the urethra and cervix are infected in 65–75% and 85–90% of cases respectively and the rectal mucosa in 25–50%. Occasionally (about 10%) infection extends from the cervix to the endometrium and Fallopian tubes. Infection of the fauces may occur in both sexes (5–15%); eye infections are seen rarely in adults. In homosexual men, who act as passive partners in anal intercourse, rectal infections also occur.

In children before the age of puberty gonorrhoea is commoner in girls than boys and those affected are usually in a low socioeconomic group. In girls the infection may follow contamination but sexual abuse is a frequent mode of transmission causing vulvovaginitis or a rectal infection. In boys the source of gonorrhoea may be homosexual contact with older men (White et al 1983).

In the neonate gonococcal conjunctivitis (ophthalmia neonatorum), acquired during birth, is potentially blinding and requires urgent treatment. In localities where gonorrhoea is common, primary care for all difficult to secure and antenatal care inadequate, then active consideration of prophylaxis for gonococcal infections in the newborn will be required, particularly gonococcal conjunctivitis.

In a small percentage of untreated cases, systemic spread gives rise to an entity known as disseminated gonococcal infection, characterized clinically by arthritis with or without skin lesions.

AETIOLOGY

The causative organism, Neisseria gonorrhoeae, exists as small Gram-negative cocci, kidney shaped and arranged in pairs (diplococci) with the long axes in parallel and the opposed surfaces slightly concave; the organisms are typically intracellular, are delicate and have exacting nutritional and environmental requirements. Media containing blood or serum, a temperature of 36–37°C and a moist atmosphere, enriched with 10% carbon dioxide must be provided to ensure growth. The organism is liable to die if separated from its host and is also readily killed by drying, soap and water, and many other cleansing or antiseptic agents.

Gonococci in pus appear in specific clusters in which they are surrounded by granules and granules derived from the host cells in which they multiplied. These clusters are called infectious units in which the cocci are probably protected against humoral defence mechanisms. Serum antibody to gonococci can be detected within a few days of infection but such antibodies are not protective with regard to mucosal reinfection.

TRANSMISSION OF INFECTION

Owing to the poor viability of the gonococcus away from the mucosal surfaces of the host, gonorrhoea is ordinarily acquired by sexual intercourse with an infected person. Gonorrhoea is highly infective and the risk for a female having intercourse with an infected male is between 60% and 90%; for a male the risk with an infected female is 20–50%. The gonococcus can be transmitted to the pharynx by orogenital contact; infection in this anatomical site is usually without symptoms.

The incubation period in the male tends to be about 3–5 days (range 2–10 days). In the female a precise incubation period is difficult to determine since approximately 70% or more of infections may cause no symptoms. Such asymptomatic infections make it possible for individuals to remain as sources of infection within the community whilst at risk themselves of developing pel-
vic inflammatory disease or disseminated infection: the risk of developing these sequelae are generally given as 10% and 1% respectively.

In young children under the age of puberty vulvovaginitis is caused more commonly by organisms other than N. gonorrhoeae. Although gonococcal vulvovaginitis can result from accidental contamination of the child with discharge when sleeping with an infected parent, sexual abuse may be the more likely mode of spread and involve more than one child in the family (White et al 1983). In the sexual abuse of boys the source of gonorrhoea may be homosexual contact with older males. Pharyngeal gonorrhoea, usually asymptomatic, may be the only anatomical site of infection in sexually abused children (Groothuis et al 1983).

During birth, a baby passing through an infected cervix may acquire gonococcal conjunctivitis of the newborn (ophthalmia neonatorum). Gonococcal conjunctivitis in older children and in adults is usually acquired by contact with fingers and/or moist towels contaminated with fresh pus.

### PATHOLOGY

Infection involves the columnar epithelium of the urethra and para-urethral ducts and glands of both sexes, the greater vestibular glands (Bartholin's), the cervix, the conjunctiva and the rectum. Infection may also be established in the soft stratified squamous epithelium of the vagina of young girls; involvement of this type of epithelium in other parts of the body such as the skin of the glans penis, cornea and mouth is extremely rare.

Inflammation induced by infection by N. gonorrhoeae of the mucosal surfaces may be mild or acute with the production of pus. A chronic inflammatory process of mucous membranes may have serious sequelae causing pelvic inflammatory disease, infertility, or an increased risk of ectopic pregnancy. In the male urethritis sometimes involving the deeper tissues may lead to urethral stricture and abscess formation.

### EPIDEMIOLOGY

Since man is the only natural host for N. gonorrhoeae the epidemiological factors that are important in gonococcal infection relate to human behavioural factors as much as to properties of the organism. Gonorrhoea is common in developed as well as developing countries. On a global scale there are in the soft stratified squamous epithelium of the vagina of young girls; it is extremely rare.

Inflammation induced by infection by N. gonorrhoeae of the mucosal surfaces may be mild or acute with the production of pus. A chronic inflammatory process of mucous membranes may have serious sequelae causing pelvic inflammatory disease, infertility, or an increased risk of ectopic pregnancy. In the male urethritis sometimes involving the deeper tissues may lead to urethral stricture and abscess formation.

Epidemiology of antibiotic resistance

Antibiotic resistance in the gonococcal results from mutations in chromosomal genes and/or from plasmid mediated beta-lactamase production.

Fully sensitive wild strains of gonococci have penicillin minimum inhibitory concentrations (MICs) of less than 0.06 mg/l. Mutations at a series of loci on the chromosome result in small additive increases in penicillin resistance resulting in isolates with penicillin MICs of 1 mg/l or greater, levels at which currently recommended doses of penicillin become ineffective. These levels of resistance are over 100 times greater than those which prevailed when penicillin therapy was introduced in the 1960s.

As these mutations exert their effect by altering the permeability of the gonococcal cell envelope, isolates with clinically significant levels of resistance to penicillin are likely to be relatively resistant to a range of antibiotics such as erythromycin, tetracycline and chloramphenicol. Resistance to tetracycline and chloramphenicol is controlled by specific loci as well as the non-specific loci.

Plasmid-mediated penicillin resistance

Plasmids are small circular pieces of DNA that can replicate within a bacterial cell independent of the chromosome. Gonococci totally resistant to penicillin due to a plasmid coding for a beta-lactamase (penicillinase) enzyme (PPNG) were first reported in 1976 (Phillips 1976, Ashford et al 1976).

Certain plasmids are considered endogenous because their guanine/cytosine ratio is indistinguishable from that of the chromosomal DNA of the gonococcus, viz. the 2.6 megadalton (MDa) plasmid, the 7.8 MDa plasmid and the 24.5 MDa plasmid. The 24.5 MDa plasmid occurs in about 7-8% of gonococcal strains although it may be as high as 40% in strains originating in south-east Asia.

Exogenous gonococcal plasmids include a 3.2 MDa plasmid in strains from Africa and Europe and a 4.4 MDa plasmid in strains from Asia and the United States. An apparently new plasmid of 2.9 MDa was described by van Embden et al (1985).

In London the prevalence of PPNG infection rose from less than 1% in 1980 to 6.5% in 1982. In 1983 there was a further increase to 8.7% but this was a far smaller rise than in the previous 2 years. In 1984 the prevalence of PPNG decreased to 6.5% (Eason 1985).

Early diagnosis, effective therapy and intensive contact tracing have undoubtedly contributed to the decrease in PPNG infections. It may also be, however, that in the absence of selective pressure, such as that provided by uncontrolled penicillin usage, PPNG isolates may have a diminished capacity to compete with non-PPNG.

Epidemiological markers (typing of gonococci)

Typing (e.g. autotyping, serogrouping and serotyping) of gonococci is important in epidemiological studies. Monoclonal antibody serotyping, although still essentially in the developmental stage, is likely to become the most widely used method of strain differentiation (Bygdeman 1987). Serovar determination has also proved useful in recognizing multiple infection and differentiating between reinfection and treatment failure.
Serovar analysis in medicolegal cases

Serovar analysis can also be used in medicolegal cases. In one instance a 2-year-old girl was infected with a serogroup WII strain of serovar combination Bcgik/Bopys. Of the two adult men suspected to be the source of the infection one was infected with a strain of the same serovar combination whereas the other was infected with a totally different serogroup of serovar combination Aedgkh/Aro. As the serovar combination Bcgik/Bopys was seen in only 2% of strains from the same city there was a high probability that the girl had acquired her infection from the man with the identical strain (Bygdeman 1987).

DIAGNOSIS OF GONORRHOEA: LABORATORY AND CLINICAL PROCEDURES

Microbiological tests are mandatory in making a diagnosis of gonorrhoea. Because of the short incubation period and high infectivity, rapid diagnosis followed by immediate treatment and contact tracing are important in the control of infection within the community.

Neisseria gonorrhoeae is a very fastidious organism and very careful techniques are necessary for the collection of specimens and their transport to the laboratory for culture and investigation. Ideally the patient is seen at a clinic with an adjacent or closely sited laboratory. Under these conditions the majority of infected patients (about 90–95% of males and 50–60% of females) can receive appropriate effective treatment on the first attendance after examination of Gram-stained smears. Cultural diagnosis of additional cases and confirmation of smear positive cases can be made within 24–72 h.

Specimens required for bacteriological examination

Microscopy

This enables a presumptive diagnosis to be made in the clinic so that appropriate treatment can be given immediately. Immediate treatment facilitates the prevention of spread of infection and progression of the disease to more serious sequelae, particularly in those patients likely to default.

A smear of secretion or discharge is prepared, dried, fixed by gentle heating and Gram-stained by standard bacteriological technique (Duguid et al 1987) but 0.1% neutral red is the preferred counterstain. The stained and dried slide is examined under a 2 mm oil immersion objective lens.

Specimens from males

In males, material for examination is obtained by inserting a sterile bacteriological loop into the everted urethral meatus and gently scraping the walls of the terminal part of the urethra. A loopful or less of the exudate obtained may be examined by microscopy of a Gram-stained smear and by culture. If anal intercourse is acknowledged or suspected a cotton wool tipped applicator stick should be passed gently and blindly into the anal canal to a distance of 5 cm; a proctoscope should be passed and if mucopus is seen it may be examined microscopically and by culture. If there has been orogenital contact with a person who may possibly have been infected, material should be obtained for culture from the tonsillar crypts or bed and pharynx.

Specimens from females

In female patients specimens for cultural investigation should be taken from the urethra (traditionally specimens are taken from the urethra after massaging from above downwards to expel any discharge from the para-urethral glands), external cervical os and cervical canal, rectum and throat. If pus is expressed from the orifice(s) of the ducts of the greater vestibular glands, this should be similarly examined. Smears should be taken from the urethra and endocervix for microscopic examination after Gram staining.

In prepubertal children vaginal cultures may be obtained uncontaminated by organisms at the introitus by gentle insertion of an auriscope or a suitable nasal speculum (e.g. Killian’s Nasal Speculum 2–2½", Downs Surgical plc, Church Path, Mitcham, Surrey CR4 3UE, England); a swab is then passed along the speculum to sample the vaginal contents. Anorectal and pharyngeal cultures should also be taken.

Tests in disseminated gonococcal infection

When disseminated gonococcal infection (DGI) is suspected the routine tests described and several blood cultures should be taken before commencing therapy. It is most important to inform the laboratory that DGI is a possible diagnosis. Fluid obtained by aspiration of a joint effusion should be similarly investigated. Although patients with suspected DGI may have no genital symptoms it is important to take anogenital (and pharyngeal) cultures as these are most likely to yield gonococci (Barr & Danielsson 1971).

Importance of culture site and number of diagnostic tests

Repeated testing of multiple sites is necessary since not all infections in women will be detected on first attendance. The efficiency of detecting gonorrhoea varies depending on factors such as the culture medium used. Ordinarily in the female two consecutive culture tests will be taken from the endocervix (and if possible the urethra), cervix and anorectum.

A ‘high vaginal swab’ in the adult female is totally inadequate for diagnosing or excluding gonorrhoea. Rectal cultures are important in the female and particularly so in screening for infection and assessing effectiveness of treatment.

If pharyngeal or anorectal infection is suspected two consecutive tests are advised in this site. Heterosexual men and women with pharyngeal gonorrhoea require higher penicillin dosage than those with uncomplicated infection. In all patients it is important to know which sites are infected in order that all infected sites can be sampled to assess the efficacy of treatment.

Culture

Immediate diagnosis must be supplemented as well as confirmed by culture if the maximum number of positive results is to be obtained. Cultures are obligatory in the diagnosis of rectal, oral, disseminated and asymptomatic infections in both sexes, and are also essential in order to determine antibiotic sensitivities and to assess treatment efficiency. Cultures are advisable when medicolegal issues require special consideration.

Culture media

There are many variations of media in use but laboratories will
find the modified New York City (MNYC) medium useful (Young 1981).

A combination of a selective and non-selective medium has been recommended to ensure the detection of gonococci with markedly increased susceptibility to antibiotics or in sampling sites such as joint fluid or blood which are normally sterile, as well as vancomycin sensitive gonococci.

Transport and culture systems

As the gonococcus is very susceptible to drying dry swabs should never be used. Best results are achieved by direct plating. Culture plates, preferably warmed beforehand to 37°, are inoculated directly with patient's secretions and immediately incubated at 36–37°C in a moist atmosphere containing 5–10% carbon dioxide.

When direct plating and immediate incubation are impracticable transport media should be used. Amies' medium is more effective than Stuart's at maintaining gonococcal viability (Human & Jones 1986) and should be used more widely. Nutrient transport and culture systems are expensive and seem to offer little advantage when the transit time is about 3–4 h.

Identification

After 24 h of incubation, plates are examined and any colonies suspected as being gonococcal are tested by the cytochrome oxidase test. In negative cultures incubation is continued for 48 h and the plate re-examined before the culture can be reported as negative. Bacteriological techniques include examination of Gram-stained smear from colony.

A definitive diagnosis has traditionally been made by carbohydrate utilization tests. In the rapid carbohydrate utilization test (RCUT) preformed enzyme is measured by adding a suspension of the overnight growth of the suspect organism to a buffered, non-nutrient solution containing the sugar to be tested and a pH indicator (Young et al 1976). Apart from rapidity, this enables identification of other neisseriae and confirmation of *N. gonorrhoeae* to be made within 24–72 h of seeing the patient.

Antibiotic sensitivity tests

Once the gonococcus has been fully identified, antibiotic sensitivity tests are carried out.

Detection of penicillinase-producing *N. gonorrhoeae* (PPNG)

This is the most important aspect of sensitivity testing and it is best to screen all isolates as soon as possible. One of the most sensitive and convenient methods uses commercially available paper strips impregnated with a chromogenic cephalosporin. When a few PPNG colonies are rubbed onto a test strip moistened with water the resulting hydrolysis of the beta-lactamase ring causes a purple colour to form around the area where the organisms were added; the test takes a few minutes (Young 1978).

Minimum inhibitory concentration (MIC)

Since the majority of patients with gonococcal infection will have been treated on the basis of a positive smear, antibiotic tests (other than those to detect penicillinase) are of little help in the initial management of the patient. Nevertheless, they are import

ant for epidemiological purposes and in planning rational therapy for use in the geographical area concerned.

Detection of antigen

The Gonozyme (Abbott Laboratories Ltd, Wokingham, Berkshire, England) test is currently the only commercial antigen detection system widely available and compares well with culture for specimens from men with urethritis giving a sensitivity range of 94–100% and a specificity range of 96–100% (Young & Reid 1987). The test is not recommended for rectal or pharyngeal specimens.

Possible alternative approaches to the diagnosis of gonococcal infection in places where laboratory resources are absent or limited have been usefully defined in a WHO technical report (World Health Organization 1978b).

CLINICAL FEATURES

The clinical features of gonorrhoea reflect the inflammatory changes induced by infection of mucosal surfaces by *Neisseria gonorrhoeae*; in some cases the inflammation may be so mild that the patient is unaware of being infected. A chronic inflammatory process of mucous membranes may have serious sequelae, however, particularly in women in whom infertility or an increased risk of ectopic pregnancy may result. An infrequent but serious complication is systemic dissemination of the organism.

Clinical features in infants and children under the age of puberty

*Purulent conjunctivitis of the newborn (gonococcal conjunctivitis of the newborn)*

The chief cause of a purulent conjunctivitis of the newborn (in the past generally referred to as 'ophthalmia neonatorum') in developed countries before the antibiotic era was *N. gonorrhoeae*, but now in such parts of the world it is more commonly caused by other organisms including *Chlamydia trachomatis* (Hobson et al 1983).
Fig. 22.33 Untreated gonococcal conjunctivitis in a 3-week-old infant. The cornea is opaque and a peripheral ulcer has developed which has perforated the cornea. The iris can be seen forming a plug at the base of the ulcer. (From World Health Organization 1986c, with permission.)

Gonococcal conjunctivitis (Fig. 22.32) generally manifests itself within 1–13 days after birth, usually by the third day. Commencing with hyperaemia and shedding of tears, a mucopurulent, sometimes blood-stained, discharge appears about a day later. The eyelids swell and profuse pus collects often under pressure in the conjunctival sac. The palpebral and bulbar conjunctiva become oedematous. If untreated corneal involvement first shows as a hazy greyish appearance due to a diffuse epithelial oedema. Opacities appear near the border of the cornea and sclera. Ulceration of the centre of the cornea (Fig. 22.33) may develop and proceed to perforation of the eyeball with loss of vision. If untreated gonococcal infection of the newborn may be associated with extracocular manifestations such as arthritis and septicaemia (World Health Organization 1986c).

Acute vulvo vaginitis in children
The parents usually notice discharge on the child's underwear and on examination a purulent vaginal discharge, with reddening and oedema of the vulva, may be found. It is usually accompanied by a gonococcal urethritis. Pelvic inflammatory disease may be seen in 60% of female children with gonorrhoea.

Oropharyngeal and rectal infection
Workers in the United States of America have described the occurrence of gonorrhoea in these sites in a number of children from lower socio-economic groups (Nelson et al. 1976) and victims of sexual abuse. Mostly infections of the pharynx and anorectum are asymptomatic (Groothuis et al. 1983, White et al. 1983).

Clinical features in the adolescent male (uncomplicated gonococcal infection)

Urethral infection
The patient complains of urethral discharge and an often mild dysuria in about 90% of cases. If infection has spread proximally to the posterior urethra there may be symptoms of frequency of micturition, urgency and painful erections. Clinical examination may reveal a reddened urethral meatus with a purulent or mucopurulent discharge. Inguinal lymph nodes may be enlarged on both sides. Examination of the urine by the two glass test will show pus in the first glass if the anterior urethra is mainly affected, or in both glasses if the posterior urethra and/or bladder is involved. Threads (cellular casts from mucus secreting submucosal glands) may be found in the urine. A considerable number (possibly as many as 15% in some localities) of males with urethral gonorrhoea have few symptoms if any (Neilsen et al. 1975).

Post-gonococcal urethritis may occur in at least 20% of cases of gonorrhoea in males adequately treated with penicillin.

Oropharyngeal infection
Infection of the pharynx by the gonococcus may produce symptoms in only about 20% of cases (Stolz & Schuller 1974), when there may be sore throat, perhaps with referred pain in the ear. Clinical examination may reveal no abnormalities, or a mild pharyngitis or tonsillitis.

Anorectal infection
Infection in this site in the male is invariably the result of a homosexual act. The majority of patients (more than two-thirds) with anorectal gonorrhoea have no symptoms of infection. In others there may be a history of pruritus and mucoid or mucopurulent anal discharge, anal pain, bleeding and tenesmus.

Proctoscopic examination may show a normal appearance, or there may be either patchy or generalized erythema of the rectal mucosa with mucopus in the lumen of the anal canal and rectum. The histology is that of a non-specific proctitis (McMillan et al. 1983).

Local complications of untreated anorectal infection include perianal and ischiorectal abscesses and anal fissures.

Local complications of gonorrhoea in the male
Inflammation and abscess formation may occur in the parafrenal glands (Tyson's glands). As the gonococcus tends not to attack the squamous epithelium of the glans penis balanitis is uncommon. Inflammation may also affect and cause abscesses in the para-urethral glands on either side of the urethral meatus. When medical help is delayed periurethral cellulitis may develop. Although rare in developed countries urethral strictures and fistulae as late complications are common in tropical countries (Osoba & Alausa 1976).

Abscess affecting the bulbourethral glands (Cowper's glands) is uncommon in countries with good medical services. The patients complain of fever, throbbing pain in the perineum, painful defaecation and frequency of micturition. Reflex spasm of the sphencter urethra may produce acute retention of urine. An abscess, which is usually unilateral, may point in the perineum.

Epididymitis is usually unilateral, when the patient complains of a painful swollen testis. On examination there may be erythema of the scrotum on the affected side; the epididymis is enlarged and tender; and there is often a secondary hydrocele. Inflammation of the testis itself is rare.

Infection of the median raphe of the penis is rare but when it occurs a bead of pus may be expressed from a duct opening on to the skin on the ventral surface of the penis.
Clinical features in the adolescent female (uncomplicated gonococcal infection)

In most cases females with gonorrhoea (70% or more) have few, if any, symptoms. They may occasionally complain of vaginal discharge, but this may be attributable to concomitant vaginitis caused by Trichomonas vaginalis. Uncommonly, inflammation of the trigone of the bladder produces urinary frequency. The sites infected in the uncomplicated cases are: cervix 85-90%, urethra 65-75%, rectum 25-50%, oropharynx 5-15%. The affected cervix may appear normal on inspection, or there may be signs of inflammation with mucopus exuding from the external os. There may be no clinical evidence of urethritis but occasionally pus may be expressed from the orifice. Rectal gonorrhoea in the female, as in the male, usually produces few symptoms. Oropharyngeal gonorrhoea in the female results from fellatio and the features are similar to those in the male.

Local complications of gonorrhoea in the female

Inflammation and abscess formation may affect the para-urethral glands including those lying externally on either side of the external meatus (Skene's glands), as well as greater vestibular glands (Bartholinits and Bartholin's abscess). The glands may be involved on one or both sides. There may be few symptoms of Bartholinits but, in the routine examination, on compressing the gland, pus may be expressed from the orifice of the duct. When an abscess forms, the patient may complain of pain in the vulva, and examination reveals a tender cystic swelling of the posterior half of the labium majus, the skin of which may be reddened. In less acute and partially treated cases a chronic inflammation may result, causing palpable thickening of the glands.

Disseminated gonococcal infection, involving both sexes

This uncommon complication, occurring in less than 1% of cases, is usually seen in women and in homosexual males in whom the infection has been asymptomatic and untreated (Graber et al 1960). Dissemination may occur from any infected site and more often during or just after menstruation and in pregnancy.

The clinical manifestations of disseminated gonococcal infection usually take the form of fever, rash and arthralgia or arthritis. The spectrum of clinical features of this complication is fairly broad but two forms possibly represent successive stages of the disease.

In the initial bacteriemic stage or form, symptoms are usually of short duration, the patient complaining of fever, rigors, joint pains and perhaps a skin rash. There are characteristic skin lesions and polyarticular arthritis involving usually the knees, wrists, small joints of the hands, ankles and elbows, without sufficient joint effusion present to allow aspiration. If obtained, the fluid from joints is sterile on culture but blood cultures are often positive for N. gonorrhoeae (Holmes et al 1971) if taken within 2 days of onset of the illness. There may be a tenosynovitis.

In the second form, involvement of one joint is usual, a considerable effusion is present and N. gonorrhoeae may be recoverable from the synovial fluid, which contains many polymorphonuclear neutrophil leucocytes. This form, sometimes called the 'septic joint stage', occurs usually after symptoms have been present for at least 4 days. A large joint, especially of the upper limb, tends to be affected, e.g. shoulder or elbow. The sternoclavicular or temporomandibular joint may also be affected. Systemic features are usually milder than in the bacteriæmic form, skin lesions are seldom found and blood cultures are usually negative for N. gonorrhoeae.

Intermediate stages of the disease may be seen and in the 'septic joint stage', if untreated the articular surfaces of the joint may be destroyed and fibrous or bony ankylosis may follow.

The skin lesions in disseminated gonococcal infection

These are usually associated with constitutional disturbance, including fever and polyarthritis. There are essentially two types of skin lesion (Ackerman et al 1965):

1. Haemorrhagic lesions
2. Vesiculopapular lesions on an erythematous base.

Both types of lesion begin as erythematous macules but in the haemorrhagic type the lesions become purpuric, especially on the palms and soles. In the second type lesions become purpuric and progress through vesicles to pustules.

The lesions of a disseminated gonococcal infection are clinically and histologically similar to those seen in meningococcal septicaemia.

Meningitis, endocarditis and pericarditis

Meningitis is an uncommon manifestation of disseminated gonococcal infection and is usually found associated with arthritis and dermatitis (Holmes et al 1971). Gonococcal endocarditis is also a rare but often lethal complication (John et al 1977).

Hepatitis

Hepatitis may occur following the bacteriaemia of disseminated gonococcal infection (Holmes et al 1971).

Perihepatitis

In both gonococcal and chlamydial infection in women acute perihepatitis usually occurs in association with pelvic inflammatory disease. An account of the condition is given more fully in the section on chlamydial infection (p. 1488). This complication is very rarely found in men (Kimball & Knee 1970). The patient complains of pain in the right hypochondrium and sometimes in the right shoulder from irritation of the right side of the diaphragm.

Acute pelvic inflammatory disease (PID)

The symptoms of acute PID usually occur during or shortly after menstruation or in the puerperium.

The patient complains usually of lower abdominal pain, often exacerbated by movement of the psoas muscle, fever, rigors, malaise, anorexia and vomiting. With the increased use of laparoscopy, it has become apparent that whilst abdominal pain is the most reliable symptom, it may be minimal in at least 5% of cases (Jacobson & Westrom 1969). Pyrexia (temperature of 38°C or greater) may be found in only about two-thirds of women with acute PID, and more commonly in cases due to N. gonorrhoeae (McCormack et al 1977).

There is usually tenderness and a variable degree of muscular guarding over the lower abdomen. Pain is elicited by moving the
cervix during bimanual examination. Palpation of the uterus and tubes is usually impossible on account of tenderness and guarding.

In about half the cases of acute PID, the white cell count is elevated (Falk 1965). The erythrocyte sedimentation rate is raised in about 75 of cases (Jacobson & Westrom 1969), especially when the salpingitis is associated with gonorrhoea (McCormack et al 1977).

Paralytic ileus presenting with abdominal distension and vomiting may occur in 1% of cases. In such cases fluid levels are noted on the plain X-ray film of the abdomen, taken with the patient in an upright position.

**Chronic pelvic inflammatory disease**

Chronic PID may be asymptomatic and undiscovered until the patient is investigated for infertility. Symptoms, when they occur, consist of intermittent lower abdominal pain or discomfort, discomfort in the groins, backache, malaise and frequent heavy menstrual periods. The tubes may not be palpable, or they may be irregularly thickened; the uterus may be retroverted and fixed.

For a fuller discussion on the subject of pelvic inflammatory disease and its treatment the reader is referred to a general text (Robertson et al 1989).

**Gonococcal conjunctivitis**

This is rare except in the newborn and presents as a purulent conjunctivitis affecting one or both eyes. If untreated, keratitis or panophthalmitis with blindness may result.

## TREATMENT OF GONORRHOEA

Since its introduction into medical practice in 1944, penicillin has been widely and successfully used in the treatment of gonorrhoea and more recently, semisynthetic penicillins and other antimicrobial agents have been added to the therapeutic armamentarium. Although the proportion of strains with reduced sensitivity to penicillin has been rising slowly since the 1960s, the problem of resistance to penicillin has become more ominous with the recent discovery of beta-lactamase-producing strains.

### Principles of treatment

The aim of treatment is to eradicate the organism from the body as quickly as possible. Ideally the treatment used should be based on the pattern of sensitivity to antibiotic and chemotherapeutic agents observed amongst the strains of the organism in the population served. Regimens of treatment should be constantly reviewed, account being taken of the results of continuous monitoring of isolates for the emergence of drug resistance.

A course of treatment with almost any antimicrobial drug to which the organism is sensitive will cure the majority of patients with gonorrhoea. Patient compliance, however, is often unsatisfactory and tablets may be inadvisably shared with a consort.

For these reasons a single large dose of antibiotic, given under supervision either orally or parenterally, is preferred as treatment of uncomplicated infection. In most cases blood and tissue concentrations of drug reach a high level and are maintained for sufficient time to eradicate the organism. Oral administration of antibiotics is preferred to intramuscular injections which are not only painful but more liable to cause hypersensitivity reactions. Single dose therapy has not proved satisfactory, however, in the treatment of oropharyngeal, male anorectal or complicated gonococcal infections.

**Benzylpenicillin and its parenteral use**

In UK practice benzylpenicillin (synonym: penicillin G), freshly

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**Table 22.33** Treatments available for uncomplicated genital gonorrhoea in adults or young persons of more than 50 kg weight or past puberty together with suggested dosage in children (as total daily dosage and number of divided doses) in localities where the prevalence of beta-lactamase-producing N. gonorrhoeae (PPNG) is low

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Single dosage for adults or young persons as specified*</th>
<th>Suggested dosage in children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily dose Nos of divided doses</td>
<td>0-4 weeks</td>
</tr>
<tr>
<td>Amoxicillin (R)</td>
<td>60 mg/kg 2</td>
<td>2</td>
</tr>
<tr>
<td>Aminocillin (R)</td>
<td>60 mg/kg 2</td>
<td>2</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>100 mg/kg (i.m. slow i.v. or oral) 2</td>
<td>60 mg/kg (i.m. slow i.v.) 4</td>
</tr>
<tr>
<td>Spectinomycin (H)</td>
<td>2 g i.m.</td>
<td>Not recommended for children or growing adolescents</td>
</tr>
<tr>
<td>Ciprofloxacin (H)</td>
<td>250 mg (oral)</td>
<td></td>
</tr>
<tr>
<td>Co-trimoxazole (H)</td>
<td>Not suitable</td>
<td>8 mg/kg (oral) 2</td>
</tr>
</tbody>
</table>

*With 1 g probenecid by mouth.

(R) = recommended regimens for those not hypersensitive to penicillin; (H) = treatment suitable for those who are hypersensitive to penicillin.
prepared in aqueous solution for parenteral use, is frequently given by intramuscular injection at regular intervals (2-4 times daily as a rule), and is also given by (the less painful) slow intravenous injection or infusion; intrathecal injection is not recommended (British National Formulary, No. 17, 1989, pp. 198-199). Intravenous use of benzylpenicillin in doses for the adult above 1.2 g (2 million units) should be administered slowly at not more than 300 mg/min to avoid irritation of the central nervous system (Reynolds 1985).

Treatment schedules

Uncomplicated infections

With *N. gonorrhoeae* (not beta-lactamase producing strains, viz. *non-PPNG*). Regimens for single dose treatment currently used in the treatment of uncomplicated gonorrhoea in adults together with suggested dosage for children are given in Table 22.33. In children the dosage should be given according to weight until 50 kg or puberty is reached. With *beta-lactamase producing N. gonorrhoeae (PPNG)*. Some antimicrobial agents, available for the treatment of PPNG infections, are shown in Table 22.34. Patients with uncomplicated genital infection should be treated with one of these:

1. If their infection is known to be caused by PPNG
2. If they are the sexual partners of such patients
3. If individuals have acquired their infection in areas of the world where the prevalence of PPNG is known or suspected to be high.

With *chromosomally mediated resistant N. gonorrhoeae (CMRNG)*. Patients (adults or those over 50 kg weight or after puberty) who fail with standard treatments or who are infected with penicillin resistant strains that do not produce beta-lactamase (CMRNG) may be treated with spectinomycin 2.0 g intramuscularly or ceftriaxone 250 mg intramuscularly (CDC 1985).

With the high in vitro resistance of gonococcal strains isolated in Bangkok to a number of antimicrobials (penicillin, tetracycline, erythromycin, trimethoprim/sulphamethoxazole, kanamycin, spectinomycin, chloramphenicol) it has been considered that the adaptation of the gonococcus to antimicrobial selective pressures may mean that single drug treatments of the condition are no longer appropriate and 'combination therapies' need to be evaluated to delay further antimicrobial resistance. The regimen

Table 22.34 Some antimicrobial agents available for the treatment of uncomplicated genital gonococcal infections caused by beta-lactamase-producing *Neisseria gonorrhoeae* (PPNG). Single dose treatment is given for adults and young persons of more than 50 kg in weight or past puberty as well as suggested dosage in children (given as total daily dose and number of divided doses). Infections in neonates and babies (up to 3 months) are considered in the text.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Single dosage in adult or young person as specified</th>
<th>Suggested dosage in children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-4 weeks</td>
<td>4 weeks - 12 months</td>
</tr>
<tr>
<td></td>
<td>Daily dose Nos of divided doses</td>
<td>Daily dose Nos of divided doses</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>2.0 g i.m.</td>
<td>Not suitable Nos of divided doses</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>1.5 g i.m. (2 doses of 750 mg into different sites, e.g. each buttock)</td>
<td>60 mg/kg/day i.v. or i.m. 2</td>
</tr>
<tr>
<td>Cefuroxime axetil ester</td>
<td>1.0 g oral</td>
<td>Not suitable Nos of divided doses</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1.0 g oral</td>
<td>Not suitable Nos of divided doses</td>
</tr>
<tr>
<td>Amoxycillin 250 mg Clavulanic acid 125 mg (Augmentin)</td>
<td>1-2 tablets three times daily by mouth No suitable oral prep.</td>
<td>Half-strength Augmentin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Augmentin Paediatric Suspension 15.0 ml (31 mg clavulanic acid + 125 mg amoxycillin 5 ml) (for 3-12 months only)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Augmentin 3 Jr Susp        15 ml (62 mg clavulanic acid + 125 mg amoxycillin 5 ml)</td>
</tr>
</tbody>
</table>

Amoxycillin 2.5 g + Augmentin 2 tablets (clavulanic acid 250 mg with amoxycillin 500 mg) + probenecid 1 g (effective as single dose)
urethritis (PGU)

Postgonococcal urethritis (PGU)

Single dose treatment, as given for uncomplicated genital gonorrhoea in adults, is generally considered to be less effective in the treatment of oropharyngeal infection in both sexes and in rectal infection in the male (Scott & Stone 1966, Odegaard & Gundersen 1973). Infection in these sites usually can be eliminated by a course of antibiotics given by mouth; either ampicillin or tetracycline (250 mg) may be given for five days; or five days plus 5-10 days (e.g. ciprofloxacin 250 mg) may be curative in rectal gonorrhoea in homosexual men (McMillan A 1985 Unpublished data).

Pharyngeal infection with PPNG has been treated successfully with cefuroxime in a dosage of 1.5 g daily by intramuscular injection for 3 days (Lindberg et al 1982). Ceftriaxone 250 mg intramuscularly once is also recommended for PPNG infections of this anatomical site (CDC 1985). Cotrimoxazole has been accorded by the Centers for Disease Control, USA, a special role for the treatment of pharyngeal infections due to PPNG (CDC 1985) but it should be remembered that cotrimoxazole is especially liable to induce cutaneous reactions in patients with the acquired immune deficiency syndrome whether or not they have Kaposis’s sarcoma (Idanpaan-Heikkila & Tuomista 1985) and that its toxic effects are at least equal to those of sulphonamides (Mandell & Sander 1985).

Postgonococcal urethritis (PGU) in the male

This complication may follow the apparent cure of gonococcal urethritis in the male and may be due to an associated chlamydial infection. In an attempt to reduce the incidence of postgonococcal urethritis, a 5-7 day course of erythromycin may be given starting on the day after giving treatment for gonorrhoea, to avoid the mutually antagonistic effects of penicillin and tetracycline. In children under the age of 12 years tetracyclines should not be given.

Complicated gonorrhoea

Epididymitis. Epididymitis necessitates rest, scrotal support and courses of the appropriate antimicrobial for 5–10 days (e.g. cotrimoxazole or doxycycline). The treatment of epididymitis caused by PPNG has not been adequately assessed but cefuroxime is advised.

Bartholinitis and abscess. The patient may need admission to hospital if the inflammation is severe and she should be treated with benzylpenicillin in the dosage given for epididymitis. In less severe cases there is usually a response to a course of oral antibiotics. If an abscess persists, this is best treated by aspiration during antimicrobial therapy or by marsupialization if this fails.

Pelvic inflammatory disease including salpingitis. Reference has already been made to pelvic inflammatory disease (p. 1484). For a fuller discussion of this subject and its treatment the reader is referred to a general text (Robertson et al 1989).

Disseminated gonococcal infection (DGI) with or without arthritis and/or skin lesions. The patient should be admitted to hospital, the affected joint(s) rested in a position of function and treatment with benzylpenicillin 600 mg (one million units) 6-hourly intramuscularly instituted. Usually the condition of the patient improves within 48 h and oral treatment may be substituted and continued for 10–14 days. In such cases the gonococci have been found to be very sensitive to penicillin. In the case of those allergic to penicillin, cotrimoxazole may be suitable in a dose of two tablets twice daily by mouth. When vomiting is troublesome cotrimoxazole may be given, diluted as an infusion, intravenously. For children over 12 years two 5 ml vials of cotrimoxazole (e.g. Bactrim for infusion, Roche; Septrin for infusion, Wellcome), each containing 80 mg trimethoprim BP and 400 mg sulphamethoxazole BP are diluted with 250 ml infusion solution (e.g. sodium chloride injection BP 0.9%) and given intravenously over a period of approximately 1.5 h. For children up to 12 years the recommended dosage is 6 mg trimethoprim and 30 mg sulphamethoxazole/kg body weight per 24 h divided into two equal doses. As a guide septrin for infusion may be given as follows: 6 weeks–6 months: 1.25 ml twice daily; 6 months–6 years: 2.5 ml twice daily; 6–12 years: 5.0 ml twice daily (ABPI Data Sheet Compendium, 1989–1990, Datapharm, London).

For the treatment of disseminated gonococcal infection due to beta-lactamase producing N. gonorrhoeae (PPNG) a number of alternative treatments are available and given for 7 days:

1. Cefuroxime 750 mg (for adolescents) given by intramuscular injection 6-hourly for 7 days (see Table 22.34 for suggested daily dosage in children).
2. Cefotaxime 500 mg (for adolescents) given by intravenous injection 6-hourly for at least 7 days (CDC 1985) (see Table 22.34 for suggested daily dosage in children).
3. Ceftriaxone 1.0 g given by intravenous injection once daily for 7 days in adults (CDC 1985).

Following these schedules of treatment it is often essential to deal with a possibly coexistent chlamydial infection by giving after wards the following:

1. Doxycycline 200 mg initially by mouth after food followed by 100 mg once daily for 7 days. Tetracycline, oxytetracycline and erythromycin may be given alternatively for 7 day courses.
2. In children less than 12 years of age erythromycin is used as tetracycline and doxycycline are not indicated.

Endocarditis and meningitis

In the preantibiotic era 4–10% of all cases of endocarditis were due to Neisseria gonorrhoeae (John et al 1977) and in one series of 38 cases encountered over a 12-year period 10 (26%) infections were due to this organism (Williams 1938). Since deterioration may occur rapidly in endocarditis despite apparently appropriate antibiotic treatment, and delay in valve replacement therapy may prove fatal, it is strongly recommended that such patients should be referred early for expert management by a cardiologist and a cardiac surgeon (Working Party of the British Society for Antimicrobial Chemotherapy 1985).

In meningitis treatment with intravenous penicillin should continue for 2 weeks.

Treatment of pregnant women with gonorrhoea

Care is needed in giving any drug in pregnancy. Penicillin is con-
sidered generally safe, and benzylpenicillin given intramuscularly in a dose of one million units 6-hourly for 12 doses is effective. Erythromycin is also considered to be safe although cure rates may be low, say 70% (Brown 1978), and absorption uncertain; a dose of 500 mg erythromycin base 6-hourly orally for 7 days is an alternative in patients hypersensitive to penicillin.

Ceftriaxone 250 mg (for adults) intramuscularly as a single dose will be necessary for PPNG infections. Pregnant women who are allergic to penicillin, cephalosporins or probenecid should be treated with spectinomycin 2.0 g intramuscularly as a single dose and be given erythromycin (base) 500 mg by mouth four times daily for 7 days (CDC 1985).

**Gonococcal conjunctivitis of the newborn (ophthalmia neonatorum)**

Also see section on chlamydial infection (p. 1489).

**PROPHYLAXIS**

Systematic detection and treatment of mothers infected with gonorrhea is a goal not attainable in many areas of the world and prophylactic measures to the newborn child are the only means of reducing the incidence of this potentially blinding disease. Although none of the presently recommended approaches for prophylaxis against gonococcal or chlamydial neonatal conjunctivitis (ophthalmia neonatorum) is completely satisfactory tetracycline hydrochloride (1%) eye ointment appears to be effective when applied after careful cleaning of the babies eyes after birth. It should be instilled into the conjunctival sac of all neonates as soon as possible after birth. Erythromycin 0.5% ophthalmic ointment — not widely available — is an alternative. Conjunctival sac instillation of 1% silver nitrate (CDC 1985) has been used for more than a century (Carl Siegmund Crede, Leipzig, published his method in 1881 — Forbes & Forbes 1971). The latter frequently causes a chemical conjunctivitis and will not protect against chlamydial infection. In areas of the world where gonococcal infections are very uncommon prophylaxis has been replaced by good primary care systems, good antenatal care and in the neonate early diagnosis and specific therapy. The prophylaxis of gonococcal conjunctivitis should not be abandoned prematurely (World Health Organization 1986c).

Combined local and parenteral therapy is necessary.

1. Local: frequent, repeated instillations of sterile normal saline into affected eye. Topical antibiotic treatment may produce sensitization reactions and should on this account be avoided.

2. Parenteral: benzylpenicillin should be given in a dosage of 30 mg/kg body weight per day in two or three divided doses by intramuscular injection until cure is obtained, generally within a week (McCracken & Eichenwald 1974).

For the treatment of neonatal conjunctivitis associated with PPNG cefuroxime in a dosage of 100 mg/kg/day by intramuscular injection in three divided doses for 7 days has proved useful (Dunlop et al 1980). In developing countries where hospital facilities are limited, kanamycin given as a single intramuscular injection of 75 mg or 150 mg given with gentamicin eye ointment (1% w/v) applied every 30 min for the first 10 h and then four times per day (Fransen et al 1984) for 3 days is effective in treating infection with PPNG and non-PPNG. Possible ototoxicity of kanamycin, however, must be taken into account.

Both parents must be examined and treated appropriately.

**TESTS OF CURE IN GONORRHOEA**

After treatment every patient should be carefully examined to ensure that the infection has been cured. Culture is necessary in men when urethritis persists. In women at least two consecutive tests of cure by culture of the urethra, cervix and rectum should be carried out.

**TREATMENT WHEN DIAGNOSIS OF GONORRHOEA IS SUSPECTED ON EPIDEMIOLOGICAL GROUNDS**

Accurate diagnosis and treatment is the approach of choice when adequate facilities exist. Treatment before diagnosis is not desirable as a routine even in contacts, except when there are special problems. For example a female contact of a known case of gonorrhoea may be treated if she is unlikely to reattend. In such a case a form of treatment known to produce high cure rates in that locality (say of 95%) may be justified. In the case of contacts of patients with an infection due to PPNG then treatment with spectinomycin or cephalosporin is justified. Follow-up is important.

**CHLAMYDIAL AND NON-SPECIFIC GENITAL INFECTIONS**

Although the aetiology of non-specific genital infections, particularly non-specific urethritis in men, is not wholly resolved chlamydial infection plays an important part. From the paediatric point of view Chlamydia is the main agent to consider and the possible roles of Ureaplasma urealyticum and Mycoplasma spp. remain controversial (Robertson et al 1989).

**CHLAMYDIA**

Organisms belonging to the genus *Chlamydia* are the cause of ocular, genital and systemic diseases in man. Three species are recognized, viz. *Chlamydia trachomatis*, *Chlamydia psittaci* and TWAR Chlamydia: TWAR chlamydiae (TW, Taiwan; ARS. acute respiratory) have recently been recognized as separate, more closely resembling *C. psittaci* than *C. trachomatis*, and may form a third species tentatively named *C. pneumoniae*. These are obligate intracellular parasites which cannot by themselves synthesize the high energy compounds adenosine triphosphate (ATP) and guanosine triphosphate (GTP) and multiply by binary fission. Now regarded as Gram-negative bacteria, *Chlamydia* contain DNA and RNA, possess enzymes, contain cell wall material, have a developmental cycle and share common antigens. *C. trachomatis* forms glycogen-containing inclusions that can be stained with iodine. DNA homology within *C. trachomatis* strains lies between 96% and 100% (Kingsbury & Weiss 1968, Becker 1978) and supports their classification as a single species. In so far as sexually transmitted diseases are concerned, *C. trachomatis* is the most important of the three species. Of the three biovars of this species known, one is found as a latent infection in mice and two which concern man have no animal reservoir, viz. *C. trachomatis biovar trachomatis* and *C. trachomatis biovar lymphogranuloma*. All chlamydiae, unique among prokaryotic cells, undergo a developmental cycle in the cytoplasm of eukaryotic cells (Ward...
1983) and the tendency of these to produce persistent infection is characteristic.

Pathology of chlamydial infection

In conjunctivitis due to chlamydiae the formation of a follicle commonly occurs, but this is a tissue reaction to irritation and is not specific. In trachoma, cellular inclusions were first described in 1907 by Halberstaedter & von Prowazek (1910). In histological sections of cervical mucosa similar inclusions appear as cytoplasmic vacuoles when examined by light microscopy and are most easily seen in columnar endocervical cells. The vacuoles may occupy nearly the entire volume of the cell and, on electron microscopy, are seen to contain numerous small spherical bodies about 1 μm in diameter; these represent infectious elementary bodies, non-infectious reticulate bodies and transitional stages (Swanson et al 1975). The pathology of the urethra and conjunctiva is not easy to study as biopsy is not justified; urethral follicles however have been seen with the operating microscope in some men with *Chlamydia*-positive NGU (Dunlop et al 1967). Changes are not necessarily attributable wholly to chlamydiae as other organisms commonly colonise the cervix. Neutrophil polymorphonuclear leucocytes are dominant in the early stages of eye infections and are similarly found in urethritis and cervicitis.

Laboratory diagnosis

Methods for the laboratory diagnosis of chlamydial infection are summarized briefly (Evans & Woodland 1983):

1. Detection of inclusion bodies by direct microscopy are too insensitive to be of practical value
2. Detection of *Chlamydia* antigen by immunocytochemical methods. Monoclonal antibodies have been used in fluorescent antibody and ELISA methods for the detection of chlamydial antigens in conjunctival, urethral and endocervical material (Taylor et al 1984, Thomas et al 1984, Pugh et al 1985). The major advantages of immunocytochemical methods over culture are that they are simpler, quicker and a little more sensitive. Problems of transport are also circumvented. A disadvantage is that non-viable organisms are likely to be detected.
3. Culture. Isolation of *C. trachomatis* on McCoy cells, irradiated or otherwise treated to stop their replication, is a sensitive method, whereas the isolation in the yolk sac of an egg is insensitive and prone to contamination (Darougar et al 1974). As irradiation is inconvenient for some laboratories alternative methods of comparable sensitivity have been described, e.g. treatment of cells with the nucleoside analogue 5-iodo-2-deoxyuridine (IDU) or with the fungal metabolite cytochalasin B (Stirling & Richmond 1977). It should be noted that culture of *C. psittaci* should not knowingly be attempted if the laboratory is not equipped for the culture of microorganisms defined as being of category B1 (Department of Health and Social Security 1978, Evans & Woodland 1983).
4. Serology. Information on antibody responses during chlamydial infections is based largely on microimmunofluorescence (micro-IF) tests (Grayston & Wang 1975). Only a few laboratories, however, offer this test at present. A fourfold rise in titre or conversion from seronegative to seropositive correlates well with the isolation of *C. trachomatis*, but this does not provide rapid diagnosis. In the neonate, passive transfer of maternal IgG antibody makes this test unreliable and diagnosis should be based on the detection of IgM.

Disease due to *C. trachomatis* biovar *trachomatis* (tropical trachoma, serovars A, B, Ba and C)

In hyperendemic tropical trachoma, due to biovar trachoma (serovars A, B, Ba and C), the organisms are spread by eye to eye transmission particularly in unhygienic conditions, affecting about $500 \times 10^6$ people and causing blindness in some $2 \times 10^7$. *Chlamydia* require rapid transmission in moist conditions and hyperendemic trachoma occurs in conditions of ‘ocular promiscuity’ — that is to say in conditions that favour the frequent, unrestricted and indiscriminate mixing of ocular contacts or of ocular discharges (Jones 1975; the word promiscuous derives from pro — for or in favour of, *miscere* — to mix, hence promiscuous implies conditions that favour mixing).

Disease due to *C. trachomatis* biovar *trachomatis* (ocular or genital infections, serovars D, E, F, G, H, I and K)

The ocular or genital infections of western countries, on the other hand, tend to occur where there is frequent mixing of genital contacts or discharges with occasional transfer to the eye (Jones 1975). These chlamydial infections, due to serovars D–K, are predominantly genital, i.e. non-gonococcal urethritis (NGU) in men, or endocervical infection in women (Robertson et al 1989) and the incidence of both adult chlamydial conjunctivitis and conjunctivitis of the newborn, with or without involvement of other sites, e.g. nasopharynx, are dependent upon the incidence of genital infections in the adult population, and in the case of infants, in their mothers (Viswanagam et al 1983).

Although mainly genital, either alone or with *N. gonorrhoeae* in women, these organisms can cause pelvic inflammatory disease and sometimes perihepatitis in the form of the Curtis–Fitz–Hugh syndrome (Robertson et al 1989). In the latter syndrome the combination of right upper quadrant abdominal pain and perihepatitis occurs in association with genital tract infection (Bolton & Darougar 1983).

There is evidence which suggests that chlamydiae may be a cause of peripheral and axial forms of human arthritis either in association with lymphogranuloma venereum or as a reactive arthritis (Keat et al 1983), as in Reiter's disease.

In the sexually active adolescent, the manifestations of genital infection are similar to the adult. For further details the reader should consult Robertson et al (1989, pp. 92–107). Paediatric infections due to *C. trachomatis* are largely confined to the neonate and young baby as a result of vertical transmission from an infected mother. The estimated attack rate is 60–70% (Harrison & Alexander 1984).

Infections in the newborn due to *Chlamydia trachomatis*

**Conjunctivitis**

Conjunctivitis is the most obvious clinical form of neonatal chlamydial infection since contamination can occur from the mother’s infected cervical excretions during birth or by shedding
of *Chlamydia* in inflammatory discharges from an infected eye in the newborn.

The increase in incidence of NGU in men and the increasing isolation rate of certain serotypes of *C. trachomatis* in some 40% or more in this condition is likely to be associated with a large number of women acting as carriers. As chlamydial infection can be asymptomatic in men with minimal signs of urethritis, and is ordinarly so in women, conjunctival infections of the newborn are to be expected.

In the United Kingdom, where prophylactic treatment of the eyes is not given at birth, clinical conjunctivitis in maternity units was found in 2.6% (71 of 2700 infants in Cambridge in 1968, and 139 of 5282 infants in Liverpool in 1980) (Hobson et al 1983).

There are difficulties in assessing the true incidence of chlamydial conjunctivitis because babies are rarely in hospital for the full incubation period of the order of 3–15 days or longer, when the inoculum is small but has been estimated to be as high as 50% of babies born to infected mothers (Harrison & Alexander 1984). In the case of gonococcal conjunctivitis diagnosis is usually made 24–48 h after birth.

**Clinical features.** The infection usually presents as a mucopurulent conjunctivitis (Fig. 22.34) which may vary from mild to severe within the incubation period already stated. The discharge may be only scanty and not obviously purulent but it is sometimes more copious and frankly purulent, or on occasions blood stained (Rees et al 1977). On examination the palpebral conjunctiva shows mild to severe infiltration and papillary hyperplasia. In its more severe form there is also oedema of the eyelids and palpebral conjunctiva, particularly of the lower lid. Signs may be minimal and inflammatory reaction apparent transitory but in some cases conjunctival scarring develops (Watson & Gairdner 1983).

In the absence of specific treatment, the course is usually benign, but may be protracted. The sight is not usually compromised although *micropannus* and conjunctival scarring may be found on long-term follow-up (Hobson et al 1983).

**Diagnosis.** The discharge should be wiped gently away from the surface of the eyelids with a swab and the lower lids everted. A cotton wool swab should be passed gently but firmly along the lower and upper palpebral conjunctiva and then agitated gently in 2SP transport medium; *excess fluid is removed by rotating the swab while gently pressing against the inside of the container. The sample should reach the laboratory within 2 h or stored at a temperature of −70°C until cultured.

Similar samples are taken from the posterior pharyngeal wall, particularly in older children and on follow-up.

The earlier the provisional diagnosis the earlier the full investigation and treatment, not only of the baby but also of the mother who is at risk of pelvic inflammatory disease (Hobson & Rees 1977) and of the sexual partner.

In the investigation of neonatal conjunctivitis the following are essential steps:

1. Microscopy of Gram-stained smear from the palpebral conjunctiva for gram-negative diplococci (*N. gonorrhoeae*).
2. Direct inoculation of selective medium with material from the palpebral conjunctiva to detect or exclude *N. gonorrhoeae*.
3. Swab from palpebral conjunctiva placed in 2SP or other chlamydial transport medium for the isolation of *Chlamydia*.
4. Swab taken in Amies transport medium for isolation of other bacterial pathogens.
5. Pelvic examination of the mother to exclude *N. gonorrhoeae*, *C. trachomatis* and other pathogens.
6. Examination of sexual partner of the mother.

**Treatment.** Once *N. gonorrhoeae* has been excluded it is justifiable to treat a mild neonatal conjunctivitis with a 0.5% (w/v) solution of neomycin prescribed as single dose sterile eye drops ('Minims' Neomycin Sulphate, Smith and Nephew Pharmaceutical Ltd, 20 units in each single dose), instilled into the eye every 4 h. Neomycin is effective against most isolates of *Staphylococcus* and some strains of *Proteus vulgaris* and *Pseudomonas aeruginosa*. It has no action against fungi, viruses or intracellular *Chlamydia*. If the conjunctivitis is marked and does not respond to neomycin then isolation of *Chlamydia* should be attempted and if this organism is discovered or suspected, 1% tetracycline eye ointment is applied (Achromycin eye ointment) along the lower lid 4-hourly on four occasions daily for 21 days (Charters & Rees 1976). Since topical treatment may well be insufficient consideration should be given to the strong case for the use of systemic treatment.

Infants with chlamydial infections frequently have concurrent pharyngeal infection which is likely to persist after topical therapy alone with eye ointments and may be a focus for reinfection of the eye (Rees et al 1981). In addition the eye infection may be associated with concurrent or subsequent pneumonia and otitis media and even myocarditis (Grayston et al 1981).

There is therefore a very strong case for systemic treatment of all infants with chlamydial conjunctivitis and erythromycin ethylsuccinate given in divided doses to a total of 40–50 mg/kg body weight per day for 2–3 weeks is reliable. There appears to be no advantage in giving systemic and topical treatment simultaneously (Hobson et al 1983).

**Fig. 22.34 Chlamydial conjunctivitis in a newborn infant.** (From World Health Organization 1986c, with permission.)

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**Notes:**

- 2SP: is 2.4 ml of a sucrose phosphate solution, i.e. 0.2 M sucrose in 0.02 M phosphate buffer pH 7.2 with 50 µg/ml of streptomycin.
- 100 µg vancomycin, and, except for 2SP to be used for conjunctival specimens, 25 units/ml of nystatin. Specimens may be stored at −70°C before culture (Gordon et al 1969).
Spread of the organism from the nasopharynx to the lower respiratory tract can result in pneumonitis, with an estimated incidence of 11–20% of babies born to infected mothers. Onset of pneumonitis occurs later than that of conjunctivitis, usually between 3 and 11 weeks, with gradual development of a 'staccato' cough, partial nasal obstruction and tachypnoea. Typically, the baby is afebrile and not systemically unwell. Chest signs may be minimal compared with the diffuse bilateral interstitial opacities and hyperinflation seen on chest X-ray.

The chest X-ray appearances, the presence of circulating IgM anti-chlamydial antibodies and demonstration of *C. trachomatis* in concurrent conjunctivitis or from the nasopharynx are all supportive evidence for the diagnosis of chlamydial pneumonitis. The presence of other respiratory tract pathogens (e.g. respiratory syncitial virus) must be excluded.

Treatment with erythromycin ethylsuccinate 25 mg/kg twice daily for 3 weeks produces a rapid response and chronic chlamydial pneumonia or recurring infection has not yet been shown to occur.

As with conjunctivitis, examination of the mother for evidence of chlamydial infection is mandatory.

Other mucosal surfaces in the newborn have been shown to be colonized by *C. trachomatis*, namely nasopharynx, rectum and vagina. It is difficult to be certain whether the isolation of *Chlamydia* from the nasopharynx of infants represents an 'established infection' or pathological condition of the pharynx rather than contamination by tears. The infection is usually light in terms of numbers of infectious units and is only clinically inapparent and without sequelae but it can persist for up to 200 days in the absence of chemotherapy (Harrison et al. 1978). Otitis media has been described in infants infected with *C. trachomatis* but the exact role of the organism in the pathogenesis of otitis media remains controversial (Mardh et al. 1989, pp. 221–232).

Rectal and vaginal infections do not appear to have clinical significance.

**Disease due to *C. trachomatis* biovar LGV**

(lymphogranuloma venereum, serovars L-1, L-2 and L-3)

Biovar LGV is sexually transmitted (serovars L-1, L-2 and L-3) and in contrast to biovar trachoma, which is pathogenic to the squamocolumnar cells of mucous membrane, it causes primarily a disease of the lymphatic tissue, involving characteristically the lymph nodes of the genitoanal region (pp. 1450–1451).

Psittacosis (p. 1429)

*C. psittaci* causes a disease (psittacosis) in parrots and certain other birds. In man the disease may arise when infected dust or droplets are inhaled; illness ranges from an 'influenza-like' syndrome to a severe illness with delirium and pneumonia.

**Atypical pneumonia due to TWAR agent**

There has been recent interest in the group of chlamydiad called TWAR agents and IgG antibodies against TWAR organisms are found in 25–45% of healthy adults. These infections may occur endemically and affect children and adults alike.

Symptoms of cough, sore throat and fever may accompany an elevated ESR, normal white blood cell count and chest X-ray ab-normality: often a single lesion, normally in one of the lower lobes. Mild asymptomatic infections occur, but in debilitated patients the infection can be severe and even fatal.

Diagnosis is difficult as the TWAR agent is difficult to culture. Serological studies using the genus-specific complement fixation test are not sensitive enough and require to be confirmed by a micro-IF test. However, in primary TWAR infections, the IgG micro-IF test can take several weeks to demonstrate a response. An IgM antibody titre may be demonstrated in 70% of cases of primary infection but not in recurrences.

Treatment by tetracycline is recommended, but satisfactory results have been obtained from treatment of TWAR pneumonia by a 2-week course of erythromycin (Mardh et al. 1989).

**STREPTOCOCCUS AGALACTIAE (BETA-HAEMOLYTIC LANCEFIELD GROUP B)**

*Streptococcus agalactiae* is the name applied to human members of group B streptococci (Parker 1978) although there are reasons to believe that they form a population group distinct from bovine group B — classified by Lancefield on the basis of cell wall carbohydrate antigens — streptococci (Ross 1978).

In the last 15 years *S. agalactiae* has been a serious cause of bacteremia and other invasive infection in the newborn within the first few days of life (p. 308). Dense vaginal colonization seems to make transmission more likely and prematurity and a prolonged labour after rupture of the membranes are important risk factors. Promiscuity in the female or in her male partner is one determinant of the organismal flora of the genital tract; in the case of group B streptococci, carriage rates of about 12–36% have been noted in the case of women attending STD clinics (Finch et al. 1976, Christensen et al. 1974). Carriage in adult women appears to be best detected by bacteriological examination of a urethral swab. Since the organism is to be found on the penis, a presumptive case for its transmission sexually can be made out.

Christensen et al. (1984) found that prolonged unregional carriage of group B streptococci in women (n = 88) who used tampons during menstruation was twice that in women who did not (respectively 49% and 24%) and thought that there was a causal relationship between the use of tampons and the persistence of Group B streptococci.

In relation to the problem of the newborn, however, positive cultures during pregnancy (6–28% recorded) may be less frequently found at delivery and a few patients may first acquire the organism at delivery. Invasive infections develop only in about 1% of colonized babies (Lancet 1984b).

Two clinical syndromes, 'early onset' and 'late onset', are recognized in the newborn. The early onset disease, although septicaemic in type, may also be meningitic whereas the late onset disease is almost always meningitic. Early onset disease may occur within 5 days of birth, but usually within the first 24–36 h. There is a close association between early onset infection in the newborn and maternal complications, particularly premature labour and prolonged period between rupture of the membranes and delivery. Infants of low birthweight tend to be affected. The late onset disease presents usually after the tenth day of life as purulent meningitis and can affect apparently healthy babies after normal labour with a mortality rate, although lower than early onset disease, of 15–20% (Ross 1978).

Prevention of early onset streptococcal infection seems possible: By using a monitor to count the respiratory rate of all newborns
infants for the first 36 h it has been noted that about 1% have a raised rate. Most of these will receive antibiotics, although only about 10% are later proved to have a streptococcal infection. With this procedure Valman & Wright (1984) have had no deaths from group B streptococci and the regimen is to be preferred to dosing all newborns indiscriminately with antibiotics.

CANDIDOSIS

Candidosis (synonym candidiasis, thrush) is a convenient generic term for infections caused by yeasts, acting as opportunistic pathogens in individuals whose host defences are impaired.

AETIOLOGY

Pathogenic yeast cells are typical of aerobic eukaryotes possessing intracellular organelles including mitochondria and ribosomes and a double membrane nucleus containing chromosomes. Taxonomically the yeasts have been mostly assigned to a single genus Candida, but molecular studies show that this 'genus' should be regarded as an artificial one comprised of non-sexually reproducing forms of members of a variety of other genera some of which have now been identified (Riggsby 1985). The most important member of the group, Candida albicans, is capable of causing the very common superficial infections of the mouth and vagina as well as widespread or more deep seated disease, viz. systemic candidosis, an important hazard in modern medical procedures such as transplantation surgery, intravenous hyperalimentation and immunosuppressive surgery (Odds 1979) as well as in the emergent epidemic retrovirus infection with human immunodeficiency viruses (HIV) leading to the acquired immune deficiency syndrome (AIDS).

The formation of germ tubes is accepted as a reliable property for identifying Candida albicans and is used in medical laboratories because of its rapidity. The germ tube of C. albicans is a thin filamentous outgrowth from the cell without a constriction at its point of origin. Presumptive identifications based on the formation of germ tubes are 95-100% accurate when compared with stricter taxonomic methods (van der Walt & Yarrow 1984, Kregen-van Rij 1984).

In the vagina in those with vaginitis C. albicans comprises a very high proportion of isolates (70-90%). Torulopsis glabrata (synonymous with C. glabrata) is less frequently found in those with vaginitis (6%) than in those without (17%) (Odds 1979).

Adherence of microorganisms to epithelial cells is a critical step in the colonization of mucosal surfaces and their subsequent capacity to cause disease. Hyphal production in candidosis in vivo is not necessarily indicative of tissue invasion and the case for the connection of yeast-to-mycelium conversion to pathogenicity remains a matter for debate.

Conditions which favour transition from saprophyte to pathogen and are relevant to vaginal candidosis particularly include pregnancy, diabetes mellitus, damage or maceration of the tissues, the use of immunosuppressive drugs, possibly also the oral contraceptive — particularly oestrogenic rather than progestagenic preparations — and oral antibiotics (Ridley 1988).

In those infected with HIV and in AIDS, there is profound damage to the cell mediated branch of the immune system and, although it is oropharyngeal candidosis spreading to cause oesophageal erosions that is most often seen, disseminated candidosis occurs rarely (Ebbesen et al 1984).

PATHOLOGY

In the mouth at least, in acute and chronic candidiasis there is invasion of the epithelium by hyphae, which grow downwards in more or less straight lines without respect for epithelial boundaries.

CLINICAL FEATURES OF CANDIDOSIS

Candidosis of the female genitalia

Vulvar pruritus which may vary from slight to intolerable is the cardinal symptom of candidosis. Burning is a common complaint, particularly upon micturition and dyspareunia, especially in the nulliparous, may be severe enough to make intercourse intolerable. Vulvar oedema is more common in vaginal candidosis in pregnancy.

Erythema of the vulva is the commonest sign of candidosis and tends to be limited to the mucocutaneous surfaces between the labia minora but may extend more widely. The vagina is abnormally reddened in about 20% of cases. If adherent patches or plaques of 'thrush' or pseudomembrane are removed the vaginal skin underneath appears erythematous and superficial ulceration with bleeding may be seen.

In contrast, primary cutaneous candidosis involves the outer parts of the labia majora and the genitoaural fold and not infrequently the mons veneris, the perianal region and inner thighs. Vulvar lesions tend to be reddened and moist with defined scalloped edges.

T. vaginalis or N. gonorrhoeae may coexist with the yeast infection and on occasions all three organisms are found together.

Candidosis of the glans penis and prepuce

Characteristic symptoms of soreness and itching of the penis, accompanied sometimes by a discharge from under the prepuce, are seen in candidosis of the penis where there may be a balanoposthitis with superficial erosions.

Neonatal candidosis

Candidosis in the newborn may involve the umbilicus, mouth and napkin areas. The maternal vagina is only one source as colonization may involve also the mouth and bowel. Attendants and environmental sources may also contribute to transmission.

Pharyngeal and oesophageal candidosis in AIDS

This usually manifests itself as dysphagia and retrosternal discomfort in patients who also have oral involvement with Candida.

DIAGNOSIS

Microscopy

Direct microscopy, to detect yeast cells or hyphae, is an essential as an 'on-the-spot' procedure in the diagnosis of candidosis of the vagina and of the glans penis. A wet preparation material is emulsified in 20% potassium hydroxide in water or dimethyl sulfoxide. Alternatively the smear is dried in air, fixed by heat
and then Gram stained; all yeasts, like all fungi, are Gram positive and detected using the oil immersion (×100) objective.

Culture

The swab of vaginal discharge, obtained from the vaginal fornices, may be sent to the laboratory in Ames transport medium. It is plated on malt agar and the yeast isolated and tested with horse serum for germ tube formation (see Aetiology). The isolate found to be germ tube positive is considered to be Candida albicans; germ tube negative isolates are sent to specialist mycology units for identification. In deep seated lesions identification to species level is desirable.

TREATMENT

Preparations widely used in the topical treatment of candidosis include the polyene antibiotics nystatin and amphotericin B and the imidazoles, clotrimazole and miconazole. Fluconazole, a new triazole antifungal agent (see reviews in Fromting 1987), is available for vaginal and oropharyngeal candidosis but in children under 16 years of age it is not recommended due to lack of clinical data in this group; it should not be used in pregnancy or in those at risk of pregnancy.

For life-threatening systemic candidosis 5-fluorocytosine and amphotericin B are available for parenteral use.

The polyene antifungal antibiotics

The polyene antibiotics nystatin and amphotericin B are effective antifungal agents that act by increasing the permeability of the fungal membrane and causing a leakage of intracellular solutes and cell death. Nystatin is prescribed as vaginal tablets, each containing 100 000 units, with instructions to insert one at night for 14 nights continuing during menstruation. The patient should wash her hands and vulva before bedtime, lie down on the bed and gently insert the tablet into the upper third of the vagina. It should be explained that the nystatin is itself yellow in colour and that the appearance of a yellow discharge is not a cause for worry. Nystatin cream (100 units/g) may be usefully applied to the skin on the vulva and, if required, to the adjacent skin. Should relapse occur the administration of oral nystatin (500 000 units four times a day for a week) together with local treatment and the use of nystatin cream in the male partner is justified.

Amphotericin B

Amphotericin B, a polyene derived from Streptomyces noursei, may be also used topically as a 3% cream (Fungilin, Squibb); it is very toxic if used parenterally and liable to cause renal damage.

The antifungal imidazoles

The antifungal activity of the antifungal imidazoles differs from that of the polyenes in that the imidazoles have a very broad spectrum of activity affecting many filamentous fungi and dermatophytes as well as yeasts (Odds 1979).

Clotrimazole (Canesten, Bayer) is supplied as a 1% cream for topical use and as 100 mg vaginal pessaries. One 100 mg vaginal tablet inserted every night for 6 nights is an effective alternative to nystatin (Highton 1973) and more pleasant to use; a shorter course of one 200 mg vaginal tablet inserted for three consecutive nights may be sufficient in the uncomplicated case (Masterton et al 1977), and the insertion of a single 500 mg vaginal tablet at night may also suffice. Sometimes (1% cases) vaginal applications of clotrimazole may cause burning sensation.

Miconazole nitrate (Daktarin, Dermonistat) is supplied as a 2% cream for topical use, as a 2% intravaginal cream (Gynodaktarin, Monistat) and as vaginal pessaries, each containing 100 mg.

Econazole (Ecostatin, Squibb) is another effective antifungal imidazole. It is supplied as pessaries containing econazole nitrate 150 mg and a cream, 1% econazole nitrate.

HERPES SIMPLEX INFECTION

HERPES SIMPLEX

Herpes simplex is an acute infectious disease, characterized by a sometimes recurring vesicular eruption, occurring anywhere on the skin, but most often on or near the lips or the genitals. Sometimes the infection involves the eye to cause a conjunctivitis with or without corneal involvement. The causative virus, herpes simplex virus (HSV), can be divided into two types on the basis of certain antigenic, biochemical and biological differences. Type 1 HSV (HSV-1) is usually isolated from lesions round the mouth or eye and transmitted by the direct contact of kissing or by droplet in cases or from carriers; type 2 HSV (HSV-2) is responsible for the majority of genital tract infections and spread is by direct contact during sexual intercourse. The very common nature of inadvertent infections, whether of HSV-1 or HSV-2, is becoming better appreciated.

HERPES SIMPLEX VIRUS (HSV) (SYNONYM, HUMAN ALPHAHERPESVIRUS)

Herpes simplex virus is a member of the sub-family Alphaherpesviridae which is characterized by the capacity to cause rapid spread of infection in cell culture resulting in mass destruction of susceptible cells (Roizman et al 1981). The mature herpes virion contains a core enclosing viral DNA in the form of a torus (Roizman & Bartson 1985). With restriction endonuclease it is possible to fingerprint HSV types. Its mode of transmission is mainly by contact of oral or genital mucosal surfaces.

NATURAL HISTORY OF HERPES SIMPLEX

In most viruses infections affecting humans the virus rapidly establishes itself in susceptible cells, is replicated and after a few days it and its progeny are eliminated by host mediated immunity. In a number of viruses, however, and in particular in herpes simplex virus and varicella-zoster virus, an elegant strategy has been evolved by which, in spite of immunity, the virus can persist in the host for life and also provide for its spread to other hosts. To understand the natural history of HSV infection it is necessary first to define and name the various events in infection. The definitions given below are based on those of Wildy and his Cambridge colleagues (1982) but modified to take into account suggestions made by Rawls (1985); clinical and virological features in herpes genitalis at various stages in its natural history are represented diagrammatically in Figure 22.35.

1. Primary infection. This is an infection which may be
asymptomatic, remain localized or become generalized in an individual who has not been previously infected with either type of HSV as shown by a lack of antibodies to herpesviruses.

2. Initial infection. This denotes the first infection of a virus type. It may be a primary infection in those without serological evidence of a previous herpetic infection or non-primary in those with evidence of previous infection. Clinically primary and non-primary initial infections may be indistinguishable on physical examination.

3. Latency. There is apparent recovery but some virus remains dormant in nervous tissue, particularly in certain sensory ganglion cells; this is latency (latent means hidden).

4. Reactivation. Virus may be reawakened spontaneously, or as a result of external stimuli so that infective virus may once again be found.

5. Recurrence. The reactivated virus may on occasion initiate a peripheral lesion in the dermatome relating to the sensory ganglion. The lesion is referred to as a recurrent lesion and the phenomenon is recurrence. Wildy et al (1982) used the term 'recrudescence' to describe this phenomenon but since the term 'recurrence' is deeply embedded in the literature it is used instead (Rawls 1985).

6. Axonal transport. The whole phenomenon requires translation of the virus from the periphery to the sensory ganglion and back again by way of, it is believed, the cytoplasm within the axon. The rate of translocation from the skin to the ganglion lies within the range of 2–10 mm/h.

7. Asymptomatic virus shedding. Sometimes the virus evidently reactivates and passes to a peripheral site but fails to cause a noticeable lesion, although it probably multiplies and can be isolated. The term asymptomatic virus shedding (Rawls 1985) is used in this situation to distinguish this event from recurrence.

The cell bodies of the sensory neurones are situated in the dorsal root ganglia and the axons of these cells pass to the dorsal columns of the spinal cord and peripherally to the skin. Each dorsal root ganglion innervates an area of skin, called the dermatome. The ganglion cell, the sensory nerve and the skin it innervates occupy the area of operation of both herpes simplex virus and varicella-zoster virus and is conveniently called the neurodermatome.

Herpes simplex is the commonest virus infection encountered in humans and as a successful parasitic agent herpesvirus has few equals. It is able to infect generally without causing serious disease, it is readily transmitted from person to person and can persist in its host.

Notwithstanding the wide variation in the severity of clinical disease in primary infection the majority appears to be asymptomatic. The severity of signs and symptoms in primary infection appears to be age related; usually asymptomatic infections occur in the 2–3 year age group and symptomatic in the adolescent or young adult. In the majority of cases of clinically apparent disease the lesions are localized to the site of inoculation. the sensory neurones innervating that site, and the lymphatics draining it. Spread to contiguous areas or transfer to more distant sites by autoinoculation can also occur. Although primary infections can occur at any site, the lesions are characteristically vesicles affecting the mucosa and/or adjacent skin of the mouth or genitals which evolve rapidly into greyish or yellowish painful shallow ulcers.

Asymptomatic virus shedding occurs in between 1% and 5% of adults in their oral secretions. In the case of genital secretions this depends upon age and sexual behaviour and may vary between 0.5% and 7.0%. Asymptomatic virus shedding is thus a substantial source of virus (World Health Organization 1985a, 1985b).

The majority of the adult population, including women of childbearing age, particularly in the lower socioeconomic groups, carry neutralizing antibody to HSV. This antibody is transferred across the placenta and confers passive immunity to the newborn child, but antibody will have disappeared by the age of 6–8 months when a primary infection can then occur in the non-immune infant. The commonest time at which the infection is acquired, particularly in lower socioeconomic groups, is between 2 and 3 years of age. Primary infections at this age are HSV-1 infections and the vast majority are subclinical; in an important early study, only between 1% and 11% of children showed some manifestation clinically, usually of stomatitis (Spence et al 1954).

Following primary infection antibody appears and usually remains at a constant level for a prolonged period, although it may decline until reinfection or reactivation may bring about an increase and stabilization of the titre. As the amounts of virus antigen produced may influence the magnitude of response, in many recurrent infections where virus replication is limited little or no increase in virus antibody may be observed.
DISTRIBUTION OF HERPESVIRUS TYPES

HSV-1 may occasionally be recovered from saliva, tears and the genital tract between attacks and regularly from lesions during attacks. In the case of HSV-2, as well as in HSV-1, asymptomatic carriers probably constitute the principal source of infection and the virus may be isolated from genital sites (cervix in the female or from the urethra in the male). Transmission is more likely when there is a lesion producing a high titre of virus. In the sexually active age groups spread occurs by sexual intercourse, kissing or direct or indirect orogenital contact.

In the case of genital lesions HSV-1 is less commonly found than HSV-2. In higher socioeconomic groups high proportions (40–90%) of young adults may have no antibody to either HSV-1 or HSV-2 whereas the corresponding figure in lower groups is only 20%. HSV-1 is associated with the mouth and stomatitis but genital infection with HSV-1 is likely to occur particularly in populations where 30% of young adults (15–24 years) are without antibody to HSV (Smith et al 1967). Orogenital contact or contact of the genitals with saliva contaminated fingers may be the means of transmitting the virus.

Recurrent lesions in genital sites are not as frequent in HSV-1 infections as in those of HSV-2 (Kawana et al 1976); this could be due to relative inefficiency of HSV-1 in establishing a latent infection of the sensory nerve cells in the sacral ganglia (World Health Organization 1985a, b). Prospective studies showed that among patients with a first episode of HSV-1 genital infection only 14% experienced a subsequent recurrence in contrast to 60% of those with an HSV-2 infection (Reeves et al 1981).

Complement fixing antibody can be detected before neutralizing antibody and conversion from absence of antibody to its presence in serum will signify a primary infection, which can occur in the genital area with either type 1 or type 2.

Using the restriction endonuclease patterns of HSV DNA to ‘fingerprint’ the virus isolates on the basis of the presence or absence of known cleavage sites it has been possible to begin to study the spread of the agent in the community (Buchman et al 1978). The possibility of transmission of HSV-1 within a nursery for the newborn has been confirmed (Linnemann et al 1978). Restriction endonuclease analysis for epidemiological tracing of HSV-1 is useful but because there is only a small number of variable cleavage sites in the HSV-2 genome caution is needed in the extrapolation of the results of HSV-2 analysis to establish proof of the source of an infection (Smith et al 1981, Smith JW 1985, Personal communication, Robertson et al 1989).

IMMUNITY IN HSV INFECTION

Herpes simplex virus persists throughout the life of the individual infected and achieves this by mechanisms which enable it to avoid host responses, viz. the ability of the virus to pass from cell to cell by a fusion process circumventing the need to emerge into the extracellular environment; to secure latent infection of a neuronal cell, by a mechanism as yet not precisely known; and to evade natural defence systems of the host, i.e. macrophages, natural killer cells and interferon.

Following infection there are both humoral and cell mediated immune responses lasting many years.

Primary infection yields a rise in antibody with titres reaching a peak in about 4–6 weeks and remaining stable afterwards. Virus specific IgM antibodies are produced in the early stages and persist for 6–8 weeks.

In individuals with pre-existing antibodies recurrence is not associated with a marked change in antibody titre.

In the newborn passively transferred maternal antibodies are often present but are gradually lost during the first 6 months of life.

PATHOLOGY

The basic lesion is one of localized necrosis. The nuclei of affected cells become swollen and the chromat in margi. A dense basophilic mass fills the whole nucleus and later retracts from the nuclear margin. Finally an eosinophilic intranuclear inclusion appears (the Lipschutz or Cowdry type A inclusion); all stages may be found in any one section. The lesions of the skin and mucous membrane are characterized by intraepidermal vesicle formation followed by ulceration, crusting and usually rapid healing.

In severe disseminated infections visceral lesions consist of parenchymal lesions of coagulative necrosis with specific intranuclear changes. In the liver and adrenals, the areas of focal necrosis can be seen with the naked eye. In early encephalitis, necrosis is a marked feature and in the adult form, there is asymmetrical softening with numerous haemorrhages on the surface of affected areas. The temporal lobes and the posterior orbital gyri are most commonly affected (Dudgeon 1970).

CLINICAL FEATURES

The very common nature of inapparent infections, whether by HSV type 1 or 2, is becoming better appreciated with increasing availability and improvement of isolation techniques. When apparent the prelatent or incubation period of HSV-1 or HSV-2 infections lies between 3 and 9 days (World Health Organization 1985a).

Primary herpetic gingivostomatitis

Primary herpetic gingivostomatitis is the commonest clinical manifestation of primary infection of HSV-1 in children between 1 and 5 years of age. The stomatitis begins with fever, malaise, restlessness and excessive dribbling. Drinking and eating are very

Fig. 22.36 Primary herpetic gingivostomatitis. (Courtesy of Professor J. C. Southam.)
painful and the breath is foul. Vesicles containing high titres of virus appear as white plaques on the tongue, pharynx, palate and buccal mucosa. The plaques are followed by ulcers with a yellowing pseudo-membrane. Regional lymph nodes are enlarged and tender (Nagington et al 1986).

An identical clinical picture (Figs 22.36 and 22.37) may be seen in adolescents and young adults who escaped infection in childhood but acquired the infection by kissing. Primary infections, including ocular, in adolescence are becoming more common in developed countries (Darougar et al 1985), so more severe clinical forms of the disease will be seen more often in this age group. Virus shedding continues for 14–21 days after the appearance of lesions caused by a primary infection (World Health Organization 1985a).

Primary herpes genitalis

Signs and symptoms of primary genital herpes tend to be more severe in women than in men and are usually those of vulvo-vaginitis accompanied by systemic symptoms of fever and malaise: local pain and dysuria can be severe. Difficulty in starting micturition or retention may be the presenting clinical feature. White plaques are present on the red swollen mucosas of the vulva, cervix and occasionally in the vagina (4%); scattered vesicles are seen on the labia (Fig. 22.38) and these may extend to involve the perianal skin and the skin of the thigh. The regional lymphatic glands are enlarged and tender and there may be a vaginal discharge. Healing tends to take place over a period of 1 or 2 weeks, but new lesions sometimes continue to develop over a period of 6 weeks.

In primary cervicitis there may be only swelling and redness of the mucosa but sometimes there is a necrotic ulceration and friability of the cervix which bleeds easily. The range of clinical effects is wide and asymptomatic cases are common.

Primary infection with systemic dissemination of HSV appears to be rare in immunocompetent adults but fulminating systemic HSV infection in pregnant women during the last trimester, although rare also, is a recognized and important clinical entity with a high mortality in both mother and fetus (Robertson et al 1989).

Early diagnosis is very important and the characteristic primary lesions, viz. vesicles or painful ulcers of the vulva with or without involvement of the cervix or of the mouth and pharynx, are seen in the majority. It should be emphasized however that the patient may not give a spontaneous history of either genital or throat lesions owing to the predominant systemic symptoms, particularly of fever and vomiting.

In primary herpes genitalis in the male the lesions can involve the glans or coronal sulcus and, if severe, there may be a phimosis with accumulation of secondarily infected exudate and occasionally a resulting necrotizing balanitis. In the homosexual the anorectum may be involved and show vesicles and/or ulceration. Anorectal pain, constipation, tenesmus, anal pruritus, difficulty in starting micturition, parasthesia in the sacral area, pain in the back of the thigh, fever and enlargement of the inguinal lymph nodes are characteristic symptoms. Herpesvirus may be acquired at other sites by contact. Other forms of herpes simplex include keratoconjunctivitis and eczema herpeticum, where infection has become superimposed on eczematous skin (Kaposi’s varicelliform eruption).

The significance of the immune responses becomes clinically evident in a variety of immunodeficiency states in which HSV infection or reactivation may lead to severe local or general disease, e.g. malnutrition, Wiskott–Aldrich syndrome, lymphoma, immunosuppressive therapy in renal transplantation and in the acquired immune deficiency syndrome (AIDS).

Complicated primary herpes genitalis

Aseptic meningitis

Fever, severe headache, malaise, photophobia, neck rigidity and the presence of Kernig’s or Brudzinski’s sign indicate meningeal involvement. There is a lymphocyte pleocytosis (5–1000/mm³) in...
the CSF which diminishes spontaneously. This complication may occur within 6–12 days of onset in 36% of women and 13% of men.

Sacral autonomic dysfunction
Sacral anaesthesia, urinary retention requiring intermittent catheterization and constipation may occur. The patient may complain of numbness and tingling in the buttocks or of perineal pain and there may be a decrease in perception of fine touch. Urinary retention and constipation may follow. In men impotence is associated with decrease in the bulbocavernous reflex.

Extragenital lesions
Extragenital herpetic lesions may occur nearby particularly in the buttocks, groin or thighs. Any site may be involved including the finger (in 8% in the Seattle series of Corey et al 1983) and conjunctivitis may also occur.

Oesophagitis
Primary herpetic oesophagitis may be encountered as a rarity in the apparently immunocompetent adult without accompanying lesions of the lip or gingivostomatitis. In HIV infection this form of HSV infection may be severe (Owensby & Stammer 1978).

Dissemination
In the rare disseminated infections in adults, HSV type 2 can be isolated from vesicular or pustular lesions that may appear anywhere on the body. In such cases HSV meningitis or encephalitis does not necessarily occur.

RECURRENT AND RECURRENT LESIONS IN HSV INFECTION
After the primary or initial infection, whether obvious or inapparent, there may be no further clinical manifestations throughout life. Certain febrile illnesses, such as malaria and pneumonia, provoke attacks in those in whom the disease is latent. Exposure to sunlight, menstruation or emotional stress may have a similar effect.

In the commonest form of herpes of the face, lips or genitals, itching or burning precedes by an hour or two the development of small, closely grouped vesicles on a sometimes slightly raised erythematous base. Ordinarily healing is complete within a week or 10 days. The shallow ulcers which develop may be very painful. Recurrent ulcers tend to be in the same region but not precisely on the same site.

After a primary genital herpes simplex due to HSV-1 recurrent episodes were reported in 55% of patients compared with 88% of patients with primary genital herpes simplex due to HSV-2 (Corey et al 1983).

COMPLICATIONS AND SEQUELAE
In the eye, repeated attacks of ulceration may result in corneal opacity (World Health Organization 1985a). Transmission to the fetus and newborn may have serious implications, which are discussed later.

DIAGNOSIS
In herpes simplex involving the genitals, it is essential to exclude other sexually transmitted diseases, particularly syphilis, gonorrhea and chlamydia infection, which may have been acquired concurrently.

Cells from lesions may contain characteristic intranuclear inclusions. Multinucleated giant cells can also be found in scrapings as for example in cervical smears. Virus particles may be also found in lesion material by electron microscopy but the different herpesviruses cannot be distinguished by microscopy alone. Viral antigens are detectable in cells using immunofluorescence or enzyme linked immune techniques. In the case of vesicular lesions diagnosis by tissue culture of lesion material is more sensitive as infectious virus is usually present. In later ulcers or in crusted lesions where the virus cannot be isolated the diagnosis can be better made by the detection of antigen (Ravls 1985).

Isolations by tissue culture are best made from vesicular fluid when present and/or exudate. Cellular debris is gently scraped from the lesions with a cotton wool applicator. The specimen obtained is then agitated in a bijou bottle containing virus transport medium (VTM) (Collee et al 1989).

Viruses isolates may be characterized in the laboratory by antigenic, biological or biochemical typing by various methods:

1. Cytopathic effect (CPE) in tissue culture cells
2. Immunofluorescent antibody test: fluorescein conjugated monoclonal antibodies which are type specific are now available
3. Restriction-endonuclease analysis: HSV-1 and HSV-2 can be separated unambiguously by restriction endonuclease analysis and the endonuclease HpaI shows the distinction most clearly (Lonsdale 1979).

Serological testing
Blood samples should be taken on first attendance and again after 10–14 days for a complement fixation test to determine whether the lesion is due to a primary or a recurrent infection. Recently it has been shown that antibody to glycoprotein G is apparently type specific and this has been utilized in measuring the patient's serological response to HSV-1 and HSV-2 (Lee et al 1985).

TREATMENT
Acyclovir
In 1978 the discovery of a new compound with both remarkable good antiviral activity and extremely low toxicity to the host cell — 9-(2-hydroxyethoxymethyl)guanine — heralded a new era in the chemotherapy of HSV infections (Schaeffer et al 1978). This compound, now known as acyclovir, has proved to be a highly potent inhibitor of HSV-1, HSV-2 and varicella-zoster and has extremely low toxicity for the normal host cells.

The high potency and selectivity of acyclovir for herpes simplex virus can be understood on the basis of a number of differences between cellular and HSV-specific enzymes. Firstly, whereas the HSV-specific thymidine kinase phosphorylates acyclovir to monophosphate, cellular thymidine kinase does not. The acyclovir monophosphate is subsequently converted to acyclovir triphosphate by cellular enzymes which persists in HSV-infected cells. The acyclovir triphosphate is a more potent inhibitor of the viral DNA polymerases than of the cellular polymerases (Elion 1982).
The drug has the potential to crystallize out in the kidney tubes. Patients should be hydrated and the acyclovir administered by slow intravenous infusion over approximately 1 h to avoid this problem. In those with impaired renal function the dosage of acyclovir should also be reduced (Blum et al 1982).

Reduced sensitivity to acyclovir in herpes simplex virus

Resistance to acyclovir has been shown experimentally to arise by mutations in the gene of the virus specified enzymes and DNA polymerase. Three cases have been described in immunocompromised patients on courses of intravenous acyclovir therapy (Crumpacker et al 1982, Burns et al 1982). Although 'wild' HSV has not been shown to be resistant, Field & Wildy (1982) considered that extreme caution should be applied to the widespread prophylactic use of acyclovir, especially at low dosage to avoid possible promotion of resistance.

Acyclovir formulations available

**Acyclovir for intravenous infusion.** Acyclovir for intravenous use is available in vials containing 250 mg sterile acyclovir as the freeze-dried sodium salt (Zovirax IV, Wellcome). It is indicated in HSV infections in immunocompromised patients and is given by slow intravenous infusion of 5 mg/kg per 1 h every 8 h in patients with normal renal function. In children (3 months - 12 years) the dose 250 mg/m² is given similarly and doubled in the immunocompromised and in herpes simplex encephalitis. Suggested dosages are shown in Table 22.35. Five days treatment is usually adequate. Reconstituted Zovirax IV has a pH of about 11 and should not be given by mouth. Severe inflammation sometimes leading to ulceration may occur if infused accidentally into the tissues extravascularly (Wellcome Medical Division 1984).

<table>
<thead>
<tr>
<th>Route</th>
<th>Acyclovir single dose in children</th>
<th>Times daily</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 weeks</td>
<td>4 weeks</td>
<td>Over</td>
</tr>
<tr>
<td>2 years</td>
<td>2 years</td>
<td></td>
</tr>
<tr>
<td>Oral*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i.v.†</td>
<td>10 mg/kg</td>
<td>5</td>
</tr>
<tr>
<td>i.v.†</td>
<td>5 mg/kg</td>
<td>5</td>
</tr>
</tbody>
</table>

* Available as tablets (200 mg) or as a suspension (200 mg/5 ml).
† Available as 250 mg vial of the sodium salt acyclovir for reconstitution and given over 1 h.

**Acyclovir for oral use.** Acyclovir is available as a pale blue shield-shaped tablet impressed with ZOVIRAX and containing 200 mg acyclovir; a suspension — one 5 ml spoonful equivalent to one Zovirax tablet — may be used to encourage compliance in case of this difficulty. The standard dose in adults is 200 mg five times daily at approximately 4-h intervals, omitting the nighttime dose. Administration should commence as early as possible during the prodromal period or when the lesions first appear. It is contraindicated in patients known to be hypersensitive to acyclovir.

In children under the age of 2 years, half the adult dose is advised; children over 2 years may be given the adult dose. Further details are available in the British National Formulary (March 1991), page 218.

In patients with severe renal impairment (creatinine clearance less than 10 ml/min), a dose of 200 mg every 12 h is recommended. In pregnancy caution should be exercised in prescribing (Wellcome Medical Division 1984) as very little information is available on Zovirax during human pregnancy.

Acyclovir does not seem to decrease the likelihood of subsequent recurrences when used to treat patients either during a primary attack or a recurrence. If given continuously in adults for periods of 3-6 months it seems to be effective but costs are high (Minder et al 1984); in the seriously affected or in the immunocompromised, however, acyclovir treatment is very effective (see paragraph below).

**Acyclovir for skin use.** Acyclovir 5% w/w in a white aqueous cream base is available as Zovirax Cream (2.0 g or 10.0 g tube) for the treatment of herpes simplex of the skin (this preparation is NOT for eye use), including recurrences of genital and labial herpes. The cream is applied five times daily at approximately 4-h intervals and this treatment should be continued for 5 days. If healing is not complete treatment may be continued for a further 5 days. Therapy should begin as early as possible after awareness of the lesion and preferably in the prodromal period (Wellcome Medical Division 1984). Patient initiated therapy with the skin cream is effective therapy when treatment is used early (Kinghorn et al 1983).

**Acyclovir for eye use.** Acyclovir 3% w/w in a white soft paraffin base is available for ophthalmic use as Zovirax Ophthalmic Ointment (4.5 g tube). A 1 cm ribbon of ointment should be placed inside the lower conjunctival sac five times daily at approximately 4-hourly intervals. Treatment should continue for at least 5 days after healing is complete (Wellcome Medical Division 1984). The acyclovir eye ointment is superior to other antiviral agents for the eye (Richards et al 1983, Collum et al 1980, 1982).

**HERPES SIMPLEX VIRUS IN NEONATAL INFECTIONS AND ENCEPHALITIS**

The very serious and often fatal infections with HSV are rare and occur as neonatal infections, acquired before or at birth, as sporadic encephalitis, acquired later in life, or as disseminated herpes simplex in the immunologically deficient. HSV is a rare cause of the aseptic meningitis syndrome.

**HERPES SIMPLEX VIRUS IN THE IMMUNOCOMPROMISED**

Acyclovir has given new hope for immunocompromised patients in whom relatively simple local herpes simplex recurrence can otherwise develop into disseminated and sometimes life-threatening conditions. Such patients may be immunosuppressed by drugs or radiation given as therapy for malignancy or to prevent rejection; or they may have impaired immune response, inborn, due to malignancy or seen in AIDS (Straus et al 1982, Prentice 1983, Anderson et al 1984, Griffin et al 1985, Pettersson et al 1985, Chou et al 1981, Saral et al 1981).

**VERTICAL TRANSMISSION OF HSV TO NEONATE FROM MOTHER AT THE TIME OF DELIVERY**

The importance of transmission by sexual intercourse and timing is illustrated by the case cited by Hanshaw & Dudgeon (1978).
The report describes the exposure of a wife, 1 week before delivery, to her husband, who had an episode of penile herpes genitalis in a recurrent form at the time of intercourse. His wife had a primary genital herpes lesion at the time of delivery and the infant, born by Caesarean section 6 h after rupture of the membranes, contracted a disseminated infection and died at 8 days of age. The risk of neonatal infection is highest in infants born vaginally to women with a primary genital HSV infection at term when about half will be infected (Nahmias et al 1971). Primary genital infections in late pregnancy are more likely to be clinically apparent with a serious outcome for mother and fetus than non-primary infections.

In recurrent genital herpes simplex, in contrast, in neonates exposed to the virus at the time of vaginal delivery the risk of infection is low. In the USA Prober et al (1987) reported that in pregnant women with a history of recurrent herpes (HSV-2) the theoretical maximal infection rate was 8% for vaginally delivered infants. His work suggested that the presence of neutralizing antibody contributed to the low infection rate.

Estimates of incidence of neonatal herpes in the United States vary between one in 30 000 and one in 3500, with a frequency greater in the lower socio-economic groups. In some countries or social groups, a major source of virus to the infant is the mother's genital tract at the time of delivery and more than 80% of HSV isolated from the newborn belongs to type 2, the common form of genital herpes virus (Nahmias et al. 1970). In the United Kingdom, in Manchester, however, in the period 1969–1973, the incidence of neonatal herpes was thought to be one in 30 000 live births; in one-third of the cases infection was acquired from a person other than the mother and in these cases HSV-1 predominated (Tobin 1975). Over an 18-month period from July 1986 the British Paediatric Surveillance Unit, under an active surveillance scheme, reported 20 cases of confirmed neonatal herpes simplex (Hall & Glickman 1988). The rarity of recognized infection makes it difficult to formulate preventive measures (Lancet 1988b). In Canada severe neonatal herpes is estimated to be one per million (World Health Organization 1985b).

It has been suggested that if viral cultures are taken from the vulva and cervix in women with suspected herpes genitalis in late pregnancy and Caesarean section carried out if HSV is cultured near the time of delivery the risk of neonatal infection can be avoided (Boehm et al. 1981, Vonvert et al. 1982) and indeed this procedure was recommended by the American Academy of Pediatrics Committee on Fetus and Newborn (1980). In some 70% of cases of neonatal herpes, however, the mother has had no signs or symptoms of the infection at the time of delivery (Whitley et al. 1980) and the shedding of virus during pregnancy but without manifest disease is known to occur (Bolognese et al. 1976, Vonvert et al. 1982).

In a study by Prober et al. (1988) of 6904 women at the time of delivery HSV-2 was recovered in 14 (0.2%). Twelve (86%) of the HSV-2 positive women had serological evidence of previous infection with HSV-2 and none of their infants contracted neonatal herpes. One of the two infants born to women with serological evidence of a primary HSV infection at the time of delivery contracted neonatal herpes. These findings showed that most infants at risk of exposure to HSV at delivery will not be identified if concern about asymptomatic shedding of virus and surveillance by culture is limited to women with a history of genital herpes infection. Most neonatal exposure to asymptomatic HSV infection at delivery is not predictable or preventable.

The advice now given is that all pregnant women, regardless of their history of genital herpes, be assessed for genital herpes at time of their admission in labour — history, current symptoms and signs of external genital lesions and questions should be asked about any sexual contacts with persons with genital herpes. If lesions consistent with genital herpes simplex are noted Caesarean section should be performed before rupture of the membranes. When cutaneous lesions are present the vagina and cervix should be examined with a speculum (Lancet 1988b, Kelly 1988). Any suspicious lesion should be noted and scrapedings sent by prior arrangement to the virologist for immunofluorescence testing. The use of acyclovir in late pregnancy is under study (Robertson et al. 1989).

The infant may acquire the infection after delivery from maternal herpes or herpes infection in one of the medical or nursing attendants (Francis et al. 1975). Men with herpes genitalis should be told of the dangers of communicating HSV particularly when untreated lesions are present and advised against intercourse with women who are pregnant.

Infants exposed to viral shedding during labour should have specimens taken for HSV isolation from the eyes, nares, pharynx, umbilicus and any abrasions or puncture sites acquired at delivery. Intravenous acyclovir should be given to the infant in primary infection in the mother if any of the infant’s cultures yield HSV or if the infant’s condition shows cause for concern. Careful follow-up is very desirable to assess the effect of therapy (Kelly 1988).

Uncertainties remain as the majority of infants born per vaginam to mothers with overt disease do not become clinically ill. In theory hyperimmune anti-HSV-2 gammaglobulin given to the neonate would be expected to be useful in preventing involvement of an infant delivered vaginally in a woman with genital HSV-2 infection. Acyclovir, however, might well become a more acceptable line of approach in such situations. Physicians who care for the newborn should consider neonatal herpes in the differential diagnosis when infants become ill during the first weeks of life regardless of the presence or absence of identifiable risk factors (Prober et al. 1988).

In the case of patients who become clinically very ill during the last trimester of pregnancy with a primary HSV infection with actual or suspected dissemination acyclovir offers an important advance in treatment. In such cases early diagnosis and early institution of treatment is important; mouth, throat, vulva, vagina and cervix should be carefully examined for characteristic vesicular or ulcerative lesions. Virological diagnosis by electron microscopy of the roof, base or fluid of any vesicle can be made rapidly but the clinical appearance may be sufficiently characteristic.

Infection in the neonate can produce a wide spectrum of clinical effects from subclinical to disseminated forms or to those localized to the central nervous system, the eyes or mouth. Increased application of virus isolation methods has shown that the incidence of subclinical infection is commoner than the serious clinical forms.

Neonatal HSV infection and its management is discussed on page 315. The serious problems of sporadic HSV encephalitis are referred to in Robertson et al. (1989).

ASEPTIC MENINGITIS

In the aseptic (or abacterial) meningitis syndrome characterized
by fever, signs of meningeal irritation, an increased leucocyte count in the CSF, but no signs of frank encephalitis, HSV is a very rare cause among the wide array of other viruses which have been implicated in the aetiology of a varying minority of cases (Lennette et al 1961, MacRae 1961).

**PAPILLOMAVIRUS INFECTION AND GENITAL WARTS**

Not only are genital warts due to human papillomavirus unsightly but their persistence and inconstant response to treatment give rise to anxiety and introspection in the patient as well as the added burden of multiple attendances. Their high prevalence, high infectivity, long prepatent period (average 3 months, range 2 weeks to 8 months) and often poor response to treatment all make effective control by therapy and contact tracing possible only to a limited extent. Until quite recently genital warts have been regarded virtually as wholly benign growths which regress spontaneously, but human papillomaviruses may not be solely causes of benign tumours of skin and mucosa, but in the longer term and possibly in association with, herpesvirus-type 2 and/or other cofactors, such as cigarette smoking, may play a part in the aetiology of cancer particularly of the cervix uteri. The case against papillomaviruses in regard to cancer of the cervix is, however, not yet proven.

In children anogenital warts appear to be more commonly reported. They cannot, however, be regarded as a definite indicator of sexual abuse but the possibility of this has to be considered by the clinician — see ‘Sexual abuse and anogenital warts in children’ below.

**AETIOLOGY**

Since papillomaviruses cannot be propagated in vitro and the quantities of viral material in anogenital lesions were so low their characterization directly from such material was not possible. Molecular cloning of wart virus DNA in bacterial plasmids or in bacteriophage lambda has now, however, enabled the preparation of quantities sufficient to develop a classification of the papillomaviruses from lesions in a variety of anatomical sites (Lancet 1983).

At the present time classification of HPV into separate types is based on nucleic acid hybridization studies; a virus is considered to be a separate type if such DNA studies reveal less than 50% homology with known virus types (Pfister 1984).

On the basis of these nucleic acid studies, more than 50 types have been reported of which at least seven are most commonly associated with mucosal lesions including those of the urogenital tract, viz. the common types HPV-6, 11, 16, 18 (Singer et al 1984) and the less common HPV-31, 33 and 35 (Beaudenon et al 1986; for discussion see Meanwell et al 1987).

Skin warts appear to be acquired from environmental sources, but genital warts are acquired mainly by sexual intercourse. The prepatent period varies between 3 weeks and 8 months with an average of about 3 months. Genital warts in one sexual partner will also be found in the other in about 65% of cases. Vulvar and penile warts are more clearly associated with transmission by sexual intercourse, while anal warts are less certainly associated with anal intercourse so their origin remains speculative (Oriel 1971a, b).

**HISTOLOGY**

Hyperkeratosis is not a feature of condyloma acuminatum of the genital area where the stratum corneum consists as a rule of only one or two layers of parakeratotic cells. In these warts acanthosis combined with extensive papillomatosis results in layers of the wart that consist entirely of sheets of hyperplastic keratinocytes showing multiple mitotic figures. As the condylomata age hyperkeratosis may develop (Oriel 1983).

**CLINICAL FEATURES**

**Genital lesions**

Various clinical types of wart can be distinguished. In men the fleshy hyperplastic warts (condyloma acuminatum), either one or many, occur most often on the glans penis and on the inner lining of the prepuce. The appearance depends upon the location, although moisture and accompanying inflammation may enhance the size and tendency to coalesce. Those in the terminal urethra have a bright red colour in particular. Hyperplastic genital warts in the female are commonly seen on the vulva and may extend to the adjacent skin (Fig. 22.39).

In children anogenital warts are being more commonly reported possibly associated with increasing incidence of the disease in the adult population (23126 cases in 1975 and 75995 in 1986 recorded by UK departments of genitourinary medicine — PHLS Communicable Disease Surveillance Centre 1989). Clinically (Rock et al 1986), they resemble those seen in adults. Their discovery is an indication for taking tests to exclude other sexually transmissible disease (see Fig. 22.40), but genital warts are not a
definite indicator of sexual abuse — see 'Sexual abuse and anogenital warts in children' below.

Cervical warts

HPV infection of the cervix frequently does not produce lesions that are recognizable by naked eye inspection. McCance et al. (1983) have found HPV-6 DNA by these techniques in over half of histologically proved cases of cervical intraepithelial neoplastic lesions. Different types (e.g. 6, 11, 16 of 18) may also be involved.

Sessile warts of the genitalia

Sessile warts, resembling plane warts on the not-genital skin, tend to be seen on the shaft of the penis and although often multiple they do not coalesce. Sessile warts do not seem to occur on the vulva.

Oral papilloma

Mouth lesions (lips, tongue, cheek, gingiva, hard palate, floor of mouth) may occasionally occur after orogenital contact with an infected partner.

Laryngeal papillomata

Papillomata of the larynx may very rarely appear within the first 6 months of life in babies born to mothers with genital warts at the time of delivery (Cook et al. 1973). Hybridization analyses have shown that the papillomaviruses found in laryngeal papillomata are the same as the papillomaviruses found to infect the genital tract. HPV-6 and HPV-11 have been identified respectively in ten and three laryngeal papilloma (Gissmann et al. 1983). By restriction endonuclease analysis of papillomavirus genomes in laryngeal papillomata four subtypes of HHV-6 (HPV-6c, HPV-6d, HPV-6e and HPV-6f) have been identified (Mounts & Shah, 1984). In juvenile onset disease intrapartum infection is most likely to be the method of transmission of the disease, so Caesarean delivery, prior to the rupture of the membranes, would be expected to provide a high degree of protection against infection. The relative rarity of transmission, however, is to be weighed against the risks of Caesarean section.

In juvenile onset disease (viz. onset of the disease before 16 years) hoarseness is the first symptom in 90% of cases. The larynx is the most common primary site but papillomata can occur in the trachea without involvement of the larynx. The laryngotracheal location presents a threat to the airway and respiratory distress or stridor can occur as a result. Persistent recurrence is a hallmark of the disease. Cases of adult onset disease can be as severe as in juveniles.

In the Copenhagen district during the 4-year period from 1980 to 1983 seven new cases of juvenile laryngeal papillomatosis (JLPP) occurred; in an at-risk population of 300 000 children under 16 years of age this gave an incidence of 0.6 per 1000 (Bonholt 1988). In the total population of 1 740 000 inhabitants 53 were operated upon for laryngeal papilloma — arising before puberty — during the 4-year study, giving a prevalence of 0.8 per 1000.

Juvenile laryngeal papillomatosis has, in areas with good specialist surgical facilities, mostly a good prognosis, i.e. short duration, low morbidity and no mortality unless malignant change occurs. Treatment has been by microsurgical removal but currently vaporization by means of carbon dioxide laser technique has been developed. Regression before puberty does not ensure recovery and more than half the adult patients in the Copenhagen study experienced recurrences between 6 and 22 years after puberty (median 21 years). Since in both pregnancy and at puberty itself JLPP may subside it has been postulated that the condition may be subject to hormonal influence. Severe diffuse papillomatosis involving the bronchi may be associated with malignancy and is itself a serious prognostic sign (Bonholt 1988).

SEXUAL ABUSE AND ANOGENITAL WARTS IN CHILDREN

Although anogenital warts in childhood cannot be regarded as a definite indicator of sexual abuse it is a possibility which requires to be taken into account by the clinician. DNA hybridization techniques have shown, for example, that perianal warts in a 5-year-old boy were associated with HPV-2, the HPV type also found in a hand wart from the same child, a finding supporting autoinoculation as the likely route of transmission (Fleming et al. 1987). Typing of HPV DNA in adults by molecular hybridization shows that HPVs are generally site specific (see Anetology) with genital warts most frequently being associated with HPV-6 and 11, and less commonly HPV-16 and 18, whereas common skin warts are most frequently associated with HPV-2; the specificity is not absolute — see 'Laryngeal papillomata'. In a study of five cases of genital warts in children (aged 2–8 years) the HPV types were the same (6, 11 and 16) as those associated with genital warts
in adults, suggesting sexual transmission; sexual abuse was thought likely to have occurred in three of these cases (Rock et al. 1986). Condylomata acuminata have been noted in a newborn when the mother had the same condition at term (Tang et al. 1978), a situation suggesting haematogenous spread or, more likely, ascending HPV infection of the maternal genital tract and selective viral infection of the anal epithelium. It is possible too that a conjunctival papilloma associated with HPV-11 reported in a child of 14 months (Lass et al. 1983) may have been similarly transmitted. Since the time between possible infection and patency may be long the attribution of the mode of transmission with certainty is often not possible.

DIAGNOSIS

Identification of the virus in the lesion is as yet seldom undertaken except for research purposes and diagnosis is based on recognizing genital warts clinically. In young persons in the case of cervical infection, colposcopy is indicated if cervical cytology smears show abnormalities.

Skin lesions of secondary syphilis, viz. condylomata lata and papular skin eruptions, require to be differentiated by dark field microscopy for T. pallidum and serological tests for syphilis.

TREATMENT

General considerations

Although anogenital condylomata acuminata tend to regress spontaneously treatment is important as, in the case of moist hyperplastic warts, spread is sometimes rapid and sepsis can be troublesome. The exclusion, detection and, if required, treatment of other sexually transmissible infections in the affected individual and the sexual contact(s) are essential first steps (Robertson et al. 1989). Although there are a wide range of procedures available there is no certain once-only treatment for warts (Bunney 1982).

Before local treatment of genital warts is started it is important to attend to any other local infection, whether sexually transmitted or not as warts tend to spread more readily in inflamed skin. Any cause of vaginitis or discharge must be discovered and eradicated.

Papillomavirus is frequently present in clinically and histologically normal skin adjacent to the condylomata acuminata. Latent HPV may be responsible for recurrences so commonly observed following all forms of therapy (Ferencyz et al. 1985).

Podophyllin

Podophyllin, an ethanol extract of rhizome and roots of the plant Podophyllum (Windholz 1983), often produces spectacular initial effects particularly when applied to hyperplastic condylomata acuminata of the genital skin. This extract contains, among other compounds, podophyllotoxin, the main active agent. The latter acts in a manner similar to colchicine and binds specifically at the same (or greatly overlapping) site to tubulin, a component protein of microtubules (Samson 1976) but in contrast to colchicine, this binding is reversible (Wilson et al. 1974). The disintegration of chromatin and arrest of mitosis produced by podophyllin and colchicine (Sullivan & King 1947) are explained by the disassembly of microtubules produced by these compounds (Samson 1976).

Clinical effects

Condyloma acuminatum rapidly and occasionally permanently undergo involution after one or two treatments with a 25% suspension of podophyllin resin (podophyllin) in liquid petrolatum (liquid paraffin) or in 95% alcohol (see review in von Krogh 1981). Painful local inflammatory reactions are sometimes a problem and washing off medication 3–24 h after application and protection of the surrounding epithelial surfaces with inert pastes, creams or ointments is advocated. It is generally recommended that a week should elapse between applications. Condyloma acuminatum show first blanching (4–8 h) and later a drying effect when the pink or red moist warts appear white, grey and dry; sometimes there was dark brown discoloration. In 4–24 h the condylomas decrease in size and in 48 h there is sometimes complete involution. Failures occur in chronic perianal condylomas where penetration was prevented by the dry keratinized surface.

Unwanted effects

Unwanted local effects of podophyllin, even with the washing of the preparation after an interval, vary from local itching, burning, tenderness and erythema to pain, swelling and minor erosions.

Podophyllin should not be used aggressively and in quantities beyond that now recommended (Stoehr et al. 1978). In children great caution is required in its use.

The present authors recommend dispensing about 0.5 ml quantities to be used for once-only applications given by trained personnel within the clinic; the use of single containers for repeated applications in the clinics is condemned as it may bring risks of cross infection. Ordinarily, a few warts should be treated at a time, the adjacent skin being protected by yellow or white soft paraffin (BNF), powder or both. It is good practice to treat, with due regard to any local reaction produced, at frequent intervals (every 3–7 days) with small amounts of 25% podophyllin in liquid paraffin or methylated spirit (not more than 0.3 ml at a time). In the case of the preparation in spirit time should be given to allow drying. The patient should be instructed to wash off the podophyllin after an interval of about 6 h.

An ethanolic solution of purified podophyllotoxin (0.5% w/v), the active substance in podophyllin resin, is now available (Warticon, Cph; Condyline, Gist-brocades) for use in men over the age of 18 years. Its use in children and women is not advised by the manufacturers although treatment in adult men with podophyllotoxin is more effective and causes fewer adverse reactions than podophyllin itself.

Electrocautery

Electrocautery with a 1% solution of lignocaine is used as a local anaesthetic and the wart removed with the cautery. The aim should be to coagulate the wart down to the basement membrane and cause minimal damage to surrounding skin. In the case of intramural warts, lignocaine gel (20 mg/ml) may be instilled into the terminal urethra and the wart cauterized after 5–10 min. Occasionally removal of warts by diathermy or cautery will require general anaesthesia and circumcision is sometimes required, particularly when there is phimosis.

Scissor excision

Under general anaesthesia the wart bearing area, in the case of
intra-ana]arts in particular, is infiltrated with saline adrenaline solution (1:300,000). The warts are then removed with scissors by cutting at the base of the wart from back to front so that exudate and blood do not obscure progress (Thomson & Grace 1978, Gollock et al 1982).

Cryotherapy
The application of liquid nitrogen (boiling point -195.8°C) to discrete warts is sometimes effective. The aim should be to freeze the wart until a halo of frozen skin is just visible at the base. A cotton tipped applicator can be immersed in a vacuum flask of liquid nitrogen and then applied to the wart, exposed and immobilized by stretching the skin between the fingers.

PROGNOSIS AND CONTACT TRACING
Whenever possible it is important to ensure that each sexual partner is examined. Although treatment of warts is far from satisfactory it is important to examine the patient to exclude other sexually transmitted diseases and to relieve anxieties by explanations. In the case of the female contact a cervical smear should be taken for exfoliative cytology examination and an explanation given about the importance of a lifetime follow-up.

MALIGNANT TRANSFORMATION OF ANAL AND GENITAL CONDYLOMATA ACUMINATA
Condylomata acuminata, cervical koilocyosis and respiratory papillomatosis appear all to be linked in that HPV-6 and HPV-11 are the commonest types seen. Malignant conversion is a rarity but in respiratory papillomatosis X-irradiation of recurrent laryngeal or tracheal papillomatosis, however, has been followed by carcinoma after intervals between 5 and 40 years.

Carcinoma in situ occurring in one of a group of anal warts has been described in a homosexual; the warts had been recurrent over a 2-year period and were shown on electron microscopy to contain papillomavirus (Oriel & Whitmer 1971).

PROTOZOAL INFECTIONS

MALADIA
Malaria is a disease of humans caused by infection with one or more of four species of protozoa of the genus Plasmodium (P. falciparum, P. vivax, P. malariae and P. ovale). It is usually acquired through the bite of an infected female Anopheles mosquito, although it may also follow the transfer of infected blood as in blood transfusion, or transplacentally or by the use of contaminated syringes. World-wide some 45 Anopheles species effectively transmit malaria, although the identity, behaviour and importance of local vectors varies widely with geographic location. The most effective vector is probably A. gambiae — a mosquito that is widely distributed in tropical Africa. P. falciparum is the most pathogenic of the malaria parasites and infections with it must always be regarded as serious and potentially life-threatening. The other three species tend to cause less serious illness, although on occasion a lethal nephropathy may complicate P. malariae infections. Malaria is essentially a haemoclastic illness and young children, partly because if their small red cell mass and partly because of their immunological naivety, are singularly vulnerable to it.

THE PARASITE LIFE CYCLE
Malaria parasites undergo a complex stage of asexual development in the human host and a stage of sexual development (sporogony) which occurs partly in man and partly in the mosquito vector (Garnham 1988).

Asexual development in man
This begins with the introduction of infective forms (sporozoites in mosquito saliva during the biting act. Sporozoites circulate for less than 60 min, eventually gaining access to parenchymal liver cells either directly or after passage through Kupffer cells. The invasion process may entail specific ligand/receptor interaction. Once within the hepatocyte, the sporozoite initiates the erythrocytic (EE) phase of asexual development during which it grows and undergoes repeated nuclear fission (schizogony) — eventually producing a cyst-like schizont filled with daughter parasites (merozoites). This phase usually proceeds without interruption and both the time taken to schizont maturity and the number of merozoites produced varies with the identity of the plasmodial species involved. P. falciparum completes its EE development fastest (about 5 days) and produces most merozoites per schizont (about 30 000). The other species are slower and less prolific, the respective values for P. malariae, for example, being about 15 days and about 15 000 merozoites. In two parasite species, P. vivax and P. ovale, some sporozoites initiate this uninterrupted EE stage of development but some do not. These latter on entering hepatocytes produce small unicellular forms (hypnozoites) which persist without development for periods varying from several weeks to many months. Eventually the hypnozoites, activated by mechanisms as yet not known, resume growth and proceed to schizont maturation and merozoite liberation. Hypnozoites are currently widely believed to give rise to the relapsing parasitaemias which characterize P. ovale and, particularly, P. vivax infections and which can occur even after drug treatment has effectively eliminated erythrocytic parasites. Hypnozoites do not develop in infections with P. falciparum and P. malariae and in these species recrudescence of parasitaemia is generally considered to be due to persistent erythrocytic infection.

Merozoites liberated from EE schizonts are shortlived and must find and enter a red blood cell within a few minutes. Within the erythrocyte each grows rapidly through ring form and uninucleate trophozoite stages eventually to form a schizont containing merozoites. On schizont rupture, the merozoites enter the bloodstream, attach to and penetrate fresh erythrocytes and again begin the cycle of erythrocytic asexual development. Attachment and penetration by merozoites are complex operations which appear to require specific ligand/receptor interaction. Erythrocyte invasion by P. vivax appears to require a ligand that is associated with Duffy blood group antigens, while attachment of P. falciparum merozoites to red cells requires one associated with sialic acid on the erythrocyte membrane. Age of the red cell also influences invasion by merozoites; P. vivax preferentially invades reticulocytes — a feature which tends to limit the density of parasitaemia attained by this species.
A serovar analysis of heterosexual gonorrhoea in Edinburgh 1986–90

H Young, A Moyes, J Ross, A McMillan
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Abstract

Objective—To analyse the frequency of different gonococcal serovars within Edinburgh, Scotland and to describe changes that occurred in the frequency of such serovars over time.

Methods—All heterosexual patients with a diagnosis of gonorrhoea confirmed on culture between January 1986 and December 1990 had their gonococcal strain serotyped. Temporal changes in the prevalence of gonorrhoea and the serovar of the isolates were analysed.

Results—Isolates of Neisseria gonorrhoeae from 1356 episodes of gonorrhoea were serotyped. Three serovars, Bajk (IB-3/IB-6), Bacejk (IB-1/IB-2) and Aedgkih (IA-1/IA-2), dominated, occurring in two-thirds of all infections. Over the study period Bajk (IB-3/IB-6) and Aedgkih (IA-1/IA-2) isolates declined in frequency in parallel with an overall fall in the prevalence of gonorrhoea but Bacejk (IB-1/IB-2) persisted at a lower but fairly constant level. Despite a fall in the number of gonococcal infections the variety of new serovars being isolated fluctuated.

Conclusions—The ability of some serovars to persist while others decline in incidence may be partially related to antibiotic sensitivities but other factors such as an ability to evade the immune response and transfer of serovars from one population group to another may also be important.

Introduction

Since the introduction of serotyping for Neisseria gonorrhoeae using monoclonal antibodies it has been possible to divide the gonococcus into subgroups which can be used to study the epidemiology of the organism. This technique has been used to demonstrate the wide variety of gonococcal serovars present and the rapid dynamic change that occurs within any one area. It has also been noted that different geographical areas may have marked differences in the serovars which are dominant despite being within reasonably close proximity.

Although this epidemiological information is available for individual regions at one point in time there is much less known about the changes in serovars over time and, more importantly, the factors responsible for observed changes. This study follows the frequency of isolation of gonococcal serovars from a defined population over a period of 5 years with the aim of defining changes that occur in the variety and frequency of serovars over time and postulates mechanisms to explain the observations.

Methods

All heterosexual patients (women admitting to male contacts and men admitting to female contacts only) with a diagnosis of gonorrhoea who presented to the Department of Genitourinary Medicine, Edinburgh Royal Infirmary between January 1986 and December 1990 were included in the study. The diagnosis of gonorrhoea was made on the basis of culture of N. gonorrhoeae on modified New York City culture medium from the urethra, rectum, endocervix and/or throat. All male patients attending the Department had a single urethral swab taken whilst female patients had urethral, rectal and endocervical swabs cultured on two separate occasions in order to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea and when the history indicated that this site had been placed at risk.

Gonococcal isolates were identified on the basis of biochemical and immunological tests and serotyping was performed using the Swedish panel of Genetic Systems reagents as has been described previously. Serovars were designated according to the descriptive nomenclature and later translated into the likely equivalent serovars based on the more widely used American Genetic Systems panel and numerical nomenclature.

The total number of isolates each quarter was noted and further subdivided into isolations from male and female patients. The number of infections due to the three most commonly isolated serovars (Bajk (IB-3/IB-6), Bacejk (IB-1/IB-2) and Aedgkih (IA-1/IA-2)) were calculated separately for each quarter. The number of different serovars present within each quarter was also counted and the location of where the infection was acquired noted.

Statistics Statistical analysis was performed using Spearman’s Rank Correlation coefficient on the Minitab PC software package.

Results

The number of infections in men and women is shown in table 1: 1356 episodes of heterosexually acquired gonorrhoea were identified...
over the five year study period. The number of episodes of heterosexually acquired gonorrhoea fell from 518 in 1986 to 115 in 1990. Thirteen protein IA serovars accounted for 711 (52%) of infections while 31 protein IB serovars accounted for 645 (48%). There were 10 IA serovars and 20 IB serovars isolated from women compared with 12 IA and 28 IB serovars from men. The higher number of serovars in male infections is in keeping with the greater number of infections observed in men (table 1). As shown in fig 1 the combined number of different IA and IB serovars has decreased from a total of 14 in the first quarter of 1986 to seven during the last quarter of 1990. In spite of the overall trend towards a decrease in the number of different serovars there is a degree of fluctuation particularly in the number of IB serovars isolated each quarter. This could be due to importation of strains.

The geographical area where the infections were acquired is given in table 2. As shown, only 64% of infections were acquired within Edinburgh and the surrounding Lothian region: other areas of Scotland (23% of infections) provided the main source of imported strains with a less significant contribution from the rest of the UK (4%) and abroad (5%). The geographical area of acquisition of infection did not vary over the study period.

Three serovars Bajk (IB-3/IB-6), Bacejk (IB-1/IB-2) and Aedgkih (IA-1/IA-2) accounted for 69% (934) of the infections over the 5 year study period. The correlation coefficients relating to these three serovars and the group of "other" serovars to the overall prevalence of gonorrhoea and to the sex of the patients from whom the serovars were isolated are given in table 3. Each of the serovars occurred with similar frequency in men and women, as would be expected in the case of heterosexually acquired gonorrhoea. Whereas the prevalence of Bajk (IB-3/IB-6) and Aedgkih (IA-1/IA-2) and "other" serovars correlates well with the fall in total gonorrhoea serovar, Bacejk (IB-1/IB-2) did not.

**Discussion**

Public awareness of the risk of contracting sexually transmitted diseases increased following the recognition of Human Immunodeficiency Virus (HIV) infection and there has

### Table 1 Number of male and female heterosexually acquired gonococcal infections in Edinburgh 1986–90

<table>
<thead>
<tr>
<th>Year</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986 1st Quarter</td>
<td>83</td>
<td>45</td>
</tr>
<tr>
<td>1986 2nd Quarter</td>
<td>79</td>
<td>55</td>
</tr>
<tr>
<td>1986 3rd Quarter</td>
<td>83</td>
<td>53</td>
</tr>
<tr>
<td>1986 4th Quarter</td>
<td>66</td>
<td>54</td>
</tr>
<tr>
<td>1987 1st Quarter</td>
<td>59</td>
<td>43</td>
</tr>
<tr>
<td>1987 2nd Quarter</td>
<td>60</td>
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<tr>
<td>1987 3rd Quarter</td>
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<td>1987 4th Quarter</td>
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<td>1988 1st Quarter</td>
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<td>1989 1st Quarter</td>
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<tr>
<td>1990 2nd Quarter</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>1990 3rd Quarter</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>1990 4th Quarter</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>789</strong></td>
<td><strong>567</strong></td>
</tr>
</tbody>
</table>

### Table 2 Geographical origin of heterosexually acquired gonococcal infections in Edinburgh 1986–90

<table>
<thead>
<tr>
<th>Geographical Area</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edinburgh</td>
<td>788</td>
</tr>
<tr>
<td>Rest of Lothian</td>
<td>88</td>
</tr>
<tr>
<td>Rest of Scotland</td>
<td>541</td>
</tr>
<tr>
<td>Rest of UK</td>
<td>55</td>
</tr>
<tr>
<td>Not UK</td>
<td>71</td>
</tr>
<tr>
<td>Unknown</td>
<td>48</td>
</tr>
</tbody>
</table>

**Figure 1** Number of different protein IA and protein IB serovars isolated over time.
Table 3 Prevalence of serovars correlated with total gonorrhoea and sex of patient

<table>
<thead>
<tr>
<th></th>
<th>Total gonorrhoea correlation coefficient</th>
<th>Sex of patient correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bajk (IB-3/IB-6)</td>
<td>0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>Bajk (IB-1/IB-2)</td>
<td>0.42*</td>
<td>0.81</td>
</tr>
<tr>
<td>Aedgk (IA-1/IA-2)</td>
<td>0.94</td>
<td>0.84</td>
</tr>
<tr>
<td>Others* serovars</td>
<td>0.78</td>
<td>0.78</td>
</tr>
</tbody>
</table>

*No significant correlation between Bajk and total gonorrhoea—all other correlations significant (p < 0.001).
†Significant correlation in the occurrence of each serovar between male and female patients (p < 0.001).

been a decline in the incidence of gonorrhoea throughout Britain over the past five years.6 Our data confirm a similar trend in the Lothian area of Scotland. Over the past 12 to 18 months there has however been an increase in homosexually acquired infection in the south of England10 which has not affected our area as yet, although there was an unsustained rise in homosexually acquired gonorrhoea in the last quarter of 1990.11 With the relatively high incidence of HIV in Edinburgh and therefore more frequent reminders of the risks of unprotected intercourse this may explain the persistent low levels of gonococcal infection in this area.

Although 43 different serovars were isolated over the 5 year period, three of these, Aedgk (IA-1/IA-2), Bajk (IB-3/IB-6) and Bajk (IB-1/IB-2), accounted for over two-thirds of all infections. While Aedgk (IA-1/IA-2) and Bajk (IB-3/IB-6) declined in parallel with the overall fall in the prevalence of gonorrhoea, Bajk (IB-1/IB-2) maintained a lower but consistent level (Fig 2). Bajk (IB-1/IB-2) accounted for 26% of 175 homosexually acquired infections between 1986 and 1990.6 It is possible that the persistent low level is due to the constant introduction of an essentially homosexual strain into the heterosexual population via bisexual men. Such a strain might be disadvantaged in spreading within the heterosexual population.

The persistence of certain gonococcal strains with the appearance and disappearance of others over time has been previously described.12 Our observed pattern of some strains declining in parallel with the overall trend with time whilst another persists despite a falling infection rate has not, however, been previously described. Possible explanations for these observations must relate either to the serovars themselves or else the population groups which they infect. It is unlikely that any serovar would remain confined to any single group, such as prostitutes, over such a long period although certain serovars are associated with infection in homosexual men.6 Alternatively the characteristics of the serovars themselves may explain the observed trends.

Although this study did not look at antibiotic sensitivities, a previous study looking at serovars in the same area13 reported that Bajk (IB-1/IB-2) had a higher minimum inhibitory concentration (MIC) for penicillin than average while Bajk (IB-3/IB-6) had a lower than average penicillin MIC. Almost all isolates of Aedgk (IA-1/IA-2) were highly sensitive to penicillin except for a small number of penicillinase producing strains.

It has been postulated that serovars within any one community die out as protective immunity develops in the population.5 It is possible that some strains have a greater ability to evade the immune system than others thus gaining a selective advantage. Although Protein I is relatively less important in the response of antibody in the serum compared with other antigens such as Protein II, pili and lipopolysaccharides it appears to be much more important in the genital antibody response of women compared with the serum antibody response.12

A greater variety in the number of different Protein IB serovars was observed than IA strains. This has been noted in other areas.5

Figure 2 Quarterly variation in individual serovars and total number of homosexually acquired infections over time.

![Graph showing quarterly variation in gonorrhoea infections](image-url)
A serovar analysis of heterosexual gonorrhoea in Edinburgh 1986–90

and may be due to a greater potential for antigenic drift in the IB strains compared to the IA strains. In this population approximately half the infections were due to IA strains and half due to IB strains. It has been observed by others that IB strains tend to be prevalent in large towns whereas IA strains occur more frequently in smaller population centres.

Despite the large fall in the incidence of gonorrhoea over the past 5 years a wide variety of serovars continued to be isolated (Fig 1). It is likely that these minor strains often represent an influx of infections from other geographical areas reflecting the youth and mobility of our population group. It may be that as the overall rate of gonococcal infection falls these new strains are unable to become endemic in the local population and therefore appear only transiently.

The serovar pattern in heterosexuals varies little between men and women (table 3). The observed pattern does, however, differ markedly from that seen in homosexual men from the same area over a similar time period where Ae (IA-4), Back (IB-1/IB-2), Baejk (IB-3/IB-6) and Bacejk (IB-1/IB-2) were the commonest serovars isolated. This difference in dominant strains between heterosexuals and homosexuals is in agreement with previous studies and the possible transfer of Bacejk (IB-1/IB-2) between different groups has already been noted.

In conclusion, we have demonstrated a changing pattern of gonococcal serovars over a 5 year period and proposed possible explanations for our observations. Three serovars predominated over the study period with one of these persisting at a constant low level while the others declined in parallel with the overall fall in the incidence of gonorrhoea.


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- Institution: £90.00/US$162.00
- Individual: £55.00/US$120.00

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EDITORIAL REVIEW

Syphilis: new diagnostic directions

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Keywords: Syphilis, serodiagnosis, HIV, recombinant proteins, polymerase chain reaction

INTRODUCTION

The development of the Treponema pallidum haemagglutination assay (TPHA) in the mid-1960s1,2 led to its widespread use in Europe as a screening test, usually in combination with a cardiolipin antigen test3-5. This combination was acknowledged as giving the best coverage for detection or exclusion of all forms of treponemal infection6, and in the United Kingdom in 1983, 86% of laboratories participating in the United Kingdom (UK) National External Quality Assessment Scheme for Microbiology employed this screening protocol7. In contrast to the stability in test protocols there was a marked change in the epidemiology of early syphilis with a significant overall decline in incidence in the United Kingdom starting in the late 1970s (Figure 1). As a result of this decrease the value of traditional routine testing of certain populations such as pregnant women6 and blood donors7 has been questioned.

Over the past few years the situation has changed and we have witnessed important potential developments in the diagnosis of syphilis8,9 as well as a renewed interest in many aspects of the disease itself10-14. Factors associated with this renaissance include: interactions between syphilis and infection with the human immunodeficiency virus (HIV)15-21; changes in the epidemiology of early syphilis22-25; production of pathogen-specific monoclonal antibodies26-30; production of treponemal antigens by genetic recombination and cloning in bacteria31-33; development of new assay formats such as enzyme immunoassay (EIA) applicable to large-scale screening and automation34 as well as methodologies such as immunoblotting35-37 and the polymerase chain reaction (PCR)38-40 more suited to confirmatory testing and problem cases.

The aim of this review is to examine how these factors have already impinged on our approach to diagnosis, or are likely to do so in future, and to re-assess the value and limitations of conventional diagnostic tests and strategies as a baseline against which to evaluate new approaches.

IMPORTANCE OF HIV

Increased transmission

A genital ulcer disease such as syphilis which disrupts the integrity of the mucosal epithelium may increase susceptibility to HIV17,41-43. As the base of a genital ulcer is likely to contain large numbers of activated lymphocytes and macrophages it provides not only a source of target cells for HIV acquisition but may also enhance transmission of HIV to uninfected sex partners via antigenic stimulation of latently HIV-infected cells resulting in activation and liberation of HIV42. There is some experimental evidence to suggest that rabbits infected with human T-cell leukaemia virus type I show protracted T. pallidum induced cutaneous chancre44, by analogy infection with HIV could lead to delayed healing of chancre thus increasing an individual’s infectivity in the case of syphilis. Control of genital ulcer disease including syphilis is therefore important in the control of HIV41,42. It is recommended that every patient with positive syphilis serology should be tested for HIV (with the patient’s informed consent) and that every HIV-positive patient should be tested for syphilis45.

Serodiagnosis

The finding that HIV-infected patients who acquire syphilis may fail to produce anti-treponemal antibodies19,46 has extremely serious implications for
the diagnosis and control of syphilis; certain of the patients described as seronegative are however more accurately classified as 'delayed seropositivity'\textsuperscript{15}. Nevertheless this finding increases the importance of demonstrating \textit{T. pallidum} in tissues as a means of diagnosing early syphilis\textsuperscript{47}. Fortunately the vast majority of HIV-infected patients who acquire syphilis appear to have a normal serological response to \textit{T. pallidum}\textsuperscript{15,45}. Indeed many HIV infected patients who acquire syphilis produce very high levels of anti-treponemal antibodies\textsuperscript{15}, this may be a result of polyclonal B-cell activation but the fact that many such patients are likely to be experiencing re-infection with syphilis\textsuperscript{48} might also contribute to high antibody levels. However, Hutchinson \textit{et al.}\textsuperscript{49} found that in patients with secondary infection and no previous syphilis the geometric mean RPR titres were significantly higher among HIV-seropositive patients than in HIV-seronegative patients.

**Serological tests for monitoring therapy**

The possibility that polyclonal B-cell activation might interfere with the reliability of treatment monitoring by quantitative cardiolipin tests has also been considered. Terry and colleagues\textsuperscript{50} found that co-existing HIV infection had no influence on post-treatment titres, at least when patients were in the early stages of HIV infection. A second report\textsuperscript{51} found that HIV-infected patients with primary syphilis when compared with HIV-negative controls were less likely to have a 4-fold or greater decrease in cardiolipin antibody titre, or seroreversion, within 6 months of treatment. Cases and controls with secondary syphilis had similar serological responses after treatment for syphilis\textsuperscript{51}. Further studies are obviously required to determine definitively whether HIV alters the serological response to therapy in patients with early syphilis.

**Serological markers for past syphilis**

It is, however, becoming increasingly apparent that serological evidence of treated syphilis may disappear after patients become infected with HIV\textsuperscript{20,52}. Specific antibody was undetectable in up to 10% of AIDS patients who previously had positive tests. Loss of treponemal antibody response has been noted within 2 years of treatment for secondary syphilis and before the onset of AIDS\textsuperscript{53}. In this case the loss of antibody response was not linked to serious humoral immune deficit since IgG responses to other infections such as rubella, cytomegalovirus, haemolytic streptococci, and staphylococci remained detectable. Clearly there is a reduction in levels of specific anti-treponemal antibody to \textit{T. pallidum} in some HIV-infected individuals as they progress through their illness. This means that negative specific serology does not necessarily exclude a past syphilis infection in patients with AIDS; the significance of this in relation to differential diagnosis in patients with neurological problems is not yet known. It also makes it important that new tests are evaluated for sensitivity and specificity against HIV-infected patients. There is some evidence that non-treponemal tests may yield high titres (≥16) in HIV-infected patients without syphilis\textsuperscript{54}.

**Alterations in the natural course of syphilis**

There are an increasing number of reports to suggest that in HIV-infected patients syphilis runs a more severe course and lesions may be more aggressive in both early\textsuperscript{55,56} and late stage disease\textsuperscript{57,58}. Neurological complications of secondary syphilis occur more frequently and at an earlier stage\textsuperscript{18,59} and may increase the risk of treatment failure\textsuperscript{13,60}. Ocular symptoms are important in HIV-infected patients with syphilis\textsuperscript{61-64} and in some cases their investigation has led to the diagnosis of syphilis\textsuperscript{65,66}. There is of course a considerable clinical overlap between the two infections with reports of secondary syphilis masquerading as AIDS\textsuperscript{67} and primary HIV infection mimicking syphilis\textsuperscript{68}.

**CHANGES IN EPIDEMIOLOGY**

Figure 1 shows the changing incidence of early syphilis in the United States, England and Wales, and Scotland. At the time of the peak levels of syphilis in the late 1970s in the United Kingdom and around 1982 in the United States the majority of cases of early infection were homosexually acquired\textsuperscript{69-72} and the overall decrease observed was associated with a decrease in homosexually-acquired syphilis which has been reported in the United Kingdom\textsuperscript{73,74}, the Netherlands\textsuperscript{25}, Sweden\textsuperscript{75}, Denmark\textsuperscript{76}, Canada\textsuperscript{77} and the United States\textsuperscript{69,70}.

**Increase in heterosexual transmission**

The marked increases observed recently in the United States have been associated with heterosexual transmission, drug abuse, and prostitution\textsuperscript{69,78-80} and syphilis, along with gonorrhoea and chancroid, is ‘especially rife in the nation’s poverty pockets’\textsuperscript{81}. With 50,223 cases reported in 1990 (a 9% increase over 1989) syphilis is now at its highest level since 1949\textsuperscript{82}; the incidence of 20 cases per 100,000 persons represents a 75% increase from 1985 and is well adrift from the 1990 objective of 7 cases per 100,000 population. There have also been reports of a resurgence in homosexually-acquired infection in areas of London\textsuperscript{84} as well as in Amsterdam\textsuperscript{25} and in the province of Manitoba\textsuperscript{77}. In general, however, the level of early syphilis is low in Europe and this means that clinical and laboratory experience with respect to diagnosis, particularly primary infection, is limited among younger doctors.
Congenital infection

In the USA there has been a dramatic spill over into the child-bearing population with between 17 and 18 cases of congenital infection per 1,000,000 live births in 1988, again outstripping the 1990 objective of 1.5 cases per 100,000 live births83. Levels in certain cities such as New York are even higher84. Although the overall trend in the United Kingdom is a decline, between 1985 and 1986, there was a small increase in syphilis in young women with a concomitant increase in congenital infection from 3 cases in 1985 to 9 in 198685.

DIAGNOSIS

As it is impossible to culture T. pallidum in vitro serological tests are the mainstay for the diagnosis of syphilis at all stages, other than, perhaps, the very early stages of the disease. T. pallidum may be detected, usually by microscopy, in the genital and rectal lesions of primary syphilis. In many cases this enables a diagnosis of primary syphilis to be made rapidly in the absence of reactive serology. A microscopic diagnosis of primary syphilis depends on the skill of the clinician in obtaining a specimen as well as expertise in darkfield microscopy. Microscopy is not suitable for examining the dry skin rash of secondary syphilis, but treponemes are plentiful in the moist lesions at mucocutaneous junctions, such as mucous patches or condylomata lata. Demonstration of T. pallidum in biopsy material has increased considerably in importance since the reports of seronegative syphilis in association with HIV infection19,87.

Demonstration of T. pallidum in lesion exudates

Darkfield microscopy

The identification of T. pallidum in lesion exudates by darkfield microscopy has long been regarded as standard practice in the diagnosis of primary syphilis: the method involves the identification of a motile spiral-shaped organism with morphology and motility characteristic of T. pallidum86. There are, however, many limitations associated with this80 including: the need to maintain treponemal motility in order to aid differentiation between T. pallidum and commensal spirochaetes that are found in the normal flora of the genital and rectal mucosal surfaces (because of interference from commensal spirochaetes darkfield microscopy may not be reliable in the case of rectal and non-penile genital lesions); maintaining viability means that potentially infectious specimens, possibly containing HIV, must be examined within 10–30 min; accurate interpretation of darkfield microscopy is dependent on a highly experienced observer—the decrease in primary syphilis means that experience in dark ground microscopy is limited; oral lesions normally abound with commensal treponemes and cannot be reliably examined; darkfield microscopes are normally only available in units dealing with STDs.

Immunofluorescent staining

The direct fluorescent-antibody staining for T. pallidum (DFA-Tp) test whereby a smear of exudate is made on a slide, fixed in acetone, and sent to the laboratory overcame the problem of examining potentially infectious material but did not solve the problem of specificity. Originally smears were stained with a conjugated syphilitic serum made specific for T. pallidum by absorption with cultivable treponemes87. In spite of the absorption stage the DFA-Tp test gave non-specific results and was less reliable than darkfield microscopy88.

Improvements in specificity awaited the use of a DFA-Tp test based on a fluorescein labelled pathogen-specific monoclonal antibody (H9-1) specific for a 47–48 kD antigen on T. pallidum subspecies28. This antibody did not react with the non-pathogenic T. phagedenis biotype Reiter, T. denticulata, T. refringens, or T. vincentii, nor with related spirochaetes such as Leptospira interrogs and Borrelia recurrentis.

In one study89 the DFA-Tp test using monoclonal antibody H9-1 evaluated on ulcer material from 30 patients with and 31 without syphilis was 100% sensitive and specific; darkfield microscopy was 97% sensitive and 77% specific. In a series of 66 patients with and 62 without syphilis the same test gave a sensitivity of 73% and a specificity of 100%; darkfield microscopy was 79% sensitive and 100% specific22. This test would therefore appear to be as sensitive as darkfield microscopy and of greater potential specificity. Like darkfield microscopy the test is also subject to sampling variation and obstruction of treponemes by debris and erythrocytes and lesions should be examined on three consecutive days before being considered negative. Because of the advantages described above, if the monoclonal antibody DFA-Tp test becomes widely available commercially, it may make darkfield microscopy redundant. A further potential advantage is the ability to examine lesion exudate simultaneously for other genital pathogens such as herpes simplex viruses and Haemophilus ducreyi.

EIA

The Visuwell® Syphilis Antigen EIA is a recently introduced commercially available (ADI Diagnostics, Canada) alternative to microscopy for detecting T. pallidum in early lesions. Antigen extracted from lesion material collected on a swab is captured by a pathogen-specific monoclonal antibody reactive with a 47 kD protein and detected by rabbit polyclonal antibody against T. pallidum. The presence of captured antigen is demonstrated by a goat anti-rabbit urease conjugate. The test yields a visually distinct endpoint and requires about one hour to run. A preliminary evaluation10 reported a sensitivity of 64% (42/66) compared with 53% (35/66) for darkfield microscopy; 28 specimens were
positive by both methods, 7 by darkfield only, 14 by EIA only, while 17 were negative by both methods. EIA was positive in 24% (11/45) of patients with non-syphilitic lesions of uncertain diagnosis; 3 of these patients subsequently seroconverted increasing the specificity to 82% (37/45): darkfield was 100% specific. A modified extraction procedure and results corrected for equivocal data increased EIA sensitivity to 76% (darkfield also increased to 76%) and specificity to 91% (manufacturer's product data).

Although EIA is sensitive, needs little expertise and costly equipment, lack of specificity would appear to be a major problem. The implications of a diagnosis of primary syphilis are such that specificity must be paramount. In examining material from genital ulcers the technical disadvantages of immunofluorescence are more than outweighed by the ability to observe organismal morphology.

Identification of T. pallidum in tissues

The report that the diagnosis of secondary syphilis in an HIV infected man with Kaposi sarcoma required biopsy of a skin lesion with silver staining to show spirochaetes19 re-awakened interest in this form of diagnosis. The Centers for Disease Control (CDC), Atlanta recommend that when clinical findings suggest syphilis, but serological tests are negative, biopsy tissue should be examined by DFA-Tp or silver staining (Warthin Starry Silver, Steiner)91. A caution is given, however, with regard to the occurrence of artifacts and interpretation of silver staining91; problems are most likely to arise when few organisms are seen—many normal tissue components, such as collagen, are also stained and may be easily confused for spiral organisms30.

Silver staining, immunofluorescence (IF) or immunoperoxidase staining methods using polyvalent rabbit anti-T. pallidum antiserum are not reliably performed as occasional tests and such methods have been mainly confined to research laboratories. Ito and colleagues92 considered current IF methods were non-specific and that, to date, tissue sections prepared from formalin-fixed tissues had not been successfully stained; they also considered that the DFA-Tp method currently used for examining chancre material is unsuitable for use with tissue. Using trypsin pretreatment of tissue sections from rabbits, hamsters and humans and serum absorbed with the Reiter treponeme they were able to demonstrate specific direct and indirect IF staining methods.

Recently T. pallidum has been demonstrated in the gastric wall of a patient who had cutaneous manifestations of secondary syphilis and gastric symptoms by use of fluorescent treponemal absorption complement and immunoperoxidase stains80. Anti-C3 IF demonstrated treponemes in 6 of 12 biopsies from 8 patients suspected of treponematosisis94; the method was considered easier and quicker than silver staining.

Demonstration of T. pallidum in tissues remains a highly specialized procedure out with the confines of all but larger centres. The methods recommended by the CDC are not without problems and much scope remains for the development of simpler and more accurate methods. The utilization of monoclonal antibodies in a commercially available DFA-Tp test offers the best immediate hope for more widespread and accurate detection of T. pallidum in biopsy material but new approaches such as adaption of solid phase immunoblotting29 and PCR99 may prove helpful in the longer term.

Reactivity of ‘pathogen-specific’ monoclonals

Recent studies suggest that monoclonal antibodies of the type used in the above test may react with organisms other than T. pallidum subspecies95,96. Dental plaque from healthy subjects and from patients with ulcerative gingivitis or chronic periodontitis was examined by an immunocytochemical technique using monoclonal antibodies against pathogen-specific determinants on 47 kD and 37 kD molecules from T. pallidum subspecies pallidum95. The results showed that the spirochaetes found in dental plaque from diseased, but not healthy, patients have antigens that are thought to be unique to pathogenic treponemes. They concluded that this close antigenic relationship suggested that T. pallidum or a closely related organism may be involved in the pathogenesis of periodontal disease. Many patients also had detectable IgG against the pathogen-specific molecules. The significance of these findings for diagnostic methods based on pathogen-specific antigens and monoclonal antibodies obviously requires evaluation and the possibility of current or recent periodontal disease should be kept in mind in the case of inconsistent findings.

SEROLOGICAL SCREENING AND SERODIAGNOSIS

Serological tests which form the mainstay of diagnosis beyond the primary stage are best considered in relation to the natural history of infection and the humoral immune response.

Natural history of untreated syphilis

The natural course of syphilis may span several decades and can present in a variety of clinical forms97,98. The prepatent period is usually about 25 days (range 9-90 days). If untreated, primary lesions will normally heal in 3-8 weeks and the disease will progress to the secondary stage, usually 6-12 weeks after contact, but occasionally as long as 12 months. Once the early infectious stage of the disease has run its course it enters the late non-infectious stage about 2 years after the initial contact. This latent (or
hidden) stage may persist for the remainder of an individual's natural life but on the other hand, the disease may, after several years or even decades, give rise to gummatous lesions (15%), cardiovascular (10%) or neurological (8%) disorders. Adequate treatment during the early stages will prevent development of late-stage infection (see importance of HIV under neurosyphilis however). Likewise detection and treatment of latent stage infection when there are no symptoms will prevent active infection which will otherwise develop in about one-third of patients. From the serological point of view it is important to appreciate that different serological tests vary widely in their ability to detect latent infection and this must be taken into account in considering screening policies. Patients are diagnosed with latent infection who are completely unaware of having experienced primary or secondary symptoms. Antibiotics prescribed for other conditions may abort or delay early stage infection, minimizing or abolishing early symptoms. Unfortunately we have no serological test to identify which patients with latent infection will develop symptoms.

**Humoral immune response**

Specific anti-*T. pallidum* IgM is detectable during the second week of infection while production of specific anti-treponemal IgG begins around the fourth week after infection and usually reaches much higher titres than those for IgM. Recently many investigators have applied immunoblotting to analyse the immune response in experimental and human infection. The IgG and IgM reactivity in patients with primary syphilis is variable, but generally the number of molecules recognized by antibodies and the intensity of reactivity, reflect the duration of clinical symptoms. The observation that by the time clinical signs develop most patients have both IgG and IgM antibody is of considerable importance in relation to screening methods such as EIA that employ an anti-treponemal IgG conjugate. Sera from patients with secondary and early latent syphilis uniformly demonstrated reactivity to 22 separate polypeptide antigens; decreased reactivity was seen in late latent syphilis. IgM reactivity was strongest in secondary syphilis and although very weak IgM reactivity did continue to persist in some patients with late latent infection it was not detectable by IF.

In a recent study nine pathogen-specific polypeptides (15, 17, 33, 37, 39, 43, 45, 47, 49 kD) were commonly recognized by the time chancre immunity developed—minor differences in the molecular masses of some of these antigens have been described by different investigators.

In primary infection a strong antibody response to a 48 kD band was reported by Baker-Zander and colleagues as well as reactivity against 6 other bands including the 12 kD molecule. In secondary and early latent syphilis the main serological response is associated with antigens of 12, 14, 33, 37, 42 and 47 kD. In late latent syphilis reactivity against the 47, 14 and 12 kD antigens predominates but there is a decreased spectrum and intensity of IgG reactivity. Therapeutic intervention at any stage of the disease causes a generalized loss of antibody against individual antigens; the rate and degree of this loss is inversely proportional to the duration of symptoms and the stage of the disease before therapy. HIV infection may also have a significant effect on the spectrum of antibodies produced. Western blot analysis of serum from a patient with secondary syphilis and asymptomatic HIV showed antibodies to far fewer antigens than are usually present in secondary sera; only three bands including that to the 47 kD antigen appeared to be of appropriate intensity.

The complexity of the immune response poses a considerable serological challenge and it is surprising that existing serological tests perform as well as they do. The complexity of the immune response poses considerable problems with regard to the use of serological tests based on single recombinant antigens.

**USE OF SEROLOGICAL TESTS**

The way in which the main serological tests are used is outlined in Table 1. In this discussion the term screening is used to refer to both 'screening' and 'case-finding' activities as is standard practice in most publications on syphilis serology. In the strict sense screening is the testing of apparently healthy volunteers from the general population for the purpose of separating them into groups with high and low probability for a given disorder whereas case-finding is a form of disease detection in which individuals seeking health care for any reason are given additional tests to detect a disease such as syphilis.

Basically syphilis serodiagnosis depends on the principle of dual level testing. Because of the low

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[Not commonly used in this way at present but possibly could be in future]
prevalence of infection, poor positive predictive values can result even when tests of high specificity (>99%) are used for screening. For example a test with 99% specificity has a positive predictive value of only 50% when the prevalence of syphilis is 1% but 91% when prevalence rises to 10%. Traditionally sera selected on the basis of a reactive screening test are checked by a confirmatory method in order to establish the treponemal nature of antibodies detected by the screening test. Screening therefore has the effect of increasing the prevalence of the disease in the reactive population. In the example given above screening has increased the prevalence of disease from 1% in the initial population to 50% in the confirmatory population and the use of a second (confirmatory) test of the same specificity (99%) increases the positive predictive value to 98% (ie only two of 100 patients with reactive sera will not have syphilis). It follows, however, that the confirmatory test must have as high a sensitivity as the screening method otherwise sera may be classified falsely as non-treponemal (this possibility is discussed under confirmatory tests).

Once sera are confirmed as treponemal, quantitative tests and/or detection of specific anti-treponemal IgM may help assess treatment status and monitor the efficacy of therapy. Laboratories performing confirmatory tests should also be able to assist with special problems such as the diagnosis of congenital infection and neurosyphilis. Not all laboratories need perform the full array of tests and it is common for sera reactive on screening (eg in a blood transfusion centre or a laboratory serving an antenatal clinic) to be referred for confirmatory testing.

Screening tests

There are three main choices: a cardiolipin antigen test; a T. pallidum antigen test; a combination of both. It is not the purpose of this review to cover details of these tests which have been reviewed elsewhere but to summarize the advantages and disadvantages of these approaches.

Cardiolipin antigen tests

Tests such as the Venereal Diseases Research Laboratory (VDRL) or Rapid Plasma Reagin (RPR) test are cheap, simple and quick to perform and can be adapted for automated testing as in blood transfusion centres. The RPR test is more suitable for large numbers of sera. In general, all behave in a similar manner with regard to the spectrum of antibodies detected. Antibody becomes detectable 7–10 days after the appearance of the primary chancre or 3–5 weeks after acquiring the infection. Sensitivity ranges from 60 to 87% in primary infection and may reach 100% in the secondary stage. Titres decline beyond the secondary stage and around 30% of patients with late syphilis are non-reactive. Cardiolipin antigen tests also tend to become negative after treatment.

Cardiolipin tests such as the VDRL when used alone provide an effective screen for early infection only and are often used in areas of high prevalence and limited resources. The RPR is particularly suitable as an immediate test and has been advocated for testing of pregnant patients, who have had no prenatal care, when presenting at an emergency department with any complaint, in the hopes of reducing the incidence of congenital syphilis.

Disadvantages of cardiolipin antigen tests include the occurrence of Biological False Positive Reactions (more accurately described as a non-treponemal cardiolipin response) in around 0.3–0.9% of all sera examined. The most serious, and often underestimated, disadvantage is the occurrence of the prozone phenomenon: i.e. false negative reactions resulting from inhibition of agglutination due to excess antibody in the serum. The prozone phenomenon is generally considered to occur in 1–2% of patients with secondary syphilis. This may be an underestimate: Spangler et al. reported 24 patients in various stages of infection whose serological tests were negative; 16 of the 24 negative tests were due to the prozone phenomenon. A policy of performing a quantitative VDRL test on any serum giving a TPHA titre >5120 resulted in a prozone rate of 8% in patients with secondary and 10% in patients with early latent infection.

The tendency of the prozone phenomenon to occur in sera with high VDRL titres could be particularly important in patients with concomitant HIV infection as sera from many such patients have unusually high antibody levels. Unusually high titres are also found in patients undergoing re-infection, even in cases of primary syphilis; the increased risk of the prozone phenomenon occurring in sera from these patients makes detection of re-infection by non-treponemal tests less reliable. False negative VDRL reactions may also occur in the cerebrospinal fluid (CSF). Feraru et al. reported 2 HIV positive patients with neurosyphilis whose initial CSF VDRL tests were negative. In one of these patients the initial negative VDRL test may have been due to the prozone phenomenon.

False negative results are important in antenatal screening. Recently 4 cases with false negative serological tests due to the prozone phenomenon were encountered in women who gave birth to infants with congenital infection. In another report there was a failure to diagnose syphilis at birth in 7 infants; 4 mothers and their infants had given negative qualitative RPR tests at delivery while 3 mothers had tested RPR negative during pregnancy and were not tested at delivery. Although serum dilution prior to testing was recommended as a routine procedure for all seronegative women in an area of high syphilis prevalence, this is not a practical solution when all patients can be screened simply and reliably with
specific anti-treponemal antibody tests such as the TPHA or EIA.

These findings suggest that, because of the prozone phenomenon, cardiolipin tests based on agglutination should not be used alone to screen for untreated early infection. As the RPR test is very rapid it is ideal for use in emergency departments. In these circumstances diluted as well as undiluted serum should be tested as part of the standard procedure. EIA using cardiolipin antigen should overcome the problems of the prozone reaction but would still represent a poor screen for late infection.

**Screening with a combination of cardiolipin and T. pallidum antigen tests**

The activities of the TPHA and VDRL tests are complementary and the combined use of the two tests provides an excellent screen for the detection or exclusion of syphilis at all stages. TPHA reactivity may be detectable around the 4th week of infection. The sensitivity in untreated primary infection ranges from 64% to 87%. The TPHA titre tends to be low in primary syphilis (80–320) but rises sharply in the secondary stage reaching 5120 or greater. The titre declines during the latent stage but invariably remains positive, often at low titre (80–1280). Titres may decline after therapy but, with the exception of patients co-infected with HIV, the test almost always remains positive.

According to Luger, the TPHA, with an overall margin of error in the range of 0.07% false positive reactions and 0.008% false negative reactions, is the most sensitive and specific method for detecting antibodies to T. pallidum. When false negative reactions occur they are usually associated with early primary infection and this is the main reason why the TPHA has not been widely used as a single screening test in laboratories serving STD clinics.

Serological detection of primary syphilis is increased by the combination of tests as they are complementary in their activity with regard to primary syphilis: e.g., the VDRL test gave a sensitivity of 72.7% compared with 70.5% for the TPHA whereas the two tests gave a combined sensitivity of 84.1%. Although several studies have suggested that antibody detected by the TPHA appears later, or is of relatively lower titre, than that detected by cardiolipin antigen tests in one large retrospective analysis of routine practice the TPHA gave a sensitivity of 74.9% (487/659) in primary infection compared with 70.4% for the VDRL in using the combination of tests, apart from increasing the detection of primary infection, the TPHA will detect high titre sera that may give a false negative reaction due to the prozone phenomenon.

Therefore although the test combination can not be faulted with regard to performance data, it requires more labour than does a single test, interpretation is subjective, and the combination does not lend itself readily to automation.

**Screening with a single T. pallidum antigen test**

**TPHA** The TPHA on its own is a good screen for syphilis at all stages beyond the early primary stage. Whether or not it should be advocated as a single screening test depends on the importance attached to early primary infection. It was suggested in 1983 that the VDRL should be withdrawn from initial testing for syphilis except where primary disease is suspected. The opposite view that neither TPHA nor cardiolipin tests should be relied upon alone in screening has also been advocated. A T. pallidum antigen test with increased sensitivity in primary syphilis would be more attractive as a single screening test.

**Captia syphilis G EIA** The development of EIA in syphilis diagnosis since its first use in 1975 has been reviewed elsewhere. The sensitivity in primary infection of an EIA to detect anti-treponemal IgG is crucial in its adoption as a single screening test. The high sensitivity of EIA using anti-human IgG conjugate has been reported with both 'in-house' and commercial EIA systems. Veldkamp and Visser detected all 32 cases of untreated primary infection while the Bio-Enza Bead test was reactive in 5 of 6 cases and all 3 cases of untreated primary infection. A double conjugate EIA for both IgG and IgM found that in infected patients a positive IgG result always accompanied a positive IgM result. It was also shown by Western blotting that by the time clinical signs develop most patients have both IgG and IgM antibody.

Preliminary evaluations of a commercially available EIA (Captia Syphilis G) gave high sensitivity in detecting anti-treponemal antibody in untreated as well as treated infection. Because the sensitivity in detecting untreated primary infection was 82% (14/17) (88.2% if equivocal results were scored positive as would happen on screening) compared with 100% for all other stages of infection, Lefèvre et al. considered that this EIA was unsuitable as a replacement for the VDRL and TPHA screening combination. A retrospective analysis of routine screening results showed that a sensitivity of 82% in detecting untreated primary infection is greater than any other single test including dark ground microscopy (76%), VDRL (73%), TPHA (71%) and the fluorescent treponemal antibody absorbed (FTA-abs) test (80%). Similar sensitivities for dark ground microscopy (78%), VDRL (70%) and TPHA (75%) in untreated primary syphilis have also been found in large scale evaluations of clinical practice. Although the combination of VDRL and TPHA tests did give slightly greater sensitivity (84%) this value was not significantly different (P > 0.8) from the 82% sensitivity of Captia Syphilis G.

There is no evidence to suggest that, in the case of primary syphilis, screening for anti-treponemal IgG by EIA is significantly less sensitive than the combination of VDRL and TPHA tests. Provided
that clinicians are aware of the ‘seronegative window’ that may exist for 1–2 weeks during the early primary infection, maintain a high index of clinical suspicion and have the facility to request additional tests (e.g. specific IgM or FTA-abs) in cases of suspect primary infection, then detection of early infection will not be compromised by using anti-treponemal IgG EIA as a single screening test. A proportion, 11% \(^{103}\) to 14.5\(\%^{116}\) of cases of early primary syphilis are seronegative in all tests at the time of presentation. A high index of clinical suspicion is the key factor in making a diagnosis of primary infection and is probably more important than the serological screening schedule used. Syphilis remains a situation where very large numbers of specimens are examined in order to detect a very small number of positive findings. In these circumstances new methods such as EIA with the potential for automated testing and computerised reporting have many attractions.

Other EIA
A competitive EIA for Treponema pallidum antibodies (CETPIA) has been produced ‘in-house’ at the Public Health Laboratory, Newcastle upon Tyne\(^ {124}\). Preliminary analysis suggests that the assay has high sensitivity and specificity. An EIA based on purified axial filaments of the reiter trepomene (AF-ELISA) was compared with an EIA using an ultrasonicate of T. pallidum (TP-ELISA)\(^ {125}\); both assays were prepared ‘in-house’. At all stages of syphilis the sensitivity of the TP-ELISA, the AF-ELISA, the TPHA and the FTA-abs test did not differ significantly, except that the AF-ELISA was less sensitive than the TPHA for treated syphilis.

As far as the author is aware none of the above EIAs are available commercially although an EIA based on a recombinant antigen TmpA\(^ {126}\) is. The TmpA EIA gave a sensitivity of 76% for primary syphilis, 100% for secondary, and 98% for early latent infection but has not been evaluated in late infection\(^ {226}\). These EIAs require more detailed evaluation before their role in syphilis serology can be assessed in relation to existing strategies.

Screening policies: population groups and tests
Although the role of antenatal screening has been questioned\(^ {8}\) the overwhelming body of opinion is that routine antenatal screening for syphilis is not only cost-effective but is a valuable public health measure that must continue, even in countries in which the disease has a relatively low prevalence\(^ {127–133}\). Likewise, opinion favours maintaining syphilis screening of blood donations\(^ {134–136}\) although there is some debate regarding which tests should be used\(^ {7}\): tests such as the VDRL tend to be used in the United States, France, and Belgium whereas in other countries such as Germany, the Netherlands, and the UK, the TPHA is often used for screening. Routine screening also has a role in certain hospitalized groups such as psychogeriatric patients\(^ {137}\) and those who are alcohol or drug abusers\(^ {138}\). It was advocated that all patients admitted to hospital with a neurological or psychiatric disorder should have syphilis serology checked as a routine, although it was not considered necessary to perform tests routinely on outpatients\(^ {139}\). Others have questioned the value of routine screening of psychogeriatric patients\(^ {140}\) and it is clear that a detailed investigation of patients is required if routine blood screening is to be justified and of value in management\(^ {137,141}\). The recent increase of early syphilis has highlighted the need for syphilis serology as part of a rapid serological screen in emergency departments\(^ {107,112}\).

In Europe it is generally accepted that the additional cases of late syphilis, including late latent, detected by screening with T. pallidum antigen tests outweighs the difficulties that may arise in differentiating between inadequately or untreated patients and those who have a well documented history of appropriate therapy\(^ {142,143}\). A careful examination of 53 individuals with negative VDRL but reactive TPHA on screening revealed seraotisitis in 4 and neurosyphilis in 2 patients\(^ {29}\). Gormsen\(^ {144}\) found that half of 97 patients with syphilitic aortitis (diagnoses confirmed at autopsy) were negative in the VDRL test. Whilst it is accepted that these are highly selected groups they nevertheless emphasize the importance of screening with T. pallidum antigen tests.

In the United States, however, treponemal tests are considered as confirmatory tests and screening relies mainly on cardiolipin tests. Indeed the case definition of latent syphilis used for public health surveillance is given as ‘no past diagnosis of syphilis and a reactive nontreponemal test, and a reactive treponemal (fluorescent treponemal antibody-absorbed [FTA-ABS]) microhaemagglutination assay for antibody to Treponema pallidum [MHA-TP] test’\(^ {145}\). The finding that HIV-infected patients who acquire syphilis may fail to produce anti-treponemal antibodies\(^ {19,46}\) combined with the tendency for serological evidence of treated syphilis to disappear after patients become infected with HIV\(^ {20,32,55}\) may act as a stimulus for the more widespread adoption of testing with T. pallidum antigen tests.

Confirmation
The Treponema pallidum immobilization (TPI) test Once the only reliable test for distinguishing between treponemal and non-treponemal cardiolipin-antibody responses, the TPI test has now been superseded by the widespread use of the FTA-abs test\(^ {146}\). The need to improve FTA-abs test performance rather than rationalize its deficiencies was also stressed\(^ {146}\). The TPI test may be negative in primary infection and this is a cause of discrepancies between TPI and FTA-abs test results\(^ {147}\). The FTA-abs test is not only more sensitive than the TPI in all stages of untreated
infection it also remains positive longer after treatment accounting for the view that the TPI fails to provide useful diagnostic information. The main role of the TPI nowadays is for research purposes.

FTA-abs
The FTA-Abs is currently the standard confirmatory test. It becomes reactive around the third week of infection and in primary infection has a sensitivity ranging from 86% to 100%. It is positive in all secondary cases and 96–100% of late stage infections. Reactivity persists after adequate therapy although the test may occasionally become non-reactive if treatment is given early in the disease.

Most laboratories use commercial kits for performing the FTA-abs test and this is an area where much greater standardization is required. The ability of four different kits to detect reactive samples varied from 83% to 95%, and the agreement with non-reactive and borderline samples ranged from 81% to 96.4%. In another study, agreement between 4 kits was 63% for treponemal sera and 50% for non-treponemal sera. Discrepancies with treponemal sera were associated with low levels of antibody characterized by TPHA titres ≤ 160 and a negative VDRL test. Discrepancies with non-treponemal sera were significantly associated with false reactivity on screening with EIA.

The FTA-abs is considered to be a very specific test. However, the test is not endowed with the high level of specificity usually accorded. The reputation for high specificity stems from the principle of dual testing described earlier, i.e the FTA-abs is used to test sera that have been pre-selected thus increasing considerably the probability that they contain anti-treponemal antibodies. The specificity of the FTA-abs test is not absolute, however, and varies from 92% to 99%. The total prevalence of false reactivity is around 1% in normal persons, but higher rates have been reported in hospital patients. It has been estimated that at a prevalence of syphilis of 1.4% around 30% of reactive FTA-abs tests would be ‘false-positives’ if the FTA-abs test was used for screening.

Reactivity only in the FTA-abs test must be treated with caution. An evaluation of 43 such patients found that only 3 had primary or treated syphilis, 21 (49%) had clinical and/or serological signs of Lyme disease, 7 (16%) had genital herpes simplex infection, and the remaining 12 (28%) miscellaneous disorders. In the same study an isolated positive FTA-abs reaction was found in 43% (13/30) of control patients with Lyme disease.

The degree of reactivity in a subjective test such as the FTA-abs is also important and can have a very significant effect on the utility of the test. Borderline reactions are common and in the United States it has been recommended that the borderline report should be eliminated. This change increased the specificity of the test from 82.5% to 88.7% but decreased the sensitivity from 100% to 99.5%. In an evaluation of 4 commercial FTA-abs kits, discounting borderline reactions resulted in a sensitivity of up to 100% with VDRL positive sera, but when VDRL negative treponemal sera were included sensitivity ranged from 74% to 90%. These findings suggest that if the CDC recommendations are applied to sera selected on the basis of screening by a treponemal test such as the TPHA (or EIA) then a significant number of treponemal sera will fail to be confirmed. This is because the TPHA is more sensitive than the FTA-abs (except during the 3rd to 4th week of infection).

As expected there is a decrease in specificity when borderline reactions are scored positive. Specificity varied from 52% to 83% when borderline reactions were scored positive and from 71% to 96% when they were scored negative. When the TPHA is used for screening this is not a major problem as the TPHA is the most specific method for the detection of antibody to T. pallidum, with false reactivity as low as 0.07%–0.9%. False reactivity in the FTA-abs, however, was significantly associated with false reactivity on screening with EIA suggesting that other tests may be more suited to confirming the treponemal nature of sera reactive on screening by EIA. Interestingly, a correlation between unexplained reactivity in the FTA-abs and EIA was noted by Veldkamp and Visser in 1975.

EIA
Although the Bio-EnzaBead test has been recommended as a suitable alternative to the FTA-abs test, it lacks sensitivity in VDRL negative sera and would not be suitable for confirming sera detected by screening with treponemal antigen tests. The Captia Syphilis G EIA has also been recommended as a confirmatory test. This test has high sensitivity in the case of VDRL negative sera and is therefore better suited to confirming the treponemal nature of sera selected by treponemal antigen tests. However, the potential for automation endows EIA with greater advantages in a screening rather than a confirmatory role.

TPHA
The TPHA test is simple to perform and would make a good alternative to the FTA-abs, particularly with regard to sera screened by EIA. All 72 cases of treated syphilis and 93 of 96 (98.3%) cases of untreated syphilis evaluated by Lefevre et al. were positive in both the TPHA and Captia Syphilis G; 3 sera from primary stage infection were positive by TPHA but negative by EIA. TPHA reactivity may be variable in primary syphilis and of 60 EIA positive sera the only one that would not have been confirmed by the TPHA was from a case of untreated primary infection. EIA positive TPHA negative sera should be tested for specific anti-treponemal IgM in order to exclude an early untreated primary infection.
Immunoblotting was appreciated as a potential diagnostic method as early as 1985. Recent reports support its value as a confirmatory test although further evaluation is required in order to define the most suitable criteria for designating a positive result. Antibodies against a 15.5 kD antigen were demonstrated in 97% (107/110) of documented cases of syphilis: reactivity was 100% of patients with secondary or early latent syphilis, both untreated and treated, 98.3% in those with late latent treated syphilis and 100% in patients with neurosyphilis. Positive blots for the 15.5 kD antigen were also obtained with 99% of 294 cases of serologically diagnosed syphilis of undetermined stage. This was a highly stringent test of sensitivity as the sera from late latent treated cases contained 8 specimens that were FTA-abs and TPHA negative. The sera from staged cases that were negative on blotting comprised 2 cases of seronegative primary infection and one case of late latent treated syphilis. The sensitivity of the test for primary syphilis was improved by using anti-human IgM conjugate rather than anti-IgG. Antibodies against the 15.5 kD antigen were absent in 47 patients with false positive reactions for syphilis and in 121 healthy blood donors.

Byrne et al. considered an immunoblot test result was positive when antibodies were demonstrated to at least 3 of 4 major antigens of 15.5, 17, 44.5, and 47 kD. Based on this definition the assay had a sensitivity of 91.7% and a specificity of 100% for clinically defined samples. A positive result was obtained in 93% (37/40) of cases of primary syphilis giving 97.5% agreement with the FTA-abs test. The high reactivity in primary infection probably results from the use of conjugate containing anti-human IgG plus IgM. Because of its high sensitivity and specificity, together with its simplicity and objectivity it was considered a good confirmatory test. Immunoblotting has also been used in the diagnosis of congenital infection.

Response to treatment

Cardiolipin antigen tests

Because there is no microbiological 'test for cure' patients treated for syphilis are assessed by serial quantitative non-treponemal tests such as the VDRL or RPR. All cardiolipin antigen tests tend to become negative after treatment, particularly in early syphilis while the rate of decline in titre depends on the stage of infection, the initial titre, and the history of previous syphilis. In interpreting a fall or rise in titre a 4-fold change is considered significant but a 2-fold change is not. In primary and secondary syphilis the titre declines approximately 4-fold in 3 to 6 months and 8-fold in 12 months. In early latent syphilis the decrease was only 4-fold at 12 months. The mean period of reactivity after treatment is 4 months for primary syphilis, 17 months for secondary, 13 months for early latent disease and 60 months for latent infection of indeterminate duration. After adequate treatment of late infection the VDRL test will show a slow decline in titre, some cases eventually becoming negative while others may remain reactive (titre < 8) for many years.

Recently, Lukehart highlighted invasion of the central nervous system by T. pallidum in 40% of patients with early syphilis, and the failure of benzathine penicillin G to provide treponemical levels in the CSF after standard therapy in both HIV-infected and uninfected patients as important questions to be addressed in relation to 'test of cure'. Studies were advocated to examine rates of titre decline and seroreversion after therapy with single-dose benzathine penicillin G compared with regimens known to eradicate T. pallidum from the central nervous system in both HIV-infected and uninfected individuals. Studies from the United States suggest that HIV-infected patients with primary syphilis when compared with HIV-negative controls were less likely to have a 4-fold or greater RPR decrease or seroreversion within 6 months of treatment. A preliminary study from London found that serological responses after treatment tended to be preserved in HIV-infected patients. It is not clear whether these differences result from different treatment regimens or from the fact that the London patients were in the early stages of HIV infection. Clearly more data are needed.

EIA for anti-treponemal IgM

An appreciable decrease in specific IgM may be interpreted as evidence of therapeutic success. Lefevre et al. considered simultaneous measurement of IgG and IgM antibodies for T. pallidum by the Captia EIAs was an efficient and simple method of confirming the diagnosis of syphilis as well as for indicating whether active disease was present. There was a good correlation between the IgM capture EIA and the 19S(IgM) FTA-abs with respect to reactivity of sera from treated patients. Although EIA was considered to be as effective as the 19S(IgM) FTA-abs test in monitoring the effect of treatment there was no serial data to support this view. Ijsselmuiden et al. followed the course of anti-treponemal IgM reactivity after treatment of 6 patients for early infectious syphilis. The quantity of IgM antibody declined in nearly all patients after treatment but remained detectable in 5 patients up to 6 months after treatment: the VDRL test became negative in 4 of the 6 patients within 5 months of treatment. Detection of specific IgM by EIA was not recommended as a replacement for the VDRL test to monitor patients treated for syphilis.

TPHA

Although a rapid fall in TPHA titre may occur after treatment for secondary syphilis, the TPHA is of little help in monitoring the efficacy of treatment.
A significant rise in titre may indicate reinfection and provides a safeguard against missing reinfection as a result of a false negative VDRL reaction due to the prozone phenomenon. Because the TPHA remains positive, usually for life in non-HIV infected patients who have been adequately treated, its use (and that of other specific tests) for screening has increased the need for tests to provide guidance on activity of infection.

Activity of infection
Quantitative VDRL testing is to some extent useful in assessing treatment status as titres > 16 are rarely found in adequately treated infections99. In theory, tests to detect specific IgM should provide a more direct indication of treatment status and response to therapy. Detection of specific anti-treponemal IgM, in patients without a history of recent treatment, suggests active disease and the need for chemotherapy. In general, the titres of specific IgM decline after adequate treatment of early syphilis and reactivity ceases within 3–9 months; reactivity may be found from 1 to 1.5 years after treatment of late disease99. The many methods available for detecting anti-treponemal IgM are discussed in detail earlier86.

Immunofluorescence with the IgM fraction of serum obtained by gel filtration or ultracentrifugation, the 19S (IgM) FTA-abs test, is considered the reference method. Failure to separate IgG and IgM fractions adequately can lead to false-positive results due to rheumatoid factors and false-negative results due to competitive inhibition of IgM binding by IgG antibodies of the same specificity for the binding site on the antigen. Separation of the IgM fraction is, however, technically demanding making simpler procedures desirable.

The immunoglobulin M solid phase haemadsorption test, (IgM)-SPHA, was one of the first specific anti-treponemal IgM tests that was simple and cheap enough to be applied on a large scale161. In this test microtitre plate wells act as solid phase for \( \mu \)-chain capture; the anti-treponemal component of the captured IgM is detected by TPHA reagents. Merlin et al.162 found that the sensitivity of the SPHA (96%) was comparable to that of the IgM-FTA-abs test (92%) but that the specificity of the SPHA (97.4%) was superior to that of the IgM-FTA-abs test (89.6%). In this study the SPHA was not compared with the ‘gold standard’ as the IgM-FTA-abs test was performed on whole serum using a mono-specific conjugate.

Early infection
Muller and Lindenschmidt163 found that the SPHA gave false negative results in more than half of patients with untreated primary syphilis and in just over a quarter of patients with untreated secondary and late infection. Poor sensitivity of the SPHA in primary syphilis has been noted by others86,105 and is a major drawback in the use of the test as an adjunct to the diagnosis of untreated primary infection.

The recently described commercially available EIA for anti-treponemal IgM (Captia Syphilis M) appears to be as sensitive as the 19S(IgM) FTA-abs test in early disease123,160. In this \( \mu \)-chain capture assay specific IgM is detected by a tracer complex comprising: T. pallidum antigen-biotinylated monoclonal antibody against T. pallidum axial filament (32–39 kD)-streptavidin conjugated horse radish peroxidase. Because of the use of the tracer complex false-positive reactions are not likely to result from rheumatoid factor160. The Captia Syphilis M test has high sensitivity in early infection: 82% (18/22) in primary, 60% (12/20) in secondary, and 53% (16/30) in latent (not stated but probably early latent) compared with 19S(IgM)-FTA-abs reactivity of 82% (primary), 70% (secondary), 60% (latent)160. High sensitivity in early infection was confirmed by Lefevre123 with values of 94% (16/17), 85% (11/13) and 64% (9/14) for primary, secondary and early latent infection, respectively. The 19S(IgM)-FTA-abs test was reactive in all primary and secondary cases and in 79% of early latent cases. High sensitivity (100%) was found for the Captia Syphilis M and 19S (IgM)-FTA-abs tests when applied to 51 cases of primary, secondary, or early latent infection166. Both tests were reactive in two sera before the screening tests (VDRL and TPHA) supporting a role for EIA for anti-treponemal IgM in suspect early primary infection. The SPHA was reactive in only 35% of the 51 patients.

All 9 patients with primary or secondary syphilis diagnosed in Edinburgh in recent years were Captia Syphilis M positive compared with only 3 of 5 patients with early latent infection103. The two patients with early latent infection and negative IgM results had received antibiotics on at least one occasion in the two years preceding the diagnosis. Previous exposure to antibiotics combined with the general decrease in the spectrum and strength of antibody response which occurs with increased duration of infection100 may contribute to poor sensitivity in late infection.

Late infection
Sensitivity was poor in late latent infection with only 3% (1/33) of sera reactive in the 19S (IgM)-FTA-abs test and none in the Captia Syphilis M123. Others have also noted that the 19S (IgM)-FTA-abs test was non-reactive in late syphilis167. The SPHA test appears to be of high sensitivity (around 95%) in late infection99,168.

The Captia Syphilis M test shows poor reactivity in neurosyphilis with reported sensitivity values of 34% (11/32)160, 67% (2/3)123 and 5% (1/20)166. The 19S (IgM)-FTA-abs test also gave poor sensitivity in the same studies with values of 72% (23/32)160, 33% (1/3)123 and 5% (1/20)166. In keeping with high sensitivity in late infection the SPHA test was reactive with all 20 neurosyphilis cases examined by Schmidt et al.106. There are no clear reasons for these differences.
Re-infection
The Captia Syphilis M test showed poorer sensitivity 53% (8/15) than the 19S (IgM)-FTA-abs test 87% (13/15) in cases of re-infection. Low sensitivity, 21.4% (9/42) was also observed in reinfection using an EIA for detecting IgM against the Reiter treponeme flagellum: no other specific IgM tests were compared. Clearly more comparative data is needed before definite conclusions can be drawn with regard to the sensitivity of IgM test during reinfection. Muller and Wollemann reported lower initial IgM SPHA titres and delayed decrease following treatment in patients with reinfections who were in the primary and secondary stage compared with those patients with primary and secondary stage disease but no previous syphilis. This may be due to a partial in vivo suppression of IgM antibodies by high titres of IgG antibodies of the same specificity which are regenerated by memory cells after the repeated contact with T. pallidum related antigens. Clearly more comparative data is needed before definite conclusions can be drawn with regard to the sensitivity of IgM tests during reinfection.

Comment
In patients with positive serological tests who lack a history of recent adequate treatment, a reactive anti-treponemal IgM test supports the need for treatment. Occasionally, however, the sera of patients who have been adequately treated will contain 8S IgM that may react in assays where immunoglobulins are not fractionated before testing. Untreated or inadequately treated infections, particularly beyond the early stages, as well as reinfections, cannot be excluded reliably on the basis of a negative anti-treponemal IgM test, and due consideration must be given to clinical findings, the history of the patient, and quantitative VDRL and TPHA tests. Little is known of the anti-treponemal IgM response in patients with coexisting HIV and this merits detailed study. New recombinant antigen tests such as the TmpA EIA may have a role in detecting active infection as well as monitoring the success of antibiotic treatment.

NEUROSYPHILIS
Neurosyphilis includes the clinical entities of asymptomatic neurosyphilis, acute syphilitic meningitis, cerebrovascular neurosyphilis, paretic neurosyphilis, and tabes dorsalis. Because invasion of the central nervous system can be detected before symptoms develop, and because the early effects of syphilis on the system can often be reversed by penicillin treatment, examinations of the CSF are important in the assessment of patients with syphilis. Invasion of the CSF is not unusual in early infection and by using the rabbit infectivity test (RIT) T. pallidum was found in 30% (12/40) patients with untreated primary or secondary infection. Concurrent infection with HIV was not associated with isolation of T. pallidum, increased numbers of CSF abnormalities, or reactive CSF serological tests for syphilis, although CSF pleocytosis was more common in HIV-infected patients. The WHO recommendations for treatment of syphilis are that a minimal benzyl penicillin concentration of 0.018 mg/l of serum should be maintained for 7-10 days in early syphilis; and penicillin-free or subtreponemical intervals should not exceed 24-30 hours. The CDC recommendation for the treatment of early syphilis with a single intramuscular (i.m.) injection of 2.4 million units of benzathine penicillin G is not followed by the Massachusetts Department of Public Health whose approach is an i.m. injection of 2.4 million units benzathine penicillin G given once a week on two consecutive weeks. Despite the recent CDC endorsement of treatment by a single injection, Musher considered that 3 doses of benzathine penicillin, 2.4 million units at weekly intervals, is probably the minimum accepted dosage for primary or secondary infection. Practice in the UK and Europe has favoured more prolonged treatment for early syphilis, e.g. 900 mg procaine penicillin daily for 10-14 days. Penicillin at conventional doses does not yield treponemical levels in the central nervous system and probably does not eradicate the infecting organisms, suggesting that it works synergistically with the host's immune response in preventing neurosyphilis.

Recently a number of case reports, mainly from the USA, have described the development of neurosyphilis in patients previously treated for early syphilis suggesting that HIV infection may alter the natural course of syphilis because of its profound effect on cell mediated immunity. Whilst the immunobiology of syphilis supports these concerns Jordan questioned whether there was sufficient evidence to support the view that neurosyphilis was occurring in these patients with unexpected frequency, an unusually short period of latency, increased severity, and a reduced responsiveness to penicillin therapy. Ruffi in 1989 commented that so far there was no statistical evidence to prove the relationship between HIV infection and increased frequency of neurosyphilis. There have been several reports (reviewed in reference) of neurosyphilis occurring after appropriate therapy in normal hosts. HIV-infection has undoubtedly increased awareness, probably also the frequency, of a phenomenon that was already recognized. The more prolonged treatments used in the UK may tip the synergistic balance in favour of the host with regard to elimination of T. pallidum, even in those patients with an immunological deficit.

Who should be tested?
In the case of patients with syphilis of uncertain duration or in the late symptomatic or late latent stage, CSF examination should be undertaken
before treatment. CSF examination should precede and guide treatment of HIV infected patients with latent syphilis present for longer than one year or for unknown duration. It has been suggested that if CSF examination is not possible, patients should be treated for presumed neurosyphilis. Hook, however, stated that the policy to treat empirically all HIV-infected patients who have syphilis with high-dosage therapy effective for neurosyphilis was of unproven benefit, expensive and time-consuming for both patients and health care providers. Dunlop considered that probenecid with penicillin or amoxycillin should be as effective in the treatment of neurosyphilis, provided treponemal antibodies are produced and maintained, as the more complicated intravenous regimen. According to Mushkr, more intensive diagnostic evaluation, perhaps including routine cerebrospinal fluid (CSF) analysis, more intensive therapy, and far more rigorous follow-up are indicated in HIV-infected patients with syphilis. When there is evidence of clinical relapse or a 4-fold rise in titre of follow-up tests CSF examination should also be undertaken.

It is unnecessary to perform routine screening tests for syphilis on the CSF of patients with symptoms referable to the central nervous system in whom there is no suspicion of syphilis. A negative T. pallidum antigen test on serum will virtually exclude active neurosyphilis and is a better screen for all forms of late syphilis than examination of the CSF. The latter should be reversed for cases selected on clinical grounds and backed by a positive TPHA on serum. It was suggested recently that a serum TPHA titre of >2560 should be considered as a criterion when selecting patients (non HIV-infected) for CSF examination. If a titre of 1280 was taken as the cut-off to allow for a safety margin this policy would still have reduced the number of lumbar punctures performed by 68%—the authors considered that apart from eliminating any potential risk to the patient there would have been a substantial financial saving as the cost of a lumbar puncture has been calculated at around 600 dollars. Perhaps a more conservative cut-off titre of >640 should be applied initially as in one series of 45 patients with active neurosyphilis titres were 640 (1), 1280 (1), 2560 (4) and >5120 (39). Neurosyphilis should be considered in the differential diagnosis of neurological disease in anyone with HIV infection.

Exclusion of neurosyphilis

A negative T. pallidum antigen test on CSF virtually excludes neurosyphilis while it is most improbable at serum or CSF titres below 320. A negative cardiolipin test (VDRL/RPR) on the other hand does not exclude neurosyphilis as 30% to 57% of patients with active neurosyphilis will give a negative result on CSF examination. More recent studies support the view that the CSF VDRL is under-sensitive in neurosyphilis. In contrast Jordan in a critical review of some of the studies on neurosyphilis suggested that the CSF VDRL test is the most appropriate diagnostic test. Detailed analysis involving parameters other than serological tests found the CSF VDRL non-reactive in 20% (9/45) of patients with neurosyphilis.

Confirmation of neurosyphilis

According to the WHO the traditional criteria for involvement of the CNS in syphilis, viz a positive VDRL test, pleocytosis, and an increased concentration of protein in the CSF are of limited value. Although the CSF-VDRL is under-sensitive in neurosyphilis a reactive test is strongly suggestive of neurological involvement. Luger reported reactivity in only 6% (4/67) of patients with adequately treated syphilis without involvement of the CNS. A cell count of >5 x 106/l and total protein concentration above 0.45 g/l indicates inflammation without disclosing its cause. These parameters are even less specific indicators of neurosyphilis in HIV-infected patients as 40–60% of such patients without syphilis have abnormalities of CSF protein or cell counts.

Measurement of antibody in the CSF, particularly by the more sensitive T. pallidum antigen tests, is of limited value without simultaneous knowledge of serum antibody and the degree of permeability of the serum-CSF barrier. Again great care must be taken in interpreting results in HIV-infected patients. CSF serology yielded 25% (4/16) false positive FTA-abs results (VDRL and TPHA negative) in patients infected with HIV (CDC stage II); there was no clinical evidence, history or serology (serum VDRL, TPHA, and FTA-abs negative) to suggest syphilis.

Quantitative TPHA test on CSF combined with albumin, IgG, and IgM estimations are helpful in suggesting diagnosis of T. pallidum specific IgG and in excluding errors that may arise from disturbed function of the blood/CSF barrier. Details of the various methods and expected values are reviewed elsewhere but they include: defining the normality or degree of impairment of the blood/CSF barrier by the ratio of CSF to serum albumin (the albumin quotient); evidence of the local production of IgG, IgM and treponemal IgG by estimating the ratio of the CSF to serum IgG concentration/the albumin quotient (the IgG index); the ratio of the CSF to serum IgM concentration/the albumin quotient (the IgM index); the ratio of CSF TPHA titre to albumin quotient (TPHA index); or by detecting oligoclonal immunoglobulin bands in the CSF.

Neurosyphilis is most probable at a TPHA index between 70 and 500 and is strongly suggested at values above 500; the previous upper limit for normal of 100 missed 3 cases of neurosyphilis giving a sensitivity of 93% (42/45). Whilst several values from patients without neurological involvement approached 70 the specificity remained at 100% (67/67) when 70 was taken as the cut-off. An
increased IgG or IgM index, or both, was found about 70 times more often in symptomatic neurosyphilis than in latent syphilis without involvement of the central nervous system. In the study by van Eijk and colleagues the IgG index gave a sensitivity of 62% (13/21) in neurosyphilis and was more sensitive than the albumin (13/21) ratio (62%). The intrathecal T. pallidum antibody (ITPA) index (ratio of CSF to serum TPHA titres/mg total IgG) demonstrated production of antibodies within the CNS in 95% of patients with neurosyphilis. The ITPA index was also raised in 25% of patients with early and 28% with late syphilis who did not have any neurological deficit: the significance of these findings is unclear.

A review of clinical and laboratory data for 9 HIV-infected patients with symptomatic neurosyphilis found 7 had a reactive CSF-VDRL test (8 of 9 tested a reactive serum VDRL/RPR test) and concluded that most, if not all, HIV-infected patients with symptomatic neurosyphilis will have elevated serum and CSF syphilis serology. Whilst acknowledging that their numbers are small, and that further experience is necessary, their conclusion that a negative serum VDRL/RPR screening test may rule out neurosyphilis in an HIV-infected patient with CNS symptomatology should be treated with caution.

Fortunately the number of cases of neurosyphilis remains low. Nevertheless there would appear to be a need for multi-centre collaborative studies based on the examination of CSF by an accepted range of tests performed in a standardized manner. Reagent kit inserts normally refer to specimens of serum or plasma but not CSF in their recommended protocols. In one study a CSF-TPHA dilution of 1:4 (serum normally 1:19) or more was taken as positive while the CSF-FTA-abs was performed at a 1:5 dilution as for serum but in phosphate buffered saline rather than sorbent. Others have used the same methods for the VDRL and FTA-abs when evaluating both serum and CSF.

CONGENITAL INFECTION

Attention has already been drawn to the marked increase in congenital syphilis in the USA and this has highlighted the need for appropriate surveillance and diagnostic tests: guidelines and case definitions have been published. Challenges relating to improved serodiagnosis and a better understanding of the immunology and pathogenesis of congenital syphilis have also been reviewed. The impact of congenital syphilis on third world countries remains dramatic: rates as high as 3200/100 000 live births have been reported in Addis Ababa; in Zambia 8.6% of infants less than 3 months admitted to hospital and 7.5% of neonates admitted to intensive care units had congenital syphilis; and in Ethiopia syphilis was the 4th most common cause of perinatal death accounting for 10% of the approximately 70 perinatal deaths per 1000 live births and nearly 5% of all postneonatal deaths. Since IgM is not transferred across the placenta the demonstration of specific anti-treponemal IgM by the 19S (IgM)-FTA-abs test confirms a diagnosis of congenital infection. This test is not widely available, however, and is considered experimental rather than a standard laboratory test.

EIA

The Captia Syphilis M EIA appears to be a simple and useful method to detect congenital syphilis: it was reactive in all 6 reported cases of congenital infection (5,110 and 1,123) but not in 31 infants (21,160 and 1,122) of mothers adequately treated before or during pregnancy: all of the infants had evidence of transplacental antibody detected by the TPHA, EIA-IgG or FTA-abs. Because this test uses a tracer complex instead of an IgG class conjugate IgM-rheumatoid factor should not interfere. Rheumatoid factor is so common in congenital syphilis that a positive latex test for rheumatoid factor on infant’s serum, in the presence of maternal syphilis, strongly suggests congenital infection. IgM rheumatoid factor measured by EIA was found in 92% of infants with congenital syphilis and elevated levels correlated with liver and renal involvement as well as the extent of the disease.

Immunoblotting

Immunoblotting using anti-human IgM conjugate to detect binding of anti-treponemal IgM to treponemal antigens is also of potential value in diagnosis. Serum from 5 congenitally infected infants with symptoms were shown to react differently from that of control infants born to normal, serofast, and biological false positive mothers. IgM antibody to the 47 and 37 kD antigens was found only in sera from infected infants. Serum from an asymptotically infected infant had a response to the 47 kD antigen but not the 37 kD antigen. The importance of the 47 kD antigen as a marker for the diagnosis of congenital infection has been confirmed by others. Lewis et al. made a more stringent interpretation of a positive blot. A typical pattern of IgM reactivity in infected infants was directed against the 47, 17 and 15.5 kD antigens with a variety of activities against the other low-molecular weight proteins. IgM reactivity was frequently seen against the 45 and 37 kD antigens and less often against the 42, 34.5, 31, and 24 kD antigens. The presence of at least 5 visually distinct IgM reactions (including specifically the 47, 17, and 15.5 kD antigen bands) was considered a reactive blot. Based on these criteria sera from 92% (23/25) of symptomatic infants diagnosed with congenital syphillis yielded positive reactions. Sera from
80 asymptomatic infants considered at risk of developing symptomatic infection were also tested and 16 exhibited IgM reaction patterns consistent with those seen in congenital infection, although in 5 cases the reactions were equivocal. On re-testing the 16 specimens using the IgM fraction obtained by serum fractionation the 5 equivocal reactors gave negative blots while the other 11 remained positive. The test was considered to be an excellent confirmatory test for congenital syphilis with superior sensitivity and specificity to the 19S (IgM)-FTA-abs test.

Serial testing of potentially infected infants is important; in the absence of infection, passively transferred antibody detected by both cardiolipin and T. pallidum antigen tests will become negative in a time period related to the initial titre, the half-life of IgG (18–23 days) and the relative sensitivity of the tests—even very high initial titres of transplacental antibody should disappear within 6 months. Serial examinations are also recommended because of the possibility that specific IgM may not be detectable until several weeks after birth due to suppression of neonatal IgM synthesis by high levels of circulating maternal anti-treponemal IgG.

Congenital and acquired syphilis may be difficult to distinguish when a child is seropositive after infancy. Signs of congenital syphilis may not be obvious and stigmata may not have developed. Data on specific anti-treponemal IgM in late stage congenital infection is inadequate.

THE POLYMERASE CHAIN REACTION (PCR)

PCR can selectively amplify the copy number of a target gene more than 10⁶-fold and has the theoretical ability to detect a single organism. Detection of T. pallidum DNA in body fluids and tissues by PCR has been investigated as a means of improving the diagnosis of congenital and neurosyphilis, conditions where serology is difficult to interpret, as well as early syphilis when a serological response may not have had time to develop. PCR is highly specific with regard to a wide range of organisms including B. burgdorferi, skin organisms, STD and CNS pathogens. The PCR tests used so far will not differentiate between T. pallidum subspecies pallidum and the closely-related T. pallidum subspecies pertenue and is of no help in differentiating between yaws and syphilis.

Although PCR has demonstrated that there might be a tendency for these two subspecies to differ in a single base pair in the TpF1-(pallidum) and TyF1-(pertenue) encoding genes it is unlikely to be a reliable differential characteristic for either subspecies.

Neurosyphilis

Hay and colleagues performed the PCR separately with two sets of 21 base primers to detect DNA sequences from the TmpA and 4D genes of T. pallidum (Table 2). Specimens of CSF were investigated and were scored positive only if both DNA sequences were detected. The CSF from 53% (10/19) patients with positive serological tests for syphilis who were being investigated for late syphilis were DNA-positive compared with only 3% (1/30) of patients with no known history of syphilis. The 10 DNA-positive patients comprised neurosyphilis (2), possible asymptomatic neurosyphilis (1), and latent syphilis (7). CSF from 28 HIV-positive patients were also tested. Fourteen of these patients had CNS disease and 7 were DNA-positive, whereas none of the 14 without CNS disease were DNA-positive. Five of the 7 DNA-positive patients had a history of syphilis giving a total reactivity of 58% (15/26) in patients with a history of syphilis. DNA equivalent to about 65 treponemes in 0.5 ml of CSF were required to give a positive reaction which could account for the relatively low sensitivity with clinical specimens.

The occurrence of treponemal DNA in CSF was also investigated by PCR amplification of a 617 base pair fragment of the 39 kD bmp gene of T. pallidum. Although purified T. pallidum DNA equivalent to a single organism could be detected the method was less sensitive when applied to CSF: after concentration using diatomaceous earth it was possible to detect about 100 treponemes in 1 ml of CSF. Prior to penicillin treatment T. pallidum DNA was detected in 71% (5/7) of patients with acute neurosyphilis, in none of 4 patients with chronic symptomatic neurosyphilis tested before treatment, and in 12.5% (2/16) of patients with asymptomatic neurosyphilis. These results may represent an underestimate of the sensitivity of PCR as the CSF specimens were collected in the pre-PCR era and were stored without special care to avoid degradation of DNA.

Unexpectedly DNA was often detected in CSF long after intravenous treatment with penicillin; in one case after 3 years. It would seem that the detection of T. pallidum DNA by PCR is of limited value in checking the efficacy of treatment for patients with neurosyphilis. DNA is a very stable biopolymer and may remain present in CSF for long periods of time after the killing of T. pallidum by intravenous penicillin therapy.

Table 2. Main recombinant antigens tested for diagnosis

<table>
<thead>
<tr>
<th>Antigen</th>
<th>kDalton</th>
<th>Method and reference</th>
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<tbody>
<tr>
<td>P6</td>
<td>47</td>
<td>Immunoblotting¹⁴</td>
</tr>
<tr>
<td>TmpA</td>
<td>44.5</td>
<td>EIA¹²</td>
</tr>
<tr>
<td>'37K'</td>
<td>37</td>
<td>RIA¹⁷</td>
</tr>
<tr>
<td>P²</td>
<td>37</td>
<td>Immunoblotting¹⁵</td>
</tr>
<tr>
<td>TmpB</td>
<td>34</td>
<td>EIA</td>
</tr>
<tr>
<td>TpD</td>
<td>29-35</td>
<td>CIE²⁶</td>
</tr>
<tr>
<td>4D</td>
<td>19</td>
<td>EIA¹⁸; RIA¹⁹</td>
</tr>
</tbody>
</table>

EIA, enzymeimmunoassay; RIA, radioimmunoassay; CIE, crossed-immunoelectrophoresis

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Congenital infection

A PCR based on primers to detect sequences of the gene encoding the pathogen-specific and highly conserved 47 kD membrane immunogen, tpp47, was routinely positive with suspensions of treponemes calculated to contain 10 or more organisms and reactive with some suspensions calculated to contain a single organism. It was also possible to amplify T. pallidum DNA from paraffin-embedded tissue. Inhibition of PCR could result from rabbit testicular tissue and human leukocytes but this could be overcome by DNA extraction. Grimprel and colleagues evaluated this PCR on a variety of clinical specimens of value in diagnosing congenital infection. PCR was 100% specific for T. pallidum when compared with the rabbit infectivity test (RIT) for all clinical materials tested. PCR was 100% sensitive for seven amniotic fluids also positive by RIT; amniotic fluid is known to contain large numbers of treponemes. Twenty newborns of mothers with syphilis were also investigated. Compared with RIT the sensitivity of PCR was 60% (3/5) for neonatal CSF and 67% (4/6) for neonatal sera: these specimens are likely to contain fewer organisms than amniotic fluids. It was concluded that PCR can be a useful adjunct in the diagnosis and clinical management of congenital syphilis as well as providing a valuable tool for investigating the pathogenesis of congenital infection. A large prospective study is required to compare the results of PCR with RIT, serological tests including demonstration of fetal anti-treponemal IgM, and clinical evaluation.

Early syphilis

The suitability of PCR for detecting DNA in various specimens in early syphilis was investigated using experimentally infected rabbits. Whole blood in heparin or EDTA (but not serum), lesion exudate, and punch biopsy as well as swabs of lesions were useful specimens. DNA was detected in whole blood in 30% (3/10) of rabbits on day 4 post-infection and in 90% (9/10) on day 8; the rabbit whose blood was negative by PCR did not develop orchitis. Of 18 serum samples from rabbits whose blood was PCR positive only one (6%) gave a positive PCR result; treponemes were thought to be trapped during the clotting process. These findings are different from those of Grimprel and colleagues who reported a reactive PCR in 45% (9/20) of serum specimens from neonates with congenital infection. All 36 swabs taken during the development of primary lesions at 10 and 18 days post-infection were positive including 13 kept at room temperature for 60 days in tightly sealed tubes. All 15 punch biopsy specimens of 18 day lesions were positive including seven stored at room temperature for 60 days. In the case of healing lesions (day 30 post-infection) biopsy specimens were better than swabs: relative sensitivities were 75% (6/8) and 63% (5/8) when tested on the day of collection compared with 83% (10/12) and 10% (1/10) when stored for 60 days before testing.

The specificity of PCR, particularly with regard to early lesions, is of great importance in view of reports that pathogen-related spirochaetes, that cause necrotizing ulcerative gingivitis, react with monoclonal antibodies once considered to be pathogen specific (T. pallidum subspecies pallidum and T. pallidum subspecies pertenue).

RECOMBINANT ANTIGENS

The production of specific T. pallidum protein antigens in Escherichia coli is ethically preferable to animal culture and would overcome the technical problems of producing large quantities of pure antigen from rabbit testes. Details of production and a list of the 25 or so T. pallidum recombinant antigens produced so far are given elsewhere. The main such antigens used in serodiagnosis are shown in Table 2: the sequence of the majority of proteins is known and all are cell envelope-associated.

Four of these antigens, P6, P2, TmpB, and TpD have been evaluated to a very limited extent. The other three antigens have been evaluated more extensively and by methods suitable for screening: TmpA (a strongly antigenic exported lipoprotein) by EIA; '37K' (function and properties not clearly defined) by radioimmunoassay (RIA); 4D (subunit of a 190 kD polymer able to form ring structures) by EIA. The sensitivity of these tests in primary syphilis and latent syphilis is summarized in Figures 2 and 3 while the specificity is shown in Figure 4. Data for the Captia Syphilis G EIAs based on conventional T. pallidum antigen EIA are given for comparison. Data are not given for secondary syphilis as all tests approach 100% sensitivity. All of the tests described also react with sera from patients with yaws and pinta and are of no value in differentiating between these infections.

Sensitivity in primary syphilis—Figure 2

With untreated patients the Captia Syphilis G is the most sensitive although there is very little difference between the various tests. Whereas all of the
primaries in the Captia evaluation were seropositive, 16% (9) of those evaluated by TmpA were seronegative and if these are excluded the sensitivity reaches 89%. Sensitivity of 4D in treated patients is similar to the sensitivity of the other tests with untreated patients. However, as the sera in the 4D evaluation were taken within 3 months of treatment this is not surprising. Coates and colleagues using RIA to determine antibody to 4D antigen found a sensitivity of only 55% in 121 untreated primaries. The higher sensitivity with the '37K' antigen when applied to a mixed group of treated and untreated patients is in keeping with the development of seropositivity after treatment.

**Sensitivity in early latent and late syphilis—**

**Figure 3**

It is more difficult to make comparisons between tests in late syphilis because of the different composition of the patient groups. The 4D data illustrates the decrease in sensitivity between early latent and late infection. Treatment status is an additional complicating factor. Whereas the early latent group were evaluated within 3 months of treatment, the treatment status and period since treatment were undocumented for the late latent and late active groups. This combined with the small number of patients may account for the apparent higher sensitivity in late latent compared with late active infection. The '37K' data shows a similar level of sensitivity between latent (unspecified but presumed late latent) and late active infection: both categories comprised treated and untreated patients. The TmpA assay has high sensitivity in untreated early latent syphilis. As antibody to TmpA drops sharply after treatment, and in general is similar to VDRL/RPR activity, it suggests low sensitivity in late infection. In a preliminary analysis, however, we found the TmpA was reactive in 70% of 13 untreated late latent infections; VDRL reactivity was 54% (unpublished data). The superiority of Captia Syphils G in detecting infection beyond the secondary stage is clearly demonstrated.

It may be that diagnostic reagents suitable for screening will require a pool of several recombinant antigens. Appropriate single antigen tests may then be used to help assess the stage of infection: eg P2 was shown by immunoblotting to have superior reactivity in primary (5/6) compared with secondary (1/5) and latent (0/6). We also need more information on antibody responses to single recombinant antigen tests during re-infection before they are considered for adoption as first line screening tests.

**Specificity of recombinant antigen tests—**

**Figure 4**

In theory, pathogen specific recombinant antigen tests should have higher specificity than tests based on sonicated *T. pallidum* prepared from rabbit testes. However, as a significant proportion of the population contains serum antibodies against antigens of the *E. coli* outer envelope, recombinant antigens must be stringently purified to remove *E. coli* proteins. The 4D antigen EIA was 100% specific when tested against 172 VDRL negative and 20 BFP sera. Using RIA about 3% of 118 normal sera were reactive. The '37K' antigen reacted with 9.5% (4/42) BFP sera and 4.6% (3/65) normal sera. TmpA reactivity was found in 0.4% (4/938) blood donors and 1.6% (3/190) STD clinic patients (all VDRL, TPHA and FTA-abs negative). We have now evaluated approximately 58,500 sera by Captia Syphils G and found 1.1% (643/57,830) of patients subsequently shown to be non-infected (VDRL and TPHA negative) had an antibody index >0.9 on screening; 0.8% (477/57,830) of the patients were confirmed as false positives (antibody index >1.0) on re-testing the same specimen. The mean antibody index for false-positive sera was 1.26 compared with 2.72 for VDRL positive and 1.80 for VDRL negative treponemal sera. These false positive reactions were not associated with any specific condition. Recently Lefebvre and colleagues confirmed the overall high specificity of the Captia Syphils G test but found it was reactive with 30% (6/20) of sera from patients with Lyme borreliosis.

The theoretical specificity of recombinant antigen tests has not been proved in practice, even in relatively small scale research evaluations. Further difficulties will be experienced in 'scaling-up' tests to large scale commercial production levels required for use in routine diagnostic laboratories. For
example in a preliminary evaluation of TmpA we obtained a specificity of 100% on testing 40 non-infected STD clinic patients; in a later large scale evaluation the sensitivity decreased to 93% (558/601) when evaluating all sera requiring serological tests for syphilis. This value is significantly higher (P<0.01) than the value determined in the preliminary studies126; these differences are almost certainly due to changes in antigen purification when ‘scaling up’ production.

CONCLUSIONS

EIA because of its suitability for automation and computerization is likely to be used increasingly as a single screening test. It is a highly sensitive test and should be useful in detecting evidence of past treponemal infection in HIV-infected patients. In suspect primary infection it can be supplemented by IgM testing on clinical request. Recombinant antigen EIAs are likely to gain acceptance as screening tests, particularly for early infection, although large scale purification procedures require optimization. Such tests may also have a role in treatment monitoring and in indicating treatment status. Limitations of the FTA-ats are becoming increasingly recognized particularly when treponemal antigen tests are used for screening. There appears to be a correlation between false reactivity in EIA and the FTA-ats. New confirmatory approaches such as immunoblotting are required to complement screening by EIA. EIA for anti-treponemal IgM is of value in determining treatment status in early infection and in diagnosing congenital infection in the newborn. More sensitive IgM tests are required to help assess treatment status in late infection. In view of the interactions between syphilis and HIV infection, all aspects of syphilis serology must be carefully monitored in co-infected patients. The significance of CSF abnormalities requires especially detailed clinical and laboratory appraisal. PCR should over the next few years increase our understanding of treponemal pathogenesis, particularly in HIV infected patients. Its role in diagnosis, however, remains to be established and results should be treated in the context of research data. Perhaps the most important factor at present is to make the best use of our existing technology and ensure that reliable testing is delivered to the populations at risk.

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(Accepted 5 August 1992)
ARTICLE


H Young and A Moyes

Gonococci were serotyped and tested for antibiotic susceptibility as described previously (Weekly Report 90/29) and designated by a numerical nomenclature based on typing with a standard panel of monoclonal antibody typing reagents.

Isolates from a total of 558 episodes of infection were examined compared with a total of 544 in 1990 (Table 1). During 1991 there were 901 cases of gonorrhoea seen at Genitourinary Medicine Clinics compared with 848 in 1990 representing an increase of 6.1% (Information and Statistics Division Scottish Health Service Common Services Agency). The proportion of serogroup IB isolates (67%) is significantly higher (P<0.05) than the 60% found in 1990 (Weekly Report 91/45). The increase occurred in all areas but was most marked in Tayside where the proportion of IB isolates increased from 40.6% to 60.3% and in Fife where it increased from 40.6% to 58%.

<table>
<thead>
<tr>
<th>Area/Laboratory</th>
<th>Number (percentage) IA</th>
<th>Number (percentage) IB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/AYR:ARL</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>AC/DUN:ARL</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>AC/GRE:CLY</td>
<td>18 (62%)</td>
<td>11 (38%)</td>
<td>29</td>
</tr>
<tr>
<td>FF/TIF:ARL</td>
<td>36 (42%)</td>
<td>50 (58%)</td>
<td>86</td>
</tr>
<tr>
<td>HG/INV:RAI</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>GG/GLA:ROY</td>
<td>43 (34%)</td>
<td>84 (66%)</td>
<td>127</td>
</tr>
<tr>
<td>GG/GLA:SOU</td>
<td>21 (27%)</td>
<td>57 (73%)</td>
<td>78</td>
</tr>
<tr>
<td>LO/EDI:STD</td>
<td>29 (22%)</td>
<td>100 (78%)</td>
<td>129</td>
</tr>
<tr>
<td>LO/EDI:ROY</td>
<td>7 (39%)</td>
<td>11 (61%)</td>
<td>18</td>
</tr>
<tr>
<td>LO/EDI:CML</td>
<td>0</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>LO/EDI:CIT</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>TY/DEE:UNI</td>
<td>16 (33%)</td>
<td>33 (67%)</td>
<td>49</td>
</tr>
<tr>
<td>TY/PER:ROY</td>
<td>11 (50%)</td>
<td>11 (50%)</td>
<td>22</td>
</tr>
<tr>
<td>TY/ANG:STR</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>187 (34%)</td>
<td>371 (66%)</td>
<td>558</td>
</tr>
<tr>
<td>1990</td>
<td>216 (40%)</td>
<td>328 (60%)</td>
<td>544</td>
</tr>
</tbody>
</table>

The number and distribution of protein IA serovars are given in Table 2. As in previous years serovar IA-2 predominated in all areas. The most significant change was a decrease in IA-6 from 10.7% in 1990 to 1.1% in 1991 (P<0.001). Two isolates of a previously unrecognised serovar were received from Glasgow Royal Infirmary. These were sent to CDC Atlanta for confirmation of the monoclonal antibody reaction pattern and were accorded the new serovar number IA-25. This is the first new IA serovar reported since 1988. Although these isolates were from a male/female contact pair, unfortunately, the source of the infection could not be traced (Personal communication: Dr Meg Weir, Department of Genitourinary Medicine, Glasgow Royal Infirmary).

H Young and A Moyes are from the Sexually Transmitted Diseases, STD Diagnostic Laboratory, Dept. of Medical Microbiology, University of Edinburgh.
Thirteen protein IB serovars were found among the serogroup IB isolates in 1991 (Table 3) compared with 16 serovars in 1990. The serovars IB-19 and IB-31 were found only in 1991 and accounted for 2.4% of the isolates. Five serovars (IB-4, IB-11, IB-14, IB-16 and IB-22) accounted for 2.7% of isolates in 1990 but were not encountered in 1991. IB-2 was the most common isolate (45.3%) and although this represented a slight overall increase from 1990 (39%) this was not statistically significant. The increase, however, was largely accounted for by Fife where IB-2 isolates increased from 24.3% to 57% (P<0.01) and IB-3 decreased from 54.1% to 24% (P<0.01). Serovar IB-6 which has been correlated with homosexually acquired infection within Lothian\(^{a}\) was most common amongst isolates from the Southern General Hospital in Glasgow where the proportion increased from 5.4% in 1990 to 26.3% in 1991 (P<0.01). In Lothian there was a significant decrease in IB-6 isolates from 16% in 1990 to 7.6% in 1991 (P<0.05). As in 1990 serovar IB-17 was isolated only within Greater Glasgow.

**TABLE 2**

Prevalence and geographical distribution of protein IA serovars (1991)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AA</th>
<th>AC</th>
<th>FF</th>
<th>HG</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>LO</th>
<th>TY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>0</td>
<td>19(100)</td>
<td>35(97.2)</td>
<td>3</td>
<td>30(69.8)</td>
<td>13(61.9)</td>
<td>28(77.8)</td>
<td>29(100)</td>
<td>157(84)</td>
</tr>
<tr>
<td>IA-5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (4.7)</td>
<td>0</td>
<td>7 (19.4)</td>
<td>0</td>
<td>7 (3.7)</td>
<td></td>
</tr>
<tr>
<td>IA-6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2.3)</td>
<td>1 (4.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IA-16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (19.4)</td>
<td>0</td>
<td>7 (3.7)</td>
<td></td>
</tr>
<tr>
<td>IA-21</td>
<td>0</td>
<td>0</td>
<td>1 (2.8)</td>
<td>8 (18.6)</td>
<td>7 (33.3)</td>
<td>1 (2.8)</td>
<td>0</td>
<td>17 (9.1)</td>
<td></td>
</tr>
<tr>
<td>IA-25</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (4.7)</td>
<td>0</td>
<td>0</td>
<td>2 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>0</td>
<td>19(100)</td>
<td>36(100)</td>
<td>3(100)</td>
<td>43(100)</td>
<td>21(100)</td>
<td>36 (100)</td>
<td>29(100)</td>
<td>187(100)</td>
</tr>
</tbody>
</table>

**TABLE 3**

Prevalence and geographical distribution of protein IB serovars (1991)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AA</th>
<th>AC</th>
<th>FF</th>
<th>HG</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>LO</th>
<th>TY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB-1</td>
<td>2</td>
<td>5(1.3)</td>
<td>4 (8)</td>
<td>0</td>
<td>5 (6.8)</td>
<td>5 (8.8)</td>
<td>9 (7.6)</td>
<td>2 (4.5)</td>
<td>32 (8.6)</td>
</tr>
<tr>
<td>IB-2</td>
<td>0</td>
<td>4(25)</td>
<td>29(38)</td>
<td>0</td>
<td>41(48.8)</td>
<td>14(24.6)</td>
<td>63(57.6)</td>
<td>12(27.3)</td>
<td>168(45.3)</td>
</tr>
<tr>
<td>IB-3</td>
<td>0</td>
<td>12(24)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9 (15.8)</td>
<td>14(11.9)</td>
<td>27(51.4)</td>
<td>79 (21.3)</td>
</tr>
<tr>
<td>IB-5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2.8)</td>
<td>2 (3.5)</td>
<td>1 (0.8)</td>
<td>0</td>
<td>3 (0.8)</td>
<td></td>
</tr>
<tr>
<td>IB-6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 (2.7)</td>
<td>15(26.3)</td>
<td>9 (7.6)</td>
<td>0</td>
<td>36 (9.7)</td>
<td></td>
</tr>
<tr>
<td>IB-7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4 (4.8)</td>
<td>2 (3.5)</td>
<td>6 (5.1)</td>
<td>1 (2.3)</td>
<td>14 (3.8)</td>
<td></td>
</tr>
<tr>
<td>IB-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1.8)</td>
<td>4 (3.4)</td>
<td>0</td>
<td>5 (1.3)</td>
<td></td>
</tr>
<tr>
<td>IB-15</td>
<td>0</td>
<td>2(36)</td>
<td>0</td>
<td>0</td>
<td>1 (1.8)</td>
<td>1 (0.8)</td>
<td>0</td>
<td>2 (0.5)</td>
<td></td>
</tr>
<tr>
<td>IB-17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (2.4)</td>
<td>0</td>
<td>2 (1.7)</td>
<td>0</td>
</tr>
<tr>
<td>IB-19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (2.4)</td>
<td>0</td>
<td>2 (1.7)</td>
<td>0</td>
</tr>
<tr>
<td>IB-26</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (3.5)</td>
<td>1 (0.8)</td>
<td>1 (2.3)</td>
<td>4 (1.1)</td>
</tr>
<tr>
<td>IB-29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (1.8)</td>
<td>1 (0.8)</td>
<td>0</td>
<td>2 (0.5)</td>
</tr>
<tr>
<td>IB-31</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (3.6)</td>
<td>0</td>
<td>1 (2.3)</td>
<td>7 (1.9)</td>
</tr>
<tr>
<td>No Type</td>
<td>0</td>
<td>0</td>
<td>3 (6)</td>
<td>0</td>
<td>1 (1.2)</td>
<td>0</td>
<td>5 (0.8)</td>
<td>0</td>
<td>5 (1.3)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2</td>
<td>16(100)</td>
<td>50(100)</td>
<td>84(100)</td>
<td>57(100)</td>
<td>118(100)</td>
<td>44(100)</td>
<td>371(100)</td>
<td></td>
</tr>
</tbody>
</table>

Again there is a highly significant difference (P<0.001) in the penicillin susceptibility of IA and IB isolates (Table 4 and 5): excluding penicillinase-producing *Neisseria gonorrhoeae* (PPNG) 96% of IA isolates had a
minimum inhibitory concentration (MIC) to penicillin of ≤0.015 mg/L, compared with 14.7% of IB isolates (P<0.001). The corresponding values for 1990 of 86% and 15% are not significantly different from the 1991 results. A total of 12 (3.4%) non-PPNG IB isolates were chromosomally mediated resistant N. gonorrhoeae (CMRNG) (MIC ≥1.0 mg/L) compared with four (1.3%) reported in 1990. This is significantly lower (P<0.001) than the 10.6% (100/940) CMRNG found when sampling isolates from St. Mary's Hospital London between 1984 and 1988.

**TABLE 4**

Penicillin susceptibility of IA Serovars (1991) No. of isolates with following MIC (mg/L)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1.0</th>
<th>≥1.0</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>141</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1(a)</td>
<td>157</td>
</tr>
<tr>
<td>IA-5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IA-6</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IA-16</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6*</td>
</tr>
<tr>
<td>IA-21</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>IA-25</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>168</td>
<td>15</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1(a)</td>
<td>186*</td>
</tr>
<tr>
<td>1990</td>
<td>181</td>
<td>12</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>6(a)</td>
<td>216</td>
</tr>
</tbody>
</table>

(a) PPNG isolates
* One isolate IA-16 not available for MIC

**TABLE 5**

Penicillin susceptibility of IB Serovars (1991) No. of isolates with following MIC (mg/L)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1.0</th>
<th>≥1.0</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB-1</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>14</td>
<td>0</td>
<td>6(a)</td>
<td>32</td>
</tr>
<tr>
<td>IB-2</td>
<td>47</td>
<td>41</td>
<td>21</td>
<td>55</td>
<td>1</td>
<td>3(a)</td>
<td>168</td>
</tr>
<tr>
<td>IB-3</td>
<td>0</td>
<td>27</td>
<td>26</td>
<td>21</td>
<td>2</td>
<td>3(a)</td>
<td>79</td>
</tr>
<tr>
<td>IB-5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IB-6</td>
<td>1</td>
<td>7</td>
<td>10</td>
<td>11</td>
<td>0</td>
<td>6(a)</td>
<td>35*</td>
</tr>
<tr>
<td>IB-7</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>1(a)</td>
<td>14</td>
</tr>
<tr>
<td>IB-8</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(a)</td>
<td>5</td>
</tr>
<tr>
<td>IB-15</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>IB-17</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>IB-19</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IB-26</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>IB-29</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IB-31</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>7*</td>
</tr>
<tr>
<td>NO TYPE</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0(a)</td>
<td>4*</td>
</tr>
<tr>
<td>TOTAL</td>
<td>51</td>
<td>90</td>
<td>79</td>
<td>116</td>
<td>12</td>
<td>21(a)</td>
<td>369</td>
</tr>
<tr>
<td>1990</td>
<td>48</td>
<td>56</td>
<td>116</td>
<td>97</td>
<td>2</td>
<td>8</td>
<td>311</td>
</tr>
</tbody>
</table>

(a) = PPNG isolates
* One IB-6 and one non-typable isolate not available for MIC
The susceptibility of isolates to cefuroxime, tetracycline, erythromycin and ciprofloxacin is summarised in Table 6. Although spectinomycin is now shown in this table only two isolates had an MIC ≥ 16 mg/L: one isolate was a non-PPNG and the other a IB-7 PPNG. Of the isolates that grew on 0.5 mg/L tetracycline, 9 were not available for re-testing: the MICs for the other isolates were ≤ 1 mg/L (22 isolates), 2 mg/L (16 isolates), and 8 mg/L (1 isolate). Apart from ciprofloxacin the levels of sensitivity were generally comparable with those reported for the 1990 isolates. In 1990 all isolates had a ciprofloxacin MIC of ≤ 0.008 mg/L whereas in 1991 ten IB isolates (7 non-PPNG and 3 PPNG) had an MIC > 0.008 mg/L. Nine of the isolates were re-tested and gave MICs of 0.015 mg/L (5 isolates), 0.03 mg/L (2 isolates), 0.120 mg/L (2 isolates). Treatment failure with ciprofloxacin may occur at MICs > 0.03 mg/L\(^5\). The range of antibiotic concentrations is being extended in 1992 to minimise the number of isolates requiring to be re-tested.

**TABLE 6**

Antibiotic susceptibility of serotype IA and IB isolates (1991)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype</th>
<th>Cumulative percentage MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>1A</td>
<td>≤ 0.015 0.06 0.12 0.5 1.0</td>
</tr>
<tr>
<td></td>
<td>1B</td>
<td>90.3 98.4 98.4 98.9 99.5(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.8 38.2 59.6 91.1 94.3(b)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>1A</td>
<td>≤ 0.02 0.10 0.5 1.0</td>
</tr>
<tr>
<td></td>
<td>1B</td>
<td>96.2 98.4 100 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.2 78.9 99.2 100</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1A</td>
<td>≤ 0.125 0.5</td>
</tr>
<tr>
<td></td>
<td>1B</td>
<td>28.5 99.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8 87.3</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1A</td>
<td>≤ 0.125 0.50</td>
</tr>
<tr>
<td></td>
<td>1B</td>
<td>50.5 98.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.7 90.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1A</td>
<td>≤ 0.002 0.004 0.008</td>
</tr>
<tr>
<td></td>
<td>1B</td>
<td>51.7 97.3 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.8 86.6 97.3</td>
</tr>
</tbody>
</table>

(a) 1 isolate MIC > 71.0 mg/L; PPNG
(b) 21 isolates MIC > 1.0 mg/L; all PPNG

The serovar and geographical distribution of PPNG stains is given in Table 7. Overall 3.9% of isolates were PPNG compared with 2.6% in 1990, 1.4% in 1989 and 2.8% in 1988: these differences are not significant. Details of plasmid analysis and auxotype will be reported separately.
TABLE 7
Serovar and Geographical Distribution of PPNG Isolates (1991)
No. of PPNG isolates/Total No. of isolates

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AA</th>
<th>AC</th>
<th>FF</th>
<th>HG</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>LO</th>
<th>TY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>0</td>
<td>0/19</td>
<td>0/35</td>
<td>0/3</td>
<td>0/30</td>
<td>0/13</td>
<td>1/28</td>
<td>0/29</td>
<td>1/157</td>
</tr>
<tr>
<td>IB-1</td>
<td>0/2</td>
<td>5/5</td>
<td>0/4</td>
<td>0</td>
<td>0/5</td>
<td>0/5</td>
<td>1/9</td>
<td>0/2</td>
<td>6/32</td>
</tr>
<tr>
<td>IB-2</td>
<td>0</td>
<td>1/4</td>
<td>1/29</td>
<td>0</td>
<td>0/41</td>
<td>0/14</td>
<td>0/68</td>
<td>1/12</td>
<td>3/168</td>
</tr>
<tr>
<td>IB-3</td>
<td>0</td>
<td>0</td>
<td>0/12</td>
<td>0</td>
<td>0/7</td>
<td>0/9</td>
<td>0/14</td>
<td>3/27</td>
<td>3/79</td>
</tr>
<tr>
<td>IB-6</td>
<td>0</td>
<td>2/4</td>
<td>0/1</td>
<td>0</td>
<td>0/7</td>
<td>4/15</td>
<td>0/9</td>
<td>0</td>
<td>6/36</td>
</tr>
<tr>
<td>IB-7</td>
<td>0</td>
<td>0</td>
<td>0/1</td>
<td>0</td>
<td>0/4</td>
<td>0/2</td>
<td>1/6</td>
<td>0/1</td>
<td>1/14</td>
</tr>
<tr>
<td>IB-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/1</td>
<td>1/4</td>
<td>0</td>
<td>2/5</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0/3</td>
<td>0/4</td>
<td>0</td>
<td>0/23</td>
<td>0/19</td>
<td>0/16</td>
<td>0/2</td>
<td>0/67</td>
</tr>
<tr>
<td>Total</td>
<td>0/2</td>
<td>8/35</td>
<td>1/86</td>
<td>0/3</td>
<td>0/127</td>
<td>5/78</td>
<td>4/154</td>
<td>4/73</td>
<td>22/558</td>
</tr>
<tr>
<td>1990</td>
<td>0</td>
<td>0/7</td>
<td>0/78</td>
<td>0</td>
<td>7/128</td>
<td>1/80</td>
<td>5/185</td>
<td>1/59</td>
<td>14/537</td>
</tr>
</tbody>
</table>

References:


Acknowledgements:

We thank our numerous bacteriological and clinical colleagues for their help and support in making this surveillance possible. Any additional laboratories that would like to participate in the surveillance programme should contact Dr. H Young Neisseria gonorrhoeae Reference Laboratory (Scotland), Department of Medical Microbiology, Edinburgh University Medical School. Edinburgh EH8 9AG (Tel 031-650-3143).

Special thanks are extended to Mrs Joan McElhinney for careful record keeping and preparation of the manuscript.

Please note:- This article has been reproduced in full this week because the second page, including tables, was not printed when it first appeared (Weekly Report 92/35).
Footnote for Table 1

Health Board Area and Laboratories submitting specimens.

Ayrshire and Arran
AA/AYR:ARL - Dept of Microbiology, Crosshouse Hospital Kilmarnock, Ayrshire.

Argyll and Clyde
AC/DUN:ARL - Vale of Leven Hospital, Alexandria, Dunbartonshire.
AC/GRE:CLY - Inverclyde Royal Hospital, Greenock.

Fife
FF/FIF:ARL - Fife Area Laboratory, Hayfield Road, Kirkcaldy, Fife.

Highland
HG/INV:RAI - Raigmore Hospital, Inverness.

Greater Glasgow
GG/GLA:ROY - Glasgow Royal Infirmary, Glasgow.
GG/GLA:SOU - Glasgow Southern General Hospital, Glasgow.

Lothian
LO/EDI:ROY - Edinburgh Royal Infirmary, Edinburgh.
LO/EDI:CML - Western General Hospital, Crewe Road, Edinburgh.
LO/EDI:CIT - City Hospital, Greenbank Drive, Edinburgh.

Tayside
TY/DEE:UNI - Ninewells Hospital & Medical School, Dundee.
TY/PER:ROY - Perth Royal Infirmary, Perth.
TY/ANG:STR - Stirling Royal Infirmary, Stirling.
Enzyme immunoassay for anti-treponemal IgG: Screening or confirmatory test?

H Young, A Moyes, A McMillan, J Patterson
Enzyme immunoassay for anti-treponemal IgG: Screening or confirmatory test?

H Young, A Moyes, A McMillan, J Patterson

Abstract

Aims: To review the performance of the Venereal Diseases Research Laboratory (VDRL) test and the Treponema pallidum haemaggulination assay (TPHA) as a combined screen for syphilis to provide a baseline for assessing screening by anti-treponemal IgG EIA.

Methods: Between 1980 and 1987 all serum samples were screened by both VDRL and TPHA tests. The FTA-ABS test was also used in suspected early primary syphilis, or when one of the other tests was positive. A positive result in a screening test was confirmed by quantitative testing. From 1988 all specimens were screened with an enzyme immunoassay (Captia Syph G) as a single screening test.

Results: Of the 44 primary, 47 secondary, and 38 early latent cases of syphilis, the VDRL and TPHA detected 32 (73%) and 31 (71%) of the primary cases; the combination detected 37 (84%). All 85 cases of secondary and early latent infection were reactive in the TPHA test, whereas the VDRL was reactive in only 68 (80%). EIA had a reported sensitivity of 82% for primary infection.

Conclusions: EIA can be used as a single screening test for detecting early syphilis because its results are comparable with those of the combined VDRL and TPHA tests. The conventional VDRL test should not be used as a single screening test.

The addition of the enzyme immunoassay (EIA) to the range of serological tests for treponematial infection offers laboratories yet greater choice in the selection of individual tests or combinations of tests for syphilis screening and diagnosis.1 A possible limitation of screening with an anti-treponemal IgG EIA is the failure to detect cases of primary syphilis. The natural humoral response to treponematial infection means that there is a period of two to three weeks when the IgG response may be absent or inadequate for serological detection.2 Although more data are required, this limitation may be more of a theoretical rather than a practical issue. Nevertheless, it is important to consider when deciding whether EIAs that detect antitreponemal IgG should be used as screening3 or as confirmatory tests.4 The potential for automation makes EIA a particularly attractive screening test as we reported in our evaluation5 of Captia Syph G (Mercia Diagnostics UK). The overall screening performance of this new anti-treponemal IgG EIA (sensitivity 98.4% and specificity 99.3%) was comparable with that provided by the widely used screening combination of the Venereal Diseases Research Laboratory (VDRL) test and the Treponema pallidum haemaggulination assay (TPHA). Unfortunately, there were two few cases of primary syphilis in our patient population to assess the efficiency of Captia Syph G in detecting primary infection. Recently, Lefèvre and colleagues evaluated the Captia Syph G in 96 patients with untreated syphilis at various stages, including 17 patients with primary stage infection as well as 63 patients with treated infection.6 The overall sensitivity of the Captia Syph G was 98.3%; the test was 100% sensitive in treated syphilis and in all stages of untreated syphilis except primary infection (sensitivity 82%). Because of its lower sensitivity in primary infection it was concluded that the Captia Syph G test did not seem to be a suitable replacement for the present screening combination of VDRL and TPHA tests but was an ideal confirmatory test.6 As this was not an “in-use” study there were no data to support the view that the VDRL and TPHA screening combination performs better than the Captia Syph G (with a sensitivity of 82% in primary syphilis) might in routine practice.

We reviewed the performance of the VDRL and TPHA screening combination over eight years of routine practice to provide a baseline for assessing screening by anti-treponemal IgG EIA.

Methods

We reviewed the notes and laboratory records for all patients with a diagnosis of untreated syphilis who attended the genitourinary medicine unit at Edinburgh Royal Infirmary between 1980 and 1990 inclusive. Between 1980 and 1987 all sera were screened by the combination of VDRL and TPHA tests.5 The FTA-ABS test was performed as an additional test whenever requested, as in the case of suspected early primary syphilis, or routinely whenever one of the screening tests was positive. A positive result in a screening test was confirmed by quantitative testing: the VDRL test was titrated to the end point whereas the TPHA titration was usually limited to a final serum dilution of 1 in 5120. Titres were expressed as the reciprocal of a
Table 1 Diagnosis of syphilis 1980-1990 in patients attending genitourinary medicine unit in Edinburgh

<table>
<thead>
<tr>
<th>Year</th>
<th>Primary syphilis</th>
<th>Secondary syphilis</th>
<th>Early latent syphilis</th>
<th>All other stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1980</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>1981</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>1982</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>1983</td>
<td>11</td>
<td>11</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>1984</td>
<td>10</td>
<td>6</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>1985</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>1986</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1987</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1988</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1989</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1990</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Primary 92% male; secondary 98% male; early latent 95% male; other stages 68% male.

Table 2 Pattern of serological tests results in 44 cases of untreated primary syphilis

<table>
<thead>
<tr>
<th>VDRL</th>
<th>TPHA</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>5</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>26</td>
</tr>
</tbody>
</table>

Results

Table 1 shows the pronounced decrease in the number of cases of syphilis diagnosed during the past decade.

During the period 1980-84 there were 208 cases of which 113 (54.3%) were early infections; between 1985 and 1989 there were 61 cases of which 21 (34.4%) were early infections: the decrease in the proportion of early syphilis is highly significant ($\chi^2 = 6.7; p < 0.01$). In 1990, however, there was a significant increase in the proportion of cases of early syphilis when compared with the period 1985-89 ($\chi^2 = 5; p < 0.05$). The pattern of serology and dark ground microscopy results for the 44 cases of primary syphilis diagnosed from 1980-87 is shown in table 2.

A total of 11 patients did not have a dark ground investigation. The overall sensitivity of dark ground microscopy was 75-8% (25 of 33) while 11-4% (five of 44) of cases were positive only by microscopy. Of the individual screening tests, the VDRL detected 72-7% (32 of 44) and the TPHA 70-5% (31 of 44). The FTA-ABS was reactive in 79-5% (35 of 44) and was the most sensitive single test. The screening combination of VDRL and TPHA tests detected 84-1% (37 of 44) of cases. The VDRL and TPHA were complementary in detecting primary infection that in five cases were TPHA positive VDRL negative, while six cases were TPHA negative VDRL positive. The sensitivity of the VDRL and TPHA tests was similar in terms of reactivity above the cut-off point for screening, but the antibody titre was higher in the case of the VDRL test than in the TPHA (table 3).

Twenty six sera (59%) gave a titre of $\geq 2$ in the VDRL test compared with 14 sera (32%) giving an equivalent titre in the TPHA ($\chi^2 = 5.5; p < 0.02$). Four of the patients with primary infection had been treated for syphilis; three of these patients had a TPHA titre of $> 64$ units and a VDRL titre of 32.

Results of quantitative tests performed following screening are given for secondary syphilis in table 4 and for early latent syphilis in table 5.

One patient with early latent syphilis was omitted from the analysis because records were missing. All 85 cases of secondary and early latent infection were reactive in the FTA-ABS and TPHA tests. Quantitative VDRL testing was performed irrespective of the screening results.

Statistical analysis was performed using the $\chi^2$ test with Yates's correction.

Table 3 VDRL and TPHA titres in 44 cases of untreated primary syphilis (1980-87)

<table>
<thead>
<tr>
<th>Titre</th>
<th>VDRL</th>
<th>TPHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases with corresponding titre in:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>32</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>64</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>44</td>
</tr>
</tbody>
</table>

VDRL titre given as units—that is, end point dilution/screening dilution: $1 = 80; 2 = 160; 64 = \geq 5120$. 

Equivocal reactions scored positive.
Table 4  VDRL and TPHA titres in 47 cases of untreated secondary syphilis (1980-87)

<table>
<thead>
<tr>
<th>Titre</th>
<th>VDRL</th>
<th>TPHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>32</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>64</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>47</td>
</tr>
</tbody>
</table>

Four sera (8%) gave a prozone reaction in the VDRL test (titres of 16, 64, 120 and 512).

Table 5  VDRL and TPHA titres in 38 cases of untreated early latent syphilis (1980-87)

<table>
<thead>
<tr>
<th>Titre</th>
<th>VDRL</th>
<th>TPHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>64</td>
<td>32</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>38</td>
</tr>
</tbody>
</table>

Four sera (10%) gave a prozone reaction in the VDRL test (titres of 32; 120; 3, and 512).

showed reactivity in 97-9% (43 of 44) of cases of secondary syphilis, but four of these sera (8.5%) gave a false negative reaction on screening, presumably due to the prozone phenomenon. Therefore the sensitivity of the VDRL based on the initial screening results was 89-4% (42 of 47). Prozone reactions also occurred in early latent syphilis: the VDRL was reactive in 79% (30 of 38) of cases after repeat testing, but there were four false negative reactions initially, resulting in a screening sensitivity of 68% (26 of 38). Apart from the greater sensitivity of the TPHA in detecting early latent syphilis, titres were also significantly higher than in the VDRL test: titres of >16 were found in 57-9% (22 of 38) TPHA results compared with 31-6% (12 of 38) VDRL tests ($x^2=4-3; p<0-05$).

Screening and quantitative results for the 14 cases of early syphilis diagnosed since the introduction of Captia Syph G as a single screening test are shown in table 6.

Table 6  Serological results for 14 cases of early syphilis detected by screening with anti-treponemal IgG enzyme immunoassay

<table>
<thead>
<tr>
<th>Stage</th>
<th>EIA-IgG</th>
<th>EIA-IgM</th>
<th>VDRL</th>
<th>TPHA</th>
<th>FTA-ABS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>3.5</td>
<td>2.1</td>
<td>12</td>
<td>160</td>
<td>Positive</td>
</tr>
<tr>
<td>Primary</td>
<td>2.5</td>
<td>2.1</td>
<td>8</td>
<td>160</td>
<td>Positive</td>
</tr>
<tr>
<td>Primary</td>
<td>2.1</td>
<td>3.2</td>
<td>9</td>
<td>80</td>
<td>Positive</td>
</tr>
<tr>
<td>Primary</td>
<td>1.7</td>
<td>1.2</td>
<td>12</td>
<td>80</td>
<td>Positive</td>
</tr>
<tr>
<td>Secondary</td>
<td>2.3</td>
<td>1.8</td>
<td>16</td>
<td>2560</td>
<td>Positive</td>
</tr>
<tr>
<td>Secondary</td>
<td>1.3</td>
<td>2.2</td>
<td>12</td>
<td>2560</td>
<td>Positive</td>
</tr>
<tr>
<td>Secondary</td>
<td>3.1</td>
<td>2.0</td>
<td>128</td>
<td>5120</td>
<td>Positive</td>
</tr>
<tr>
<td>Secondary</td>
<td>4.1</td>
<td>1.3</td>
<td>128</td>
<td>5120</td>
<td>Positive</td>
</tr>
<tr>
<td>Secondary</td>
<td>4.5</td>
<td>2.3</td>
<td>64</td>
<td>5120</td>
<td>Positive</td>
</tr>
<tr>
<td>Early latent</td>
<td>3.3</td>
<td>1.5</td>
<td>12</td>
<td>2560</td>
<td>Positive</td>
</tr>
<tr>
<td>Early latent</td>
<td>3.7</td>
<td>1.1</td>
<td>16</td>
<td>5120</td>
<td>Positive</td>
</tr>
<tr>
<td>Early latent</td>
<td>1.4</td>
<td>2.9</td>
<td>2</td>
<td>640</td>
<td>Positive</td>
</tr>
<tr>
<td>Early latent</td>
<td>4.2</td>
<td>0.0</td>
<td>128</td>
<td>5120</td>
<td>Positive</td>
</tr>
<tr>
<td>Early latent</td>
<td>2.5</td>
<td>0.3</td>
<td>0</td>
<td>160</td>
<td>Positive</td>
</tr>
</tbody>
</table>

IgG and IgM results expressed as an antibody index. Secondary with VDRL titre of 128 gave a prozone.

All four patients with primary infection gave positive tests for anti-treponemal IgG and were also reactive in the VDRL, TPHA, and FTA-ABS tests. Specific anti-treponemal IgM was found in 85-7% (12 of 14) of the patients: the two patients with early latent infection and negative IgM results had received antibiotics on at least one occasion in the two years preceding the diagnosis.

Discussion

In the United Kingdom the annual number of cases of syphilis declined between 1980 and 1986. Recently, however, there has been a resurgence of heterosexually acquired syphilis in London while over the past year a similar phenomenon has been observed in Edinburgh (table 1). In Amsterdam the declining trend was reversed in 1987 when increases in heterosexually acquired infection were observed. In the United States the increase in heterosexually acquired infection observed in recent years has been associated with a very steep increase in congenital infection. These findings substantiate the case for antenatal screening, and the value of which has been questioned in recent years. The potential for concomitant infection with the human immunodeficiency virus (HIV) in both the treponemal antibody response and the clinical course of syphilis is an additional factor to consider in the selection and use of serological tests for syphilis.

The above changes in the epidemiology of syphilis necessitate a reappraisal of diagnostic methods. In spite of the recent increases, screening or case finding for syphilis involves very large numbers of specimens being examined to detect a very small number of positive findings. In these circumstances new methods such as EIA, with the potential for automated testing and computed reporting, have many attractions. Before replacing existing screening tests with a new protocol it is important to establish that the performance of the new method is comparable with that of the existing procedures.

The Captia Syph G gives high specificity and is sensitive in detecting anti-treponemal antibody in untreated as well as treated infection. Because the sensitivity in detecting untreated primary infection was 82% (compared with 100% for all other stages of infection), Lefevre et al considered that this EIA was unsuitable as a replacement for the VDRL and TPHA screening combination. Our results show, however, that a sensitivity of 82% in detecting untreated primary infection is greater than any other single test, including dark ground microscopy (76%), VDRL (73%), TPHA (71%) and FTA-ABS (80%). Similar sensitivities for dark ground microscopy (78%), VDRL (70%), and TPHA (75%) in untreated primary syphilis have also been reported. Although the combination of VDRL and TPHA tests did give slightly greater sensitivity (84%), this value is not significantly different (p > 0-05) from the 82% sensitivity of EIA. The high sensitivity of EIA using anti-human IgG conjugate has been
reported with both "in-house" and commercial EIA systems. Veldkamp and Visser detected all 32 cases of untreated primary infection, while the Bio-Inza Bead test was reactive in five of six cases and all three cases of untreated primary infection. In our study all four primary cases diagnosed since 1988 were reactive in the EIA for IgG. A double conjugate EIA for both IgG and IgM found that in infected patients a positive IgG result always accompanied a positive IgM result. It was also shown by western blotting that by the time clinical signs develop most patients have both IgG and IgM antibody.

Routine serological screening will always fail to detect some cases of early primary infection. For example, in our study (table 2), 11% of cases were negative in all serological tests including the FTA-ABS. Anderson et al. reported that 14.5% of 939 patients with primary syphilis were seronegative in all tests at the time of presentation. A high index of clinical suspicion is the key factor in making a diagnosis of primary infection and is probably more important than the serological screening schedule used.

Compared with the seronegative "window" in early primary syphilis, the occurrence of false negative results in secondary and early latent syphilis when very high antibody titres are found (tables 4 and 5) is a more serious and underestimated limitation of certain serological screening tests. The prozone phenomenon is generally considered to occur in 1–2% of patients with secondary syphilis.2,24 Spangler et al. however, reported 24 patients in various stages of infection whose serological tests were negative; 16 of the 24 negative tests were due to the prozone phenomenon. Our findings (tables 4 and 5) that 8% of patients with secondary and 10% of patients with early latent infection gave a false negative result in the VDRL test due to the prozone phenomenon means that such tests should not be used alone to screen for untreated early infection.

The high rate of prozone reactions reported in our study may be related to our routine policy of performing a quantitative VDRL test of any serum giving a TPHA titre of ≥5120. The tendency of the prozone phenomenon to occur in sera with high VDRL titres could be particularly important in patients with concomitant HIV infection as sera from many such patients have unusually high antibody titres.13,22 Unusually high titres are also found in patients undergoing reinfection, even in cases of primary syphilis (table 3). The increased risk of the prozone phenomenon occurring in sera from these patients makes detection of reinfection by non-treponemal tests less reliable. False negative VDRL reactions may also occur in the cerebrospinal fluid. Feraru et al. reported two HIV positive patients with neurosyphilis whose initial cerebrospinal fluid VDRL tests were negative. In one of these patients the initial negative VDRL test may have been due to the prozone phenomenon. False negative results are also important in antenatal screening. Recently four cases with false negative serological tests due to the prozone phenomenon were encountered in women who gave birth to infants with congenital infection.14 Although serum dilution before testing was recommended as a routine procedure for all seroconverters in women and in areas of high syphilis prevalence,21 in our opinion this is not a practical proposition when all patients can be screened simply and reliably with specific antitreponemal antibody tests such as the TPHA or EIA.

Our results also indicate that detection of anti-treponemal IgG followed by testing for specific IgM antibodies by EIA is an efficient and simple screening method for detecting syphilis and indicating whether active disease is present. In our series all nine patients with primary or secondary syphilis were IgM positive compared with only 60% (three of five) of cases of early latent infection. Lefevre also found that the correlation between detection of anti-treponemal IgM and active disease was high 90% (27 of 30) in primary and secondary infection but lower 64% (nine of 14) in early latent infection. The lower detection rate of specific IgM in early syphilis could be due to a combination of previous antibiotics for other conditions (as in the two negative cases in our series) and the general decrease in the spectrum and strength of antibody response which occurs with increased duration of infection.20

We conclude that in the case of primary syphilis there is no evidence to suggest that screening for anti-treponemal IgG by EIA is significantly less sensitive than the combination of VDRL and TPHA tests. Provided that clinicians are made aware of the "seronegative window" that may exist for one to two weeks during early primary infection, they maintain a high index of clinical suspicion, and have the facility to request additional tests, such as specific IgM or FTA-ABS, in cases of suspect primary infection, then there are many practical advantages and benefits in screening with a single test that lends itself readily to automation and computed report generation, thus overcoming problems of subjective interpretation and transcriptional errors.

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ORIGINAL ARTICLE

Anogential non-gonococcal Neisseriae: prevalence and clinical significance

J G McKenna BSc MRCP1*, R J Fallon MD FRCPath2, A Moyes FIMLS3 and H Young PhD MRCPath3

1Genitourinary Medicine Unit, Royal Infirmary of Edinburgh, 2Department of Medical Microbiology, Edinburgh University, and 3Meningococcus Reference Laboratory (Scotland), Ruchill Hospital, Glasgow, UK

Summary: Over a 13-year period non-gonococcal neisseriae (NGN) were isolated from 114 of 88,670 patients (0.13%) screened for anogential gonorrhoea at a Genitourinary Medicine Unit. During the same period there were approximately 9000 anogential gonococcal infections (1%). The prevalence of NGN was 0.09% (27/31,500) in women, 0.04% (20/52,800) in heterosexual men and 1.5% (67/4370) in homosexual men: the differences in prevalence between women and heterosexual men (P<0.01) and between heterosexual patients and homosexual men (P<0.001) are highly significant. Neisseria meningitidis was isolated most frequently and accounted for 85% (99/114) of the NGN. Whenever possible, N. meningitidis was serogrouped and its occurrence correlated with patient symptoms. Eleven of 18 heterosexual men who had meningococci isolated from their urethras had urethritis but co-existing chlamydial infection was excluded in only 5. None of 9 women with cervical colonization had clinical evidence of pelvic inflammation. Only one of 49 men with rectal colonization had proctitis. The management of anogential NGN infection is discussed in relation to our findings and those of previously published studies.

Within each patient group the prevalence and incidence of anogential NGN were similar at the beginning and end of the study period indicating that levels have not been influenced by the advent of AIDS.

Keywords: Neisseria sp., Moraxella sp., prevalence, urogenital diseases, rectal diseases

INTRODUCTION

The selective culture media used in screening for gonococcal infection allows the growth of Neisseria meningitidis and N. lactamica as well as gonococci and such organisms are frequently isolated from the pharynx. Occasionally N. meningitidis and other non-gonococcal neisseriae (NGN) are isolated from an anogential site and it was proposed in 1942 that in certain circumstances, N. meningitidis in the urogenital tract may be pathogenic. Since then meningococci have frequently been implicated in case reports of urethritis, proctitis, vulvovaginitis and pelvic inflammatory disease (PID). Most epidemiological data on anogential N. meningitidis colonization is derived from studies in homosexual men but there are no long-term studies comparing the prevalence of N. meningitidis in homosexual and heterosexual men and women.

In this study we have determined retrospectively the prevalence of anogential N. meningitidis and the other NGN in homosexual and heterosexual men and women over a 13-year period. Whenever possible we have correlated bacteriological and clinical findings and compared results between index cases and partners.

MATERIALS AND METHODS

Altogether 88,670 new and 'return new' patients attending the Genitourinary Medicine Unit, Royal Infirmary of Edinburgh over the period 1978–1990 were screened for gonorrhoea as described previously. All isolates of oxidase positive Gram negative cocci were identified by the rapid carbohydrate utilization test; in addition gonococci were serogrouped by the Phadebact Monoclonal GC Test and N. meningitidis were confirmed and serogrouped by coagglutination at the Meningococcus Reference Laboratory [Scotland]. The case notes of all patients with an anogential NGN were reviewed retrospectively and the clinical features at...
the time of colonization noted. Patients were subdivided into their sexual orientation on the basis of their given history, though full data pertaining to the specific sexual practices of the individual was not sought. Data pertaining to other bacteriological investigation and contact action, if undertaken, were also noted. Chlamyrial diagnosis was not undertaken routinely in all patients.

In order to determine if there had been a change in prevalence and incidence of anogenital NGN during the AIDS era, data were compared for the 3-year period at the beginning (1978–80) and the end (1988–90) of the study.

Statistical analysis was by the Chi-square method with Yates' correction.

RESULTS

Over the 13-year period NGN were isolated from an anogenital site in 114 of 88,670 patients (0.13%). During the same period there were approximately 9000 anogenital gonococcal infections (10%). The prevalence of NGN was 1.5% (67/4370) in homosexual men and this was significantly higher than the level of 0.06% (47/74300) found in heterosexual men and women (P < 0.001). The prevalence of NGN in women was 0.09% (27/31,500) and this was significantly higher than the 0.04% (20/52,800) found in heterosexual men (P < 0.01). N. meningitidis was isolated most frequently and accounted for 85% (99/114) of the NGN.

Of the 20 NGN isolates from heterosexual men, 18 were N. meningitidis and two were Moraxella (Branhamella) catarrhalis. Details of urethral meningococcal colonization in heterosexual men are given in Table 1.

Eleven men (55%) had urethritis, but culture for co-existing chlamydial infection was carried out in only 5 and these were negative. Urethral smears from 5 patients showed typical Gram-negative diplococci (GNDC), and 3 were treated for presumptive gonorrhoea before the results of culture were available.

Ten female partners of these 20 men with urethral NGN were screened subsequently, but only one yielded a strain of N. meningitidis from the cervix. She was asymptomatic and therefore not treated. Anogenital NGN were isolated from 27 women (Table 2).

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Number of isolates</th>
<th>Urethritis</th>
<th>Gram negative diplococci on smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Z</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-groupable</td>
<td>6</td>
<td>4*</td>
<td>2</td>
</tr>
<tr>
<td>Not available</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>11</td>
<td>5</td>
</tr>
</tbody>
</table>

*Includes one patient with co-existent N. gonorrhoeae

Eighteen women, one with N. gonorrhoeae infection, were colonized with N. meningitidis and 9 with other NGN. NGN other than meningococci tended to be associated with urethral or rectal rather than cervical colonization; they were present in 6 of 18 rectal cultures, 4 of 6 urethral cultures but in only one of 9 cervical cultures. Nine women yielded NGN from the cervix; 5 were asymptomatic; 2 had a pruritic vaginal discharge from an accompanying candidal vulvitis and 2 patients had a vaginal discharge for which no other cause could be found. One of these patients had GNDC on the cervical smear and was treated presumptively for gonorrhoea. Culture subsequently yielded N. meningitidis Group C. Her contact was found to have an asymptomatic urethral gonococcal infection. A further 4 male partners of the female patients with cervical NGN were screened but none had NGN isolated from the urethra.

Sixty-seven homosexual men had anogenital NGN. The sites of isolation are shown in Table 3.

Seven (54%) of the 13 patients with urethral colonization had symptoms of urethritis. However, chlamydial exclusion was undertaken in only 2 patients. One patient was labelled as presumptive gonorrhoea on the basis of GNDC on microscopy. Culture revealed the same Group C N. meningitidis from his partner's rectum and throat.

Fifty-five patients (82%) had rectal colonization (Table 3), including 2 with a dual infection. Only one had symptoms of proctitis, but neither chlamydia nor herpes simplex virus grew on culture.

Contact data identified 13 partners of 11 patients. All but two of these partners were colonized with meningococci, usually involving the throat. In two instances urethral colonization with meningococci in the index case was accompanied by rectal colonization with meningococci of the same serogroup in the partner. Serogrouping was performed on 39 of the 49 meningococcal isolates colonizing the rectum. The distribution of serogroups: 1 (2.5%) group A; 15 (38.4%) group B; 3 (7.7%) group C; 2 (5.1%) group Y; 1 (2.5%) group Z' and 17 (43.5%) auto/non-agglutinable was similar to that found in heterosexual patients with group B being the most prevalent serogroup.

Within each patient group the prevalence and incidence of anogenital NGN were similar at the beginning and end of the study. Values for the period 1978–80 and 1988–90 were 1.11% (8/721) and 0.98% (9/915) in homosexual men; 0.048% (6/12,346) and 0.037% (4/10,596) in heterosexual men; and 0.06% (4/6636) and 0.07% (5/7327) in women.

DISCUSSION

Unlike gonorrhoea, the prevalence and incidence of anogenital NGN have not declined during the AIDS era. Homosexual men had the highest frequency of colonization with anogenital NGN, with the rectum being colonized in 55 (82%) of 67 cases. While this
One were 2 partner's pharynx.

Patient data; be either saliva from the individual would persist in suggested with urethral N. meningitidis. This in part borne out by the contact data; 14 partners were identified, and all but 2 were colonized with N. meningitidis.

Despite the number of patients colonized with NGN, only one had an associated proctitis, which resolved without treatment. In view of these findings and those of other authors, NGN appear to have at most a limited role in producing a clinically apparent proctitis and their presence requires no immediate treatment.

Oro-genital contact ('oral sex') is another common sexual practice, yet colonization of the urethra with NGN occurred less frequently than colonization of the rectum; this suggests that NGN have difficulty in producing a lasting colonization of the urethral mucosa.

Likewise, although an aggressive cervicitis with pelvic inflammation has been described in association with the isolation of N. meningitidis these reports preceded the recognition of Chlamydia trachomatis as an important genital tract pathogen. None of our 9 women with cervical colonization had evidence of pelvic inflammation. Eight women with NGN were contacts of male patients with urethral gonorrhoea, yet only one had a gonococcal cervical infection. There may therefore be a protective effect in having an anogenital NGN, possibly through derived local immunity, the 'bacteriocins' of Volk et al. or the growth inhibitor described by Dubreuil et al.
Again the ability of NGN to cause urethritis is difficult to assess because many of the case reports were published before C. trachomatis was recognized widely as a pathogen. Even today the association between meningococci and urethritis remains difficult to investigate because chlamydial diagnostic tests are not undertaken routinely in men with urethritis. However, in the presence of an NGN, 11 (55%) of 20 heterosexual men had a urethritis, 5 of whom showed GNDC on a urethral film. Thirteen homosexual men had a urethral NGN; 7 (54%) of these had urethritis. However chlamydial exclusion was undertaken on only 7 (5 heterosexual and 2 homosexual) patients.

A literature review has provided at least 40 cases of male urethral colonization with NGN3-13,17-19,24,25, 21 of them through homosexual13 or bisexual26 contact. Urethritis was described in 31 of these patients (75%). GNDC were noted on urethral smear on 17 occasions3-8,10-13,18, and when present were always associated with urethritis in that patient. All were treated as presumptive gonorrhoea. Many of the patients3-8,11,13,18 were treated with penicillin alone and had clinical resolution of their symptoms, evidence against an associated chlamydial urethritis.

Our findings and those of published studies support the role of NGN in producing urethral infection in the case of a susceptible individual; all serogroups, including non-groupable (and hence doubtfully pathogenic) N. meningitidis, appear capable of doing this (Table 1). We therefore advocate treating urethral NGN if symptoms are present; the majority of such patients may already have been treated for either gonococcal or nongonococcal urethritis on the basis of a Gram-stained smear. Whenever possible all men with NGN should be tested for C. trachomatis and initial treatment should be directed towards chlamydia if found to be present. Treatment of a cervical isolate is more problematical as evidence for their association with PID is scant, and the urethra of the male partner is unlikely to become colonized. Indeed, the cervical isolate can disappear spontaneously within a week (unpublished observations), but the more cautious physician may wish to treat the more persisting meningococcal cervical isolates, particularly with its association with meningococcal septicaemia in 2 patients27, and a fatal meningococcal septicaemia in a neonate whose mother had asymptomatic vaginal colonization28.

Although there are a few reports3,7,10-12 where fellatio has produced a meningococcal urethritis in men our data suggests that NGN become established more readily in the rectum than in the urethra or cervix. The practice of "rimming" is well suited to spreading a primarily pharyngeal organism such as N. meningitidis to an anogenital site. The serogroups of the anogenital N. meningitidis isolates in our study are similar in pattern to those of the pharyngeal isolates from a general population29 adding support to this mode of transmission. Additional support derives from the recent report that rectal gonorrhoea can be acquired from a pharyngeal source through oro-anal contact without penetrative anal intercourse occurring30.

Acknowledgment: We would like to thank Dr A McMillan and his Consultant colleagues for permission to report their patients.

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Edited by A D Farr
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Typing of *Neisseria gonorrhoeae* by auxotype, serovar and lectin agglutination

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(Accepted 5 January 1993)

Abstract: A total of 267 strains of *Neisseria gonorrhoeae*, comprising 129 serogroup IA and 138 serogroup IB, isolated in Edinburgh over a two-year period were analysed to assess the discrimination given by three typing methods: auxotyping; serotyping using the Genetic Systems (GS) and Pharmacia (PH) monoclonal antibody panels; and lectin agglutination. Each typing system was assessed individually and in combination. Serotyping subdivided the strains into 14 GS and 18PI4 serovars. Auxotyping (Aux) yielded 11 separate auxotypes while lectin agglutination yielded 22 different reaction patterns (LP). The standard auxotype/GS serovar (A/S) classification system yielded 37 classes. Lectin agglutination allowed further subdivision of the main A/S classes. AEIU/IA-2 strains, which accounted for 70% of IA strains, yielded nine different lectin patterns (A/S/LP classes). Likewise, lectin agglutination allowed subdivision of the main IB A/S classes. NR/IB-1, NR/IB-2 and NR/IB-3, which accounted for 28%, 38% and 20% respectively of the IB strains, yielded 7, 7 and 6 A/S/LP classes respectively. It was concluded that lectin agglutination is a useful adjunct to the standard A/S classification system for studying the micro-epidemiology of gonococcal infection.

Key words: Epidemiologic methods. Neisseria gonorrhoeae. Serotyping.

Introduction

Epidemiological typing is important in the control of infectious diseases. As ‘contact tracing’ is part of the overall strategy for the control of gonorrhoea, typing of isolates may be particularly valuable. Gonococcal typing allows the recognition of new strains; detection of strains associated with antibiotic resistance; differentiation between reinfection and treatment failure; and comparisons between strains in medico-legal cases.1

Distinct stable nutritional profiles of gonococci grown on chemically defined media containing or lacking selected compounds is termed auxotyping, and was first described in 1973.2,3 Since then, auxotyping has been used for the epidemiological study of *Neisseria gonorrhoeae*.4–10 Although only a limited number of auxotypes are encountered, the gonococcus has been shown to be heterogeneous with respect to distribution and prevalence of auxotypes.7 Temporal changes in auxotype pattern have also been observed.11,12 Auxotyping on its own, however, is of limited value.1,7

Serological classification of *Neisseria gonorrhoeae* using monoclonal antibodies raised to epitopes on the gonococcal outer membrane protein I is a powerful tool in studying gonococcal epidemiology.1,13–15 Monitoring of serovar patterns from geographical areas around the world has shown marked geographical variations which may allow the origin of outbreaks to
be studied. A combination of auxotype and GS serovar, the so-called auxotype-serovar (A/S) classification, greatly enhances the discrimination given when either method is used alone. Nevertheless there are limitations in the A/S system, and further sub-division would be helpful.

Lectins are natural proteins of non-immune origin that react with sugar residues. They have been used in the study of cell surface carbohydrates of bacteria and can detect inter-strain variation in cell wall carbohydrate composition. The interaction between lectins and members of the genus *Neisseria* has been well documented in a number of studies, and evaluated for identification and epidemiological typing.

In this study we analyse the discrimination given by auxotyping, serotyping and lectin agglutination used singly and in combination, to assess their potential value in the micro-epidemiology of gonorrhoea.

**Materials and methods**

**Bacterial strains**

A total of 267 clinical isolates of *N. gonorrhoeae* isolated from patients attending the Genitourinary Medicine Unit, Edinburgh Royal Infirmary during 1988 and 1989 were included in the study. The isolates were identified as *N. gonorrhoeae* by the rapid carbohydrate utilisation test (RCUT) and the Phadebact Monoclonal GC test: 129 isolates belonged to serogroup IA and 138 to serogroup IB.

**Serotyping**

Serotyping was performed as previously described using two different systems: the Pharmacia (PH) panel of monoclonal antibodies and the nomenclature described by Bygdeman; and the Genetic Systems (GS) panel of monoclonal antibodies and the nomenclature described by Knapp et al.

The PH antibodies with corresponding control strains were supplied by Dr S. Bygdeman, Karolinska Institute, Sweden; and the GS antibodies with corresponding control strains by Dr C. Ison, St Mary's Hospital, London.

**Auxotyping**

The nutritional growth profiles of the isolates were determined using the defined media and method of Copley and Egglestone. Strains were classified according to their requirement for proline (P), arginine (A), hypoxanthine (H) and uracil (U). Citrulline was substituted as an alternative to arginine and the requirement for citrulline (C) noted. Non-requiring strains were coded NR.

**Lectin agglutination**

Sixteen animal and plant lectins obtained from Sigma Ltd were used at the concentrations described previously. Gonococcal suspensions were made from overnight cultures and boiled for 10 min. Lectin agglutination tests were performed as described previously and results expressed as reaction patterns: a total of 22 lectin patterns (LPs) were given by nine of the lectins, as shown in Table 1.

**Results**

The discrimination given by each method and combination(s) of methods is given in Table 2. The degree of discrimination tended to increase in accordance with the number of methods employed,
and ranged from 11 to 22 classes for a single method, 29 to 60 for two methods, 55 to 85 for three methods, and 99 for all four methods.

Axotyping and lectin agglutination were valuable additions to serotyping with respect to subdivision of both IA and IB isolates (as illustrated in Tables 3 and 4). The data shown in these tables are based on subdivision of GS serovars, as this forms the basis of the widely used A/S classification system. Axotyping alone is of limited value as AHU strains accounted for 72% (93/129) of IA isolates. Serotyping alone is also of limited value as serovar IA-2 accounted for 86% (111/129) of IA isolates. Serovar IA-2 could, however, be subdivided into six A/S classes and 18 A/S/LP classes (Table 3). The remaining three IA serovars could be divided into eight A/S classes and 12 A/S/LP classes.

Likewise, axotyping and lectin agglutination were valuable in sub-dividing the common IB serovars (Table 4). Based on axotyping 64.5% (89/138) strains were NR, while serogroups IB-1, IB-2 and IB-3 accounted for 28.3%, 37.7% and 19.6% of the 138 IB isolates respectively. Serovar IB-1 could be divided into five A/S classes and 14 A/S/LP classes, serovar IB-2 into seven A/S classes and 15 A/S/LP classes, and serovar IB-3 into three A/S classes and nine A/S/LP classes.

Table 2. Discrimination given by each method and combination(s) of methods

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*The same auxotype or lectin pattern can occur in IA and IB isolates. GS=Genetic Systems serovar; PH=Pharmacia serovar; Aux=Auxotype; LP=Lectin pattern.

Discussion

Serotyping is the simplest and most widely used typing system for the epidemiological study of gonococcal infection. For many years different panels of serotyping antibodies have been used. More recently the GS panel and nomenclature advocated by Knapp et al. has been adopted as the standard primary typing panel. The use of a standard panel is valuable in allowing core comparisons, as in macro-epidemiological studies between different geographical areas. For more detailed epidemiological work auxotyping and GS serotyping have been combined (the A/S classification system) and used widely.

As similar discrimination was obtained in this study when auxotyping was combined with either the PH serotyping panel (39 A/S classes) or the standard GS panel (37 A/S classes), there is no reason to change the serotyping panel in the standard dual classification. The combination of auxotyping and lectin agglutination, however, produces even greater discrimination (58 classes). However, because so much basic data have already been accumulated with regard to the A/S system it is likely to remain the accepted dual classification system. For example, in a worldwide study of 1433 gonococcal isolates, Knapp et al. reported 107 A/S classes, while in Seattle 57 A/S classes were identified among 425 gonococcal isolates. In Europe, a two-year study of women in Heidelberg, Germany, found gonococcal isolates were distributed across 56 A/S classes. The finding of 37 A/S classes in the 267 isolates from Edinburgh suggests that we are encountering a good cross-section of the global gonococcal population.

There are, however, certain correlations between auxotype and serovar and these can limit the value of A/S classification when applied to detailed epidemiological studies. For example, in our study AHU/IA-2 was the predominant class accounting for 33.7% of the total isolates and 96.8% of AHU strains. A similar correlation was recognised in the worldwide study of Knapp et al., who reported that serovar IA-1/IA-2 accounted for 98% of AHU strains. Correlation between serovar AHU and IA-2 was also found in studies from Stockholm, London and Bristol.

There are, however, geographical differences in the prevalence of AHU/IA-2 strains. AHU/IA-2 accounted for less than 10% of 425 isolates from Seattle and 11.9% of the 630 isolates from two
Table 3. GS serovar, auxotype and lectin pattern for 129 serogroup IA isolates

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Table 4. GS serovar, auxotype and lectin pattern for 138 serogroup IB isolates

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London hospitals. The higher prevalence 33.7% (90/267) of AHU/IA-2 strains encountered in Edinburgh may be due to the fact that we routinely test all isolates rather than a sample of the total population.

The strong correlation between AHU and IA-2 makes sub-division by lectin agglutination particularly valuable. As shown in Table 3, AHU/IA-2 strains could be divided into nine LP classes. AHU/IA-2/LP2 accounted for 71% of the isolates and probably represented the endemic core strain population: the remaining eight LP classes accounted for 10%, 8%, 4%, 2% and 1% (x4) respectively, and most likely represented the micro-epidemic strain.

The correlation between serovar and the MR auxotype which was found in 64.5% of our strains also limits the value of A/S classification. The MR auxotype predominated within each of the common IB serovars, representing 74.4% (29/39) of IB-1, 51.9% (27/52) of IB-2 and 70.3% (19/27) of IB-3 isolates respectively. In terms of the total isolates NR/IB-1, NR/IB-2 and NR/IB-3 represented 10.9% (29/267), 10% (27/267) and 7.1% (19/267), respectively. A single A/S class such as NR/IB-3 may, however, account for a much higher proportion of the total population: in a study of 179 prostitutes in Heidelberg NR/IB-3 strains predominated, accounting for 24% (43/179) of the total isolates.

Using lectin agglutination (Table 4) the 29 NR/IB-1 strains could be divided into seven LP classes: NR/IB-1/LP1 31%; LP2 17%; LP4 10%; LP6 14%; LP8 14%; LP11 10%; and LP17 3%. Similarly, the 27 NR/IB-2 strains could be divided into six LP classes with NR/IB-2/LP2 accounting for 63% of the strains, while the 19 NR/IB-3 strains could also be divided into six LP classes with NR/IB-3/LP1 accounting for 42% of the strains. Again, these subdivisions may represent core and micro-epidemic or transient strain populations.

The finding that penicillin sensitivity of IB-1 strains demonstrates bimodal distribution suggests this group is heterogeneous and further sub-division is required. Sub-populations also exist within serovar IB-2. Although IB-2 strains have been associated with homosexually-acquired infection, sub-division on the basis of PH serotyping has shown that these correlations are significantly stronger with certain sub-populations of IB-2 strains. Serovar IB-6 also shows strong correlation with homosexually-acquired infection, and the finding that 80% (8/10) of strains are NR/LP1 supports the view that this represents a core strain within the local homosexual population.

In conclusion, we advocate that for detailed microbiological studies, lectin agglutination should be used to supplement the A/S system. To maximise the benefit that can be derived from this approach the adoption of a standard panel of lectins is essential. PH serotyping provides very good discrimination with regard to IB strains, and could be added as an additional method for studying gonococcal populations that have a very high proportion of IB strains.

References


10. Vazquez F, Palacio V, Vazquez JA et al. Gonorrhoea in women prostitutes: clinical data and auxotypes,
32 Sigma Chemical Company Limited, Fancy Road, Poole, Dorset BH17 7NH, England, UK.
ARTICLE


H. Young and A. Moyes

Gonococci were serotyped and tested for antibiotic susceptibility as described previously (Weekly Report 90/29) and designated by a numerical nomenclature based on typing with a standard panel of monoclonal antibody typing reagents.

Isolates from a total of 612 episodes of infection were examined compared with a total of 558 in 1991 (Table 1). During the same period the number of cases of gonorrhoea seen at Genitourinary Medicine Clinics decreased from 856 in 1991 to 616 in 1992 (Information and Statistics Division Scottish Health Service Common Services Agency).

As shown in Table 1 the proportion of serogroup IB isolates (52%) is significantly lower (P<0.001) than the 66% found in 1991. This difference is due to the large proportion of IA isolates (78%) in Grampian (isolates from Grampian were not tested in 1991). The proportion of IB isolates in Grampian (22%) is atypical of the other larger centres where IB accounts for around 70% of all isolates.

The number and distribution of protein IA serovars are given in Table 2. Although Grampian produced a large number of IA isolates they were extremely homogenous with IA-2 accounting for 98% of all isolates. This homogeneity tends to suggest endemic transmission of a successful "core strain" rather than frequent importation of new strains from other localities. The new serovar IA-25 discovered in Glasgow in 1991 (two isolates) was found in three patients in Lothian in late May/early June 1992. Serovar IA-21 was found only in Glasgow.

Fifteen protein IB serovars were found among the serogroup IB isolates in 1992 (Table 3) compared with 13 serovars in 1991. The serovars IB-10, IB-16 and IB-18 were found only in 1992 and accounted for 2.8% of the isolates. Serovar IB-15 was not found in 1992 but accounted for 1.1% of isolates in 1991. As in previous years IB-2 was the most common isolate. There was however a significant decrease (P<0.01) in IB-2 isolates between 1991 (45.3%) and 1992 (36%). The decrease in IB-3 isolates from 21.3% in 1991 to 7% in 1992 was also highly significant (P<0.001) as was the increase in IB-1 isolates from 8.6% in 1991 to 26% in 1992. The decrease in IB-3 isolates was particularly marked in Tayside - from 61.4% in 1991 to 14% in 1992 (P<0.001). As in 1990 and 1991 serovar IB-17 was isolated only in Greater Glasgow.

Again there is a highly significant difference in the penicillin susceptibility of IA and IB isolates (Tables 4 and 5): excluding penicillinase-producing Neisseria gonorrhoeae (PPNG) 88.5% of IA isolates had a minimum inhibitory concentration (MIC) to penicillin ≤0.015 mg/L compared with 11.8% of IB isolates (P<0.001). The corresponding values for 1991 of 96% and 14.7% are not significantly different from the 1992 results.

A total of 22 (7.2%) non-PPNG IB isolates were chromosomally mediated resistant N. gonorrhoeae (CMRNG) (MIC ≥1.0 mg/L) compared with 3.4% in 1991 and 1.3% in 1990. The 1992 level is significantly higher (P<0.05) than in 1991 and approaches the level of 10.6% (100/940) CMRNG found when sampling isolates from St.Mary's Hospital London between 1984 and 1988. The increase in CMRNG between 1991 and 1992 was associated with strains belonging to serovar IB-1 (0% to 2.6%) and serovar IB-2 (0.3% to 1.6%). The incidence of CMRNG has not increased amongst isolates in Avon monitored between 1988 and 1991.

The susceptibility of isolates to cefuroxime, tetracycline, erythromycin and ciprofloxacin is summarised in Table 6. For each antibiotic there was a decrease between 1991 and 1992 in the number of isolates in the most susceptible category. Although spectinomycin is not shown in this table only one isolate had an MIC >16 mg/L: this was a test-of-cure culture for a IB-7 PPNG first isolated in December 1991.

H. Young and A. Moyes, Scottish Neisseria gonorrhoeae Reference Laboratory, Department of Medical Microbiology Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG
The serovar and geographical distribution of PPNG strains is given in Table 7. Overall 3.9% (24/612) of isolates were PPNG; this level is identical to that in 1991 (22/558). In 1991 however only 1 of the 22 isolates was of serogroup IA whereas 12 of the 24 PPNG isolates in 1992 were IA. Full details of plasmid analysis and auxotype will be reported separately. Data provided by Dr Janina Harvey (Consultant Venereologist, Falkirk District Royal Infirmary) concerning a small cluster of PPNG infection in Central Region suggested that some PPNG strains may lose their resistance plasmids spontaneously. On 17 August 1992 a IB-1 PPNG was isolated from a female patient and on 2 September a similar strain was isolated from a male contact: both isolates had a ciprofloxacin MIC of 0.06mg/L and were non-requiring (NR) on auxotyping. On 10 September a IB-1 non-PPNG with ciprofloxacin MIC of 0.06mg/L and NR auxotype was isolated from a male while a IB-1 PPNG with a ciprofloxacin MIC of 0.06mg/L and NR auxotype was isolated from a female contact on 11 September. On receipt in the Reference Laboratory the latter isolate gave a weak reaction for penicillinase using the chromogenic cephalosporin test while the MIC for penicillin was 0.06mg/L. All organisms obtained on subculture were penicillin sensitive but had a ciprofloxacin MIC of 0.06mg/L. A repeat culture from the same patient received on 17 September was IB-1 non-PPNG with a ciprofloxacin MIC of 0.06mg/L and NR auxotype. Others have reported that PPNG strains may lose the β-lactamase encoding plasmid and it is important to take this into account when contact tracing is undertaken during a PPNG outbreak.

Seven ano-genital isolates of Gram negative diplococci (GNDC) proved to be non-gonococcal neisseriae (NGN). These included five isolates of *N.meningitidis* (two male rectal isolates, one female rectal isolate, one male urethral isolate, and a genital isolate - sex of patient not given) and two isolates of *Moraxella* (Brachmella) *catarrhalis* (one male urethral and one female rectal isolate). Although the absolute prevalence of ano-genital NGN has not increased in recent years owing to the decrease in gonorrhoea, NGN have increased as a proportion of the total ano-genital GNDC. This may decrease the positive predictive value of certain identification methods.

References:


Acknowledgements:

We thank our numerous bacteriological and clinical colleagues for their help and support in making this surveillance possible. Any additional laboratories that would like to participate in the surveillance programme should contact Dr H Young *Neisseria gonorrhoeae* Reference Laboratory (Scotland), Department of Medical Microbiology, Edinburgh University Medical School, Edinburgh EH8 9AG (Tel 031-650-3143).

Special thanks are extended to Mrs Joan McElhinney for careful record keeping and preparation of the manuscript.
TABLE 1

Source of specimens, prevalence and geographical distribution of IA and IB serotypes (1992)

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<th>Number IB</th>
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<td>7(87%)</td>
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<td>10(53%)</td>
<td>9(47%)</td>
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<td>13(59%)</td>
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<td>DG/DMF: ROY</td>
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<td>7(64%)</td>
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<td>FG/FF: ARL</td>
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<td>9(90%)</td>
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Footnote for Table 1

Health Board Area and Laboratories submitting specimens.

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### TABLE 2

Prevalence and geographical distribution of protein IA serovars (1992)

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<td>3(1%)</td>
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<td>4(100)</td>
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<td>7(100)</td>
<td>109*[100)</td>
<td>22(100)</td>
<td>23(100)</td>
<td>8(100)</td>
<td>2(100)</td>
<td>31(100)</td>
<td>28(100)</td>
<td>289*[100)</td>
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(a) 1 IA-2 not included (GLA:VIC)
(b) 2 Serogroup IA isolates not available for typing (GR)
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<tr>
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<th>AC</th>
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<th>DG</th>
<th>FF</th>
<th>FV</th>
<th>GR</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>HG</th>
<th>LN</th>
<th>LO</th>
<th>TY</th>
<th>TOTAL</th>
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<td>3(23%)</td>
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<td>13(36%)</td>
<td>9(36%)</td>
<td>6(19%)</td>
<td>8(15%)</td>
<td>12(23%)</td>
<td>1</td>
<td>1</td>
<td>21(32%)</td>
<td>2(10%)</td>
<td>82(26%)</td>
</tr>
<tr>
<td>IB-2</td>
<td>5(28%)</td>
<td>9(69%)</td>
<td>5</td>
<td>18(50%)</td>
<td>3(8%)</td>
<td>19(61%)</td>
<td>13(25%)</td>
<td>5(10%)</td>
<td>2</td>
<td>0</td>
<td>25(38%)</td>
<td>10(48%)</td>
<td>114(36%)</td>
</tr>
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<td>2(6%)</td>
<td>1(3%)</td>
<td>6(12%)</td>
<td>5(10%)</td>
<td>0</td>
<td>0</td>
<td>4(6%)</td>
<td>3(14%)</td>
</tr>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4(6%)</td>
<td>0</td>
</tr>
<tr>
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<td>1(3%)</td>
<td>1(3%)</td>
<td>9(17%)</td>
<td>10(19%)</td>
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<td>0</td>
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<td>2(10%)</td>
</tr>
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<td>0</td>
<td>1(3%)</td>
<td>4(11%)</td>
<td>2(7%)</td>
<td>7(14%)</td>
<td>3(6%)</td>
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<td>0</td>
<td>6(9%)</td>
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<td>0</td>
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<td>1(3%)</td>
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<td>0</td>
<td>0</td>
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<td>1(2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
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<td>0</td>
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<td>13(4%)</td>
</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>1(2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(2%)</td>
<td>1(5%)</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(2%)</td>
<td>0</td>
</tr>
<tr>
<td>IB-31</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>2(4%)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>1(3%)</td>
<td>1(2%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(2%)</td>
<td>2(10%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>18(100)</td>
<td>13(100)</td>
<td>7(100)</td>
<td>36(100)</td>
<td>19(100)</td>
<td>31(100)</td>
<td>52(100)</td>
<td>52(100)</td>
<td>3(100)</td>
<td>1(100)</td>
<td>66(100)</td>
<td>21(100)</td>
<td>319*(100)</td>
</tr>
</tbody>
</table>

* 1 IB-1 not included (GLA:VIC)
### TABLE 4
Penicillin susceptibility of IA Serovars (1992)

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<tr>
<th>Serovar</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1</th>
<th>&gt;1.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
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<td>IA-2</td>
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<td>0</td>
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<td>244</td>
</tr>
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<td>0</td>
<td>3</td>
<td>3</td>
</tr>
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<td>0</td>
<td>6</td>
<td>0</td>
<td>8</td>
<td>15</td>
</tr>
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<td>0</td>
<td>0</td>
<td>11</td>
<td>11</td>
</tr>
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<td>0</td>
<td>0</td>
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<td>1</td>
<td>1</td>
</tr>
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<td>0</td>
<td>0</td>
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<td>13</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td><strong>0</strong></td>
<td><strong>7</strong></td>
<td><strong>0</strong></td>
<td><strong>12</strong></td>
<td><strong>290</strong></td>
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<td>168</td>
<td>15</td>
<td>0</td>
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<td>1</td>
<td>1</td>
<td>186</td>
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</table>

(a) PPNG isolates
* One isolate not available for MIC:
* One isolate not serotyped not included

### TABLE 5
Penicillin susceptibility of IB Serovars (1992)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1</th>
<th>&gt;1.00</th>
<th>Total</th>
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</thead>
<tbody>
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<td>13</td>
<td>31</td>
<td>6</td>
<td>7</td>
<td>83</td>
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<td>9</td>
<td>57</td>
<td>5</td>
<td>2</td>
<td>114</td>
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<td>0</td>
<td>23</td>
</tr>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>IB-6</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>12</td>
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<td>0</td>
<td>26</td>
</tr>
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<td>IB-7</td>
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<td>0</td>
<td>21</td>
<td>5</td>
<td>1</td>
<td>27</td>
</tr>
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<td>IB-8</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
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<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
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<td>0</td>
<td>0</td>
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</tr>
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<td>1</td>
<td>0</td>
<td>3</td>
</tr>
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<td>1</td>
<td>0</td>
<td>2</td>
</tr>
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<td>5</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
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<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>46</strong></td>
<td><strong>67</strong></td>
<td><strong>137</strong></td>
<td><strong>20</strong></td>
<td><strong>14</strong></td>
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<td>90</td>
<td>79</td>
<td>116</td>
<td>12</td>
<td>21</td>
<td>369</td>
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</tbody>
</table>

(a) 12/14 PPNG; two IB-1 isolates chromosomally resistant
(b) All PPNG
TABLE 6

Antibiotic susceptibility of serotype IA and IB isolates (1992)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype</th>
<th>Cumulative percentage MIC (mg/L)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td></td>
<td></td>
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<tr>
<td>IA</td>
<td>≤0.015</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>85%</td>
<td>93%</td>
<td>93%</td>
</tr>
<tr>
<td>IB</td>
<td>11%</td>
<td>26%</td>
<td>47%</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IA</td>
<td>≤0.02</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>93%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>IB</td>
<td>11%</td>
<td>56%</td>
<td>94%</td>
</tr>
<tr>
<td>Tetracycline</td>
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</tr>
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<td>IA</td>
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<td>0.5</td>
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</tr>
<tr>
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<td>1%</td>
<td>92%</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>1%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
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<td></td>
<td></td>
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<tr>
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<td>≤0.125</td>
<td>0.5</td>
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<td>29%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>IB</td>
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<td>56%</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>5%</td>
<td>63%</td>
<td>100%</td>
</tr>
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<td>5%</td>
<td>66%</td>
<td>96%</td>
</tr>
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</table>

(a) 12 isolates MIC > 1.0 mg/L; all PPNG
(b) 14 isolates MIC > 1.0 mg/L; 12 PPNG
### TABLE 7

Serovar and geographical distribution of PPNG isolates (1992)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AC</th>
<th>AA</th>
<th>DG</th>
<th>FF</th>
<th>FV</th>
<th>GR</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>HG</th>
<th>LN</th>
<th>LO</th>
<th>TY</th>
<th>Total</th>
</tr>
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<tbody>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>3/3</td>
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<td>0/6</td>
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<td>1/21</td>
<td>0/2</td>
<td>5/82</td>
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<td></td>
</tr>
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<td>0/9</td>
<td>0/5</td>
<td>0/18</td>
<td>0/3</td>
<td>2/19</td>
<td>0/13</td>
<td>0/5</td>
<td>0/2</td>
<td>0</td>
<td>0/10</td>
<td>2/114</td>
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<td>4/7</td>
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<td>0/1</td>
<td>0/4</td>
<td>0/2</td>
<td>1/7</td>
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<td>0/6</td>
<td>0/1</td>
<td>1/27</td>
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<td>Others</td>
<td>0/16</td>
<td>0/10</td>
<td>0/6</td>
<td>0/38</td>
<td>0/10</td>
<td>0/113</td>
<td>0/41</td>
<td>0/47</td>
<td>0/8</td>
<td>0/2</td>
<td>0/36</td>
<td>0/34</td>
<td>0/363*</td>
</tr>
<tr>
<td>Total</td>
<td>0/30</td>
<td>0/22</td>
<td>0/11</td>
<td>0/70</td>
<td>2/26</td>
<td>3/142</td>
<td>5/74</td>
<td>9/75</td>
<td>0/11</td>
<td>0/3</td>
<td>3/97</td>
<td>2/49</td>
<td>24/612</td>
</tr>
</tbody>
</table>

- **1991**: 8/35 0/2 1/86 0/127 5/78 0/3 4/154 4/73 22/558

* Two non-PPNG isolates (GLA:VIC) not included in sub-totals

... not listed in 1991
SEROTYPE PATTERNS OF GONOCOCCAL INFECTION IN CONTACT PAIRS


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Key words: Gonorrhoea - Serotyping - Serovar - Monoclonal antibodies - Sexual contacts

The use of gonococcal serovars in studying the epidemiology of Neisseria gonorrhoeae is well established. Most studies assume that the isolated serovar remains stable in vivo indefinitely. This study was designed to observe the correlation between serovars isolated in patients naming each other as sexual contacts. The overall rate of discordant transmission episodes was 12% (26/220). There were however significantly more discordant transmission episodes for partners of patients infected with IB serovars than IA serovars: 19% (23/124) versus 3% (3/96) - p < 0.01. The overall prevalence of serogroup IB isolates although significantly higher at 53% (p < 0.01) was thought insufficient to account for the correlation between discordant pairs and serogroup IB infection. Reasons considered for the discrepancies in contact pairs included: problems of partner reporting involving inaccurate or incomplete information; technical problems with reagents; culture induced phenotypic variation in antigenic expression or differences in the in vitro recognition of epitopes; and antigenic differences resulting from genetic mutation within the Protein I gene. It was concluded that carefully planned and controlled prospective studies involving duplicate parallel testing of isolates from patients and their well documented partners are needed to assess the extent to which these various factors contribute to discordancies in serovars isolated from contact pairs. Serotyping should be combined with other methods such as auxotyping for detailed microepidemiological studies involving partner notification.

INTRODUCTION

Gonococcal serotyping is a well established technique to subdivide Neisseria gonorrhoeae into serovars using monoclonal antibodies directed at epitopes on the outer membrane of the organism (14). This technique is simpler, cheaper and less time consuming than characterising gonococci on the basis of nutrient requirements i.e. auxotyping. The epidemiological applications include identifying associations between sexual orientation and serovar (4, 18) and between site of infection and serovar (5). Certain serovars (1B-5/7 (8, 16) are associated with resistance to antibiotics and the spread of these strains through a community can be charted using serotyping (7, 8, 16). In addition different geographical areas have marked variation in the dominant serovars isolated (17), which themselves vary with time (9, 17).

Of crucial importance in using serovars as an epidemiological tool is the stability of the serovar within an individual and his or her sexual partner over time. A certain degree of genetic variation must occur in order to have produced the different serotypes but most studies assume that the serovar is stable indefinitely. The aim of this study was to assess the concordance of serovars between patients who named each other as sexual contacts.

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METHODS

All patients with a diagnosis of gonorrhoea who presented to the Department of Genitourinary Medicine (GUM), Edinburgh Royal Infirmary, between January 1987 and December 1989 were included in the study. The diagnosis of gonorrhoea was made on the basis of culture of N. gonorrhoeae on modified New York City culture medium from the urethra, rectum, endocervix and/or throat. All male patients attending the Department had a single urethral swab taken whilst female patients had urethral, rectal and endocervical swabs cultured on two separate occasions in order to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea and when the history indicated that this site had been placed at risk.

Gonococcal isolates were identified on the basis of biochemical and immunological tests and serotyping was performed using the Swedish panel of Genetic Systems (GS) reagents and the Pharmacia (Ph) reagents as has been described previously (18). The reproducibility of serotyping was validated by testing multiple isolates from patients with infection at more than one site. Serovar patterns involving weak reaction were always checked and scored by the same technologist throughout the study period.

All sexual contacts named by the patient were contacted initially by the patient with support from trained health advisors who, when necessary, followed up the contact by phone, letter, or visit to the contact’s home address. Serovars from patients who attended the GUM clinic and had named each other as sexual contacts were compared retrospectively.

Each contact of an infected index case was taken as a separate transmission episode. Discordant serovar pairs were further analysed to determine which epitopes differed between infected contacts.

Statistical analysis was performed using the Epi-Info PC statistical package. The chi-square test was used to compare the number of discordant transmission pairs associated with serogroup IA and IB isolates. The time frame for clinic attendance of sexual contacts for concordant and discordant pairs was compared by the Mann-Whitney confidence interval and test.

RESULTS

A total of 932 gonococcal infections were identified over the 3 year study period. These consisted of 194 matching transmission pairs and 27 discordant transmission pairs. The mean time between the patient and contact attending the clinic was 5 days for concordant pairs and 7 days for discordant pairs: this is not a statistically significant difference (Mann-Whitney confidence interval and test - 95% confidence interval for 2 day difference - 1 to 4 days; P = 0.16). In 490 (53%) cases no contact could be traced. On one occasion the partner of a patient with a serogroup IA isolate (Aedgk/Arost) was infected with a serogroup IB isolate (Bacejk/Brypust). This discordant transmission episode was excluded from further analysis as it was considered to indicate infection from an additional source. The overall rate of discordant transmission episodes was 12% (26/220). There were however considerably more discordant transmission episodes for partners of patients infected with IB serovars than IA serovars: 19% (23/124) versus 3% (3/96) (P < 0.01). The overall prevalence of serogroup IB isolates was also significantly higher at 53% (P < 0.01).

The major serovars and epitope differences for the discordant serovar pairs are shown in Table 1. With the GS panel of monoclonal antibodies against protein IB differences between partners were found most often with “k”, “c” and “e” while with the Ph panel single antibody differences tended to be associated with “p” and “r”. In addition the epitope combination “yus” was lost or gained on four occasions. In contrast to the discrepancies in contact pairs, multiple isolates (cervix and rectum 89; cervix and throat 23; rectum and throat 6; urethra and throat 36; cervix, rectum and throat 23; urethra, rectum and throat 3) from all but one of 180 patients were identical. The one difference, urethra (Aedgk/Arost) and throat (Bacejk/Brypust) was considered to indicate dual infection.

TABLE 1. - Discordant Transmission Episodes.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serovar isolated from:</th>
<th>Contact</th>
<th>Epitope differences</th>
<th>GS panel</th>
<th>Ph panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedgk/Arost</td>
<td>Aedgk/Arost</td>
<td>-ih</td>
<td>Aedgk/Arost</td>
<td>-ikh</td>
<td>-o</td>
</tr>
<tr>
<td></td>
<td>Aedgk/Arost</td>
<td>-ik</td>
<td>Aedgk/Arost</td>
<td>-ikh</td>
<td>-o</td>
</tr>
<tr>
<td>Bacejk/Brypust</td>
<td>Bacejk/Brypust</td>
<td>-k</td>
<td>Bacejk/Brypust</td>
<td>-k</td>
<td>-s</td>
</tr>
<tr>
<td></td>
<td>Bacejk/Brypust</td>
<td>-k</td>
<td>Bacejk/Brypust</td>
<td>-j</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Bacejk/Brypust</td>
<td>-c</td>
<td>Bacejk/Brypust</td>
<td>-ye, +o</td>
<td>-y, +o</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>-r</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>-r</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>-r</td>
</tr>
<tr>
<td>Bajk/Bropt</td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>+kus</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>-p</td>
<td>Bajk/Bropt</td>
<td>-p</td>
<td>-r</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>Bajk/Bropt</td>
<td>+c</td>
<td>+r</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>-p</td>
<td>Bajk/Bropt</td>
<td>-p</td>
<td>-r</td>
</tr>
<tr>
<td></td>
<td>Bajk/Bropt</td>
<td>-r</td>
<td>Bajk/Bropt</td>
<td>-r</td>
<td>-r</td>
</tr>
</tbody>
</table>

+ epitope added; - epitope lost.
DISCUSSION

An identifying marker that remains stable over time is essential to classify gonococci for epidemiological study. Mutation of the gene which codes for the major outer membrane protein (Protein I), which forms the basis for serotyping, must occur in order to have produced the variety of serovars which are currently observed. The factors controlling such mutation are unknown but serovar specific humoral immunity may have a role in the control of infection (2, 3, 13) and therefore may also act to encourage mutation to different antigenic serotypes through natural selection. This mechanism is more obviously important in the antigenic variation observed in gonococcal pill structure (11).

The level of discordant serovars between contact pairs (3% for serogroup IA and 19% for serogroup IB) suggests that serotyping alone may be of limited value in contact tracing and confirmation of transmission. The reasons for serovar discrepancies in contact pairs could be due to one or more of the following: problems of partner reporting resulting from inaccurate or incomplete information provided by patients; technical problems such as inconsistency in individual reagents and interpretation of weak reactions; culture induced phenotypic variation in antigenic expression or differences in the in vitro recognition of epitopes using available reagents; and antigenic differences resulting from genetic mutation within the Protein I gene. The extent to which each of these factors contributes to discrepancies is difficult to assess but all may be involved to some extent.

It is accepted that patients will not always provide complete information with respect to all of their partners. The patient with a serogroup IA infection whose named partner had a serogroup IB infection strongly suggests that an additional partner was involved. The fact that this did not occur more often suggests either that the overall level of information provided by patients is fairly accurate or that patients infected with a particular serogroup tend to mix preferentially with a pool of individuals who are infected with isolates of the same serogroup. The higher percentage of IB infections combined with the greater variety of IB serovars (12) would mean that the effect of undisclosed partners would result in more discordant transmission pairs being noted in the case of IB infections. The fact that approximately 85% of IA isolated belong to serovar Aedgkii/Arost would tend to minimize the likelihood of detecting additional partners if the undisclosed partner was also infected with a serogroup IA isolate.

A Swedish study (1) involving a smaller number of sexual contact pairs reported a discordant rate of 5% (4/84). In one of the pairs there was a difference in serogroup between the two partners while additional sexual partners were known for the remaining three pairs (all serogroup IB isolates). Isolates from only one of the four pairs differed by a single epitope. The four pairs in the Table involving the loss or gain of the multiple epitopes "yus" are associated with the most prevalent IB serovars in this locality (12) and these discrepancies may well be due to undisclosed partners. If these four pairs are excluded the discordant rate for IB serovars is reduced from 19% to 15%.

It is considered that single epitope differences are most likely to be due to factors such as reagent inconsistencies, antigen expression or recognition or possibly mutational change. As shown Table certain epitopes are more likely to differ between partners than others - "k", "c" and "e" in the GS panel and "p" and "r" in the Ph panel. In a previous study (12) where we tested gonococcal isolates with two different GS panels (the Swedish and American panels) we found that "k" and "c" were discordant more than other epitopes although the level of discrepancy for the commonly isolated serovars was only 0.7%. The reagent reactive with epitope "c" has been reported to produce variable reactions due to a tendency to precipitate during the coagglutination reaction (6). Although we did not perform blind duplicate testing of all isolates in the present study the discordant results from testing multiple isolates in the same patient suggests that poor reproducibility is unlikely to account for the major portion of the observed discrepancies.

The majority of discrepancies in contact pairs may therefore be due to changes occurring in the particular serovar during the period between testing the isolate from the patient and his/her partner. However, the time period for contacts attending the clinic was not significantly different between matching and discordant transmission pairs. Whether these differences are due to changes in antigen expression or recognition induced by the host or to actual mutational changes is impossible to tell from the present study. Sandstrom et al. (15) proposed a model of antigenic drift in gonococci based on proposed epitope changes in observed serovars. Comparison of this model with our results for protein IB serovars reveals some correlation with many predicted mutations being observed in the serovar pairs i.e. BacekJ/Bryust → BacekJ/Bryust; BajkJ/Bropt → BajkJ/Bropt; BajkJ/Bropt → BajkJ/Bropt; BajkJ/Bropt → BajkJ/Bropt.

It is also possible that the same epitopes may be the most likely to exhibit culture induced changes in expression/recognition.

In conclusion we have demonstrated that there is an appreciable rate of discordance in the serovars isolated from patients and their named contacts. The reasons for these discrepancies can not be adequately defined from this study.

Clearly there is a need for carefully planned and controlled prospective studies involving duplicate parallel testing of isolates from patients and their well documented contacts.

In the meantime our results reinforce the view that serotyping should be combined with other methods (e.g. auxotyping) for microepidemiological studies such as partner identification (10).
Acknowledgement

We are grateful to Alison Curry for her meticulous work in helping to collect the data.

REFERENCES


Comparative Evaluation of AccuProbe Culture Identification Test for Neisseria gonorrhoeae and Other Rapid Methods

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Received 19 January 1993/Accepted 4 May 1993

The AccuProbe chemiluminescent culture identification test for Neisseria gonorrhoeae (Gen-Probe Inc., San Diego, Calif.) was assessed in a comparative evaluation with other rapid methods by using 269 isolates of oxidase-positive, gram-negative diplococci. Chemiluminescence was read with a PAL luminometer, and results were expressed as PAL light units (PLUs): the cutoff value for a positive identification was 1,500 PLUs. All 200 isolates of gonococci confirmed by carbohydrate utilization and serotyped with monoclonal antibodies were identified correctly by AccuProbe on initial testing. The API Quadferm system (Bio Merieux, Marcy l'Etoile, France) identified 95% (n = 190) of the gonococci correctly on initial testing and 99.5% (n = 199) on repeat testing, while the Phadebact Monoclonal GC test (Kara Bio Diagnostics AB, Huddinge, Sweden) identified 95.5% (n = 191) of the gonococci on both initial and repeat testing; 8 of the Phadebact-negative isolates were all of the same rare serovar (serovar 1B-17). The mean PLU for the gonococcal isolates was 9,014 (range 2,264 to 10,845) compared with a mean of 51 (range 8 to 109) for the 69 nongonococcal isolates. We conclude that the AccuProbe culture confirmation test provides a rapid and accurate objective means of identifying cultured N. gonorrhoeae isolates.

Several commercial identification systems based on a variety of biochemical and immunological methods are available for the rapid identification of Neisseria gonorrhoeae (3). Recently, DNA probe technology has created new diagnostic approaches for use in the clinical laboratory (8, 20, 23). Several tests have now been developed for the direct detection of target sequences of N. gonorrhoeae either in patient exudates (5, 6, 13, 18, 19) as a means of noncultural diagnosis or in cultured organisms (1, 11, 12, 16, 22) to provide confirmatory identification. Probes based on target sequences of chromosomal DNA have been evaluated most extensively as identification tests, but with a few exceptions (11), they have given mixed results with specificity (1, 16, 22) and sensitivity (22). DNA probes can also be derived from rRNA (4), and this approach has been used in the AccuProbe culture identification test for N. gonorrhoeae (Gen-Probe Inc., San Diego, Calif.). The AccuProbe test uses a chemiluminescent labelled single-stranded DNA probe that is complementary to gonococcal rRNA. Test bacteria are lysed to release the rRNA, which, in the case of gonococci, combines with the DNA probe to form a stable DNA-RNA hybrid. A selection reagent differentiates between the nonhybridized and the hybridized probes. The labelled DNA-RNA hybrids are measured in a Gen-Probe luminometer, and the results are expressed as relative light units (RLUs). A prototype AccuProbe identification test demonstrated 100% sensitivity and specificity (12). We report a comparative evaluation of a commercially available Gen-Probe kit with other rapid methods for the identification of N. gonorrhoeae.

MATERIALS AND METHODS

A total of 269 isolates of oxidase-positive, gram-negative diplococci were included in the study. These comprised strains submitted to the Scottish Gonococcal Reference Laboratory for gonococcal serotyping and antibiotic susceptibility testing, unselected oxidase-positive, gram-negative diplococci isolated in our laboratory from cultures of anogenital and throat specimens from patients undergoing screening for gonococcal infection or from cultures of throat swabs that were requested for routine culture, and a strain of Neisseria cinerea from the National Collection of Type Cultures (NCTC 10294). All isolates were subcultured onto modified New York City medium (25) lacking selective antibiotics, incubated overnight at 37°C, and used as the inoculum for the following identification procedures, which were performed in parallel.

The rapid carbohydrate utilization test (RCUT) was performed by using in-house reagents as described previously (25, 28), but microwell strips rather than individual tubes were used. Each strip included wells for glucose, maltose, sucrose, lactose, fructose, and ampicillin in a buffer containing phenol red pH indicator. Results were read after 3 h of incubation at 37°C. The Phadebact Monoclonal GC test (Kara Bio Diagnostics AB, Huddinge, Sweden) was performed according to the manufacturer’s instructions. This coagglutination test uses two reagents comprising pools of murine monoclonal antibodies that are reactive with epitopes on isolates with proteins IA (WI reagent) and IB (WII/III reagent), respectively. A boiled suspension of the test organism was tested against each reagent, and results were read within 1 min as described previously (26). The API Quadferm system (Bio Merieux, Marcy l’Etoile, France) is a standardized system for the rapid identification of Neisseria and Branhamella species and the determination of penicillinase activity. Each test strip contains seven microwells comprising a control well and glucose, maltose, lactose, sucrose, DNase, and penicillinase test wells.

Test strips were inoculated, incubated, and read after 2 h according to the manufacturer’s instructions. The AccuProbe N. gonorrhoeae culture confirmation test (Gen-Probe) was performed according to the manufacturer’s instructions. For each specimen, a 1-μl loopful of cells or several (three to four) small colonies was transferred to a probe reagent tube.

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containing 50 μl of lysis reagent; the loop was twirled in the lysis reagent to remove the cells. Hybridization reagent (50 μl) was then added and the tubes were capped, mixed by vortexing, and incubated for 15 min at 60°C. After the addition of 300 μl of selection reagent, the tubes were vortexed, incubated for 5 min at 60°C, and read in a PAL/AccuLDR luminometer (Gen-Probe). The values were reported as PAL light units (PLUs). Samples with PLUs of >1,500 were considered positive, those with PLUs of 1,200 to 1,499 were considered equivocal, and those with PLUs of <1,200 were considered negative. Serotyping of suspect gonococcal isolates was performed as described previously (15) by using the Genetic Systems panel of monoclonal antibodies (9). Strains that were nontypeable with this panel were typed with the Pharmacia panel of monoclonal antibodies (15).

RESULTS

The results of testing the 269 isolates are summarized in Table 1. Because the Phadebact and AccuProbe tests can differentiate only between gonococci and nongonococcal organisms, the actual identity of each nongonococcal isolate is not given. The 200 isolates that gave positive reactions by the AccuProbe and RCUT methods were confirmed as *N. gonorrhoeae* with monoclonal antibodies. The isolates included 68 serovar IA strains (34 IA-2, 3 IA-4, 3 IA-5, 5 IA-6, 17 IA-16, 4 IA-21, 2 IA-25) and 132 serovar IB strains (24 IB-1, 26 IB-2, 14 IB-3, 3 IB-5, 12 IB-6, 8 IB-7, 4 IB-8, 3 IB-15, 3 IB-16, 8 IB-17, 3 IB-19, 1 IB-24, 5 IB-26, 2 IB-29, 3 IB-31, and 13 nontypeable with Genetic Systems reagents but serovar Bx/Av with Pharmacia reagents). The mean PLU for the 200 gonococcal isolates was 9,014 (range, 2,264 to 10,845). Of the 10 gonococcal isolates not identified correctly by Quadferm on initial testing, 6 were identified as *Neisseria meningitidis*, 2 as *Moraxella catarrhalis*, 1 as the *Neisseria sicca-Neisseria subflava-Neisseria mucosa* group, and 1 no identity. On retesting, nine isolates were identified correctly as *N. gonorrhoeae*, while the strain identified initially as a member of the *N. sicca-N. subflava-N. mucosa* group gave the same identity. All nine Phadebact-negative gonococcal isolates remained negative on repeat testing; eight isolates were serovar IB-17 and 1 isolate was serovar IA-16. Because no nongonococcal organisms were identified as *N. gonorrhoeae*, the positive predictive value for all four tests with respect to identifying gonococci was 100%. The negative predictive value with respect to identifying an isolate as nongonococcal was also 100% for AccuProbe and RCUT. The negative predictive value for Quadferm was 87.4% on the first test (10 false-positive nongonococcal identifications) and 98.6% on repeat testing (one false-positive nongonococcal identification). The negative predictive value for Phadebact was 88.5% for both initial and repeat testing (nine false-positive nongonococcal identifications).

The mean PLU was 51 (range, 8 to 109) for the 69 nongonococcal isolates. The RCUT identified the 69 nongonococcal isolates as *N. meningitidis* (n = 22), *Neisseria lactamica* (n = 20), *Neisseria perflava* (n = 5), and *M. catarrhalis* (n = 22); the *N. cinerea* isolate was wrongly identified as *M. catarrhalis*. The mean PLUs for the various organisms were 46.4 for *N. meningitidis*, 49.5 for *N. lactamica*, 52.6 for *N. perflava*, 56.6 for *M. catarrhalis*, and 42 for one isolate of *N. cinerea*. Quadferm results agreed with the RCUT results for all of the *N. meningitidis* and *N. lactamica* isolates and for 8 of the *M. catarrhalis* isolates; no identification was obtained for the discordant isolate with Quadferm. Because additional culture tests were not undertaken, four of the five *N. perflava* isolates were identified within the *N. sicca-N. subflava-N. mucosa* group; the fifth isolate was identified as *M. catarrhalis*. No identification was obtained for the strain of *N. cinerea* with Quadferm (*N. cinerea* is not listed in the Quadferm identification table).

**DISCUSSION**

Not only did the AccuProbe test give 100% sensitivity and specificity but it also showed excellent discrimination between gonococci and nongonococcal organisms with respect to PLUs. No isolate gave a PLU result in the equivocal range. The mean PLU for gonococci was 6 times (range, 1.5 to 7.2 times) the cutoff value of (1,500) for a positive result read on the PAL luminometer. The cutoff value (1,200) for a negative result was 23.5 times (range, 11 to 150 times) the mean PLU for nongonococcal organisms. These results confirm the 100% sensitivity and specificity found in the evaluation of the prototype AccuProbe kit by using the Leader I luminometer (12). The differentiation was, however, slightly greater in the latter study; the mean RLU for gonococci was 21.7 times (range, 1.7 to 33.4 times) above the cutoff (50,000) for a positive result, while values for nongonococcal organisms ranged from 181.8 times (for a strain of *N. meningitidis*) to 5.6 times (for a strain of *N. cinerea*) below the cutoff. Harada et al. (7) reported a significant correlation between the AccuProbe result in terms of RLUs and the number of gonococcal organisms (*r* = 0.96); the threshold of detection was 5 × 10⁸ CFU per tube. The sensitivity of detection of gonococci was not influenced by the presence of other bacteria (7), although endogenous RNase activity may decrease the RLU by 43 to 71% when cultures are incubated for 96 h (12). This decrease did not result in a decrease in the RLUs given by any of the gonococci below the cutoff. Nevertheless, the manufacturer recommends that colonies should be less than 48 h old.

Because the strains evaluated in the present study include isolates that were negative by other test systems, the absolute sensitivity of AccuProbe is particularly reassuring. Serovar IB-17 has previously been reported to be negative in the Phadebact Monoclonal GC test (26). However, because the prevalence of this serovar is low in most geographical areas (14, 27), the performance of the Phadebact Monoclonal GC test is generally in excess of 99% rather than 95.5% as reported here. The reason for the poor initial identification of *N. gonorrhoeae* by Quadferm is unclear, because all nine gonococcal isolates that were misidentified initially were identified correctly on repeat testing. Although it was not used in the present study, the Syva Micro Trak fluorescent-antibody identification test was negative for the very rare serovar IB-24 (14). Two penicillinase-producing isolates of
**N. gonorrhoeae** serovar IB-19 were also found to be negative by the Syva test (24), even though a high degree of sensitivity was obtained with serovar IB-19 during initial trials.

The absolute specificity of AccuProbe is also reassuring because of difficulties that have been encountered in differentiating between *N. cinerea* and *N. gonorrhoeae* (2, 10). In one case, this resulted in proctitis associated with *N. cinerea* being misdiagnosed as a gonococcal infection in an 8-year-old boy (4). The failure of some strains of meningococci to give a positive reaction in certain biochemical test systems (21) has resulted in colonization with "maltose-negative" meningococci being misdiagnosed as pharyngeal gonorrhoea (17). The high sensitivity and specificity of AccuProbe suggest that the same test principle may be of considerable value in detecting gonococci in patient exudates. Preliminary reports of direct detection in high-prevalence (21 to 24%) populations with the Gen-Probe PACE assay gave a sensitivity of approximately 90 to 93% and a specificity of 99% (5, 6). In a comparison of Gen-Probe PACE 2 with transported Gen-Probe swabs and conventional culture based on JEM-BEC plates incubated overnight prior to spending a minimum of 2 days in the mail system, 2.7% of specimens were positive by the probe method and 0.6% were positive by culture; indirect evidence suggested that the probe-positive, culture-negative specimens were false-negative cultures (13). Evaluations involving optimal culture procedures and low-prevalence populations are, however, lacking. The limit of detection of 5 × 10⁵ CFU per tube by AccuProbe is severalfold in excess of the mean number of gonococci found in cervical aspirates (1 × 10⁶ CFU/ml) and is similar to the minimum of the culture-positive range (5 × 10⁵ to 8 × 10⁵) (29). This supports the probability of good sensitivity, provided that an adequate sample is obtained.

We conclude that the AccuProbe culture confirmation test provides a rapid (total time, 30 min, with 5 to 10 min of hands-on time) and accurate objective means of identifying cultured *N. gonorrhoeae*. We have not evaluated the test using primary cultures, but the finding of Harada et al. (7) that detection sensitivity was not influenced by the presence of other bacteria suggests that this procedure should give similar results. Like immunological test systems, the probe test does not characterize nongonococcal neisserial isolates to the species level. We do not consider this to be an important factor in test selection in those laboratories whose main aim is to detect or exclude patients with gonococcal infections.

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THE CENTRAL AFRICAN JOURNAL OF MEDICINE

ORIGINAL ARTICLES

Teenage obstetric and gynaecological problems in an African city

ME DUNCAN1, G TIBAUX2, A PELZER3, L MEHARI1, J PEUTHERER1, H YOUNG1, Y JAMIL1, S DAROUCHAR1, P PIOT1, E ROGGEN1

SUMMARY

Objective: To measure the prevalence of sexually transmitted diseases (STD), pelvic inflammatory disease (PID), cervical cancer, pregnancy and use of contraception in teenagers, and to determine socio-economic factors associated with these conditions to aid planners of medical services and promotion of sexual health.

Subjects: 181 Ethiopian teenagers and 1845 women aged 20 to 45 years for comparison.

Setting: Gynaecological outpatient department, antenatal, postnatal and family planning clinics, in two teaching hospitals and a mother and child health centre in Addis Ababa, Ethiopia.

Methods: Results of serologic tests for STD, clinical evidence of PID, and cervical cytology were analysed against socio-economic factors.

Results: In teenagers early age at first marriage/coitus, more common in those of rural origin, was associated with poverty, a greater number of lifetime sexual partners, and prostitution: 40 pc were first sexually active before the menarche. Prevalence of seropositivity to specific STD pathogens was; Treponema pallidum (TPHA) 21 pc, Neisseria gonorrhoeae (gonococcal antibody test: GAT) 40 pc, genital chlamydiae 51 pc, hepatitis B virus 36 pc, herpes simplex virus (HSV-2) 32 pc, and Haemophilus ducreyi 16 pc: 92 pc of teenagers were seropositive to one or more STD's, STD seroprevalence was higher in those with more than one sexual partner, those sexually active by age 15 (very high in those sexually active by age 12), those involved in prostitution and those attending the family planning clinic. Forty three pc had clinical evidence of PID; one married at age 10 had invasive cervical cancer by age 18; 40 pc of teenagers were pregnant compared with 25 pc of those aged 20 to 45; 21 pc attended for family planning; of regular FPC attenders 81 pc were GAT seropositive.

Conclusion: Despite legislation early age of sexual debut is common, STD and PID are widely prevalent, the pregnancy rate in adolescents is high and contributes to the national population growth rate. Action is required at family, medical and governmental level to encourage cultural acceptance that marriage and sexual activity should not occur before the age of 16 years, with education appropriate to culture to prevent STD. Similar studies are recommended in other countries to establish a baseline for informed strategy regarding
prevention of STD and health education.

INTRODUCTION

In industrialised countries adolescents are becoming sexually active younger and have more sexual partners. There was more than a 400 pc increase in gonorrhoea from the mid 1950's to the early 1980's, the greatest rise being in women younger than 20. Studies of teenagers attending city clinics for sexually transmitted disease (STD) have shown higher rates of STD in adolescents than in all other age groups. Early age at first coitus, first marriage or first childbirth has been recorded as significant in the aetiology of cervical cancer (CC). Doubling of the rate of CC was noted in those with first coitus before the age of 16. In Europe a teenager behaviour syndrome has been described in which it has been shown that the earlier the sexual debut the greater the likelihood of the girl having more sexual partners, leaving home, and supporting herself by prostitution. Recent reports of the sociological consequences of early sexual activity have been reported by investigating journalists in the popular rather than the medical and scientific press.

In sub-Saharan Africa urbanisation has eroded the social structures that in the past regulated sexual behaviour. Reports from South Africa show that two thirds of pregnancies amongst those aged 16 or younger, in a rural setting, were in unmarried adolescents. In an urban study only one of 175 (0.6 pc) teenage primigravidae attending hospital was married, and 26 pc (36/138) of this group for whom data were available had STD.

Little is known of the patterns, or consequences in teenagers, of early sexual behaviour in Ethiopia, although first coitus before menarche, occurring between 14 and 15 years of age, is a risk factor for STD, pelvic inflammatory disease (PID) and CC in later life. Prevalence rates for STD in that country are considerably higher than reported earlier and elsewhere in Africa.

Child marriage, for many an economic necessity, was and is widely practiced in developing countries. Teenage obstetric and gynaecological problems have not been investigated or analysed except as part of an overview of maternal morbidity and mortality where the very young primigravida has been shown to be particularly at risk. A high prevalence of STD among the teenage population could cause serious sequelae such as PID, ectopic pregnancy and infertility. These conditions would place an increasing burden on health services of countries which today have 60 to 70 pc of the population aged 15 or less. An analysis of the medical requirements of teenagers is essential to both the planners and providers of health services and to health educationalists.

The aim of this study was to analyse data from teenagers, from an international collaborative study of 2 111 women, regarding socio-economic aetiological factors for STD, PID, cervical cancer, pregnancy and contraception. Such data would provide invaluable basic information to both planners of medical services and educationalists in promotion of sexual health, not only in Africa, but also in industrialised nations where early teenage sexual activity is posing major problems with regard to the prevention of HIV transmission.

MATERIALS AND METHODS

Patients: After giving their informed consent, 181 Ethiopian teenagers, aged 14 to 19 years, were recruited from the clinics in Addis Ababa: 83 (46 pc) from the gynaecological outpatient department (GOPD), 31 (17 pc), 49 (27 pc) and 18 (10 pc) teenagers from routine family planning (FPC) antenatal (ANC) and postnatal clinics (PNC) respectively. Selection was as follows: women from GOPD were first attenders, women from routine FPC, ANC and PNC were the first to register on study days. Statistical comparison was from 1 845 women aged 20 to 45 who were subjected to the same examination and data collection.

Permission to carry out this study and export patient data and clinical samples for analysis, was obtained from the Ethiopian Ministry of Health.

Epidemiological and socio-economic data: For each patient data were obtained using a questionnaire completed by an Ethiopian female assistant. These included: clinic attended, residence (urban/rural), age, age at first marriage, age at first coitus and relation of this to menarche, sequential number of husbands/sexual partners (polyandry was not practised), last marital status/profession (single/married/divorced/widow/housemaid/prostitute: the categorisation according to last marital status/profession was mutually exclusive e.g. a divorced woman working as a prostitute was classified as a prostitute, similarly a widow working as a seller of local beer was classified as a seller. The women described themselves thus: ‘I was
married now I am a 'bargirl', thus we used the last marital status/profession for statistical analysis), family monthly income, parity, total number of pregnancies, and use and type of contraception.

Clinical data: These data resulted from a full gynaecological examination. Particular attention was paid to the condition of the cervix and for evidence of infection in the urethra, salpinges and Bartholin glands (USB) which were inspected (UB) and palpated (USB).

Cytology: Cervical smear preparations were made and transported to Liège where they were stained by Papanicolaou stain and read: excessive numbers of polymorphonuclear (PMN) cells in the smears were indicative of cervical infection.

Serologic tests: Blood was collected from all women by venepuncture; serum was separated and stored at -20°C until testing by micromethods became available. Sera were not aliquotted, but were transported on dry ice to the collaborating laboratories in turn, where they were stored at -20°C until tested; in Edinburgh for syphilis using TPHA and hepatitis B virus (HBV) in London for Herpes simplex virus (HSV2) and Chlamydia trachomatis D-K (CTD-K) and Lymphogranulomavirus venereum 1-3 (LGV) in Copenhagen for Neisseria gonorrhoeae (gonococcal antibody test: GAT),21 and in Antwerp for Haemophilus ducreyi.22,23

Statistical methods: Statistical analysis was made using the Chi-square and Cochran-Mantel-Haenszel General Association Statistics44 to determine the significance level of any kind of association found between seropositivity for specific STD and other recorded data for the various groups of patients; odds ratio (OR) estimates and 95% confidence interval (c.i.) were computed using the latter statistic.

RESULTS

The age, age at first coitus, last marital status/profession, number of husbands/sexual partners and parity/number of pregnancies are shown in Table 1.

The age range for sexual debut was 7 to 19 years. Forty pc had first coitus before their menarche which occurred between ages 14 and 15 years. Girls from the countryside were married and first sexually active younger than those from the city: 64 pc compared with 55 pc being sexually active by age 15. Age at first coitus was earlier (p = 0.01) in poorer women: 75 pc of those sexually active age 12 or less had a monthly family income of < 50 Ethiopian Birr (50EB = 25SUS), while

Table I: Personal details of teenagers studied.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>(pc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>34</td>
<td>19</td>
</tr>
<tr>
<td>18</td>
<td>76</td>
<td>42</td>
</tr>
<tr>
<td>19</td>
<td>55</td>
<td>31</td>
</tr>
<tr>
<td>Age at first coitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>13-15</td>
<td>65</td>
<td>38</td>
</tr>
<tr>
<td>&gt;16</td>
<td>75</td>
<td>44</td>
</tr>
<tr>
<td>(Missing data = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first coitus in relation to the menarche</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before menarche</td>
<td>63</td>
<td>40</td>
</tr>
<tr>
<td>After menarche</td>
<td>94</td>
<td>60</td>
</tr>
<tr>
<td>(Missing data = 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status/profession</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single/student</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Married</td>
<td>89</td>
<td>49</td>
</tr>
<tr>
<td>Divorced</td>
<td>52</td>
<td>18</td>
</tr>
<tr>
<td>Widow</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Housemaid</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Prostitute</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>(Missing data = 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of husbands/sexual partners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>113</td>
<td>66</td>
</tr>
<tr>
<td>2-5</td>
<td>58</td>
<td>22</td>
</tr>
<tr>
<td>&gt;5</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>(Missing data = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity/number of pregnancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>84</td>
<td>47</td>
</tr>
<tr>
<td>1</td>
<td>68</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>&gt;3</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>(Missing data = 2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*(14(8 pc) had one or more abortion/ectopic pregnancies).*
married women and 45 pc of divorcees were first sexually active at age 16 or older (p = 0.001). The number of sexual partners decreased significantly (p < 0.001) with increasing age at first coitus: the percentage of women having only one sexual partner being 33 pc, 60 pc and 86 pc respectively for those with a sexual debut age of 12 or younger, 13 to 15 and 16 and older.

Sexually transmitted disease: One hundred and sixty seven (92 pc) of teenagers were seropositive to one or more STD (Figure I). Prevalence of seropositivity to specific STD was: syphilis (TPHA) 21 pc; gonorrhoea (GAT) 40 pc (IgG ≥ 1/320 indicating recent, active or recurrent infection was present in 18 pc); genital chlamydiae (CTD-K and LGV) 51 pc (IgG ≥ 1/64 or IgM indicating active genital chlamydial infection were present in 36 pc); HBV 36 pc; HSV2 32 pc; and H. ducreyi 16 pc.

Analysis of STD seropositivity by age at first coitus showed a decrease in STD seropositivity with increase in age at first coitus (Table II): of 94 known to be

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**Figure I: Distribution of teenagers according to their sero-negativity or cumulative sero-positivity to one or more sexually transmitted diseases. Sero-neg = sero-negativity i.e. no seropositivity for any STD.**

<table>
<thead>
<tr>
<th>Number of STDs seropositive</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>181</td>
</tr>
</tbody>
</table>

---

**Sero-Neg = Sero-Negativity = no seropositivity for any STD.**

237
Table II: Seropositivity for sexually transmitted disease and diagnosis of pelvic inflammatory disease, according to age at first coitus.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. in group</th>
<th>Age at first coitus</th>
<th>13–15</th>
<th>&gt; 15</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 13</td>
<td>13–15</td>
<td>&gt; 15</td>
<td></td>
</tr>
<tr>
<td>Syphilis (TPHA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>146</td>
<td>24</td>
<td>58</td>
<td>64</td>
<td>0.01</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>31 (21 pc)</td>
<td>10 (42 pc)</td>
<td>13 (22 pc)</td>
<td>8 (12 pc)</td>
<td>0.01</td>
</tr>
<tr>
<td>Gonorrhoea (GAT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>146</td>
<td>23</td>
<td>57</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>n positive</td>
<td>59 (40 pc)</td>
<td>13 (57 pc)</td>
<td>28 (49 pc)</td>
<td>18 (27 pc)</td>
<td>0.01</td>
</tr>
<tr>
<td>Genital Chlamydiae (CT D-K &amp; LGVI-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>148</td>
<td>25</td>
<td>58</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>n positive</td>
<td>75 (51 pc)</td>
<td>17 (68 pc)</td>
<td>34 (59 pc)</td>
<td>24 (37 pc)</td>
<td>0.01</td>
</tr>
<tr>
<td>HBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>148</td>
<td>25</td>
<td>58</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>n positive</td>
<td>54 (36 pc)</td>
<td>8 (32 pc)</td>
<td>25 (43 pc)</td>
<td>21 (32 pc)</td>
<td>NS</td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>148</td>
<td>25</td>
<td>58</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>n positive</td>
<td>48 (32 pc)</td>
<td>11 (44 pc)</td>
<td>18 (31 pc)</td>
<td>19 (19 pc)</td>
<td>NS</td>
</tr>
<tr>
<td>H. ducreyi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>143</td>
<td>23</td>
<td>56</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>n positive</td>
<td>22 (15 pc)</td>
<td>3 (13 pc)</td>
<td>11 (20 pc)</td>
<td>8 (12 pc)</td>
<td>NS</td>
</tr>
<tr>
<td>Cumulative seropositivity*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>169</td>
<td>30</td>
<td>64</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>n seropositive</td>
<td>157 (93 pc)</td>
<td>30 (100 pc)</td>
<td>62 (97 pc)</td>
<td>65 (87 pc)</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>PID (BUS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n examined</td>
<td>168</td>
<td>29</td>
<td>64</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>n positive</td>
<td>71 (42 pc)</td>
<td>16 (55 pc)</td>
<td>32 (50 pc)</td>
<td>23 (31 pc)</td>
<td>NS</td>
</tr>
</tbody>
</table>

n = number; NS = No statistically significant association; TPHA = Treponema pallidum haemagglutination assay; GAT = Gonococcal antibody test; CT D-K = C. trachomatis D-K; HBV = Hepatitis B virus; HSV-2 = Herpes simplex virus type 2; PID = Pelvic inflammatory disease; B = Bartholin; U = Urethritis; S = Salpingitis; Cumulative seropositivity* = seropositive for one or more STD.

sexually active by age 15 only two were STD seronegative.

Teenagers involved in prostitution, compared with those who were not, had a significantly higher prevalence of syphilis, gonorrhoea and genital chlamydial infection, but not HBV, HSV2 or chancroid. All prostitutes were seropositive to one or more STD (Table III). The increased risk (OR) of gonorrhoea, syphilis and genital chlamydial infection in those engaged in prostitution compared with those who were not was 72,1, 10,6 and 5,5 respectively.

There was significantly more STD amongst those with more than one partner (Table IV). The increased risk of infection (OR) in those with more than one sexual partner, compared with those still married to their first husband, was 4,1; 2.9,2.6 and 2.0 for syphilis,
Table III: Analysis of sexually transmitted disease, pelvic inflammatory disease and cervical cancer by last marital status/profession.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No in group</th>
<th>Non Prostitutes n (pc)</th>
<th>Prostitutes n (pc)</th>
<th>OR (p value)</th>
<th>95 pc c.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis (TPHA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>157</td>
<td>139</td>
<td>18</td>
<td>10.64</td>
<td>4.14-27.32</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>34(22 pc)</td>
<td>22 (16 pc)</td>
<td>12 (67 pc)</td>
<td>(&lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td>Gonorrhoea (GAT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>157</td>
<td>139</td>
<td>18</td>
<td>72.05*</td>
<td>4.2-1221.84*</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>65 (41 pc)</td>
<td>47 (34 pc)</td>
<td>18 (100 pc)</td>
<td>(&lt; 0.01)</td>
<td></td>
</tr>
<tr>
<td>Genital Chlamydiae (CT D-K &amp; LGV-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>159</td>
<td>141</td>
<td>19</td>
<td>5.52</td>
<td>1.71-17.86</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>82 (52 pc)</td>
<td>67 (48 pc)</td>
<td>15 (83 pc)</td>
<td>(0.015)</td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>159</td>
<td>141</td>
<td>18</td>
<td>1.50</td>
<td>0.56-4.04</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>57 (36 pc)</td>
<td>49 (35 pc)</td>
<td>8 (44 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>159</td>
<td>141</td>
<td>18</td>
<td>1.10</td>
<td>0.39-3.13</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>50 (31 pc)</td>
<td>44 (31 pc)</td>
<td>6 (33 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td>H.ducreyi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>15*</td>
<td>116</td>
<td>18</td>
<td>1.65</td>
<td>0.50-5.52</td>
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<tr>
<td>n positive</td>
<td>24 (16 pc)</td>
<td>20 (15 pc)</td>
<td>4 (22 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td>Cumulative seropositivity**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>180</td>
<td>161</td>
<td>19</td>
<td>3.83</td>
<td>0.22-66.84</td>
</tr>
<tr>
<td>n seropositive</td>
<td>166 (92 pc)</td>
<td>147 (91 pc)</td>
<td>19 (100 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td>PID (BUS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>179</td>
<td>160</td>
<td>19</td>
<td>2.50</td>
<td>0.96-6.56</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>77 (43 pc)</td>
<td>65 (41 pc)</td>
<td>12 (63 pc)</td>
<td>(0.06)</td>
<td></td>
</tr>
</tbody>
</table>

Non prostitutes = married, single, divorced, widowed, maid. OR = odds ratio; n = number; NS = No statistically significant association. TPHA = Treponema pallidum haemagglutination assay; GAT = Gonococcal antibody test; CT D-K = C. trachomatis D-K; HBV = Hepatitis B virus; HSV-2 = Herpes simplex virus type 2; PID = Pelvic inflammatory disease; B = Bartholinitis; U = Urethritis; S = Salpingitis.

*As all the prostitutes tested were GAT positive, the odds ratio is from the assumption of a small percentage of negative: technically a "statistically guestimate". **Cumulative seropositivity = seropositive for one or more STD.

Gonorrhoea, genital chlamydial infection and PID respectively.

Pelvic inflammatory disease (PID): Details for one patient were missing. Overall 77/180 (43 pc) had clinical signs of PID with palpably thickened Bartholin glands, urethra or salpinges with/without pain on palpation. Nine (5 pc) had evidence of Bartholinitis largely associated with GAT seropositivity (eight were tested and positive and one was not tested); 44 (24 pc) had urethritis and 41 (23 pc) had salpingitis. The twofold increased risk of PID with very young age at first coitus, prostitution and having more than one sexual partner is detailed in Tables II, III and IV.

Cervical cancer and abnormal cytology: One 18 year old, first married at age 10, had stage II cervical cancer confirmed by typical cancer cells in the cytolo-
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. in group</th>
<th>Number of husbands</th>
<th>OR</th>
<th>95 pc c.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2 and more</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p value)</td>
<td></td>
</tr>
<tr>
<td>Syphilis (TPHA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>148</td>
<td>95</td>
<td>53</td>
<td>4.14</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>34 (23 pc)</td>
<td>13 (14 pc)</td>
<td>21 (40 pc)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Gonorrhoea (GAT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>148</td>
<td>95</td>
<td>53</td>
<td>2.91</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>62 (42 pc)</td>
<td>31 (32 pc)</td>
<td>31 (58 pc)</td>
<td>(&lt; 0.001)</td>
</tr>
<tr>
<td>Genital Chlamydiae (CT D-K &amp; LGVI-3)</td>
<td>150</td>
<td>96</td>
<td>54</td>
<td>2.57</td>
</tr>
<tr>
<td>n tested</td>
<td></td>
<td></td>
<td></td>
<td>1.28-5.15</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>78 (52 pc)</td>
<td>42 (44 pc)</td>
<td>36 (67 pc)</td>
<td>(&lt; 0.05)</td>
</tr>
<tr>
<td>HBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>150</td>
<td>96</td>
<td>54</td>
<td>1.16</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>55 (37 pc)</td>
<td>34 (35 pc)</td>
<td>21 (39 pc)</td>
<td>(NS)</td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>150</td>
<td>96</td>
<td>54</td>
<td>1.09</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>49 (33 pc)</td>
<td>32 (33 pc)</td>
<td>17 (31 pc)</td>
<td>(NS)</td>
</tr>
<tr>
<td>H. ducreyi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>146</td>
<td>94</td>
<td>52</td>
<td>1.20</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>23 (16 pc)</td>
<td>14 (15 pc)</td>
<td>9 (17 pc)</td>
<td>(NS)</td>
</tr>
<tr>
<td>Cumulative seropositivity*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>171</td>
<td>113</td>
<td>58</td>
<td>1.59</td>
</tr>
<tr>
<td>n seropositive</td>
<td>159 (93 pc)</td>
<td>104 (92 pc)</td>
<td>55 (95 pc)</td>
<td>(NS)</td>
</tr>
<tr>
<td>PID (BUS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n examined</td>
<td>170</td>
<td>112</td>
<td>58</td>
<td>1.99</td>
</tr>
<tr>
<td>n diagnosed (pc)</td>
<td>72 (42 pc)</td>
<td>41 (36 pc)</td>
<td>31 (54 pc)</td>
<td>(&lt; 0.04)</td>
</tr>
</tbody>
</table>

OR = odds ratio; n = number; NS = No statistically significant association.
TPHA = Treponema pallidum haemagglutination assay; GAT = Gonococcal antibody test; CT D-K = C. trachomatis D-K; HBV = Hepatitis B virus; HSV-2 = Herpes simplex virus type 2; PID = Pelvic Cumulative seropositivity* = seropositive for one or more STD.

ogy smear. A second 18 year old with first coitus age 16 had dysplasia (CIN2/3) on cytology. Cytology showed no cancer cells in 154, but 61 (36 pc) had excessive numbers of polymorphonuclear cells indicative of cervical infection, and 27 (16 pc) had Trichomonas vaginalis in the smear reflecting a heavy trichomonal vaginal infection.

Pregnancy: Of 181 teenagers 40 pc were pregnant. More pregnant women (58 pc) were seen amongst teenagers whose first coitus was at age 16 or older (p = 0.01). Eighty five pc of pregnant teenagers were seropositive for one or more STD. There were significantly (p = 0.001) less gonorrhoea (26 pc) in pregnant than in non pregnant teenagers (52 pc), while 47 pc of PNC attenders were GAT positive.

Family planning and use of contraception: Thirty eight (21 pc) attended for family planning check-ups and advice, 31 of them at the FPC. Of these teenagers 26 (14 pc) used contraception: 24 (13 pc) oral contraception and two (1 pc) had IUCD. All 38 were seropositive for one or more STD. Of 157 women tested for GAT serology, 21/26 (81 pc) attending the FPC for advice.
Table V: Comparison of behavioural factors, sexually transmitted disease and pelvic inflammatory disease in sexually active adolescents aged 14 to 19 and women 20 to 45.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. in group</th>
<th>Age 14-19</th>
<th>Age 20-45</th>
<th>OR (p value)</th>
<th>95 pc c.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at first coitus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total group</td>
<td>1,944</td>
<td>169</td>
<td>1,775</td>
<td>0.70</td>
<td>0.51-0.96</td>
</tr>
<tr>
<td>Up to 15 years</td>
<td>1,235</td>
<td>94 (56 pc)</td>
<td>1,141 (64 pc)</td>
<td>(0.025)</td>
<td></td>
</tr>
<tr>
<td><strong>Family planning</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>2,026</td>
<td>181</td>
<td>1,845</td>
<td>0.62</td>
<td>0.43-0.90</td>
</tr>
<tr>
<td>n FPC (pc)</td>
<td>593 (29 pc)</td>
<td>38 (21 pc)</td>
<td>555 (30 pc)</td>
<td>(0.01)</td>
<td></td>
</tr>
<tr>
<td><strong>Pregnant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>2,026</td>
<td>181</td>
<td>1,845</td>
<td>2.02</td>
<td>1.47-2.77</td>
</tr>
<tr>
<td>n pregnant (pc)</td>
<td>527 (26 pc)</td>
<td>72 (40 pc)</td>
<td>455 (25 pc)</td>
<td>(&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>Syphilis (TPHA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>1,763</td>
<td>157</td>
<td>1,606</td>
<td>0.48</td>
<td>0.32-0.71</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>624 (35 pc)</td>
<td>34 (22 pc)</td>
<td>590 (37 pc)</td>
<td>(&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>Gonorrhoea (GAT)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>1,776</td>
<td>157</td>
<td>1,619</td>
<td>0.46</td>
<td>0.33-0.66</td>
</tr>
<tr>
<td>n positive</td>
<td>1,043 (59 pc)</td>
<td>65 (41 pc)</td>
<td>978 (60 pc)</td>
<td>(&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td><strong>Genital Chlamydiae (CT D-K &amp; LGVI-3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>1,171</td>
<td>159</td>
<td>1,612</td>
<td>0.62</td>
<td>0.45-0.86</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>1,099 (62 pc)</td>
<td>82 (52 pc)</td>
<td>1,017 (63 pc)</td>
<td>(0.004)</td>
<td></td>
</tr>
<tr>
<td><strong>Hepatitis B virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>1,785</td>
<td>159</td>
<td>1,626</td>
<td>0.89</td>
<td>0.63-1.24</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>686 (38 pc)</td>
<td>57 (36 pc)</td>
<td>629 (39 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td><strong>HBS-2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>1,771</td>
<td>159</td>
<td>1,162</td>
<td>0.71</td>
<td>0.50-1.01</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>682 (38 pc)</td>
<td>50 (31 pc)</td>
<td>632 (39 pc)</td>
<td>(0.06)</td>
<td></td>
</tr>
<tr>
<td><strong>H. ducreyi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>1,757</td>
<td>154</td>
<td>1,603</td>
<td>0.76</td>
<td>0.48-1.19</td>
</tr>
<tr>
<td>n positive (pc)</td>
<td>339 (19 pc)</td>
<td>24 (16 pc)</td>
<td>315 (20 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td><strong>Cumulative seropositivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n tested</td>
<td>2,062</td>
<td>181</td>
<td>1,845</td>
<td>0.72</td>
<td>0.40-1.29</td>
</tr>
<tr>
<td>n seropos. (pc)</td>
<td>1,907 (94 pc)</td>
<td>167 (92 pc)</td>
<td>1,740 (94 pc)</td>
<td>(NS)</td>
<td></td>
</tr>
<tr>
<td><strong>PID (BUS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n examined</td>
<td>2,008</td>
<td>180</td>
<td>1,828</td>
<td>1.46</td>
<td>1.07-1.99</td>
</tr>
<tr>
<td>n diagnosed (pc)</td>
<td>1,031 (51 pc)</td>
<td>77 (43 pc)</td>
<td>954 (52 pc)</td>
<td>(0.02)</td>
<td></td>
</tr>
</tbody>
</table>

n = number; NS = No statistically significant association; OR = odds ration; FPC = Family planning clinic attendees' TPHA = Treponema pallidum haemagglutination assay; GAT = Gonococcal antibody test; CT D-K = C.trachomatis D-K; PID = Pelvic inflammatory disease; B = Bartholinitis; U = Urethritis, S = Salpingitis; Cumulative seropositivity = seropositive for one or more STD.
had evidence of gonococcal infection: in contrast only 33 pc of non FPC attenders were GAT positive (p < 0.001).

Infertility: Only four women (2.2 pc) attended because of infertility, although one teenager had been married and divorced three times because of childlessness.

Comparison of teenagers with women aged 20 to 45 years: Prevalence rates for STD and PID were a little lower in teenagers than in older women. Of all the women seen, teenagers attended more for pregnancy than women aged 20 to 45; in contrast more women aged 20 to 45 attended for family planning advice than teenagers (Table V).

DISCUSSION

In industrialised countries in the early twentieth century menarche occurred between 15 to 16 years, and marriages occurred at a younger age than today. Because of economic and social factors the age of the menarche has fallen while the age for first marriage has increased, creating an increasing gap between the age of biological sexual maturity and preferred age for marriage. In contrast in developing countries the age at first marriage/coitus is lower while the age at menarche is considerably higher, although with improving nutrition and education the age at menarche is starting to fall. Earlier reports have shown the age at menarche in Ethiopia to be 15, falling slightly to 14 to 15 in this study. In Nigeria, where the per capita income is three times greater than that in Ethiopia, the age of menarche has now fallen to 13.8.

The exposure of sexually active teenagers to STD pathogens reported here is very high: overall 91 pc, with 100 pc of those attending for FPC, having serological evidence of one or more STDs in contrast to 41 pc of sexually active urban American adolescent FPC attenders. The prevalence of syphilis, gonorrhoea, genital chlamydial infections as well as PID was significantly higher in those sexually active before age 15, and very much higher in those sexually active by age 12 all of whom had been exposed to at least one STD.

Infertility was not a particular problem at the time of assessment, only affecting four (8 pc) of those who attended for neither obstetric nor contraceptive purposes. Eighteen pc and 36 pc had high titres indicative of gonorrhoea or genital chlamydia respectively. With 23 pc of teenagers having clinical evidence of salpingitis, it is predicted that infertility will become a major problem in Ethiopian women in the future as it is elsewhere. Reports from Kenya, where tubal infertility accounts for 70 pc of GOPD time, are similar. STDs are more common in adolescents; women with infertility due to tubal factors had an earlier age at first coitus, compared with a group of healthy controls (16,7 years), and more infertile women were sexually active before the menarche. Those sexually active at a younger age were more likely to have multiple sexual partners, be exposed to sexually transmitted disease and to have run the risk of an unwanted pregnancy, possibly terminating in abortion.

Our results show not only an increased prevalence of STD and PID in prostitutes (Table III), but a significantly higher prevalence of these conditions in those who had more than one sexual partner (Table IV). The prevalence of STD in those with only one sexual partner, their first husband, is a measure of male promiscuity or previous sexual experience of these men. Cumulative percentages of our teenage group show 18 pc sexually active by age 12, 23 pc by age 13, 40 pc by age 14 and 56 pc by age 15. This very young age at sexual debut is comparable to that reported from Kenya where 42 pc of girls age 12 to 13 had had at least one sexual exposure and where the mean age for coitus for girls was 14,9 years in rural areas and 16 years in urban areas.

There is, however, one important difference between the two countries: at the time of data collection, according to culture, all but 12 (7 pc) of the Ethiopian girls were married as virgins and hence first sexually active within marriage, whereas because of the sexual revolution in Kenya many first sexual encounters reported were pre-marital and transient. More recently (reported in 1990) as a result of an increasing breakdown of ethnic traditions, 24 pc of female high school students in Ethiopia are sexually active and hence at risk of acquiring not only classical STDs but HIV.

If evidence of exposure to gonorrhoea (GAT) is taken as a measure of sexual activity and subsequent exposure to HIV infection it must be a matter of concern that 100 pc of teenagers engaged in prostitution were GAT positive, as were 81 pc of FPC attenders. That 26 pc of pregnant teenagers are GAT seropositive must interest paediatrics and obstetricians because of the risk to the baby of ophthalmia neonatorum and to the mother of gonococcal puerperal infection. The fact that 47 pc of PNC attenders were GAT seropositive
could indicate some self selection for "routine" PNC examination, possibly because of symptoms of perineal infection.

The high percentage (40 pc) of pregnancy in teenage clinic attenders we report is almost twice the rate in the 20 to 45 year olds - showing that in Ethiopia as in Kenya, which has a similarly high population growth rate (3.6 pc), a disproportionately large number of all pregnancies occur in adolescents.29

The data for this study were collected in the early years of the revolution before population mobilisation and nationalisation of urban and rural land had affected the culture of the people. The prevalence rates for STD and PID we report must be of concern to health authorities especially as there have been reports of an increase in STDs, gonorrhea in particular, from 1982 to 1989.31,32

In most countries, including Ethiopia, marriage is unlawful before 16, also the age of consent. Recently, however, Sweden and Holland (with certain provisos) have lowered the age of consent to 12 on the grounds that biological maturity occurs at that age, sexual activity is likely and contraception is advised. In other countries including the UK and Zimbabwe, and chiefly to allow the provision of contraceptives to young adolescents, pressure groups are recommending reduction of the age of consent to 13. In many other countries the age of consent law is not enforced.

The gynaecological problems of teenage sexual activity reported here are based on an analysis of teenagers attending hospital and mother and child welfare clinics, and not on a population survey. Nonetheless, it is clear that despite legislation early age of sexual debut is common; STDs are widely prevalent; the pregnancy rate in adolescents is high and contributes to the national population growth rate. Of the group of teenagers using FPC, 100 pc had serological evidence of at least one STD, and 81 pc had evidence of exposure to gonorrhoea, indicating a high rate of partner change or promiscuity of male partners of that age group.

Similar studies regarding teenage sexual practise and gynaecological and obstetric problems are urgently required elsewhere in Africa to establish a baseline for informed strategy. Action is required at family as well as government level to encourage cultural acceptance that marriage (and sexual activity) should not occur before age 16. Health education, appropriate to culture, showing early teenage sexual activity as an important risk factor for STD, PID, (unwanted) teenage pregnancy and cervical cancer should be given in schools and to youth organisations.

ACKNOWLEDGEMENTS

We thank the staff and patients of St Pauls and Black Lion Hospitals, and Lidetta Clinic for their co-operation; Dr Philippa Wilson for help in collecting data; Drs I Lind and K Reimann and the Neisseria Department of the Statens Seruminstitut, Copenhagen for detecting antibodies to Neisseria gonorrhoeae. We acknowledge with thanks financial assistance from Allied Medical Group for serological tests; The Wellcome Trust for travel and secretarial expenses for MED; and SAREC (Grant SPE-AIDS-HN-03-AV), Stockholm, Sweden for support for PP and ER. We thank Mrs M Pearce for secretarial help.

REFERENCES


A SOCIOECONOMIC, CLINICAL AND SEROLOGICAL STUDY IN AN AFRICAN CITY OF PROSTITUTES AND WOMEN STILL MARRIED TO THEIR FIRST HUSBAND

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Abstract—The aim of this paper was to compare women involved in prostitution with a group of women still married to their first husband and reporting having had only one sexual partner, in order to ascertian what factors if any contributed to women going into prostitution or staying still married to their first husband, their only sexual partner, and thereafter to compare clinical and serological aspects of the gynaecological conditions of the women in these two groups.

The role of prostitutes in transmission of sexually transmitted diseases (STD) is widely recognised. Socioeconomic factors determining whether a woman will drift into prostitution or have a stable first marriage are largely unknown as are prevalence rates of STD, pelvic inflammatory disease (PID) and cervical cancer in these women. A socioeconomic, clinical and serologic study is reported for 2111 Ethiopian women attending teaching hospitals and maternal and child health clinics in Addis Ababa. Analysing basic demographic data of three groups of women: (i) 278 engaged in prostitution, (ii) 730 still married to their one and only sexual partner, and (iii) 1103 single, widowed, divorced or married to their second or subsequent partner. Thereafter groups (i) and (ii) were compared and contrasted with regard to further socioeconomic, clinical and serological associations. The most significant socioeconomic associations for women in prostitution were low income (95% had <50 Ethiopian birr [<U.S. $25] per month), ethnic group, and the timing of first coitus in relation to the menarche (81% were first married by age 15), in that order. Women still married to their first sexual partner had higher income, higher age at first marriage and longer duration of marriage. Sero-prevalence rates of STD in prostitutes were high: gonorrhoea 88%, genital chlamydiae 78%, syphilis (TPHA) 62%, HSV2 and HBV 46%, and chancroid 19%. 67% had PID and 27% cervical cancer. In comparison, rates for women married to their first and only sexual partner were: gonorrhoea 40%, genital chlamydiae 54%, syphilis (TPHA) 19%, HSV2 33%, HBV 35%, chancroid 13%, PID 47% and cervical cancer 1%. While the very high prevalence of STD in women involved in prostitution is not unexpected, the high rate of STD in women still married to their first and only sexual partner is indicative of male promiscuity. Control of prostitution and diseases spread by it, together with education of both men and women is a national priority.

Key words—Africa, prostitution, monandrous (married) women, sexually transmitted disease, cervical cancer

INTRODUCTION

The implications of an epidemic of AIDS virus infection in Kenya are sobering. If urban prostitutes constitute a major reservoir of AIDS virus in such African capitals as Nairobi, Kigali, and Kinshasa we may expect that the virus will continue to be spread throughout the African continent by heterosexual men transferring infection from one community of urban prostitutes to another [1].

Prostitutes are a major reservoir/source of sexually transmitted diseases (STD) in Nairobi [2, 3]. More recently prostitutes have been shown to play a major role in the heterosexual transmission of HIV in developing countries [1, 3–6], both within the countries concerned and to international travellers [7]. The real issue in heterosexual transmission of STD is prostitution since it involves both men and women. It is the act of prostituting or going to a prostitute which is important—risky behaviour rather than a risk group. With increasing urbanisation, travel to work, travel for work and, more recently, tourism, prostitution originally proscribed by many African cultures [8, 9] has become an accepted fact in the late 20th century, even where it is officially prohibited [10]. In an epidemiological study of urethritis in Ibadan, it was shown that while 47% of affected men had casual partners, for a further 21% their regular sexual partner was a prostitute [11]. Twice as many single men visited prostitutes as

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†Deceased.
married men (30% compared with 14%). It has been observed "In many developing countries, prostitutes constitute the majority of 'promiscuous woman pool': as this group is so important in epidemiology it deserves a closer study" [12]. Reported reasons for women going into prostitution include poverty [8], socioeconomic reasons [13], divorce [8, 12], and "one child sterility" [12], although 27% of prostitutes in Lagos are childless [8]. Amongst urban prostitutes in Kenya 44% were divorced or separated women [12] as were 40% of Nigerian city prostitutes, 33% because of unhappiness at home [8].

Despite its key role in the heterosexual transmission of STD and HIV, there have been remarkably few medico-socioeconomic studies of the causes for and medical sequelae of prostitution. Education, promotion of the practice of having one faithful partner for life, together with barrier contraception for those who are unable or unwilling to stick to one partner [14] are recommended for the control of HIV and STD.

The aim of this paper was to compare women involved in prostitution with a group of women still married to their first husband and having had only one sexual partner, in order to ascertain what factors if any contributed to women going into prostitution or staying still married to their first husband, their only sexual partner, and thereafter to compare clinical and serological aspects of the gynaecological conditions and problems of the women in these two groups.

The data presented here are from a larger international collaborative study to investigate the prevalence of STD and cervical cancer in women attending hospital and health centre clinics in Addis Ababa, Ethiopia, and the possible socioeconomic causal factors of these conditions. The protocol for recording data was set up according to the purpose of this main study.

**MATERIALS AND METHODS**

**Patient population**

We studied 2111 Ethiopian women—53% were symptomatic gynaecological outpatients (GOPD) and 47% were asymptomatic women attending routine Family Planning (FPA), Antenatal (ANC) and Postnatal (PNC) clinics in Addis Ababa, Ethiopia, and the possible socioeconomic causal factors of these conditions. The protocol for recording data was set up according to the purpose of this main study.

**Marital details**

Twenty women were 'single' (of these a few had been married before as they gave a different age for age at first marriage and age at first coitus). Data for age at first marriage was missing for 104; 99% of other women married as virgins. Child betrothal/marriage was frequently practised, sometimes the girl living with her mother-in-law until she was considered old enough for consummation of marriage. Marriage practices in Ethiopia and acceptance of prostitution are described elsewhere [16].

In this study we were interested in comparing the socioeconomic and medical aspects of women of two contrasting sexual behaviours: those with a stable marriage to their one and only sexual partner, their first husband (1H/1PWSM = 1 husband/sexual partner women still married) and those with > 5 sexual partners. It appeared that only these two groups could be established with a sufficient degree of certainty, based on cultural knowledge, for statistical comparison. The other categories showed much more variable sexual behaviour: for example we noted that a few divorced couples attended for contraceptive advice (this may have been just a precaution on the part of those who had been divorced because they refused to bear any more children); others stated that they had been married—divorced—remarried, or married—divorced—local beer [taita] seller, thus we could not record with certainty the exact number of sexual partners. Preliminary statistical analysis showed that these other categories grouped together formed a
third, intermediate group between the 1H/PWSM and those in prostitution, probably due to the fact that it includes women with rather different sexual behaviour especially with regard to age at first coitus and number of husbands. As a consequence this analysis of basic demographic data—age, ethnic group, religion, origin of residence, age at first marriage, and age at first coitus and relation of sexual debut to the menarche—showed the women divided into three groups:

(1) those still married to their one and only sexual partner, their first husband (1H/PWSM), having been married as virgins.
(2) those who were involved in prostitution, and
(3) the remainder: singles, widowed, divorced or married but to their second or subsequent husband.

For analysis of the remaining socioeconomic data—monthly family income, duration of marriage, sexual life duration, parity and total number of pregnancies—use of contraceptives, self-history of STD, clinical findings and results of serological tests, we compare and contrast only two groups:

(1) those still married to their one and only sexual partner, their first husband, having been married as virgins (1H/PWSM), and
(2) those who were involved in prostitution.

Women involved in prostitution

We have accepted the traditional definition of prostitution as selling sexual favours for money. In Ethiopian culture at the time of the study, premarital sexual activity was not acceptable. A girl had to be a virgin at the time of her first marriage. There was thus almost no path to prostitution from being single and a virgin. Girls/women would go into prostitution only after a broken marriage, as a result of widowhood, or possibly after running away from an incompatible marriage (frequently to a much older husband), and destitution, to support themselves (and their children) financially in a country where there was no social security. In Ethiopian culture three subgroups were recognised: bargirls, (professional) prostitutes and talla sellers.

In the study we use these three sub-groups of women involved in prostitution:

(i) Bargirls who are the youngest are employed by bar owners to serve (as waitresses) in bars, and to provide the physical needs of their customers. These women are paid a monthly wage irrespective of their work load. Most of the bars are advertised by coloured strip lights and the name of the bar, some have a bead curtain in the doorway.

(ii) Prostitutes. These women, the professional prostitutes (seit aderi), are self-employed and put a coloured (red, blue, green) light in the window of their house. The youngest stand (and solicit) in front of their doorway or in the streets. The older women, better known, sit at home and wait for their clients. The higher class prostitutes would also accompany their clients to night clubs.

(iii) Talla sellers. Talla [local beer] is made by many Ethiopian women as a beverage for general consumption and special occasions. The art of preparing talla is learned in childhood and the early teens; however, women who call themselves talla sellers work from home, many of them sitting outside their house selling beer to passers-by returning from local markets or advertising their produce by an upturned tin can on a stick in front of their house. These women, frequently widows or divorcees, are among the oldest and poorest of those involved in prostitution. Not all talla sellers are categorically involved in prostitution as some married women may supplement a low family income by making and selling talla. Seven out of 132 talla sellers (5%) reported having become talla sellers without explicitly stating that they were separated from their husbands. We left them in the talla seller group as there was a high probability, but not absolute certainty, that they were involved in prostitution.

In the study the categories marital status/prostitution were thus mutually exclusive.

Epidemiological and socioeconomic data

For each patient data were obtained using a questionnaire completed in privacy by an Ethiopian female assistant. Random checks were made on these data at the time of the clinical examination to ensure accurate completion of the questionnaires. These data included: ethnic group, religion, residence (urban/rural), clinic attended, age, age at first marriage, age at first coitus and relation to this to menarche, sequential number of husbands/sexual partners (polyandry was not practised), last marital status/profession (single/married/divorced/widow/housemaid/bargirl/talla seller/prostitute), family monthly income, parity, total number of pregnancies, use and type of contraception, and chief complaint/reason for attending the hospital/clinic.

Clinical data

These data resulted from a full gynaecological examination. Particular attention was paid to the condition of the cervix and for evidence of infection in the urethra, salpinges and Bartholin glands (USB) which were inspected (UB) and palpated (USB).

Cytology

Cervical smear preparations were made and transported to Liège where they were stained by Papanicolaou stain and read: excessive numbers of polymorphonuclear (PMN) cells in the smears was indicative of cervical infection.

Serological data is from results of tests in Ethiopia for syphilis using VDRL and FTA; in Edinburgh for syphilis using TPHA [17] and hepatitis B virus (HBV)
were bargirls, some form of or widowed while of Health. export samples, was using involved in women analysis of Association Statistic tors interaction ible.

Permission for the study, including permission to export all patient data, cytology slides and serum samples, was obtained from the Ethiopian Ministry of Health.

RESULTS
Of 2111 women interviewed, 278 (13%) stated that while they had at one time been married, single, widowed or divorced that they were now engaged in some form of prostitution. Of these 41 (15%) were bargirls, 105 (38%) were prostitutes and 132 (47%) were makers and sellers of local beer [talla]; 730 (35%) women in the study were married to their one and only sexual partner, their first husband; the remaining 1103 (52%) were single, widowed, divorced or married but to their second or subsequent husband.

1. Analysis of basic demographic data of the whole study population
Comparison of these three groups—women in prostitution, women married to their first husband and 'others'—is highly significant against most socioeconomic factors.

1.1. Distribution by age (Table Ia). There was a correlation between present age of the women and their marital status/profession (P < 0.001). More women married to their first husband (1H/PWSM) were younger than 34 years, while there were more women in prostitution in age groups 35-49 and over 50 years.

1.2. Distribution by Ethnic group, religion and origin (Table 1 (b,c,d)). Ethnic group, religion and origin are associated with prostitution (P < 0.001). More (77%) women in prostitution were Amharas, the majority (95%) being Ethiopian Orthodox and coming from the city, while very few (3%) were Gurage women 36% of whom are Moslems coming mainly from the countryside. Statistical analysis shows that ethnic group is a stronger factor than religion for this association. The proportion involved in prostitution was higher (P = 0.01) in those coming from the city compared with rural areas.

Table 1. Analysis of socioeconomic factors against (i) Women married to their first husband; (ii) women in prostitution (bargirls, prostitutes and sellers of tanna [local beer]); (iii) others (women married to their second or subsequent husband, single, widows, divorcees)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Married (1 Husband)</th>
<th>Prostitute</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Age</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>79 (10.9)</td>
<td>19 (6.9)</td>
<td>83 (7.6)</td>
<td>181 (8.6)</td>
</tr>
<tr>
<td>20-34</td>
<td>333 (73.2)</td>
<td>183 (66.6)</td>
<td>705 (64.3)</td>
<td>1421 (67.8)</td>
</tr>
<tr>
<td>35-49</td>
<td>108 (24.8)</td>
<td>63 (23.0)</td>
<td>272 (24.8)</td>
<td>443 (21.1)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>8 (11.1)</td>
<td>9 (3.3)</td>
<td>36 (3.3)</td>
<td>53 (2.5)</td>
</tr>
<tr>
<td>Total</td>
<td>728 (100.0)</td>
<td>274 (100.0)</td>
<td>1096 (100.0)</td>
<td>2098</td>
</tr>
</tbody>
</table>

(Missing Data = 13)

<table>
<thead>
<tr>
<th>(b) Ethnic group</th>
<th>Married (1 Husband)</th>
<th>Prostitute</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amhara</td>
<td>314 (43.1)</td>
<td>215 (77.3)</td>
<td>694 (63.1)</td>
<td>1223 (58.1)</td>
</tr>
<tr>
<td>Oromo</td>
<td>103 (14.4)</td>
<td>31 (11.2)</td>
<td>181 (16.5)</td>
<td>317 (15.0)</td>
</tr>
<tr>
<td>Tigré</td>
<td>77 (10.5)</td>
<td>17 (6.1)</td>
<td>82 (7.5)</td>
<td>176 (8.4)</td>
</tr>
<tr>
<td>Gurage</td>
<td>190 (26.1)</td>
<td>9 (3.2)</td>
<td>119 (10.8)</td>
<td>318 (15.1)</td>
</tr>
<tr>
<td>Other</td>
<td>43 (5.9)</td>
<td>5 (2.2)</td>
<td>23 (2.1)</td>
<td>72 (3.4)</td>
</tr>
<tr>
<td>Total</td>
<td>729 (100.0)</td>
<td>278 (100.0)</td>
<td>1099 (100.0)</td>
<td>2106</td>
</tr>
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(Missing Data = 5)

<table>
<thead>
<tr>
<th>(c) Religion</th>
<th>Married (1 Husband)</th>
<th>Prostitute</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethiopian Orthodox</td>
<td>594 (85.1)</td>
<td>262 (95.3)</td>
<td>1000 (9.17)</td>
<td>1856 (89.9)</td>
</tr>
<tr>
<td>Moslem</td>
<td>104 (14.9)</td>
<td>13 (4.7)</td>
<td>91 (8.3)</td>
<td>208 (10.1)</td>
</tr>
<tr>
<td>Total</td>
<td>698 (100.0)</td>
<td>275 (100.0)</td>
<td>1091 (100.0)</td>
<td>2064</td>
</tr>
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</table>

(Missing Data = 47)

<table>
<thead>
<tr>
<th>(d) Origin</th>
<th>Married (1 Husband)</th>
<th>Prostitute</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>580 (81.1)</td>
<td>245 (89.1)</td>
<td>893 (82.7)</td>
<td>1718 (83.0)</td>
</tr>
<tr>
<td>Rural</td>
<td>135 (18.9)</td>
<td>30 (10.9)</td>
<td>187 (17.3)</td>
<td>352 (17.0)</td>
</tr>
<tr>
<td>Total</td>
<td>715 (100.0)</td>
<td>275 (100.0)</td>
<td>1080 (100.0)</td>
<td>2070</td>
</tr>
</tbody>
</table>

(Missing Data = 41)
1.3. Age at first marriage and first coitus and relation to the menarche (Fig. 1). Twenty women were single: data for age at first marriage was missing for 104; 99% of other women were married as virgins. Five percent had first coitus later than first marriage; for the remainder age at first marriage and age at first coitus have the same value. We present here age at first marriage.

Eighty-one percent of those involved in prostitution were married by age 15; in contrast to 47% of those still married to their first husband (1H/PWSM). Forty-two percent of prostitutes were married under the age of 13, compared with 9% of those married to their first husbands (1H/PWSM). Of the whole study group, 66% of those marrying over the age of 18 were still married to their first husband (1H/PWSM) while only 4% were involved in prostitution. A similar picture was seen with the analysis of age at first coitus.

More (67%) women in prostitution had been sexually active before the menarche than women (31%) still married to their first husband (1H/PWSM). Detailed analysis shows that 71% prostitutes, 66% talla sellers and 61% bargirls had been sexually active before the menarche.

2. Comparison of women in prostitution and women with one husband

The following factors contrast prostitutes with women still married to their first husband (1H/PWSM).

2.1. Duration of marriage (Table 2a). More (48%) women entering prostitution were married for less than 5 years compared with 33% still married to their first husband (1H/PWSM), although 15%, 10% and 6.0% of widowed or divorced women entered prostitution after 11, 16 or 21 years of marriage respectively. Detailed analysis shows that 77% of bargirls, 50% of prostitutes and 39% talla sellers were married for less than 5 years.

2.2. Income (Table 2b). Only 58% prostitutes and 82% married women stated details of family income. Income for women involved in prostitution was essentially earnings through their profession, except for talla sellers, whose main income was from selling beer (talla). Married women were able to state the earned income of their husbands and themselves (if they were working). In some cases the husband’s occupation was a guide to the family income. Where the husband’s income was unknown, and his wife received only housekeeping money the income was regarded as ‘missing data’. 95% women involved in prostitution had an income of <50 EB per month compared with 42% women married to their first husband (1H/PWSM). Detailed analysis shows that while 95% bargirls, 96% prostitutes and 94% talla sellers had a monthly income of <50EB; 5% bargirls, 42% prostitutes and 45% talla sellers had <10EB per month.

2.3. Sex life duration (Table 2c). Thirty percent of women in prostitution had a sexual life >20 years compared with 14% women married to their first husband (1H/PWSM), reflecting earlier age at first coitus and rather older age of those in prostitution.

2.4. Parity and total number of pregnancies (Table 2d, e). The association between women in prostitution and women still married to their first husband (1H/PWSM), with both parity and total number of pregnancies suggested that primary infertility was not a major problem and hence a cause for divorce for women who subsequently went into prostitution; that 3% more pregnancies than married women (1H/PWSM) had had only 1 or 2 pregnancies may indicate that one or two child secondary infertility may have been a factor for breakdown of marriage and drift into prostitution.

2.5. Use and method of contraception (Table 2f). Forty-three percent of women in prostitution used contraception compared with 33% married women (1H/PWSM): 40% sex workers using contraceptive pills and 2% an intrauterine contraceptive device (IUCD) compared with 25% and 6% respectively of women still married to their first husband (1H/PWSM). Detailed analysis showed 73% bargirls, 45% prostitutes and 30% talla sellers used contraceptives.

2.6. History of STD. Self history of some form of STD was more frequent in women in prostitution (44% 105/237) than in women still married to their first partner (12% 72/591) (P < 0.001).

2.7. Gynaecological problems and conditions of prostitutes and women married to their first husband. Fifty percent of the women involved in prostitution attended for gynaecological problems, 40% for family planning advice or check-up, and 10% for ante¬natal or postnatal care, in contrast to 44%, 26% and 30% respectively for women still married to their first
husband (1H/PWSM) (Table 2g). The higher prevalences of STD, pelvic inflammatory disease (PID) and cervical cancer seen in sex workers compared with married women (1H/PWSM) together with increased risk for these conditions are shown in Table 3. Only 1% women in prostitution and 9% women married to their first husband (1H/PWSM) had no serological evidence of STD, while 16% and 13% respectively had been exposed to all six STD (Table 4).

Table 2. Analysis of women married to their first and only sexual partner and women involved in prostitution showing the percentage distribution of women with regard to (a) duration of sexual life, (b) parity, (c) total number of pregnancies and (d) use of contraceptives

<table>
<thead>
<tr>
<th>Factor</th>
<th>Married (1 husband)</th>
<th>Women in prostitution</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Years married</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>240 (33)</td>
<td>130 (48)</td>
<td>370 (37)</td>
<td></td>
</tr>
<tr>
<td>6-10</td>
<td>169 (23)</td>
<td>54 (20)</td>
<td>223 (22)</td>
<td></td>
</tr>
<tr>
<td>11-15</td>
<td>157 (21)</td>
<td>41 (15)</td>
<td>198 (20)</td>
<td></td>
</tr>
<tr>
<td>16-20</td>
<td>92 (13)</td>
<td>27 (10)</td>
<td>119 (12)</td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>61 (8)</td>
<td>16 (6)</td>
<td>77 (8)</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>11 (2)</td>
<td>1 (1)</td>
<td>12 (1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>730</td>
<td>269</td>
<td>999</td>
<td></td>
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</tbody>
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(Missing Data = 9)

(b) Family monthly income in Ethiopian Birr (1EB = $1 U.S.)

<table>
<thead>
<tr>
<th>Value</th>
<th>&lt;10</th>
<th>10-50</th>
<th>50-100</th>
<th>100-500</th>
<th>&gt;500</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>71 (12)</td>
<td>182 (30)</td>
<td>88 (14)</td>
<td>203 (34)</td>
<td>58 (10)</td>
<td>712</td>
</tr>
<tr>
<td>(Missing Data = 244)</td>
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<td></td>
<td></td>
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</table>

(c) Sex life duration (yr)

<table>
<thead>
<tr>
<th>Value</th>
<th>&lt;10</th>
<th>10-19</th>
<th>20</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>350 (49.2)</td>
<td>260 (36.5)</td>
<td>102 (14.3)</td>
<td>712</td>
</tr>
<tr>
<td>(Missing Data = 32)</td>
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(d) Parity

<table>
<thead>
<tr>
<th>Value</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3-5</th>
<th>6-10</th>
<th>&gt;10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>119 (16.3)</td>
<td>121 (16.6)</td>
<td>120 (16.5)</td>
<td>226 (31.0)</td>
<td>129 (17.7)</td>
<td>14 (1.9)</td>
<td>729</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td></td>
<td></td>
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</table>

(e) Total number of pregnancies

<table>
<thead>
<tr>
<th>Value</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3-5</th>
<th>6-10</th>
<th>&gt;10</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>100 (13.7)</td>
<td>116 (15.9)</td>
<td>124 (17.0)</td>
<td>221 (30.3)</td>
<td>142 (19.5)</td>
<td>26 (3.6)</td>
<td>729</td>
</tr>
<tr>
<td>(Missing Data = 4)</td>
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<td></td>
<td></td>
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</table>

(f) Method of contraception

<table>
<thead>
<tr>
<th>Method</th>
<th>Pill</th>
<th>IUCD</th>
<th>Sterilised</th>
<th>Other</th>
<th>None</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>177 (25.6)</td>
<td>45 (6.4)</td>
<td>5 (0.7)</td>
<td>5 (0.7)</td>
<td>475 (67.2)</td>
<td>707</td>
</tr>
<tr>
<td>(Missing Data = 32)</td>
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(g) Clinic attended

<table>
<thead>
<tr>
<th>Clinic</th>
<th>GOPD</th>
<th>FPC</th>
<th>ANC</th>
<th>PNC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%)</td>
<td>318 (43.6)</td>
<td>189 (25.9)</td>
<td>185 (25.4)</td>
<td>37 (5.1)</td>
<td>729</td>
</tr>
<tr>
<td>(Missing Data = 1)</td>
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</tr>
</tbody>
</table>

GOPD = Gynaecological Out Patient Department; FPC = Family Planning Clinic; ANC = Antenatal Clinic; PNC = Postnatal Clinic.
Table 3. Analysis of women married to their first and only sexual partner, and women involved in prostitution, showing the serological evidence of exposure to sexually transmitted disease, and diagnosis of pelvic inflammatory disease and cervical cancer

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Tested No.</th>
<th>Affected No. (%)</th>
<th>Tested No.</th>
<th>Affected No. (%)</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis (TPHA)</td>
<td>634</td>
<td>133 (20%)</td>
<td>240</td>
<td>149 (62%)</td>
<td>6.8</td>
<td>5.1-9.3</td>
</tr>
<tr>
<td>Gonorrhoea</td>
<td>640</td>
<td>258 (40%)</td>
<td>242</td>
<td>213 (88%)</td>
<td>10.9</td>
<td>7.5-15.6</td>
</tr>
<tr>
<td>Chlamydia (D-K &amp; LGV)</td>
<td>635</td>
<td>342 (54%)</td>
<td>242</td>
<td>189 (78%)</td>
<td>3.1</td>
<td>2.2-4.3</td>
</tr>
<tr>
<td>HSV2</td>
<td>635</td>
<td>209 (33%)</td>
<td>242</td>
<td>111 (46%)</td>
<td>1.7</td>
<td>1.3-2.3</td>
</tr>
<tr>
<td>HBV</td>
<td>641</td>
<td>225 (35%)</td>
<td>244</td>
<td>113 (46%)</td>
<td>1.6</td>
<td>1.2-2.2</td>
</tr>
<tr>
<td>Chancroid</td>
<td>630</td>
<td>85 (13%)</td>
<td>237</td>
<td>46 (19%)</td>
<td>1.5</td>
<td>1.0-2.3</td>
</tr>
<tr>
<td>PID (BUS)</td>
<td>720</td>
<td>322 (45%)</td>
<td>275</td>
<td>171 (62%)</td>
<td>2.0</td>
<td>1.5-2.7</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>723</td>
<td>7 (1.0%)</td>
<td>270</td>
<td>8 (2.9%)</td>
<td>3.1</td>
<td>1.2-8.1</td>
</tr>
</tbody>
</table>

Odds Ratio shows the increased risk for prostitutes compared to women married to their first husband.

CT = C. trachomatis (D-K & LGV).
HSV2 = Herpes simplex virus 2.
HBV = Hepatitis B virus.
PID = Pelvic inflammatory disease.

Gonococcal antibody titres \( \geq 1/320 \) were shown in 35%, 46% and 71% of talla sellers, prostitutes and bargirls respectively [26], while genital chlamydial IgG \( \geq 1/64 \) or IgM were recorded in 58%, 71% and 75% of talla sellers, prostitutes and bargirls respectively [27]. These titres indicate active/present infection.

2.8. Multivariate analysis. In the above analysis we found that the most significant socioeconomic factors contrasting the women in prostitution with those still married to their first and only sexual partner, their first husband (1H/PWSM) were: ethnic group, religion, age, age at first marriage, income, timing of first coitus in relation to the menarche, parity and number of years of marriage. A multivariate logistic regression model was fitted on these data excluding patients with missing data for one of the above mentioned variables. The stepwise logistic regression procedure enters variables in the model one by one in decreasing order of significance and stops the process when the entry of a new variable does not improve the goodness of fit test. In that process the most significant factors associated with prostitution were found to be income, ethnic group, the timing of first coitus in relation to the menarche, number of years of marriage, and age at first marriage, in that order. All these variables were entered with highly significant \( P \) value (\( P < 0.001 \)). When these factors are in the model the remaining variables, religion and parity, do not reach the significance value (associated value of \( P > 0.1 \)).

DISCUSSION

We have shown that there are significant differences between women with a stable first marriage (1H/PWSM) and those involved in prostitution with regard to all socioeconomic factors as well as the clinic attended, the reason for hospital attendance, past history of STD, prevalence of STD, PID and cervical cancer, and the use and method of contraception.

The analysis of socioeconomic factors leading to either prostitution or to stable first husband marriage (1H/PWSM) is highly complex as will be apparent from the following discussion, and will be considered in order of significance as found from the multivariate analysis.

Data for family income was missing for 244 (29%) of the women studied. Reasons for this lack of information are chiefly ignorance of husband's income and the nature of employment as casual labour normally paid on a daily basis with most of the earnings being spent on food for the next 24 hr. Women in prostitution, apart from the bar girls who were paid a basic monthly wage with free board and lodgings, were mostly self-employed and used their earnings to support themselves and their children.

Table 4. Comparison of women involved in prostitution and women with stable first marriage showing the numbers seronegative or seropositive to one or more of the STD tested (syphilis, gonorrhoea, genital chlamydia, genital herpes, hepatitis B and chancroid)

<table>
<thead>
<tr>
<th>Number of STDs seropositive</th>
<th>Seronegativity</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostitutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>4</td>
<td>17</td>
<td>27</td>
<td>54</td>
<td>83</td>
<td>48</td>
<td>45</td>
<td>279</td>
</tr>
<tr>
<td>(%)</td>
<td>1.4</td>
<td>6.1</td>
<td>9.7</td>
<td>19.4</td>
<td>29.9</td>
<td>17.3</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>Married women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>66</td>
<td>126</td>
<td>192</td>
<td>148</td>
<td>69</td>
<td>34</td>
<td>95</td>
<td>750</td>
</tr>
<tr>
<td>%</td>
<td>9.0</td>
<td>17.3</td>
<td>26.3</td>
<td>20.3</td>
<td>9.4</td>
<td>4.7</td>
<td>13.0</td>
<td></td>
</tr>
</tbody>
</table>

Sero-negativity = no sero-positivity for any STD.
Thus they were asked to state what their monthly ‘family’ income was. Essentially these women should be regarded as being on subsistence income. A few better off professional prostitutes could have been reluctant to state their earnings for personal reasons. Despite the high percentage of missing data, it is unlikely that it caused a bias in interpretation of the results.

The stated monthly family income reflects the economic status of the women at the time of the study. Low income of prostitutes may be both a result of and cause for prostitution—95% of prostitutes, compared with only 42% women married to their first husband (1H/PWSM), coming from the lowest income groups. Poverty was also related to very young age at first marriage and first coitus [28, 29]. The association between age at first marriage and family income is highly significant ($P < 0.001$; details not shown in the tables): 76% of those first married by 12 or younger had a monthly income of 50 EB or less, and 69% of those first married aged 13–15 years of age had a monthly family income of 50 EB or less. In contrast, 61% of those first married aged 19 or older had a monthly family income of 100 EB or more. For those first married aged 16–18 years, approximately half (53%) were in the lower income group, while 47% had monthly family incomes of 100 EB.

While ethnic group and religion can appear to be significantly associated with prostitution, in this study the multivariate analysis shows that ethnic group is the dominant factor not only because it has the best significance test value, but also because when ethnic group enters the model the religion variable loses almost all its significance. This may reflect that the study was carried out in a predominantly Amhara area of the country where 95% Amhara women were Ethiopian Orthodox. A larger study involving similar numbers of women from different ethnic groups in their own cultural environment would be required to confirm or refute this observation.

We have already shown that Amhara women from the lower income groups marry earlier and hence are sexually active younger than other women [28, 29]. The earlier age of marriage of Amharas is recorded elsewhere [16], and is substantiated by the observation (unpublished) that large numbers of young Amhara women from Gojam and Begemdir provinces are treated for vesico-vaginal fistulae (childbirth injuries) in Addis Ababa. Ethnic factors also determine the livelihood of widows and divorcees: Gurage women are more likely to become merchants and traders while younger Amhara women from the rural areas find employment in bars and as maid servants and older women tend to become sellers of local beer and prostitutes.

Arranged marriage of young girls, not infrequently to older men, is widely practised in many developing countries, with a variable time lapse before consummation of marriage. In certain circumstances in Ethiopia, arranged marriage could be more accurately equated with a betrothal ceremony. In this study 99.4% were first married as virgins and the age at first coitus was for the majority the age at first marriage. Thirty-eight percent of prostitutes and 8% women still married to their first husband (1H/PWSM) experienced their sexual debut aged 12 years or younger while 20% prostitutes and 54% women with a stable first marriage (1H/PWSM) had first coitus aged 16 or older.

Divorce subsequent to one or two child infertility may explain the shorter duration of marriage of prostitutes and may have led to the mother supporting herself and her child(ren) by prostitution. One pre-pubertal 13 year old before becoming a barmaid had been married and divorced 3 times on grounds of childlessness.

Early age of marriage has in itself been shown to have certain socioeconomic consequences. It has been reported elsewhere that the earlier the first marriage the less stable that marriage, the greater risk of breakdown of marriage, divorce and remarriage, multiple partners and eventually the young woman running away from home and her older husband seeking a better way of life in the cities, the breakdown of the marriage is by prostitution [16, 29, 30], or working as a housemaid. The analysis of our results is consistent with this observation. Moreover we have shown that the early age at first coitus is significantly associated with poverty: women with older age at first marriage/coitus being chiefly those with higher income [28, 29].

There is evidence of increased genococcal infection in those married and sexually active younger. This is due to anatomical, physiological and immunological immaturity of the genital tract of the child or young teenager as well as to behavioural factors of her partner [15].

In general self-reported history of STD is under-reported [1]. In Ethiopia there was little or no social stigma associated with having had either syphilis or gonorrhea, both diseases having been highly prevalent since the early twentieth century [31], only 44% prostitutes gave a history of STD although 98.6% had serological evidence of at least one STD. Reasons for this under-reporting include:

(i) the asymptomatic nature of early infection, women presenting late for treatment;
(ii) in the pre-HIV/AIDS era, in common with other African countries, injections frequently of penicillin, and often with streptomycin, were the common treatment for pelvic infections—a woman was likely to have been treated for PID without the underlying STD being diagnosed;
(iii) STD is widespread and with a limited health budget (0.36 EB per person per annum) [16] notification and contact tracing cannot be practised.
When the age factor is analysed against the whole study group it is of interest that the group of women including the divorcees, widows and those married to their second or subsequent husband form a highly significant intermediate group between those who are still married to their first husband and those involved in prostitution. Widows or divorcees, because of poverty, were likely to go into prostitution, many within this group being makers of local beer.

As expected the prevalence rates and titres of STD were very high in sex workers. Barrier contraception was rarely used being contrary to local custom. Thus among women in prostitution prevalence rates of STD were high resulting in 62% having PID. Several serological tests used in the study measured overall exposure to infection [21,27] rather than present active infection although in industrialised countries the high titres of antibodies detected would represent active infection.

The high prevalence of gonorrhoea, 88% overall exposure, in Ethiopian prostitutes is in contrast to 10% recorded for Ibadan, Nigeria, where prostitutes took prophylactic antibiotics [11], but compares with 86% reported from 15–25 year old prostitutes from Rwanda [32]. The overall prevalence, 78%, for genital chlamydial infection is rather less than the 94% and 100% prevalence for prostitutes in Iran [33] and Algiers [34]. The prevalence of IgG to H. ducreyi we report for women still married to their first husband (13%) is higher than that reported for pregnant women in Kenya (9%) [21].

The prevalence rate of PID in Ethiopian prostitutes (62%) compares with 67% for prostitutes in Lagos who had signs and symptoms of STD in the previous 6 months [8]. The prevalence of HSV2 and HBV in the Ethiopian study is similar to that (46%) reported from Somalia [35] and 43% for South African black prostitutes [36].

The unexpectedly high STD prevalence rates in women still married to their first husband (Tables 3 and 4) is indicative of a male factor or male promiscuity [14].

Control of gonorrhoea and other STDs and their sequelae requires a greater recognition of the problems by health services and at a governmental level [37] by understanding the causes for prostitution [12], providing regular checks for prostitutes [12], recognising patterns of sexual behaviour, and obtaining reliable data on that (difficult in industrialised countries and a far greater problem in Africa [38]), and education of prostitutes [38]. However by targeting policies to prostitution and female sex workers, as has chiefly been the case worldwide, the most important issue has been largely overlooked both in industrialised and developing countries, for example: “A promiscuous female pool (PFP) with many silent infections, infects a larger number of promiscuous males; these men feed back gonorrhoea (STD) into the PFP and may also infect a non-promiscuous secondary contact, usually the wife” [12, 39].

Some communities have double standards in sexual matters. Young men are readily permitted, even encouraged, to indulge in promiscuous sexual activity with prostitutes. Urethral discharge is regarded as a sign of adolescence or evidence of sexual potency, while young girls are expected to remain chaste. Even after marriage the same double standards pertain [40]. In Ethiopia a commonly held belief was that an adolescent male was not a man until he had had syphilis (unpublished observation). In other African countries the religious practices of polygamy and purdah place some restrictions on women but do not restrict the men from promiscuous sexual behaviour. The men infect their wives. Infections lead to marital disharmony and divorce, and the unfortunate woman has no livelihood apart from prostitution [41]. These observations by African researchers on male promiscuity or male factor apply equally elsewhere.

Male factor was first described in industrialised countries in studies on cervical cancer and the number of sexual partners. In married women with cervical cancer who claimed not to have had a sexual partner other than their husbands, the number of sexual partners of the husbands was found a significant risk factor for cervical cancer [42]. This male promiscuity which has been called the 'male factor' [43] is clearly more likely to occur with prostitution and with child marriage, where a child is usually married to a sexually experienced man.

Differences in methods of contraception used by the two groups resulted from medical policy that the IUCD would not be fitted for women known to be engaged in prostitution. Workers at FP clinics had noted in women in prostitution a high prevalence of both STD and salpingitis. Fitting an IUCD for a woman who had clinical evidence of old salpingitis almost always resulted in recurrent acute salpingitis, necessitating immediate removal of the IUCD (Duncan M. E. and Mehari L. unpublished observation). By issuing the pill for a month at a time, it was possible to provide early treatment for symptomatic STD and PID at FP clinics. Those of the high risk group who failed to avail themselves of free contraceptive advice were chiefly the talla sellers, the oldest and poorest of those in prostitution.

The earlier policy of using oral contraceptives, now recognised as causing cervical ectropion thus exposing the transformation zone and the endocervical epithelium to bacterial and viral agents and leading to increased CC and risk of HIV transmission, is now being replaced by education and supply of free condoms. While it may be claimed that oral contraception emancipated the woman, use of condoms as barrier contraception is slower to gain acceptance because of the need for male compliance and acceptability.

Prevalence of cervical cancer in prostitutes was three times that in married women with one husband, although not statistically significant because of small
numbers; prostitution may be a factor for cervical cancer in talla sellers who share the combined risk factors of age and multiple sexual partners [44].

**Control of prostitution**

Teenage pregnancy may interrupt schooling and further training with no job prospect other than prostitution, and child marriage causes the run-away-from-home syndrome. While control and limitation of prostitution by education may be supported by medical opinion, national awareness and action is required to support existing legislation thus raising the age of marriage/consent to 16 years and preferably to 18 years, and to encourage the practice of sound ethnic traditions and taboos which prohibit sexual activity prior to marriage and also relationships between older men and young girls. Further action could prohibit/limit the activity of women in bars and nightclubs as was tried in China with effective although short lived drastic reduction of prostitution [45].

Education of girls has already been shown to increase the age at marriage as girls quickly become more interested in schooling than in early marriage, working in the home and in the fields. Moreover education of girls results in a significant decrease in maternal, infant and perinatal mortality, and increased acceptance of family planning [46,47]. A review of recent studies in Ethiopia, however, reveals disturbing developments amongst high school students: 53% of male and 24% of female students were reportedly sexually active, 60% with 2-5 partners, while nearly half of those currently engaged in prostitution had given up their education and turned to that profession as their only means of self support [48]. Indeed in some towns there is a small, newly emerging group of women involved in prostitution, namely *temarereoch* [female students] who are resorting to prostitution to pay for the cost of their continuing education, books and clothes (unpublished observation). STDs constitute a serious health problem in Ethiopia, the magnitude of which has increased steadily from the early 1980s and is likely to increase further in the near future [48].

Education of women and girls alone is not enough because male promiscuity is so evident in transmission of STD. Adult men and teenage boys must be educated, by whatever means are socially and culturally acceptable, regarding their role in prevention of STD. While the most effective and feasible control measures in the near term appear to be educational, supported by treatment campaigns targeted at high risk groups, control of STD in the long term will require major socioeconomic changes that result in greater stability of marriages and greater security of single women and juveniles together with changes in attitudes toward family planning, prostitution and STD [48,49].

**Acknowledgements** — We thank the staff and patients of St Pauls and the Black Lion Hospitals and Lidet Clinic for their co-operation and Dr Philippa Wilson for her assistance in collecting data. We thank the staff of NAMRU-5, Addis Ababa, Ethiopia, for the initial syphilis tests and storage of sera; Drs I. Lind and K. Reimann and the Neisseria Department of the Statens Seruminstitut, Copenhagen for testing sera for antibodies to *Neisseria gonorrhoea*. We acknowledge with thanks financial assistance received from Allied Medical Group [United Medical Enterprises] for the cost of the serological tests, the Welcome Trust for travel and secretarial expenses for M.E.D. and SAREC, Stockholm, Sweden for support for P.P. and E.R. We thank Mrs M. Pearce for secretarial help.

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assistance. Dr Camarda was supported by a fellowship on the FLAIR No. 6 Programme ‘Prevention and control of potentially pathogenic microorganisms in poultry and poultry meat processing’.

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Rapid identification of pathogenic neisserias using the Identicult-Neisseria test

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DST/57: received 24 March 1994 and accepted 11 April 1994

A. MOYES, G. CHRONAS AND H. YOUNG. 1994. A rapid enzymatic method using chromogenic substrates for the rapid identification of pathogenic neisseria (Identicult-Neisseria, Scott Laboratories Inc., CA, USA) was tested in parallel with the rapid carbohydrate utilization test (RCUT) and the Phadebact Monoclonal GC Test against 198 consecutive clinical isolates of oxidase-positive Gram-negative diplococci (118 Neisseria gonorrhoeae, 76 N. meningitidis and four N. lactamica). On initial testing the Identicult-Neisseria gave a 95% overall concordance (97-5% N. gonorrhoeae, 90-8% N. meningitidis) with the RCUT and Phadebact tests; the corresponding figures after repeat testing were 98% overall concordance (98-3% N. gonorrhoeae, 97-4% N. meningitidis). Two of the three strains of N. gonorrhoeae mis-identified as N. meningitidis on primary testing were also mis-identified on repeat testing. Seven strains of N. meningitidis were mis-identified on initial testing (six as Moraxella catarrhalis and one as N. lactamica) and two on repeat testing (both as Mor. catarrhalis). We conclude that the Identicult-Neisseria is not sufficiently reliable for the culture confirmation of gonococci and meningococci.

INTRODUCTION

Laboratory identification of Neisseria species has traditionally depended upon carbohydrate degradation tests. In conventional tests such as cysteine tryptic agar 24-48 h growth is required before the detection of acid production (Morello et al. 1985). Commercially available rapid methods for the culture confirmation of Neisseria gonorrhoeae include: immunological tests such as fluorescent monoclonal antibody staining (Ison et al. 1988; Welch and Cartwright 1988) and coagglutination using monoclonal antibodies raised to epitopes on the gonococcal outer membrane protein I (Young and Moyes 1989); carbohydrate utilization tests for the detection of preformed enzymes (Young et al. 1976); radiometric detection of 14C-labelled gas production (Boyce et al. 1985); and more recently nucleic acid hybridization tests using a chemiluminescent single-stranded DNA probe (Naher et al. 1989; Sprott et al. 1989; Ridderhof et al. 1990; Young and Moyes 1993). Disadvantages have, however, been associated with some of these methods. Fluorescent antibody-negative (Moyes and Young 1989; Walton 1989) and coagglutination-negative strains have appeared (Young and Moyes 1989), a large inoculum is required for carbohydrate utilization tests while radiometric methods pose the problem of disposal of radioactive waste.

D’Amato et al. (1978) first described the use of chromogenic substrates for the identification of pathogenic Neisseria species. The Identicult-Neisseria identification system (Scott Laboratories Inc., Carson, CA, USA) utilizes three chromogenic substrates, impregnated on filter paper, to detect preformed enzymes associated with N. lactamica, N. meningitidis and N. gonorrhoeae. Neisseria lactamica is identified by the presence of beta-D-galactosidase (BDG), N. meningitidis by the presence of gamma-glutamyl-aminopeptidase (GAP) while N. gonorrhoeae is differentiated from N. meningitidis by the presence of prolylaminopeptidase (PAP) and absence of GAP. None of these enzymes is present in Moraxella catarrhalis.

The aim of this study was to evaluate the Identicult-Neisseria for routine clinical use by comparing it with the rapid carbohydrate utilization test and the Phadebact Monoclonal GC test (Karo Bio Diagnostics AB, Huddinge,
Sweden), both of which are currently employed in our laboratory.

**MATERIALS AND METHODS**

**Bacterial strains**

One hundred and ninety-eight consecutive isolates of oxidase-positive Gram-negative diplococci isolated from patients attending the Genitourinary Medicine Unit, Edinburgh Royal Infirmary were included in the study. The patient samples were inoculated directly onto modified New York City medium (MNYC) (Young 1979) and forwarded to the laboratory for incubation at 37°C in an atmosphere enriched with 5% carbon dioxide. The isolates were identified by the rapid carbohydrate utilization test (RCUT) (Young et al. 1976) and the Phadebact Monoclonal GC Test (Young and Moyes 1989). The strains comprised 118 isolates of *N. gonorrhoeae* (30 serogroup 1A and 88 serogroup IB), 76 isolates of *N. meningitidis* and four isolates of *N. lactamica*. On 66 occasions (37 *N. gonorrhoeae*, 28 *N. meningitidis*, one *N. lactamica*) sufficient growth was obtained on the primary isolation plate after 24 h incubation for all tests to be performed. The remaining 132 isolates (81 *N. gonorrhoeae*, 48 *N. meningitidis*, three *N. lactamica*) were subcultured onto non-selective medium (MNYC medium lacking antibiotics) to provide sufficient growth.

**Identicult-Neisseria**

Suspect colonies were taken from the MNYC medium with an inoculating loop and smeared onto the three test areas (A, B and C) of the test strip containing the chromogenic substrates. The test areas were first pre-moistened with a few drops of reagent 1 supplied with the kit. The strip was incubated at 37°C in an aerobic atmosphere and read after 10 min. A positive reaction (blue green colour formation) in area A containing substrate for BDG requires no further action; if there is no colour change a few drops of a second reagent are added to area B containing substrate for GAP and area C containing substrate for PAP. Positive reactions in B (purple colour formation) and C (red colour formation) should occur within 10 s. Expected results are shown in Table 1.

**Serotyping**

Serotyping of the gonococcal strains was performed using the Genetic Systems and Pharmacia panels of monoclonal antibodies as previously described (Moyes and Young 1991).

**Testing of discrepant isolates**

Strains showing discrepancies after subculture onto non-selective medium and retesting with RCUT and Identicult-Neisseria were tested by API Quadferm (Biomérieux, Marcy l’Etoile, France).

**Statistical analysis**

The Chi-squared test was used for all statistical analysis.

**RESULTS**

There was 100% agreement between the RCUT and the Phadebact Monoclonal GC test in differentiating between gonococcal and non-gonococcal isolates. Of the 66 isolates tested from the primary culture six discrepancies occurred between the RCUT and Identicult-Neisseria system. Three strains of *N. gonorrhoeae* were identified as *N. meningitidis* and three strains of *N. meningitidis* were identified as *Mor. catarrhalsis* by the Identicult-Neisseria system. An additional four discrepancies occurred in the 132 isolates subcultured before testing; all four discrepancies were in strains of *N. meningitidis*, three of which were identified as *Mor. catarrhalsis* and one as *N. lactamica* by the Identicult-Neisseria system. All 81 subcultured isolates of *N. gonorrhoeae* were correctly identified by both systems.

On repeat testing of the six discrepant primary isolates, the three mis-identified *N. meningitidis* strains were correctly identified as *N. meningitidis* by the Identicult-Neisseria

<table>
<thead>
<tr>
<th>Organism</th>
<th>A (beta-D-galactosidase)</th>
<th>B (gamma-glutamylaminopeptidase)</th>
<th>C (prolyl-aminopeptidase)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Neisseria lactamica</em></td>
<td>Blue/green</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>—</td>
<td>—</td>
<td>Red</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>—</td>
<td>Purple</td>
<td>or red</td>
</tr>
<tr>
<td><em>Moraxella catarrhals</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

—, No colour change.
system (Table 2). The three mis-identified *N. gonorrhoeae* strains were identified as *N. gonorrhoeae* (one) and *N. meningitidis* (two); these two strains were confirmed as gonococci by API Quadferm and reactivity with gonococcal serotyping antibodies. On repeat testing of the four mis-identified *N. meningitidis* strains from the subcultured isolates two strains were identified correctly as *N. meningitidis* but two strains were again mis-identified as *Mor. catarrhals* by Identicult-Neisseria, these two strains were confirmed as *N. meningitidis* by API Quadferm.

Both GAP and PAP enzymes were detected in 20 of the 76 strains of *N. meningitidis*; GAP only was detected in 54 strains while neither enzyme was detected in the two isolates mis-identified as *Mor. catarrhals*. Of the 118 gonococcal isolates, 116 were positive for PAP only; the two isolates mis-identified as meningococci were positive for both PAP and GAP. The 118 gonococcal isolates belonged to 17 different serovar combinations. The three mis-identified *N. gonorrhoeae* isolates belonged to three distinct serovars, namely: IB-2/Bpyust, IB-7/Bopyst and IB-8/Bpyut.

**DISCUSSION**

In this study we found that the Identicult-Neisseria only gave a 95% overall concordance (97.5% *N. gonorrhoeae*, 90.8% *N. meningitidis*) with the RCUT and the Phadebact Monoclonal GC test on initial testing of the 198 clinical isolates. Discrepancies were, however, higher when testing primary isolates (9%) than subcultures (3%); this difference just fails to reach significance at the 5% level ($\chi^2 = 3.37; P > 0.05 < 0.1$).

All three discrepant *N. gonorrhoeae* isolates on initial testing and two of the three isolates on repeat testing (Table 2) gave positive reactions for both GAP and PAP. The three discrepant *N. meningitidis* isolates on initial testing and two of the four isolates on repeat testing all gave a positive reaction for GAP after subculture. Insufficient production of GAP in a few strains of *N. meningitidis* on primary culture using MNYC medium has been described previously by Wood and Young (1986); the correct enzyme profile being obtained after subculture.

Table 2 Discrepancies between rapid carbohydrate utilization test (RCUT) and Identicult-Neisseria

<table>
<thead>
<tr>
<th>Isolate from</th>
<th>First test result</th>
<th>Repeat test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary plate</td>
<td>Subculture</td>
<td>RCUT</td>
</tr>
<tr>
<td>NG(3)</td>
<td>NM(3)</td>
<td>NG(3)</td>
</tr>
<tr>
<td>NM(3)</td>
<td>NM(4)</td>
<td>NM(3)</td>
</tr>
<tr>
<td>NM(4)</td>
<td>MC(3), NL(1)</td>
<td>NM(4)</td>
</tr>
</tbody>
</table>


In a similar study of 100 fresh clinical isolates (82 *N. gonorrhoeae*, nine *N. meningitidis*, five *N. lactamica*, four *Mor. catarrhals*) Hosmer et al. (1986) reported 100% correlation between the Neisseria-Identicult, fermentation tests on Columbia agar slants and the Phadebact Monoclonal GC test. Sperry et al. (1986) found 97% correlation between the Neisseria-Identicult and MNYC from a total of 104 isolates (54 *N. gonorrhoeae*, 40 *N. meningitidis*, eight *N. lactamica*, two *Mor. catarrhals*). The three discrepancies comprised two strains of *N. meningitidis* that gave a weak reaction for GAP and one strain of *N. meningitidis* that gave no reaction for GAP; all three strains, however, gave positive reactions for PAP. Janda and Sobieski (1988) reported that the Identicult-Neisseria test correctly identified 98.9% of 90 *N. gonorrhoeae* strains, 98.3% of 60 *N. meningitidis* strains, 96.2% of 26 *N. lactamica* strains and 100% of 36 *Mor. catarrhals* strains. They found that eight *N. subflava* strains which grew on modified Thayer-Martin agar were PAP-positive and were misidentified as *N. gonorrhoeae* by the Identicult-Neisseria.

In a comparison of the Identicult-Neisseria with the Neisseria/Haemophilus Identification Test Kit, Hughes et al. (1987) experienced limitations in the identification of 157 pathogenic *Neisseria* and *Mor. catarrhals* isolates. Incubation of the Identicult-Neisseria at 35°C for 10 min resulted in 99% of the isolates being identified while an alternative incubation protocol at 22°C for 20 min resulted in only 92% of the isolates being identified. We found that 26.3% of the *N. meningitidis* strains were positive for both GAP and PAP; this is considerably lower than the 92.5% reported by Sperry et al. (1986) who advocated that *N. meningitidis* strains be regarded as PAP-positive. The higher incidence of positivity for PAP with the Identicult-Neisseria over other chromogenic substrate tests was attributed to the higher sensitivity of the filter paper method. The two strains of gonococci which gave a positive GAP reaction in our study suggest that this may be over-sensitive.

The Gonocheck identification system uses similar chromogenic substrates, albeit in a different format from the Identicult-Neisseria. Several comparative studies have been made of the Gonocheck system and the specificity has
ranged from 86.7 to 100% (Wellborn et al. 1984; Philip and Garton 1985; Wood and Young 1986; Dillon et al. 1988).

The rapid isolation and identification of N. gonorrhoeae is important in the control and treatment of gonococcal infections. The increase in extra-genital gonococcal infection (Young and Reid 1987) has reinforced the need to differentiate gonococci from other non-gonococcal Neisseria from different anatomical sites. The drive for quick and accurate results has promulgated the development of rapid tests for the identification of N. gonorrhoeae. The Identicult-Neisseria identification test certainly meets the requirements for a rapid test, with an incubation time of only 10 min. It is, however, only intended for use in the identification of pathogenic Neisseria sp. grown on selective medium. If non-selective medium is used, non-pathogenic Neisseria such as N. subflava may grow leading to misidentifications (Dillon et al. 1988; Janda and Sobieski 1988). Depending on the sample site and sexual orientation of the patient isolation of non-gonococcal Neisseria may vary from 0-4 to 11-4% (Young and Reid 1987).

With the difficulties also observed with carbohydrate degradation tests (Philip and Garton 1985) it may be that due to the biochemical heterogeneity of the gonococcus we have reached the limits of specificity with conventional tests and may have to look in future to the molecular biologists to provide an identification test of absolute sensitivity and specificity.

On the basis of our results we conclude that the Identicult-Neisseria test when used alone is not sufficiently reliable for the culture confirmation of gonococci and meningococci.

REFERENCES


Immunofluorescence (IF) has a longer history in routine use than many other immunoassays, especially in regard to early direct diagnosis of respiratory virus infections following the pioneering work of Gardner and McQuillin [5] in Newcastle, England. Specificity of assays and the range available improved greatly with the development of monoclonal antibodies reacting with defined epitopes. Pools of monoclonal antibodies may be employed for a single virus or bacterial species, covering different epitopes to minimise problems of strain variation, or to screen for any one of a range of viruses, for instance in the centrifugation-
enhanced ‘shell vial’ technique for respiratory viruses [6]. The IF methods are still very important in rapid direct detection of infected cells in virology, or organisms (Pneumocystis carinii), particularly for single samples or small numbers of investigations. The sensitivity of immunofluorescence for antibody determination has been very useful over the years, but more sensitive and less subjective measurements of antibody are now appropriate. Methods which can be readily automated are also preferred for large scale screening for antibody or antigen.

Immunoaassays vary in sensitivity and specificity, as do all laboratory investigations. The inherent sensitivity (percentage of true positives shown as positive) and specificity (percentage of true negatives shown as negative) are features of the test product. However, in the clinical setting the usefulness of a test depends on the predictive values of the results and these vary with the prevalence of the disease concerned in the population under study. For example, with a prevalence of 1% (not uncommon in a screening situation) a test with a specificity of 95% would result in only 17 correct positive results out of every 100 positives, i.e. 83 out of 100 would be false positives: a specificity of 99% would result in half the positive results being false positives whilst a specificity of 99.9% would produce only 1 false positive in every 10 positive results (Figure 1a). Therefore in screening low prevalence populations a test with high specificity is required in order to reduce the burden of confirmatory testing of positive results which turn out to be false. As shown in Figure 1a, as the prevalence increases so does the positive predictive value such that at a disease prevalence of 50% a test kit with a specificity of 95% would give only 5 false positive out of every 100 positive results.

The opposite situation occurs in the case of the negative predictive value which decreases as the disease prevalence increases (Figure 1b) – this is because more true positives means fewer true negatives but more false negatives. The actual number of false negatives is related to the sensitivity of the test. For example in a population with a disease prevalence of 1% a test with a sensitivity of 95% gives a negative predictive value of 99.95% and would result in 5 false negatives per 10,000 negative results: a sensitivity of 99% gives a negative predictive value of 99.99% (1 false negative per 10,000 negative results) while a sensitivity of 99.9% gives a negative predictive value of 99.999% (1 false negative per 100,000 negative results).

Clearly a knowledge of the positive and negative predictive values is required to determine the accuracy of a test in a particular situation [7]. Unfortunately sensitivity usually shows an inverse correlation with specificity and taking account of the disease prevalence a balance must be drawn between these characteristics to minimise the number of infections likely to be missed while at the same time ensuring that the number of confirmatory tests required is not too high. However, in blood donor screening, although the disease prevalence is low, false negative results are critical and in this situation a relatively large proportion of unproductive confirmatory tests must be accepted. Although occasional false negative results may not be considered quite so important in other situations (e.g. screening for sexually transmitted diseases) samples which are non-reactive in screening immunoaassays are generally not subjected to repeated or alternative assays (unless there are other grounds for suspicion of infection) so it is important that screening tests have as high a sensitivity as possible in keeping with a level of specificity compatible with an acceptable level of confirmatory testing. A confirmatory test (unless involving a specific blocking reaction) should generally be of a different format from the screening test and be of equivalent or higher sensitivity to minimise the number of true positives that will not be confirmed. Because the disease
Figure 1a: Effect of prevalence on Positive Predictive Value for test results with kits of differing specificity.

![Graph showing the effect of prevalence on positive predictive value.](image)

Specificity 99.9%   Specificity 99%   Specificity 95%

If Sensitivity 100% - Negative Predictive Value 100%

Figure 1b: Effect of prevalence on Negative Predictive Value for test results with kits of differing sensitivity.

![Graph showing the effect of prevalence on negative predictive value.](image)

Sensitivity 99.9%   Sensitivity 99%   Sensitivity 95%

If Specificity 100% - Positive Predictive Value 100%
prevalence has been increased by screening, a useful confirmatory test need not necessarily be of overall higher specificity but it is important that it does not show the same pattern of false reactivity as the screening test. For example, as discussed above, at a disease prevalence of 1% a test with a specificity of 99% has a positive predictive value of 50%. When this group of sera is taken for confirmatory testing the disease prevalence has therefore increased to 50% and a confirmatory test with a specificity of 99% (the same as the screening test) will give a positive predictive value of 99% resulting in only 1 false positive per 100 positive confirmatory tests.

Reactivity in a screening assay requires to be confirmed by additional testing of the same sample and in some circumstances by examination of a second sample. In the context of sexually transmitted disease, confirmation (or use of a test which does not produce false positives, such as culture) is important because of the implications for contact tracing and investigation.

Screening immunoassays are available in a confusing multitude of formats, based on different antigen or antibody sources and utilising a variety of detection systems. The enzyme label has largely superseded the radioactive one for routine use, on grounds of safety, longer shelf life and acceptable sensitivity, particularly when amplified detection systems are used. The developments in molecular biology have made it possible to produce proteins expressed from recombinant DNA in various systems (bacteria, yeasts, insect cells and others), to manufacture synthetic oligopeptides. These defined antigens are incorporated in a huge range of assays, and play a significant role in the daily screening for antibodies to HIV1/2, for instance. Hepatitis C virus assays are unique in that no native antigens have been available, but, since the virus was obtained by molecular cloning, an increasing number of structural or non-structural antigens have been expressed or synthesised. The careful choice of antigens to be included in a screening assay is essential to detection of infections at different stages or where more than one strain of virus may be responsible.

Confirmatory tests for antigen (such as for C. trachomatis) may be assays of the same format as the screen using simple blocking (neutralisation) with a monoclonal antibody. More complex assays have come into general use in referral centres offering confirmation of the presence of antibodies to HIV or hepatitis C. Originally the HIV antibody tests were based on infected cell or viral lysate preparations, and the transfer of viral proteins, separated by electrophoresis in a gel, to a membrane (Western blot) enabled reactions with individual viral proteins to be ascertained. Variations on this method of presenting viral proteins separated by size are commercially available for a range of viruses and bacteria as standardised strips. Hepatitis C virus posed the necessity to use totally recombinant proteins (or some synthetic peptides) in an equivalent test format so that antibodies to a range of viral antigens could be detected, hence the recombinant immunoblot (RIBA) or line assays, with the proteins applied directly to the membranes. Detailed guidelines on the interpretation of the results obtained with HIV immunoblotting are published [8].

The introduction of automation for immunassay procedures in medical microbiology is now proceeding apace, although far behind that in clinical biochemistry. Cost-effective, labour saving methods are valuable, particularly where a rapid turn around time provides the clinician with the relevant result in time to influence patient care [9]. The need for continuing medical and scientific supervision of the choice, application and interpretation of these methods is even greater than when 'catch-all' methods based on culture were the norm. Simultaneously, there is a great expansion in simple rapid tests which can be carried out without any but the most basic equipment, ideal for field use, or near-patient testing. Provision and
supervision of such tests should be in conjunction with an accredited laboratory, where advice on safe handling, limitations of the tests and confirmatory or further investigations can be obtained [10].

This first part of the Immunoassay Kit Directory Series B on Infectious Diseases is devoted to Genitourinary Infections. The range of infections included are summarised in Table 1. The majority of these infections were chosen on the basis that they are frequently or occasionally transmitted through sexual contact [11], and that immunoassay kits were available for detection of the infection. Some of these infections are thought of almost exclusively as sexually transmitted disease (gonorrhoea and syphilis) while others are recognised as having a significant blood-borne spread also

Table 1 Genitourinary Infection: *pathogens included in Series B Part 1 of the Immunoassay Kit Directory

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Main clinical presentations</th>
<th>Common or serious sequelae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlamydia trachomatis (serovars D–K)</td>
<td>Urethritis, cervitis</td>
<td>Neonatal infection; pelvic inflammatory disease, ectopic pregnancy, infertility, arthritis</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Primary infection usually subclinical; occasional mononucleosis</td>
<td>Congenital infection; in immunocompromised – colitis, pneumonitis, retinitis, hepatitis</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>Hepatitis</td>
<td>Chronic hepatitis, cirrhosis, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Hepatitis</td>
<td>Chronic hepatitis, cirrhosis, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Hepatitis</td>
<td>Chronic hepatitis, cirrhosis</td>
</tr>
<tr>
<td>Hepatitis D virus</td>
<td>Hepatitis (simultaneous HBV required)</td>
<td>Recurrent episodes; neonatal infection</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Ulcers/vesicles – lower genital tract; mouth or pharynx; skin</td>
<td>AIDs</td>
</tr>
<tr>
<td>Human immuno-deficiency virus</td>
<td>Immunodeficiency</td>
<td></td>
</tr>
<tr>
<td>Human T-cell lymphotropic virus</td>
<td>Leukaemia, lymphoma, tropical spastic paresis</td>
<td></td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>Urethritis, cervicitis</td>
<td>Neonatal infection, pelvic inflammatory disease, ectopic pregnancy, infertility, disseminated infection</td>
</tr>
<tr>
<td>Streptococcus haemolytic group B (Strepto-</td>
<td>Asymptomatic carriage common in genital and gastrointestinal tracts of adults</td>
<td>Septicaemia and meningitis in newborn; post-partum septicaemia</td>
</tr>
<tr>
<td>coccius agalactiae)</td>
<td>Syphilis</td>
<td>Congenital infection; neurological and cardiovascular disease</td>
</tr>
<tr>
<td>Treponema pallidum</td>
<td></td>
<td>Pyelonephritis</td>
</tr>
<tr>
<td>Urinary tract pathogens</td>
<td>Urinary tract infection frequency and/or dysuria</td>
<td></td>
</tr>
</tbody>
</table>

*(Adapted from reference 11). Information was sought on kits for Candida albicans, Gardnerella vaginalis, Haemophilus ducreyi, human papilloma virus and Trichomonas vaginalis. Although a few kits were available for C. albicans they were for use in systemic infection.
(HIV). In some cases, spread by sexual contact is much less important in transmission than other means of exposure (hepatitis A), or is still not clearly confirmed as a mode of spread (hepatitis C).

References


GONORRHOEA IN ABERDEEN: A SEROVAR ANALYSIS

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Department of Medical Microbiology, University of Edinburgh, Teviot Place, Edinburgh

Abstract: The serotyping of gonococcal isolates has enabled detailed studies of the epidemiology of gonorrhoea in different geographical areas. This study reports the patterns of infection found in 1992 in Aberdeen and relates these to a variety of clinical parameters. 140 cases of gonorrhoea were isolated the majority of which belonged to one strain (IA-2) in contrast to the diversity of strains seen in other Scottish cities. Most isolates were highly sensitive to penicillin and only 3 penicillinase producing strains were found. A low rate of infections in homosexual men is reported again in contrast to the situation seen in other geographically close cities. Compared to the rest of Scotland Aberdeen has a high prevalence of gonorrhoea.

Keywords: Gonorrhoea, serotyping, clinical features, sexual orientation, antibiotics

Introduction

The ability to serotype Neisseria gonorrhoeae using monoclonal antibodies has permitted detailed epidemiological studies of gonococcal infection to be performed over the past seven years.1-4 Different strains of N. gonorrhoeae have been demonstrated between different geographical areas5 and within one area over time6-8. The factors involved in producing these geographical and temporal variations are as yet unknown. Serovars isolated from groups within a population, such as homosexual men, also exhibit different serovar patterns. We have previously shown that serovars isolated from gay men in Edinburgh not only are different when compared to heterosexually acquired infections, but also that within the homosexual group dynamic change occurs in the prevalent serovar pattern.9 An association between clinical presentation and gonococcal serotype10 as well as the changing serovar patterns in different areas may help to provide the explanation for these variations. Serovar analysis can also provide useful information on the spread of antibiotic resistance within a particular area.11-12

This prospective study was designed to observe and report the patterns of gonococcal serotypes isolated in Aberdeen over a one year period with regard to clinical features and penicillin sensitivity.

Methods

All patients with a diagnosis of gonorrhoea presenting to the Department of Genitourinary Medicine at Woolmanhill Hospital, Aberdeen between January and December 1992 were included in the analysis. The diagnosis of gonorrhoea was made on the basis of culture of N. gonorrhoeae on selective and non selective media from the urethra, rectum, endocervix and/or throat. All male patients had a single urethral swab taken whilst female patients had urethral and endocervical swabs cultured on two separate occasions to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea and when the history indicated that this site had been placed at risk. Rectal cultures were taken routinely from men who gave a history of homosexual contact and in women in whom the history suggested that this site had been placed at risk. Isolates were confirmed as N. gonorrhoeae by the

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Department of Medical Microbiology, Forsterhill, Aberdeen and forwarded to the Scottish Neisseria gonorrhoeae Reference Laboratory for serotyping and penicillin susceptibility testing. Serotyping was performed using the American panel of monoclonal antibodies as has been described previously.13 Minimum inhibitory concentrations (MICs) of penicillin were determined by an agar plate dilution method3 using a series of plates incorporating 0.015, 0.06, 0.12, 0.5 and 1.0 mg/l of penicillin: MICs less than or equal to 0.12 mg/l were classified as sensitive and isolates with MICs greater than or equal to 0.5 as reduced susceptibility.

Information was obtained from the case notes of patients with a diagnosis of gonorrhoea on sex, date of birth, sexual orientation, antibiotic sensitivity, date of diagnosis, locality of acquisition of infection, presence of other sexually transmitted diseases (STDs) and presence of symptoms. The data on serovar prevalence was then analysed with respect to the clinical information and penicillin sensitivity.

The data was entered into the database (Borland Software) database programme and statistical analysis was performed using chi-square on the Epinfo statistical package (WHO public domain software).

Results

A total of 140 cases of gonorrhoea were diagnosed in 138 patients (two patients had two separate infections during the year). The characteristics of the patients are summarised in Table I. Serovar data was available in 139 patients (99%).

The incidence of isolated serovars is shown in Table II. For most of the analysis the three commonest serovars (IA-2, IB-1 and IB-2), which together accounted for 130/140 (93%) of infections, were analysed separately whilst the other serovars were analysed collectively. The quarterly incidence of serovars is shown in the Figure. All male infections were urethral with the exception of one rectal infection. Fifty-one female infections involved the endocervix alone and 15 were dual infections of endocervix and urethra.

Serovars were divided into those acquired within Aberdeen and those acquired outwith the city as shown in Table III. There were no significant differences demonstrated between serovars with regard to the location acquired. With regard to asymptomatic infections there was again no significant differences evident between serovars although serovar IA-2 showed a trend to being more asymptomatic in men than other serovars (IA-2 asymptomatic in 6/30 cf. 1/17 for other serovars).
42/140 (30%) of patients had an additional STD isolated whilst attending with gonorrhoea.

The distribution of penicillin sensitivities for different serovars is demonstrated in Table IV. All IA-2 infections were highly penicillin sensitive while all IB-1 infections showed reduced sensitivity. Of the six IB-1 infections two were acquired locally, one outwith Aberdeen and three were of unknown origin. The 3 'other' serovars with reduced sensitivity (MIC=0.5mg/l) were two IB-7 infections and one IB-00. Three penicillinas producing strains (PPNG) were isolated consisting of two IB-2 infections and one IB-6 infection. Two of these infections were acquired outwith Aberdeen with the remaining one of unknown origin. One patient with PPNG was an offshore oil worker, one was unemployed and the occupation of the third was unknown.

**Discussion**

Over the 12 month study period gonococcal infections in Aberdeen were dominated by serovar IA-2 which accounted for three quarters of all infections. Only two other serovars (IB-2 and IB-1) were isolated on more than two occasions. This contrasts to the situation in many cities in the UK, including Glasgow, Edinburgh and Newcastle, where a greater variety of serovars is evident. A preponderance of serogroup IA infections is usually seen in smaller towns compared to a higher incidence of IB infections in larger cities which may reflect the greater diversity of serovar types of the IB group.

Over the study period however the number of IA-2 infections declined progressively with the appearance of six IB-1 infections in the last quarter of the year. Whilst IA-2 infections were universally sensitive to penicillin all the IB-1 isolates had reduced susceptibility. Whether this is an important determinant of serovar prevalence is unclear however. Although resistance to antibiotics may confer a selection advantage in areas where inappropriate antibiotics or inadequate doses of antibiotics are used there is little correlation between the resistance level of a gonococcal strain and its success within a population. This may reflect decreased cell membrane permeability which although protecting against the effects of antibiotics also reduces the absorption of nutrients.

The routine treatment of gonorrhoea in Aberdeen in 1992 was ciprofloxacin 250mg stat which was introduced three years ago. The present study found only non-PPNG infections with an MIC to penicillin of over 1mg/l (2%) the majority of which were acquired outwith the city itself. Whether the change in antibiotic policy has led to a reduction in resistance to penicillin by limiting its use in this group of patients cannot be determined from the present study but remains a possibility.

Only three PPNG infections were found of which were acquired outwith the city (the origin of one was unknown) which suggests that penicillinase producing infections are not endemic in Aberdeen at present.

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### Table I: Characteristics of Patients with Gonorrhoea

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-20</td>
<td>46</td>
</tr>
<tr>
<td>21-25</td>
<td>41</td>
</tr>
<tr>
<td>26-30</td>
<td>29</td>
</tr>
<tr>
<td>31-35</td>
<td>11</td>
</tr>
<tr>
<td>over 35</td>
<td>11</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>69</td>
</tr>
<tr>
<td>Female</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sexual Orientation of Men</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterosexual</td>
<td>51</td>
</tr>
<tr>
<td>Homosexual</td>
<td>1</td>
</tr>
<tr>
<td>not known</td>
<td>17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>108</td>
</tr>
<tr>
<td>IB</td>
<td>31</td>
</tr>
<tr>
<td>not known</td>
<td>1</td>
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</tbody>
</table>

### Table II: Distribution of Serovars in Aberdeen

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Heterosexual</th>
<th>Homosexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>105</td>
<td>-</td>
</tr>
<tr>
<td>IA-6</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>IA-00*</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>IB-1</td>
<td>6</td>
<td>-</td>
</tr>
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<td>IB-2</td>
<td>19</td>
<td>-</td>
</tr>
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<td>IB-3</td>
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</tr>
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<td>IB-6</td>
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<tr>
<td>IB-7</td>
<td>1</td>
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</tr>
<tr>
<td>IB-10</td>
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<td>IB-00</td>
<td>1</td>
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<tr>
<td>Not typed</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td>1</td>
</tr>
</tbody>
</table>

*classified by serogroup but unreactive with monoclonal antibody panel

---

### Table III: Locality of Acquisition of Infection

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Aberdeen</th>
<th>Outwith Aberdeen</th>
<th>Not Recorded</th>
</tr>
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<tbody>
<tr>
<td>IA-2</td>
<td>36</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>IA-6</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>IA-00</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>IB-1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IB-2</td>
<td>9</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>IB-3</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>IB-6</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>IB-7</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>IB-10</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IB-60</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not typed</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>30</td>
<td>59</td>
</tr>
</tbody>
</table>

### Table IV: Penicillin Sensitivity of Isolated Serovars of Non-PPNG Isolate

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Penicillin MIC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>0.015mg/l</td>
<td>105</td>
</tr>
<tr>
<td>IA-6</td>
<td>0.06mg/l</td>
<td></td>
</tr>
<tr>
<td>IA-00</td>
<td>0.12mg/l</td>
<td></td>
</tr>
<tr>
<td>IB-1</td>
<td>0.5mg/l</td>
<td></td>
</tr>
<tr>
<td>IB-2</td>
<td>1mg/l</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>2mg/l</td>
<td></td>
</tr>
</tbody>
</table>

---

**Fig. 1 Relative frequencies of Serovars in Aberdeen 1992**

![Graph showing relative frequencies of Serovars in Aberdeen 1992](image-url)
Only one homosexually acquired gonococcal infection was evident. This contrasts with Edinburgh where 48% of male gonococcal infections are in gay men and Newcastle were the percentage is 51%. Certain serovars, usually of the IB group, tend to be associated with homosexually acquired infection. Therefore the high incidence of IA-2 infections suggests that the low incidence of homosexual infections is genuine and not due to a failure to classify male patients as gay. This difference may be partially explained by gay men choosing to live in larger cities which provide greater anonymity and a social ‘gay scene’.

The differences in serovar prevalence observed in Aberdeen compared to other nearby cities may also reflect differing herd immunity. Although the humoral immune response mounted against a gonococcal infection is not thought to protect against reinfection there is some evidence for a serovar specific immune response which makes it less likely that an individual will become reinfected with the same serovar twice. If this were to occur on a population scale the prevalence of a particular serovar would depend on the sexually active population’s immunity and may therefore vary between districts and over time.

In many areas of Britain the incidence of gonorrhoea has declined markedly over the past decade presumably as a result of widespread media campaigns encouraging ‘safe sex’ i.e. the use of condoms and reduction in number of partners.

More recently there have been reports of an increase in homosexually acquired infections in England and Wales and Scotland. Although it is not possible to comment on the temporal changes in gonorrhoea from this study the gonococcal prevalence rate per 100 000 in the age group 16 to 45 is high in Aberdeen at 58/100 000. This compares to 25/100 000 in Edinburgh (91 infections in a population of 350 474 (population data from Information and Statistics Division, SHHD)) and 37/100 000 in Glasgow during 1992 (153 infections in population of 408 759) and suggests that further education campaigns may be necessary in Grampian.

Aberdeen exhibits an interesting serovar pattern with a strong predominance of IA-2 infections unlike other geographically close areas. There also appears to be a very low incidence of homosexually acquired infections. Compared to other cities in Scotland Aberdeen has a relatively high prevalence of gonorrhoea.

Acknowledgements: Thanks are extended to Dr. Hamish McKenzie and the technical staff of the Department of Medical Microbiology, Foresthill, Aberdeen for sending isolates to the Scottish Gonococcal Reference Laboratory.

References
The recent article by Rowbottom et al. concerning an outbreak of a penicillin sensitive strain of Neisseria gonorrhoeae in Sydney men prompts us to report the recent emergence of a new strain of infection in the gay men attending the genitourinary medicine clinic in Edinburgh, Scotland.

In 1993 we were aware of an increase in the number of infections with serovar 1A-6 in gay men. Over the 4 year period 1990 to 1993 infections with 1A-6 accounted for 4-5% (23/508) of all cases of gonorrhoea in Edinburgh. In gay men between 1990-92 1A-6 infections only accounted for 3-4% (5/147) of infections but in 1993 this increased with 21% (7/33) of homosexually or bisexualy acquired infections (p = 0.02, Fishers exact test). The prevalence of 1A-6 in the heterosexual population did not alter significantly (1990-92:10/260, 7-6%; 1993:11/422, 4-5%).

Classification of infections as homosexually acquired was based on the patients' self reported behaviour but additional confirmation was provided by the high male:female sex ratio (19:4) with no 1A-6 infections diagnosed in women in 1993. The sites of infection were also consistent with increased homosexual acquisition with rectal or pharyngeal infections accounting for 5 of the 8 1A-6 infections in 1993 compared with 5 out of 15 1A-6 infections in 1990-92.

An association between the serovar isolated and sexual orientation is well recognised.1,2 1A-12/2 infections are commonly seen in heterosexual patients whilst 1B strains are commoner in gay men.4 Thus the recent increase in incidence of infections with 1A-6 in gay men is unusual.

There are a number of possible explanations for the observed change in serovar pattern. Increased resistance to penicillin may provide a selective advantage in the gay population and serovars isolated from gay men tend to have a reduced sensitivity to penicillin.5 Although 1A strains are usually more sensitive to penicillin than 1B isolates,6 a change in penicillin sensitivity in 1A-6 strains was evident between 1990-92 and 1993 with a decrease in the proportion of isolates with an MIC of < 0.5 mg/l from 93% (14/15) to 12% (1/8) (p = 0.01). Although such resistance may be an advantage where antibiotic pressure is high there is generally poor correlation between the level of resistance to antibiotics and prevalence of a serovar6 possibly as a result of an associated impaired uptake of nutrients.

One possible source for this strain is the Far East where 1A-6 infections are common.10 The isolation of all seven homosexual isolates in the first six months of 1993 would support this hypothesis. Interestingly although all seven homosexually acquired infections were acquired locally, the one heterosexual infection in 1993 was acquired in the Fair East raising the possibility that this individual was actually bisexual.

Thus, although uncommon, 1A serogroup infections can be associated with both an outbreak of homosexually acquired infection and with reduced penicillin sensitivity.

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The value of primary colposcopy in genitourinary medicine

Moss and colleagues1 have reviewed their use of "primary colposcopy"—that is colposcopy used as a screening test—in a population of genitourinary medicine (GUM) clinic attenders. They appear to suggest that they have demonstrated a need for such screening and suggest that such screening would be cost effective. I would like to raise some doubts.

They report only the results of "primary colposcopy" in 1,338 women who had "an abnormal transformation zone". We are not told how many women in the total were performed under this regime, the majority of which might be assumed to have been normal. It would appear that a very large number of colposcopies had to be performed to detect 15 cases of cervical intraepithelial neoplasia (CIN) grade 2/3; there is no evidence that the current national screening policy would not have detected these lesions on subsequent cytology and before the development of invasive disease. It has already been shown that colposcopy as a screening tool will detect about three times as many lesions as cytology, but this does not add any additional weight to the role of cervical cytology.
Use of Nontreponemal Tests in the Diagnosis of Syphilis

Following the recent article by Jurado et al regarding the prozone reaction in secondary syphilis, we would like to report our experience of the potential problems associated with the use of nontreponemal antibody tests to screen for treponemal infections.

Report of Cases. Case 1. A 47-year-old man presented with a history of progressive cerebellar signs. There was no history of syphilis. The routine nontreponemal IgG enzyme immunoassay screening test (Cappita Syphilis G) showed a positive result with an antibody index of 3.12. Confirmatory testing revealed a negative qualitative VDRL test result, a positive Treponema pallidum hemagglutination assay titer of 10-240, a positive fluorescent treponemal antibody absorbed test result, and a positive nontreponemal IgM enzyme immunoassay (Cappita Syphilis M) with an antibody index of 1.2. Repeated VDRL testing, using diluted serum samples, gave a titer of 64, suggesting that the initial false-negative result was attributable to the prozone phenomenon and human immunodeficiency virus antibody testing that yielded negative results.

Case 2. A 60-year-old man was admitted with dysarthria and left-sided signs of weakness, brisk reflexes, and ataxic movements. Initial syphilis screening gave a reactive nontreponemal IgG enzyme immunoassay (antibody titer, 2.96). Confirmatory testing revealed a negative qualitative VDRL, a T pallidum hemagglutination assay titer of 20-480, a positive fluorescent treponemal antibody absorbed test result, and a negative IgM enzyme immunoassay (antibody index, 0.5). The VDRL test performed on a diluted serum sample confirmed that the false-negative result was attributable to a prozone reaction, the true VDRL titer being 128. The results of the human immunodeficiency virus antibody test were negative.

Comment. The use of cardiolipin antigen tests (rapid plasma reagin test and VDRL) to screen for syphilis is common in the United States. However, these tests are less sensitive than are treponemal antigen tests such as the T pallidum hemagglutination assay and treponemal enzyme immunoassay and are also subject to false-negative reactions caused by excess antibody in early syphilis, ie, the prozone phenomenon. A prozone reaction occurs in 1% to 2% of all cases of syphilis, but, in patients with a T pallidum hemagglutination assay titer of 5120 or over, up to 10% may exhibit a prozone reaction.4 The prozone reaction has also been associated with failure to diagnose syphilis in pregnant women and the subsequent delivery of congenitally infected infants.5 High antibody titers associated with concomitant human immunodeficiency virus infection offer the potential for a high prozone reaction rate,6 but the possibility of a prozone reaction must also be appreciated in patients with late-stage syphilis as in these patients. As cardiopalin tests are very rapid, they are ideal for use in emergency departments, but, in these circumstances, it would be prudent to test diluted, as well as undiluted, serum samples as part of the standard procedure.

Jonathan D. C. Ross, MRCPath
Hugh Young, PhD, MRCPath
Edinburgh, Scotland


In reply

Ross and Young indicate, as did Haslett and Lavery in their letter to the editor published in the July 25th, 1994, issue of the Archives,1 that the prozone phenomenon in syphilis is being recognized more frequently. We agree with Ross and Young that case 1 probably represents a case of the prozone phenomenon, as the patient's infection was clearly beyond the primary stage (the late nontreponemal seroconversion seen in primary syphilis would have been another possible explanation). Because of the stated low prevalence (1% to 2%) of the prozone phenomenon in syphilis,2,3 we believe that its inclusion as part of the standard nontreponemal testing is probably not warranted; rather, prozone should be considered in the setting of a clinically highly suspicious case with a negative undiluted nontreponemal test result. However, the prevalence of the prozone phenomenon needs to be reevaluated in the setting of the more sophisticated and technically improved nontreponemal tests used presently and in the context of the current increase of syphilis in the midst of the human immunodeficiency virus epidemic. We do not believe that treponemal tests, although being more sensitive than nontreponemal tests, should be used for screening purposes. They should be reserved as confirmatory tests and for the detection of syphilis in tertiary disease, where nontreponemal tests are notoriously insensitive (false-negative in up to 30% to 50% of cases).

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Pathobiology and Immunobiology of Neisseriaceae

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Carlos Oropeza
Pathobiology and Immunobiology of Neisseriaceae
Proceedings of the VIII International Pathogenic Neisseria Conference

First edition, 1994

Instituto Nacional de Salud Pública
Av. Universidad 655, Col. Santa María Ahuacatitlán
62508 Cuernavaca, Morelos, México

Printed and made in Mexico
Impreso y hecho en México

ISBN 968-6502-13-0
Infectivity and Clinical Features of Gonorrhoea: A serovar analysis

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Introduction

The ability to serotype Neisseria gonorrhoeae on the basis of Protein I epitopes has permitted a variety of epidemiological studies to be performed (1). These have included comparisons between differing geographical areas (2,3,4) and, to a lesser extent, longitudinal studies of serotype prevalence within individual centres (5). Such studies have demonstrated that a widely differing variety of serovars can be found in geographical areas of even close proximity and that there is dynamic interchange of serovars within any one area over short time periods. The cause of these fluctuations in gonococcal serovar prevalence has not been defined but probably represents the interplay between characteristics of the organism and the host.

Results

To determine if differences in the infectivity of serovars (as assessed by the number of infected sexual contacts) and clinical presentation of infection with particular serovars contribute to the ability of individual serovars to survive
within a population all patients attending the regional Genitourinary Medicine (GUM) clinic at Edinburgh Royal Infirmary, Edinburgh, Scotland with a diagnosis of gonorrhoea between January 1990 and December 1991 were studied. Gonorrhoea was diagnosed on the basis of a positive culture on modified New York City medium: gonococci were confirmed by immunological and sugar utilization tests. Specimens were routinely taken from the urethra and pharynx in men (plus rectum in homosexual men) and urethra, pharynx, endocervix and rectum in women. Serotyping was performed against the American panel of monoclonal antibodies as described previously (6).

The presence or absence of presenting symptoms including dysuria, urethral or vaginal discharge and abdominal pain was noted. For each gonococcal serovar the total number of contacts named, traced and infected was also analysed. Statistical analysis was performed using the chi-squared test with Yates correction where appropriate on the Epi-info statistical package.

336 patients who named 435 sexual contacts were included in the analysis, comprising 238 men (with 336 named contacts) and 96 women (with 99 named contacts). 209 patients were heterosexual (with 267 named contacts) and 108 were homosexual (with 155 named contacts) - the sexual orientation of the remaining 19 patients was unknown. 181 (42%) contacts were traced and screened for gonorrhoea in the GUM clinic.

Table 1 shows the number of contacts named, traced and infected for each serovar. A minor serovar was defined as one which was isolated less than 10 times over the two year study period. Serovar 1A-2 was significantly more infectious than average (p=0.04). There was no statistically significant difference between the sexual contact tracing rates for each serovar. No significant differences were found in the transmission rate of serovars with respect to sexual orientation. The presence of symptoms associated with each serovar is illustrated in Table 2. Serovar 1A-2 is significantly more likely to be asymptomatic than average (p=0.03) dysuria occurring in 14/61 (23%) patients with serovar 1A-2 infection compared to the overall figure of 163/336 (48%), p<0.01.
Table 1  Infectivity of Serovars

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. Contacts Named</th>
<th>No. Contacts Traced</th>
<th>No. Contacts Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A-2</td>
<td>75</td>
<td>32 (43%)</td>
<td>29 (91%)*</td>
</tr>
<tr>
<td>1A-6</td>
<td>14</td>
<td>9 (64%)</td>
<td>7 (78%)</td>
</tr>
<tr>
<td>IB-1</td>
<td>28</td>
<td>15 (54%)</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>IB-2</td>
<td>168</td>
<td>60 (36%)</td>
<td>45 (75%)</td>
</tr>
<tr>
<td>IB-3</td>
<td>46</td>
<td>20 (43%)</td>
<td>14 (70%)</td>
</tr>
<tr>
<td>IB-6</td>
<td>35</td>
<td>14 (40%)</td>
<td>12 (86%)</td>
</tr>
<tr>
<td>Minor Serovars</td>
<td>131</td>
<td>57 (43%)</td>
<td>43 (75%)</td>
</tr>
<tr>
<td>Total</td>
<td>435</td>
<td>181 (42%)</td>
<td>137 (75%)</td>
</tr>
</tbody>
</table>

*p < 0.05

Table 2  Presence of Symptoms with different Gonococcal Serovars

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Asymptomatic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A-2</td>
<td>21 (34%)*</td>
<td>61</td>
</tr>
<tr>
<td>1A-6</td>
<td>3 (27%)</td>
<td>11</td>
</tr>
<tr>
<td>IB-1</td>
<td>7 (32%)</td>
<td>22</td>
</tr>
<tr>
<td>IB-2</td>
<td>22 (17%)</td>
<td>127</td>
</tr>
<tr>
<td>IB-3</td>
<td>6 (19%)</td>
<td>32</td>
</tr>
<tr>
<td>IB-6</td>
<td>10 (38%)</td>
<td>26</td>
</tr>
<tr>
<td>Minor Serovars</td>
<td>6 (10%)*</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>75 (22%)</td>
<td>336</td>
</tr>
</tbody>
</table>

*p < 0.05

Discussion

We have demonstrated that serovar 1A-2 is more infectious than other serovars as assessed by the likelihood of infecting sexual contacts (p = 0.04) but other
References


Gonococcal infection in Edinburgh and Newcastle: serovar prevalence in relation to clinical features and sexual orientation

J D C Ross, A Wardropper, M Sprott, A Moyes, H Young

Abstract

Aims—The variable distribution of gonococcal serovars in different areas is well recognised but the factors that are important determinants of serovar prevalence are less clear. The aim of this study was to identify relevant clinical variables by comparing serovar prevalence in two cities over the same time period.

Methods—A prospective analysis of serovar prevalence was made between January and December 1992 in Edinburgh and Newcastle with respect to age, sex, sexual orientation, antibiotic sensitivity and presence of symptoms.

Results—224 infective episodes of gonorrhoea were studied. The serovar distribution varied between the two cities with serovar 1B-1 being more common in Edinburgh (20/91 cf. 4/133, p < 0-01) and serovar 1B-6 more common in Newcastle (26/133 cf. 2/91, p < 0-01). Serovar 1A-2 was associated with heterosexual infection (35/114 in heterosexuals cf. 0/85 in homosexuals, p < 0-01) and was more sensitive to penicillin than average (39/59 1A-2 strains highly penicillin sensitive cf. 98/184 for all other strains, p < 0-01) whilst 1B-6 was mostly acquired through homosexual contact (22/26 cf. 63/142 for all other strains, p < 0-01) and tended to show reduced penicillin susceptibility (13/28 1B-6 strains less penicillin sensitive cf. 45/195 for all other strains, p < 0-01). Infection with serovar 1A-2 was significantly less often symptomatic in heterosexuals than average (15/33 asymptomatic 1A-2 infections cf. 17/59 for all other serovars, p = 0-015). Subgroup analysis of male heterosexual infections confirms an association between asymptomatic infection and serovar 1A-2 (2/14 asymptomatic 1A-2 infections cf. 1/72 for all other serovars, p = 0-02). The distribution of infections over the year differed between the cities.

Conclusions—A variety of factors including penicillin sensitivity and virulence may be important in determining the prevalence of gonococcal serovars within a given area.

(Genitourin Med 1994;70:35–39)

Introduction

The ability to serotype *Neisseria gonorrhoeae* using monoclonal antibodies has permitted detailed epidemiological studies of gonococcal infection over the past seven years.1–4 A varying distribution of serovars has been demonstrated both geographically5,6 and temporally.7,8 Factors involved in producing geographical, temporal and sexual orientation associated differences in serovar prevalence are as yet unknown. Serovars isolated from groups within a population also differ with respect to serovar pattern. We have previously shown that serovars isolated from gay men in Edinburgh not only are different when compared with heterosexually acquired infections, but also that within the homosexual group dynamic change occurs in the prevalent serovar pattern.9 The association between clinical presentation and gonococcal serotype may help to provide the answer to this question. This prospective study was designed to observe and compare the patterns of gonococcal serotypes isolated in two cities in the United Kingdom with respect to their clinical features.

Methods

All patients presenting to the Departments of Genitourinary Medicine at Edinburgh Royal Infirmary or Newcastle General Hospital between January and December 1992 were analysed. The diagnosis of gonorrhoea was made on the basis of culture of *N. gonorrhoeae* on modified New York culture medium from the urethra, rectum, endocervix and/or throat. All male patients had a single urethral swab taken whilst female patients had urethral and endocervical swabs cultured on two separate occasions to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea and when the history indicated that this site had been placed at risk. Rectal cultures were taken routinely from men who gave a history of homosexual contact and in all women in Edinburgh. In Newcastle rectal cultures were taken when the history indicated that the site had been placed at risk, in those admitting to casual contacts and in contacts of patients known to be infected with gonorrhoea.

Gonococcal isolates were identified on the basis of biochemical and immunological tests and serotyping was performed using the American panel of monoclonal antibodies as described previously.10 Minimum inhibitory concentrations (MICs) of penicillin were determined by an agar plate dilution method11 using a series of plates incorporating 0-015, 0-06, 0-12, 0-5 and 1-0 mg/l of peni-
cillin: MICs less than or equal to 0.12mg/l were classed as sensitive and isolates with MICs greater than or equal to 0.5 as reduced susceptibility.

Information was obtained from the casenotes of patients with a diagnosis of gonorrhea on sex, sexual orientation, antibiotic sensitivity, date of diagnosis and presence of symptoms. The data on serovar prevalence in Edinburgh and Newcastle were then analysed with respect to the clinical information. The data were entered into the DBase (Borland Software) database programme and statistical analysis was performed using chi-square on the Epinfo statistical package (WHO public domain software).

### Results

In Edinburgh, 91 episodes of gonorrhoea in 91 patients were analysed. Gonococcal serovar data was available in 90 (99%) cases, sexual orientation in 86 (95%), antibiotic sensitivity in 91 (100%), date of diagnosis in 91 (100%) and details of symptoms in 85 (93%). Newcastle had 133 episodes of gonorrhoea in 126 patients. Serovar data was available in 133 cases (100%), sexual orientation in 125 (94%), antibiotic sensitivity in 132 (99%), date of diagnosis in 133 (100%) and details of symptoms in 120 (90%).

For analysis the four commonest serovars, which accounted for 73% of infections (1A-2, 1B-1, 1B-2 and 1B-6), were looked at separately whilst the other "minor" serovars were grouped together. Serovar incidence is shown in Table 1. No significant differences were seen in the age (p = 0.24), sex ratio (p = 0.24), sexual orientation (p = 0.43) or ratio of 1A to 1B infections (p = 0.17) in infections in Edinburgh compared with Newcastle (table 2). The relative frequency of gonococcal serovars in the two cities is shown in fig 1. Serotype 1B-1 infection was significantly more common in Edinburgh while 1B-6 infection was seen more frequently in Newcastle over the 12 month study period (table 1). The distribution of infections through the year differed between Edinburgh and Newcastle with the peak number of infections occurring in the 2nd quarter in Newcastle (p = 0.05) (fig 2).

Serovar 1A-2 was associated exclusively with heterosexual infection (fig 3). 1B-2 infections were significantly associated with
homosexually acquired infection (fig 3) but also occurred in heterosexual patients. Whilst in Newcastle 21 of 24 infections with 1B-6 were homosexually acquired, in Edinburgh 1B-6 occurred only twice (one heterosexual infection and one homosexual). Serovars 1A-2 and 1B-2 were more sensitive to penicillin than average (p < 0.01) and serovar 1B-6 and the “minor” serovars tended to be less susceptible (p < 0.01) (table 3). Penicillinase producing Neisseria gonorrhoeae (PPNG) were isolated uncommonly in our population: three isolates from Newcastle and four isolates from Edinburgh. Serovar 1B-5 was isolated on a total of six occasions (four Edinburgh, two Newcastle) but was associated with PPNG in four cases (two Edinburgh, two Newcastle). Genital (urethral or endocervical) infection with serovar 1A-2 in heterosexual patients was associated with no symptoms (table 4) and a subanalysis of genital infection in male heterosexuals also showed an association between asymptomatic infection and serovar 1A-2 (2/14 asymptomatic male genital infection for serovar 1A-2 cf. 1/72 for all other serovars, p = 0.02). The age distribution of infected patients did not differ for individual serovars.

### Discussion

The observed pattern of gonococcal serovars varies between Edinburgh and Newcastle although geographically the cities are not far apart. Certain serovars, such as 1A-2 and 1B-2, are observed in both cities whilst others predominate in one area only: 1B-6 in Newcastle and 1B-1 in Edinburgh, despite the patients being similar with respect to age, sex and sexual orientation. As has been previously reported some serovars are associated with certain sexual behaviour patterns and we found 1A-2 to be exclusively homosexually acquired while 1B-6 was usually acquired through homosexual contact, although 1B-2 was found in substantial numbers of both homosexual and heterosexual patients. Although serovar 1B-6 was isolated only very infrequently in Edinburgh in 1992, compared with Newcastle, previous reports have shown that this serovar was prevalent in gay men in Edinburgh in 1991 and was also increasing in prevalence in Glasgow during the same time period. It has been postulated that serovars may “cross over” from homosexual to heterosexual populations or vice versa via bisexual men. It is also possible that certain gonococcal strains have a selective advantage in gay men by, for example, being able to thrive more successfully in the rectal environment.

Plummer et al. and Buchanan et al. have suggested that there may be a serovar specific immune response which gives partial protection against reinfection with the same gonococcal strain. This implies that the success of a “new” strain within any particular community would depend on that community’s previous exposure and may explain the differences observed in this and other studies which have compared serovar patterns in different geographical areas of even relatively close proximity.

Over the past decade the incidence of gonorrhoea has fallen dramatically in many parts of the Western world although there has recently been an increase reported in England and Wales. No such increase has thus far occurred in Edinburgh, with the exception of an unsustained rise in homosexually acquired gonorrhoea, but the observed decline in the incidence of gonorrhoea has plateaued (unpublished data). Widespread media campaigns directed at preventing HIV infection over the same time period would therefore appear to have affected sexual behaviour as measured by the incidence of other sexually acquired infections although this effect is of short duration. If serovar characteristics themselves were important in determining their prevalence within a population then selective pressure on gonococcal strains...
may be expected to increase as the overall incidence of gonorrhoea declines with the emergence of a few successful serovars. We have however, observed a comparatively large number of different serovars with relative success occurring in only a few. This implies that either there is a continual influx of unsuccessful strains from other geographical areas or else population factors are also important. We have previously shown that certain serovars can persist at low levels within a population whilst others appear only transiently which lends support to the importance of population behaviour in determining a serovar’s success.

Homosexual populations often have infections with serovars that are uncommon in the heterosexual population and this is likely to be, at least partially, to relatively infrequent sexual mixing via bisexual men.

The presence of different serotypes of N. gonorrhoeae is presumably as a result of the antigenic heterogeneity which occurs secondary to genetic mutation and indeed a number of “evolutionary trees” have been proposed. The relevance of this “antigenic drift” to changing serovar patterns over time is not known as the rate of mutation in vivo has never been assessed. One way of estimating this is by comparing serovar patterns in named sexual contacts although this has given conflicting results with some authors finding a high correlation between partners and others less so.

The quarterly frequencies of gonococcal isolation in Edinburgh, peaking in the 1st and 3rd quarters, were the reverse of that observed in Newcastle. The pattern seen in Edinburgh follows that reported in the whole of Scotland in the past which was postulated to be secondary to the increased mobility of the young sexually active population during holiday periods.

The absence of symptoms would in theory be advantageous to a serovar in natural selection by increasing the chance of more contacts being infected prior to detection and treatment. Serovar 1A-2 produces less symptoms than average in heterosexually acquired genital infections in men and is also one of the most successful strains in both Edinburgh and Newcastle. Although Crawford et al. found that the AHU auxotype of gonorrhoea was less likely to produce symptoms a recent article by Horner et al. found no difference in the symptoms caused by 1A and 1B serogroups. The possible explanations for the disparity between our results and those of Horner may relate to either the different populations studied or methodological differences. Serotype 1A-2 was the predominant 1A serovar in our population and if lack of symptoms were related to this particular serovar rather than serogroup 1A in general this may bias the serogroup analysis in areas where 1A-2 is less prevalent. There are also a number of potential flaws in the analysis of Horner’s results. The number of patients included for most of their analysis is small (55) and 15% of the patients who completed questionnaires were not serogrouped. The analysis of presence or absence of symptoms was based on 91 patients in 68 of whom the information appeared to have been obtained from questionnaire while in 36 the notes were used which makes valid interpretation difficult. No comparison between data obtained from case notes and questionnaire were made. The association between symptoms and serotype is further complicated by the observation that symptoms associated with gonococcal infection may not necessarily remain static over time and an increase in the incubation period from 1932 to 1989 has been reported.

In common with other studies we found serogroup 1A more sensitive to penicillin than serogroup 1B. In particular serovar 1A-2 was universally sensitive although others have demonstrated that this strain can become chromosomally resistant with one study showing 6/278 (2.2%) of 1A-2 infections to be CMRNG. Despite this sensitivity to antibiotics, infections with 1A-2 are very successful in the community indicating that there may be some selective advantage associated with this trait.

The results of this and other studies indicate a complex interaction between the characteristics of the individual, the population and the gonococcal strain itself in determining the prevalence of a particular serovar in the community. Further research at both the epidemiological and molecular levels will be required before these issues can be resolved.
Gonococcal infection in Edinburgh and Newcastle: serovar prevalence in relation to clinical features and sexual orientation


ARTICLE

GONOCOCCAL INFECTIONS IN SCOTLAND, 1993

H. Young and A. Moyes

Isolates from a total of 464 episodes of infection were examined during the year at the Scottish *Neisseria gonorrhoeae* Reference Laboratory (SNGRL). The distribution of these isolates and corresponding incidence of gonococcal infection by Health Board is shown in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>Health Board</th>
<th>(Code)</th>
<th>Population</th>
<th>No of cases</th>
<th>Cases/100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argyll &amp; Clyde</td>
<td>(AC)</td>
<td>443118</td>
<td>8</td>
<td>1.81</td>
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<td>Ayrshire &amp; Arran</td>
<td>(AA)</td>
<td>374752</td>
<td>13</td>
<td>3.47</td>
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<td>Borders</td>
<td>(BR)</td>
<td>102141</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dumfries &amp; Galloway</td>
<td>(DG)</td>
<td>147036</td>
<td>8</td>
<td>5.44</td>
</tr>
<tr>
<td>Fife</td>
<td>(FF)</td>
<td>344590</td>
<td>43</td>
<td>12.48</td>
</tr>
<tr>
<td>Forth Valley</td>
<td>(FV)</td>
<td>272077</td>
<td>21</td>
<td>7.72</td>
</tr>
<tr>
<td>Grampian</td>
<td>(GR)</td>
<td>502863</td>
<td>115</td>
<td>22.87</td>
</tr>
<tr>
<td>Greater Glasgow</td>
<td>(GG)</td>
<td>951219</td>
<td>109</td>
<td>11.46</td>
</tr>
<tr>
<td>Highland</td>
<td>(HG)</td>
<td>200608</td>
<td>8</td>
<td>3.99</td>
</tr>
<tr>
<td>Lanarkshire</td>
<td>(LN)</td>
<td>563448</td>
<td>7</td>
<td>1.25</td>
</tr>
<tr>
<td>Lothian</td>
<td>(LO)</td>
<td>743700</td>
<td>83</td>
<td>11.16</td>
</tr>
<tr>
<td>Orkney</td>
<td>(OR)</td>
<td>19338</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Shetland</td>
<td>(SH)</td>
<td>22429</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tayside</td>
<td>(TY)</td>
<td>393792</td>
<td>49</td>
<td>12.45</td>
</tr>
<tr>
<td>Western Isles</td>
<td>(WI)</td>
<td>31048</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td><strong>5112159</strong></td>
<td><strong>464</strong></td>
<td><strong>9.08</strong></td>
</tr>
</tbody>
</table>

This represents 76% of the 612 isolates examined in 1992 and is in keeping with a decrease in the number of gonococcal infections rather than a decrease in the proportion of isolates being forwarded to the reference laboratory. Although the annual figure is not available the number of cases of gonorrhoea seen at Genitourinary Medicine Clinics in the first six months of 1993 was only 67% of the cases reported in the first six months of 1992 (Information and Statistics Division, Directorate of Information Services, NHS in Scotland). The incidence of infection in Grampian (22.87/100,000) was significantly higher than in other areas and more than twice that of the whole country (9.08/100,000). Grampian is also different from the other major areas of infection in that 73% of infections were due to serogroup IA isolates (Figure 1) compared with 50% for the whole country; the corresponding values for 1992 were 78% and 48%.

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Figure 1

Distribution of IA and IB serotypes of *N. gonorrhoeae* by Health Board area: Scotland, 1993

The number and distribution of protein IA serovars are given in Table 2. Although seven different serovars were found IA-2 predominated accounting for 92% of the IA isolates. In spite of the high number of IA isolates in Grampian they were extremely homogeneous (96% IA-2) suggesting endemic transmission of a successful "core strain" rather than frequent importation of new strains from other localities. Serovar IA-6 accounted for 28% of IA serovars in Lothian compared with 5.3% overall. Within Lothian this serovar was associated with homosexually acquired infection; all eight infections were in men, seven of whom were homosexual. During 1992 serovar IA-21 was found only in Glasgow, where it accounted for 45% of IA isolates from the Royal Infirmary and 13% from the Southern General; during 1993 it was not found anywhere in Scotland.

### TABLE 2

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AC</th>
<th>AA</th>
<th>DG</th>
<th>FF</th>
<th>FV</th>
<th>GR</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>HG</th>
<th>LN</th>
<th>LO</th>
<th>TY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>5</td>
<td>12(100%)</td>
<td>2</td>
<td>23(96%)</td>
<td>8</td>
<td>80(96%)</td>
<td>4</td>
<td>14(88%)</td>
<td>7</td>
<td>4</td>
<td>21(72%)</td>
<td>29(97%)</td>
<td>209*(91.7%)</td>
</tr>
<tr>
<td>IA-3</td>
<td>1</td>
<td>0</td>
<td>1(4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3(1.3%)</td>
</tr>
<tr>
<td>IA-4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(1%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(0.4%)</td>
</tr>
<tr>
<td>IA-6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(1%)</td>
<td>1</td>
<td>1(6%)</td>
<td>0</td>
<td>0</td>
<td>8(28%)</td>
<td>1(3%)</td>
<td>12(5.3%)</td>
</tr>
<tr>
<td>IA-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(0.4%)</td>
</tr>
<tr>
<td>IA-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(1%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(0.4%)</td>
</tr>
<tr>
<td>IA-16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(16%)</td>
<td>0</td>
<td>0</td>
<td>1(0.4%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6</td>
<td>12(100%)</td>
<td>2</td>
<td>24(100%)</td>
<td>8</td>
<td>83(100%)</td>
<td>6</td>
<td>16(100%)</td>
<td>8</td>
<td>4</td>
<td>29(100%)</td>
<td>30(100%)</td>
<td>228*(100%)</td>
</tr>
</tbody>
</table>

(a) 1 IA-2 not included (GLA:VIC)
(b) 1 Serogroup WI not available for typing (GR)
Eight protein IB serovars were found among the serogroup IB isolates in 1992 (Table 3) compared with 15 serovars in 1992. Serovar IB-32 which accounted for 1.7% of IB infections was found only in 1993. Serovars IB-5, IB-10, IB-16, IB-17, IB-26, IB-29 and IB-31 were absent in 1993 but accounted for 11.5% of IB isolates in 1992. As in previous years IB-2 was the most common isolate accounting for 59.2% of isolates in 1993 compared with 35.7% in 1992: this is a highly significant difference (X²=8.7; P<0.01). There has been a further significant decrease in IB-3 isolates to 1.7% from 7.2% in 1992 (X²=29.9; P<0.001) and 21.3% in 1991. IB-1 isolates showed a highly significant decrease to 1.7% reversing the increase from 8.6% in 1991 to 25.7% in 1992.

**TABLE 3**

Prevalence and geographical distribution of protein IB serovars of *N.gonorrhoeae* : Scotland, 1993

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AC</th>
<th>AA</th>
<th>DG</th>
<th>FF</th>
<th>FV</th>
<th>GG</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>HG</th>
<th>LN</th>
<th>LO</th>
<th>TY</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(3%)</td>
<td>1(2%)</td>
<td>1(3%)</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IB-2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>13(68%)</td>
<td>7(54%)</td>
<td>17(55%)</td>
<td>39(74%)</td>
<td>11(34%)</td>
<td>0</td>
<td>0</td>
<td>35(65%)</td>
<td>12(63%)</td>
<td>138(59.2%)</td>
</tr>
<tr>
<td>IB-3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(3%)</td>
<td>1(2%)</td>
<td>0</td>
<td>0</td>
<td>2(4%)</td>
<td>0</td>
<td>4(1.7%)</td>
</tr>
<tr>
<td>IB-6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(11%)</td>
<td>5(38%)</td>
<td>7(23%)</td>
<td>4(8%)</td>
<td>10(31%)</td>
<td>0</td>
<td>2</td>
<td>4(7%)</td>
<td>3(16%)</td>
<td>40(17.2%)</td>
</tr>
<tr>
<td>IB-7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(11%)</td>
<td>0</td>
<td>3(10%)</td>
<td>2(4%)</td>
<td>3(9%)</td>
<td>0</td>
<td>0</td>
<td>7(13%)</td>
<td>3(16%)</td>
<td>20(8.6%)</td>
</tr>
<tr>
<td>IB-8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(6%)</td>
<td>6(11%)</td>
<td>5(16%)</td>
<td>0</td>
<td>0</td>
<td>3(6%)</td>
<td>0</td>
<td>17(7.3%)</td>
</tr>
<tr>
<td>IB-19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(3%)</td>
<td>0</td>
<td>0</td>
<td>1(3%)</td>
<td>0</td>
<td>0</td>
<td>1(2%)</td>
<td>0</td>
<td>3(1.3%)</td>
</tr>
<tr>
<td>IB-32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(5%)</td>
<td>1(8%)</td>
<td>0</td>
<td>0</td>
<td>1(3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(5%)</td>
<td>4(1.7%)</td>
</tr>
<tr>
<td>IB-00*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(5%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(4%)</td>
<td>0</td>
<td>0</td>
<td>3(1.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>19(100%)</td>
<td>13(100%)</td>
<td>31(100%)</td>
<td>53(100%)</td>
<td>32(100%)</td>
<td>0</td>
<td>3</td>
<td>54(100%)</td>
<td>19(100%)</td>
<td>233(100%)</td>
</tr>
</tbody>
</table>

* Non-typable
(a) IB-2 not included (GLA:STO)

Reasons for these changes are unknown but may be accounted for in part by the finding that an increasing proportion of homosexually acquired infections are now attributable to serovar IB-2 while homosexually acquired infection also accounts for an increasing proportion of all gonococcal infections. The disappearance of IB-17 which is non-reactive in the Phadebact Monoclonal GC Test for confirming the identity of gonococci is of interest as this serovar has persisted in Glasgow since surveillance commenced in 1988. During this period the only other occurrence in Scotland was a single isolate in Lothian in 1989.

**TABLE 4**

Percentage distribution of IB-17 serovars of *N.gonorrhoeae* isolates in Glasgow, 1988-1993

<table>
<thead>
<tr>
<th>Year</th>
<th>Southern General</th>
<th>Royal Infirmary</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>9.7</td>
<td>9.9</td>
</tr>
<tr>
<td>1989</td>
<td>9.3</td>
<td>14.7</td>
</tr>
<tr>
<td>1990</td>
<td>3.6</td>
<td>7.6</td>
</tr>
<tr>
<td>1991</td>
<td>10.5</td>
<td>4.8</td>
</tr>
<tr>
<td>1992</td>
<td>25.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1993</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
TABLE 5
Penicillin susceptibility of IA Serovars N. gonorrhoeae:
Scotland, 1993

<table>
<thead>
<tr>
<th>Serovar</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1</th>
<th>≥2.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>199</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>210</td>
</tr>
<tr>
<td>IA-3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IA-4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IA-6</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>IA-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IA-9</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IA-16</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>202</td>
<td>6</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>4</td>
<td>229*</td>
</tr>
</tbody>
</table>

1992: 246 25 0 7 0 12 290

*One serogroup WI isolate not available for MIC or typing
(a) 3/4 PPNG; IA-6 isolate chromosomally resistant (MIC=2)
(b) All PPNG

TABLE 6
Penicillin susceptibility of IB Serovars of N. gonorrhoeae:
Scotland, 1993

<table>
<thead>
<tr>
<th>Serovar</th>
<th>≤0.015</th>
<th>0.06</th>
<th>0.12</th>
<th>0.5</th>
<th>1</th>
<th>≥2.0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IB-1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>IB-2</td>
<td>31</td>
<td>23</td>
<td>20</td>
<td>48</td>
<td>11</td>
<td>6</td>
<td>139</td>
</tr>
<tr>
<td>IB-3</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>IB-6</td>
<td>1</td>
<td>6</td>
<td>19</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>IB-7</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>11</td>
<td>2</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>IB-8</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>IB-19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IB-32</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>IB-00</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>42</td>
<td>47</td>
<td>79</td>
<td>17</td>
<td>14</td>
<td>333*</td>
</tr>
</tbody>
</table>

1992: 36 46 67 137 20 14 320

*One isolate (IB-19, non PPNG) not available for MIC
(a) 12/14 PPNG; two IB-2 isolates chromosomally resistant (MIC=2)
(b) 12/14 PPNG; two IB-1 isolate chromosomally resistant (MIC=2)
Again there was a highly significant difference in the penicillin susceptibility of IA and IB isolates (Tables 5 and 6). Excluding penicillinase-producing *Neisseria gonorrhoeae* (PPNG), 89.4% of IA isolates had a minimum inhibitory concentration (MIC) to penicillin $\leq 0.015$ mg/L compared with 15.4% of IB isolates ($P<0.001$). The corresponding values for 1992 were 88.5% and 11.8%. A total of 19 (8.3%) non-PPNG IB isolates were chromosomally resistant *Neisseria gonorrhoeae* (CMRNG) as defined by a penicillin MIC $\geq 1.0$ mg/L; corresponding values for previous years were 7.2% (1992), 3.4% (1991) and 1.3% (1990).

**TABLE 7**

Categories of antibiotic susceptibility for 462 *N. gonorrhoeae* isolates: Scotland, 1993

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number (and percentage) of isolates in corresponding category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
</tr>
<tr>
<td>Penicillin</td>
<td>$\leq 0.06$ mg/L</td>
</tr>
<tr>
<td></td>
<td>285 (62%)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>$\leq 0.06$ mg/L</td>
</tr>
<tr>
<td></td>
<td>352 (76%)</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>$\leq 0.008$ mg/L</td>
</tr>
<tr>
<td></td>
<td>452 (98%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>$\leq 0.25$ mg/L</td>
</tr>
<tr>
<td></td>
<td>282 (61%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>$\leq 0.5$ mg/L</td>
</tr>
<tr>
<td></td>
<td>429 (93%)</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>$\leq 32$ mg/L</td>
</tr>
<tr>
<td></td>
<td>46 (99.8%)</td>
</tr>
</tbody>
</table>

(a) 15/18 PPNG  
(b) 2/10 PPNG  
(c) 10/40 TRNG (including 5 also PPNG)

*Note: Categories are arbitrary with regard to ciprofloxacin. A resistant category for ciprofloxacin has not yet been defined but clinical failures have been associated with MICs $\geq 0.05$ mg/L.3*

The susceptibility of isolates to various antibiotics according to categories of susceptibility2,3 is given in Table 7. In general, the categories susceptible, intermediate and resistant are related to predictive values for clinical cure using recommended dosages for uncomplicated urogenital infection: susceptible (>95% efficacy); intermediate (>90-95% efficacy); and resistant (<85 - 90% efficacy)4. As shown in Table 7 the vast majority of isolates are within the susceptible and intermediate categories. Penicillin resistance is strongly associated with PPNG strains while tetracycline resistance is more commonly chromosomal: 0.4% of isolates were
chromosomally resistant to penicillin, 5.2% were chromosomally resistant to tetracycline and 0.25% chromosomally resistant to both antibiotics. With regard to plasmid mediated resistance, 2.2% of isolates were PPNG, 1.1% TRNG, and 1.1% were both PPNG and TRNG. These levels are much lower than found by sentinel surveillance of strains isolated in the USA during 1991: 1.8% of isolates were chromosomally resistant to penicillin, 7.2% were chromosomally resistant to tetracycline, 4.6% chromosomally resistant to both antibiotics, 11% of isolates were PPNG, 5.7% TRNG, and 2.1% both PPNG and TRNG. Of the 10 ciprofloxacin resistant isolates three were serovar IB-8 and one was serovar IB-19. As the total number of IB-8 and IB-19 isolates tested was only 18 this represents a level of ciprofloxacin resistance of 22% for these serovars compared with 1.4% for all other serovars.

**TABLE 8**

Serovar and geographical distribution of PPNG isolates: Scotland, 1993

<table>
<thead>
<tr>
<th>Serovar</th>
<th>AC</th>
<th>AA</th>
<th>DG</th>
<th>FF</th>
<th>FV</th>
<th>GR</th>
<th>GLA:ROY</th>
<th>GLA:SOU</th>
<th>HG</th>
<th>LN</th>
<th>LO</th>
<th>TY</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>0/5</td>
<td>0/12</td>
<td>0/2</td>
<td>0/23</td>
<td>0/8</td>
<td>1/80</td>
<td>0/4</td>
<td>0/14</td>
<td>0/7</td>
<td>0/4</td>
<td>1/21</td>
<td>0/29</td>
<td>2/210a</td>
</tr>
<tr>
<td>IA-8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/1</td>
</tr>
<tr>
<td>IB-1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/4</td>
</tr>
<tr>
<td>IB-2</td>
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<td>1/1</td>
<td>0/2</td>
<td>0/13</td>
<td>0/7</td>
<td>0/17</td>
<td>2/39</td>
<td>1/11</td>
<td>0</td>
<td>0</td>
<td>0/35</td>
<td>0/12</td>
<td>4/139b</td>
</tr>
<tr>
<td>IB-7</td>
<td>0</td>
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<td>0</td>
<td>1/2</td>
<td>0/3</td>
<td>1/2</td>
<td>0/3</td>
<td>0</td>
<td>0</td>
<td>1/7</td>
<td>2/3</td>
<td>5/20</td>
<td></td>
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<td>0/1</td>
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<td>0</td>
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<td>1/2</td>
<td>0/6</td>
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<td>1/17</td>
</tr>
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<td>IB-19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1/1</td>
<td>0</td>
<td>0</td>
<td>1/3</td>
</tr>
<tr>
<td>Others</td>
<td>0/1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/16</td>
<td>0/5</td>
<td>0/70</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0/8</td>
<td>1/13</td>
<td>1/8</td>
<td>1/43</td>
<td>0/21</td>
<td>4/115</td>
<td>3/59</td>
<td>1/48</td>
<td>0/8</td>
<td>0/7</td>
<td>2/83</td>
<td>2/49</td>
<td>15/464</td>
</tr>
</tbody>
</table>

1992 0/30 0/22 0/11 0/70 2/26 3/142 5/74 9/75 0/11 0/3 3/97 2/49 24/612

(a) 1 non-PPNG isolate (GLA:VIC) not included in sub-totals
(b) 1 non-PPNG isolate (GLA:STO) not included in sub-totals

The serovar and geographical distribution of PPNG strains is given in Table 8. Overall 3.2% (15/464) of isolates were PPNG, a level similar to that of 3.9% reported in 1992. Only three of the 15 PPNG isolates belonged to serogroup IA compared with 12 of 24 PPNG isolates in 1992. This difference is attributable to the lack of IA-6 isolates which accounted for eight PPNG in 1992.

Full details of the 15 PPNG strains isolated during 1993 are given in Table 9. The combination of serotype, auxotype and plasmid profile shows a highly heterogeneous group of 13 different isolates suggesting transient importation rather than endemic spread. Control and prevention of subsequent spread of PPNG strains is important as they tend to be acquired in areas (parts of Africa and the Far East) where HIV is also common and could be acquired at the same time. The epidemiological data suggests two sets of "contact pairs". An IB-2 isolate with identical characteristics was isolated from each of a male and a female patient attending Glasgow Royal Infirmary in June, while an IB-7 isolate with identical characteristics was isolated from a male in Tayside and a female in Fife in October (the latter patient represented a double infection as a penicillin sensitive serovar IA-2 strain was isolated from her cervix at the same time). The majority of isolates contained a conjugative plasmid, either in the form of the 24.5 Mdal plasmid (eight isolates) or the 25.2 Mdal plasmid (five isolates) which also codes for high level tetracycline resistance.
TABLE 9
Serovar, auxotype and plasmid profile for 15 PPNG isolates:
Scotland, 1993

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Auxotype</th>
<th>Plasmid present (Mdal)</th>
<th>Area</th>
<th>Date Received</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2</td>
<td>Hyp Pro</td>
<td>3.2; 2.52; 2.6</td>
<td>Grampian</td>
<td>1/4/93</td>
<td>F</td>
</tr>
<tr>
<td>IA-2</td>
<td>Non-requiring</td>
<td>4.4; 24.5; 2.6</td>
<td>Lothian</td>
<td>29/6/93</td>
<td>M</td>
</tr>
<tr>
<td>IA-8</td>
<td>Pro</td>
<td>4.4; 25.2; 2.6</td>
<td>Grampian</td>
<td>8/11/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-1</td>
<td>Non-requiring</td>
<td>3.05; 24.5; 2.6</td>
<td>Grampian</td>
<td>31/5/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-2</td>
<td>Pro</td>
<td>4.4; 24.5; 2.6</td>
<td>Greater Glasgow</td>
<td>7/1/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-2</td>
<td>Pro</td>
<td>3.2; 24.5; 2.6</td>
<td>Ayrshire &amp; Arran</td>
<td>1/4/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-2</td>
<td>Non-requiring</td>
<td>4.4; 24.5; 2.6</td>
<td>Greater Glasgow</td>
<td>10/6/93</td>
<td>F</td>
</tr>
<tr>
<td>IB-7</td>
<td>Pro</td>
<td>3.2; 25.2; 2.6</td>
<td>Greater Glasgow</td>
<td>10/6/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-7</td>
<td>Non-requiring</td>
<td>4.4; 24.5; 2.6</td>
<td>Tayside</td>
<td>1/4/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-7</td>
<td>Non-requiring</td>
<td>4.4; 2.6</td>
<td>Tayside</td>
<td>12/10/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-7</td>
<td>Non-requiring</td>
<td>4.4; 2.6</td>
<td>Fife</td>
<td>6/10/93</td>
<td>F</td>
</tr>
<tr>
<td>IB-8</td>
<td>Non-requiring</td>
<td>4.4; 25.2; 2.6</td>
<td>Greater Glasgow</td>
<td>5/11/93</td>
<td>M</td>
</tr>
<tr>
<td>IB-9</td>
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<td>4.4; 24.5; 2.6</td>
<td>Grampian</td>
<td>25/8/93</td>
<td>F</td>
</tr>
<tr>
<td>IB-19</td>
<td>Hyp Pro</td>
<td>4.4; 25.2; 2.6</td>
<td>Dumfries &amp; Galloway</td>
<td>4/10/93</td>
<td>F</td>
</tr>
</tbody>
</table>

Hyp : Hypoxanthine requiring
Pro : Proline requiring

Seven ano-genital isolates of Gram negative diplococci (GNDC) isolated from patients proved to be non-gonococcal strains of *Neisseria* (NGN). These included five isolates of *N.meningitidis* (two male rectal isolates, two male urethral isolates, and one female rectal isolate), one cervical isolate of *N.lactamica* and one male rectal isolate of *Moraxella catarrhalis*. Although the absolute prevalence of ano-genital NGN has not increased, owing to the decrease in gonorrhoea, NGN strains have increased as a proportion of the total ano-genital GNDC isolates. In 1993 NGN accounted for approximately 10% of all ano-genital GNDC isolated from patients attending the Genitourinary Medicine Unit at Edinburgh Royal Infirmary. This has important implications for diagnosis as it will decrease the predictive value of certain identification methods.

References:

Acknowledgements:

Special thanks are extended to Mrs Joan McElhinney for careful record keeping and assistance with the preparation of this manuscript.

We thank our numerous bacteriological and clinical colleagues for their on-going help and co-operation in submitting isolates to the reference laboratory; in particular, the following laboratories which submitted specimens:

Argyll and Clyde (AC)
AC/DUN:ARL Vale of Leven Hospital, Alexandria, Dumbartonshire
AC/GRE:CLY Inverclyde Royal Hospital, Greenock
AC/PAI:RAB Dept of Bacteriology, Royal Alexandra Hospital, Paisley

Ayrshire and Arran (AA)
AA/AYR:ARL Dept of Microbiology, Crosshouse Hospital, Kilmarnock, Ayrshire

Dumfries and Galloway (DG)
DG/DMF:ROY Dumfries and Galloway Royal Infirmary, Dumfries

Fife (FF)
FF/FIF:ARL Fife Area Laboratory, Kirkcaldy, Fife

Forth Valley (FV)
FV/FAL:ROY Falkirk & District Royal Infirmary, Falkirk
FV/STI:ROY Stirling Royal Infirmary, Stirling

Greater Glasgow (GG)
GG/GLA:ROY Royal Infirmary, Glasgow
GG/GLA:SOU Southern General Hospital, Glasgow
GG/GLA:VIC Victoria Infirmary, Glasgow

Grampian (GR)
GR/ABD:GHB University of Aberdeen, Foresterhill, Aberdeen

Highland (HG)
HG/INV:RAI Raigmore Hospital, Inverness

Lanarkshire (LN)
LN/LAN:LAW Law Hospital, Carluke
LN/LAN:MON Dept of Microbiology, Monklands Hospital, Airdrie

Lothian (LO)
LO/EDI:CIT City Hospital, Edinburgh
LO/EDI:CML Central Microbiological Laboratories, Western General Hospital, Edinburgh
LO/EDI:ROY Royal Infirmary, Edinburgh
LO/EDI:STD Genito-urinary Medicine Clinic, Royal Infirmary, Edinburgh

Tayside (TY)
TY/ANG:STR Stracathro Hospital, Brechin
TY/DEE:UNI Ninewells Hospital & Medical School, Dundee
TY/PER:ROY Perth Royal Infirmary, Perth
Pathobiology and Immunobiology of Neisseriaceae

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Carlos Oropeza
Pathobiology and Immunobiology of Neisseriaceae
Proceedings of the VIII International Pathogenic Neisseria Conference

First edition, 1994

Instituto Nacional de Salud Pública
Av. Universidad 655, Col. Santa María Ahuacatitlán
62508 Cuernavaca, Morelos, México

Printed and made in Mexico
Impreso y hecho en México

ISBN 968-6502-13-0
Pharyngeal gonorrhoea: an increase in clinical and microbiological significance?

H. Young, A. Moyes
Scottish Gonococcal Reference Laboratory, Department of Medical Microbiology Edinburgh University Medical School, Edinburgh EH8 9AG

J.D.C. Ross
Department of Genitourinary Medicine, Edinburgh Royal Infirmary, Edinburgh EH3 9YW

Introduction

Recent reports that rectal gonorrhoea can be acquired in the absence of penetrative anal intercourse (1,2) challenge the tenet that male rectal gonorrhoea reflects unsafe sexual behaviour (3). The importance of alternative routes of transmission such as oro-anal or digito-anal contact (1) is dependent on a high level of pharyngeal infection. Although HIV infection can be acquired through fellatio, and transmission may be facilitated by co-existing Neisseria gonorrhoeae infection (4), fellatio is generally viewed as a "safe" sexual practice. It is therefore possible that pharyngeal carriage and transmission of a wide range of sexually acquired pathogens, including N. gonorrhoeae, may be increasing (4) at a time when the incidence of gonorrhoea is showing a dramatic reduction (5).

Results

To determine if there has been an increase in the level of pharyngeal gonorrhoea we examined the results of throat cul-
tures taken during 1991 and compared this data with pharyngeal colonisation in homosexual and heterosexual patients throughout the period 1986-1991. All men and women attending the regional Genitourinary Medicine (GUM) clinic between 1986 and 1991 from whom Neisseria gonorrhoeae was cultured at a diagnostic test were included. Whenever possible specimens were taken routinely from urethra and pharynx in men (plus rectum in homosexual men) and urethra, pharynx, endocervix and rectum in women. Specimens were plated directly onto modified New York City medium and incubated immediately in a carbon dioxide enriched atmosphere. All oxidase-positive Gram-negative diplococci were tested by both immunological (Phadebact Monoclonal GC test) and biochemical (rapid carbohydrate utilisation test) methods (6). Statistical analysis was by the chi-square test performed using the Minitab Statistical package.

During 1991 there were 25 new episodes of gonorrhoea (any site) in women, 48 in heterosexual men and 54 in homosexual men: throat cultures had been taken at the initial visit from 16 (64%) of infected women, 36 (75%) heterosexual men and 52 (96%) of homosexual men. Pharyngeal colonisation with gonococci and meningococci for the patients with gonorrhoea in whom throat cultures were taken is shown in Table 1.

The highest rate of pharyngeal gonorrhoea (52% in total) was found in homosexual men. This was significantly higher (P < 0.001) than the rate in heterosexual men (17% in total). There were no significant differences between other patient groups. The gonococcal carriage rate in homosexual men was significantly higher (P < 0.001) than the meningococcal carriage rate (25%). There was no statistically significant difference in the gonococcal and meningococcal carriage rates in heterosexual patients.
Table 1  
Neisserial Throat Colonisation: 1991

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number (and percentage) with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ng</td>
</tr>
<tr>
<td>Women (n = 16)</td>
<td>5(31)</td>
</tr>
<tr>
<td>Heterosexual men (n = 36)</td>
<td>5(14)</td>
</tr>
<tr>
<td>Homosexual men (n = 52)</td>
<td>21(40)</td>
</tr>
</tbody>
</table>

Ng = Neisseria gonorrhoeae; Nm = Neisseria meningitis; NNI = No Neisseriae isolated

To determine if there had been an increase in pharyngeal gonorrhoea over the time period when ano-genital infection was decreasing we analysed data from the preceding five years. The pharyngeal colonization rates shown in Table 2 are based on the number of patients in whom multiple sites (urethra and throat in heterosexual men; urethra, rectum and throat in homosexual men; urethra, cervix, rectum and throat in women) were tested. The majority of patients with gonorrhoea are however included: over the six year period 87% (range 75-97) of heterosexual men, 80% (range 71-90) of homosexual men and 66% of women (range 64-87) were tested at multiple sites. During this period heterosexually acquired gonorrhoea in patients tested at multiple sites decreased from 445 (166 female and 279 male) in 1986 to 52 (16 female and 36 male) in 1991. Homosexually acquired infection which had shown a large decrease prior to 1986 decreased further throughout 1987-89 before increasing during 1990 and 1991.
Table 2  Pharyngeal Gonorrhoea: 1986-91

<table>
<thead>
<tr>
<th>Year</th>
<th>Women</th>
<th>Heterosexual men</th>
<th>Homosexual men</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>14.5%(24/166)</td>
<td>7.8%(22/279)</td>
<td>29.3%(12/41)</td>
</tr>
<tr>
<td>1987</td>
<td>22.5%(27/120)</td>
<td>6.3%(13/207)</td>
<td>16.7%(3/18)</td>
</tr>
<tr>
<td>1988</td>
<td>17.1%(14/82)</td>
<td>12%(13/108)</td>
<td>37.9%(11/29)</td>
</tr>
<tr>
<td>1989</td>
<td>8.7%(4/46)</td>
<td>5.1%(3/59)</td>
<td>10%(1/10)</td>
</tr>
<tr>
<td>1990</td>
<td>10.3%(3/29)</td>
<td>16.1%(9/56)</td>
<td>33.3%(13/39)</td>
</tr>
<tr>
<td>1991</td>
<td>31.3%(5/16)</td>
<td>16.6%(6/36)</td>
<td>55.6%(25/45)</td>
</tr>
<tr>
<td>Total</td>
<td>16.8%(77/459)</td>
<td>8.9%(66/745)</td>
<td>35.7%(65/182)</td>
</tr>
</tbody>
</table>

This increase was accompanied by high levels of pharyngeal infection. Comparing the two year periods 1986-87 and 1990-91 shows a significant increase in the overall level of pharyngeal gonorrhoea in homosexual ( P < 0.02 ) and heterosexual ( P < 0.05 ) men but not in women ( P > 0.9 ). Over the six year period infection of the throat as the only site of infection occurred in 13.7% (25/182) of homosexual men: this was significantly higher ( P < 0.001 ) than the 0.94% (7/745) in heterosexual men and 0.65% (3/459) in women. The cumulative results for pharyngeal colonisation with gonococci and meningococci for the period 1986-91 are summarised in Table 3.

Over the six year period the rate of pharyngeal gonorrhoea in homosexual men was significantly higher ( P < 0.001 ) than the level in women which was in turn significantly higher ( P < 0.001 ) than in heterosexual men. There was no significant difference in the meningococcal carriage rate between the three groups. In homosexual men there was no significant difference in the gonococcal and meningococcal carriage rates.
whereas the meningococcal carriage rate was significantly higher than the gonococcal carriage rate in heterosexual men (P < 0.001) and women (P < 0.001).

Table 3 Neisserial Throat Colonisation: 1986-91

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Percentage of patients with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ng</td>
</tr>
<tr>
<td>Women (n = 459)</td>
<td>15.7</td>
</tr>
<tr>
<td>Heterosexual men (n = 745)</td>
<td>79</td>
</tr>
<tr>
<td>Homosexual men (n = 182)</td>
<td>29.1</td>
</tr>
</tbody>
</table>

Dual pharyngeal colonisation with gonococci and meningococci was significantly higher in homosexual men than in heterosexual men and women (P < 0.001).

Discussion

There has been an increase in pharyngeal gonorrhoea in homosexual and heterosexual men attending the GUM clinic in Edinburgh during the period 1986-91. The actual levels recorded are higher than those reported previously (7,8,9). Gonococci are easily grown from expectorated saliva suggesting transmissibility and providing another reason for ensuring effective treatment (10). Patients with exclusive pharyngeal gonorrhoea who deny oro-genital contact may have been infected by strictly oral-oral contact (11). Pharyn-
geal infection has been reported as the predominant site of infection in prepubertal household contacts of children with gonorrhoea (12). These data support the view that male rectal gonorrhoea does not necessarily reflect "high risk" sexual behaviour with regard to acquisition of HIV (1,2). The frequency of positive meningococcal cultures from the ano-rectum of homosexual men also supports the likelihood of salivary transmission of gonococci (13).

An increase in pharyngeal gonorrhoea is also of significance in relation to ensuring adequate treatment (5,14), increased opportunity for the development of systemic infection (15), and a potential increase in mis-diagnosis resulting from problems which sometimes occur in differentiating gonococci and meningococci (6,16). The high level of dual colonisation with gonococci and meningococci increases the potential for confusion in the identification of neisseriae from the throat. The increase in dual infection may also be of molecular epidemiological significance in providing an environment for genetic exchange including the potential for transfer of resistance determinants from gonococci to meningococci.

Our results support the view of Murray et al (4) that health education should advise the use of condoms during fellatio and the safety of oral sex should be questioned.

References


Penicillin susceptibility testing of penicillinase producing Neisseria gonorrhoeae by the E test: a need for caution

H. Young, A. Moyes and A. Hood

Scottish Gonococcal Reference Laboratory, Department of Medical Microbiology, Edinburgh University Medical School, Edinburgh EH8 9AG, Scotland, UK

The penicillin MICs of 52 clinical isolates of penicillinase producing Neisseria gonorrhoeae (PPNG) and five reference strains were tested by agar dilution and the E test. All 52 PPNG were scored resistant (≥2 mg/L) by agar dilution whereas only 78.8% (41/52) and 82.7% (43/52) were classified as resistant by normal (0.016–256 mg/L) and low range (0.002–32 mg/L) E test MICs respectively. Isolates with an E test penicillin MIC ≥0.19 mg/L should be checked by a specific test for β-lactamase production.

Introduction

The E test (AB Biodisk, Solna, Sweden) is a new simple method for determining minimum inhibitory (MIC) values. The E test consists of a thin, inert, non-porous plastic strip with a predefined exponential gradient of antibiotic immobilised on one side and a MIC reading scale (mg/L) marked on the other side. MIC determinations by E test and standard reference methods for a wide range of organisms report excellent agreement, generally in excess of 90% (Yeung, Ng & Dillon, 1993). The few studies that have been reported for gonococci (Lind, Berthelsen & Bentzon, 1990; Olsson-Liljequist et al., 1991; Sanchez Barrett & Jones, 1992; Melby, Jorgensen & Steinbakk, 1991; Hitchcock, 1993) have shown an overall correlation (±1 log₂ dilutions) between E test and agar dilution penicillin MICs ranging from 85% (Olsson-Liljequist et al., 1991) to 100% (Hitchcock, 1993). However, very few of the above studies have included a significant number of penicillinase producing Neisseria gonorrhoeae (PPNG). Although there are specific methods to test for PPNG not all laboratories test all isolates, many testing only penicillin resistant isolates or those associated with treatment failure. As the E test is a simple and convenient method to obtain MIC values it could be used as a method to select resistant strains for further testing for β-lactamase activity. It is therefore important to ensure that PPNG will be scored resistant to penicillin by the E test method. In view of the small numbers of PPNG included in previous E test evaluations of gonococci we considered it worthwhile to evaluate the E test for penicillin susceptibility testing of PPNG using the blood containing medium that we use routinely for agar dilution MIC determinations (Young et al., 1990).
Methods

Fifty two clinical isolates of penicillinase-producing *N. gonorrhoeae* (PPNG) representing at least 25 different strains as determined by serotyping, auxotyping and plasmid analysis were included in the study. The five World Health Organisation (WHO) reference strains A, B, C, D, E (kindly supplied by Dr Inga Lind, WHO Collaborating Centre for Reference and Research in Gonococci, Statens Seruminstitut, Copenhagen) which are used routinely in our laboratory to control antibiotic susceptibility testing were also tested. Minimum inhibitory concentrations (MICs) were determined simultaneously by an agar dilution method (Young et al., 1990) and by the E test (AB Biodisk) using the same inoculum, which was prepared by suspending an overnight culture in saline to a turbidity equivalent to a 0.5 McFarland standard. Modified New York City (MNYC) medium, comprising Difco GC base, lysed horse blood, yeast dialysate and glucose, but lacking selective antibiotics was used for both agar dilution and E test susceptibility determinations. Penicillin (Mast Laboratories, Bootle, UK) was incorporated into a series of plates at the following concentrations: 0.015, 0.06, 0.12, and doubling concentrations from 0.5 to 256 mg/L. E test MICs to penicillin were determined using both the normal range antibiotic strip (0.016–256 mg/L) and the low range strip (0.002–32 mg/L). A single strip was placed on a plate which had been inoculated over the entire surface using a swab charged with the standard suspension. For agar dilution the inoculum was applied with a multi-point inoculator which delivered 1 µL per pin. Plates were incubated for 24 h at 37°C in a 5% carbon dioxide enriched atmosphere. E test MICs were determined by reading the value at the point of intersection between the zone edge and the E test strip. The agar dilution MIC was defined as the lowest concentration of antibiotic which completely inhibited growth of the inoculum: on a few rare occasions one or two colonies were observed on a spot but these were discounted in scoring the MIC. Statistical comparisons were made by the Chi Square test, two sample t test using the Minitab PC software package.

Results and discussion

The agar dilution penicillin MICs for the WHO control strains A to E were <0.015, 0.06, 0.5, 2 and 8 mg/L respectively and fell within the expected range for each strain (Lind et al., 1984). Based on the categories of susceptible (MIC < 0.06 mg/L) and resistant (MIC ≥ 2.0 mg/L) as defined by Ringertz, Rylander & Kronvall (1991) and Anonymous (1990) strains A and B were correctly scored as susceptible, C as less susceptible and D and E as resistant. With E test, strains A (MIC 0.016 mg/L with normal range strip and 0.006 mg/L with low level strip) and B (0.032 mg/L with both normal and low range strips) were correctly scored susceptible and strain C was correctly scored less susceptible (MIC 0.125 mg/L with the normal range strip and 0.094 mg/L with low range strip). Strain D (MIC 0.5 mg/L with normal and low range strips) and the PPNG strain E (MIC 1.5 mg/L with the normal range strip and 1.0 mg/L with low range strip) were scored less susceptible rather than resistant.

The distribution of penicillin MICs for the 52 clinical isolates of PPNG tested by agar dilution and E test are given in the Table. The MIC₅₀ value for the 52 PPNG was 16 mg/L for agar dilution and 4 mg/L for both the normal range and low range E tests: the corresponding MIC₅₀ values were 128, 24 and ≥ 32 mg/L. The mean penicillin MIC was 37.4 mg/L (range 2–128) for agar dilution and 6.37 mg/L (range 0.5–32) for the
The mean MIC value for the low range E test could not be calculated as 23% (12/52) of the isolates had an MIC outwith the range of the strip (>32 mg/L). On the basis of the agar dilution MIC all 52 isolates were classified as resistant (MIC ≥ 2.0 mg/L) whereas only 78.8% (41/52) and 82.7% (43/52) were classified as resistant by normal and low range E test MICs. As shown in the Table the low range E test correlated better with high agar dilution MICs than did the normal range E test: the proportion of isolates with an MIC ≥ 32 was 40% (21/52) for agar dilution which was significantly higher \( \chi^2 = 3.6; P < 0.05 \) than the 23% (12/52) for the low range E test strip which was in turn significantly higher \( \chi^2 = 10.6; P < 0.01 \) than the 2% (1/52) for the normal range strip.

The high concentrations of penicillin associated with the upper end of the penicillin gradient may diffuse into the medium at a rate (concentration gradient) in excess of that which can be inactivated by the amounts of β-lactamase produced by certain strains of PPNG. As a result of this the MIC measured by E test would depend on the dynamics between the amount of β-lactamase produced by a particular strain, the extent to which the diffusing penicillin was inactivated, and the amount of penicillin remaining to inhibit the growth of the organism: in the case of strains producing low levels of β-lactamase, the MIC measured by E test would tend to reflect the level of susceptibility of penicillin corresponding to the chromosomally mediated resistance of the strain.

The percentage of PPNG in our study, 23% (12/52) that exceeded the upper MIC range of the low test strip (32 mg/L) was significantly lower \( \chi^2 = 25.03; P < 0.001 \) than the 80% (24/30) of the PPNG isolates tested in an earlier study (Yeung et al., 1993) suggesting that the PPNG population in our study is more diverse with a greater proportion of strains producing lower levels of β-lactamase.

Whilst it could be argued that the discrepancy in the high level penicillin MICs found in PPNG isolates is academic there is potentially considerable clinical significance as 21.2% (11/52) and 17.3% (9/52) were classified as less susceptible rather than resistant.

### Table. Penicillin MIC distribution for 52 penicillinase producing Neisseria gonorrhoeae determined by agar dilution and E test

<table>
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<tr>
<td>2</td>
<td>2</td>
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by normal and low range E test MICs respectively. We disagree with the statement, based on five PPNG isolates with MIC values given as >0.64 mg/L only, that E test "shows a high correlation in the detection of PPNGs" (Hitchcock, 1993).

In conclusion E test is a simple and convenient method of determining MICs and is likely to be increasingly widely used for susceptibility testing of gonococci. Ideally β-lactamase testing should be performed on all gonococcal isolates, but in laboratories where this is not routine practice, isolates with an E test penicillin MIC ≥ 0.19 mg/L should be checked by a specific test for β-lactamase production.

References

(Received 17 January 1994; revised version accepted 13 June 1994)
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A preliminary evaluation of a prototype Western blot confirmatory test kit for syphilis

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1Department of Medical Microbiology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland and 2Clinical Microbiology Division, Institute of Medical and Veterinary Science, Adelaide, South Australia 5000

Summary: A prototype Western blot kit was evaluated as a confirmatory test for syphilis using 131 sera characterized by other serological tests for syphilis. There were 114 treponemal sera (including 94 cases of early syphilis, 83 of which were untreated) and 17 non-treponemal problem sera (11 gave false positive reactions on screening with the TmpA recombinant antigen enzyme immunoassay (EIA), 3 gave false positive fluorescent treponemal antibody absorbed (FTA-abs) tests, and 3 false positive Captia Syphilis G EIA results). Based on the manufacturer's criteria of reactivity in multiple bands for designating a positive result the Western blot test gave a sensitivity of 99.1% (113/114) and a specificity of 88.2% (15/17) when indeterminate reactions were scored positive and 98.2% (112/114) and 100% (17/17) when indeterminate reactions were scored negative. Sensitivity was high in both treated and untreated infection. Corresponding sensitivities for the TPHA and FTA-abs when equivocal reactions were scored negative were 97.5% (111/114) and 99.1% (113/114). The high sensitivity of the FTA-abs in this study is probably due to the large number of untreated primary infections. Our results with the Western blot confirm earlier studies using 'in-house' test systems and, support a role for a commercial Western blot test in the confirmatory diagnosis of syphilis. Further studies are required to confirm the high specificity and sensitivity of the kit in a larger series including a wider variety of non-treponemal cases as well as patients with untreated and treated infection.

Keywords: Syphilis, syphilis serodiagnosis, Western blot

Since the fluorescent treponemal antibody absorbed (FTA-abs) test replaced the Treponema pallidum immobilization test during the mid-1970s and early 80s, it has been accepted as the standard confirmatory test for syphilis. The FTA-abs test becomes positive around the third week of infection: sensitivity is, however, higher in early infection (86–100% in primary and 100% in secondary) than in late stage infection (96–100%). Although specificity of the FTA-abs test is high, it is not absolute with reported values varying from 92–99%. Whereas the prevalence of false reactivity is around 1% in normal persons higher rates have been reported in hospitalized patients. The FTA-abs is a highly subjective test and the degree of reactivity accorded to the test result can have a very significant effect on the utility of the test. For example, the CDC recommendation that the borderline report should be eliminated increased the specificity of the test from 82.5% to 88.7% with a minimal decrease in sensitivity from 100% to 99.5%. Unfortunately this recommendation, which is based on testing sera selected by screening with a cardiolipin antigen test such as the Rapid Plasma Reagin (RPR), can not be adopted in those areas where the incidence of early infectious syphilis is low and sera are selected by screening with a treponemal test such as the Treponema pallidum haemagglutination assay (TPHA) or Captia Syphilis-G enzyme immunoassay (EIA) as it would result in a decrease in sensitivity of 6–19% depending on the FTA-abs kit used. Marked variation in the quality of commercial FTA-abs kits is another significant limitation in the use of the FTA-abs as a confirmatory test. Because of an association between unexplained reactivity in the FTA-abs test and EIA, the general decrease in specificity of the
FTA-abs resulting from the need to include borderline reports when sera are selected by screening with a treponemal test is enhanced when EIA is used for screening. Clearly there is scope for improvement in confirmatory testing.

Western blotting has been used to analyse the immune response in experimental and human syphilis10-12 and was first appreciated as a potential diagnostic tool in syphilis serology as early as 198513. Although recent reports14,15 have supported its value as a confirmatory test, up until now, no commercial test systems have been available. We report an evaluation of a prototype Western blot kit supplied by Centocor UK Ltd, Surrey. The main emphasis of the study was to evaluate test performance in untreated infectious syphilis in a population area (Northern Territory, Australia) with a very high level of infectious disease as well as obtain preliminary data on late stage infection in an area (Scotland) with well characterized late stage infection but a low level of infectious disease.

MATERIALS AND METHODS

The 133 patients included in this study are a subgroup of patients examined as part of a collaborative evaluation of new screening tests. They attended either the Genitourinary Medicine Unit Edinburgh Royal Infirmary or Rural Aboriginal Community Clinics in Australia. Sera from the Aboriginal Communities were tested in the Clinical Microbiology Division, Institute of Medical and Veterinary Science in Adelaide while the sera from Edinburgh were tested in the Department of Medical Microbiology, Edinburgh University Medical School. Patients were classified as treponemal or non-treponemal on the basis of conventional serological tests for syphilis (RPR, TPHA, FTA-abs) clinical signs and symptoms, and history of previous syphilis infection. 114 patients were classified as treponemal, 17 as non-treponemal while in 2 cases there was insufficient information to classify the patients accurately. The stage of infection and treatment status for the 114 patients with syphilis are given in Table 1. Of the 93 untreated cases, 35 had had a previous episode of syphilis, 53 had no previous history while in 5 cases the previous history was unknown. The 17 non-treponemal cases comprised 11 sera giving false positive reactions on screening with the TmpA recombinant antigen EIA16, 3 giving a false positive FTA-abs and 3 a false positive Captia Syphilis-G EIA17.

The following serological tests were performed according to manufacturer’s instructions by experienced staff at each centre using the same batch of kits or reagents for each test: RPR test (Commonwealth Serum Laboratories, Parkville, Victoria, Australia); TPHA (Fujirebio, Japan); Captia Syphilis-G (anti-treponemal IgG) EIA (Centocor UK Ltd, Surrey); FTA-abs using the following reagents—T. pallidum antigen smears and sorbent (Biomerieux, Lyon, France), fluorescein conjugated anti-human globulin (Wellcome, Kent, UK) titrated to an acceptable working dilution (1 in 400).

The prototype Western blot kit provided by Centocor UK Ltd is a qualitative assay for the detection and identification of IgG specific antibodies to T. pallidum in human serum. The kit contains all reagents required to perform the test including wash buffers and diluent, 24 nitrocellulose antigen strips (T. pallidum proteins separated according to molecular weight by SDS polyacrylamide electrophoresis are transblotted to the nitrocellulose membrane which is washed and blocked before cutting into strips), alkaline phosphate conjugated anti-human IgG, substrate solution, positive and negative control sera and three incubation trays each with 8 channels. Sera were tested at a dilution of 1 in 101 (10 μl + 1 ml) according to the manufacturer’s instructions. Positive and negative control sera were included in each run. The manufacturer’s criteria for test validation and interpretation were as follows. Validation: there should be no bands present on the negative control strip while the following bands must be present on the positive control strip: 14 kDa (Dalton), 15 kDa (14/15 bands may appear as one), 33 kDa, 39 kDa, 44 kDa, 46 kDa, and 48 kDa (44/46/48 bands may appear as one). Interpretation: Negative—no specific bands; Indeterminate—one specific band; Positive—multiple (>2) specific bands corresponding to positive control bands (Figure 1). The molecular weight of the antigens for reactive bands was estimated by reference to the positive control strip and a ‘positive control guide’ which provides a diagrammatic illustration of the position of the various bands.

Results for all other tests were scored negative, positive, or equivocal by the following agreed criteria: RPR—positive (small or large black aggregates of antigen), negative (smooth clear or grey colour with or without slight roughness), equivocal (intermediate between negative and positive), titre (reciprocal of final serum dilution giving a positive reaction); TPHA—positive (>50% agglutination at final serum dilution of 1 in 80), negative (no agglutination, or a trace only, at 1 in 80 and <50% agglutination at 1 in 40), equivocal (>50% agreement).
bands was non-reactive in multiple bands (Table that included run been tested one of the control strips positive on and runs on A total

RESULTS

A total of 139 Western blot tests were performed in 13 runs on sera from 133 patients (4 patients had 2 tests and one patient had 3 tests). No bands were present on any of the negative control strips. Twelve of the 13 positive control strips gave the appropriate bands: one of the positive control strips was reactive only in the 44/46/48 kDa band region. The sera that had been tested in this run were re-tested in a further run that included valid positive and negative control strips. The majority of sera were reactive with multiple bands (Table 2). Excluding the serum that was non-reactive in blotting the mean number of bands was 5 (range 1 to 12): the mean was 5.3 for all early infections and 3.7 for late stage infections.

An estimate of the frequency of reactivity of the 114 treponemal sera with various antigens is given in Table 3—it was sometimes difficult to identify specific molecular weight bands with absolute certainty as band positions varied slightly between positive control strips. As shown highest reactivity was found in the 14–15 kDa region and the 46–48 kDa region: 87.7% (100/114) of the sera were reactive when bands in the 14–15 regions were combined while 98.3% (112/114) were reactive when bands in the 46–48 region were combined. The sensitivity (equivocal reactions scored positive) of Western blot and other serological tests for syphilis is given in relation to treatment status in Table 4. The percentage of positive reactions that were scored as equivocal with each test (indeterminate for Western blot was RPR (0.9%), TPHA (3.5%), Capita-G (0.9%), FTA-abs (0.9%), and Western blot (0.9%).

The patient with a negative Western blot result was a case of untreated primary syphilis: the RPR and

Table 2. Distribution of multiple band reactivity amongst 114 treponemal sera

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<td>18</td>
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<td>4</td>
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<tr>
<td>12</td>
<td>2</td>
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<tr>
<td>Total</td>
<td>114</td>
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Table 3. Frequency of reactivity of 114 treponemal sera with various antigens

<table>
<thead>
<tr>
<th>Antigen kDa</th>
<th>Reactivity with corresponding antigen</th>
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<tbody>
<tr>
<td></td>
<td>Number</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>14/15</td>
<td>98</td>
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<tr>
<td>17</td>
<td>4</td>
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<td>32</td>
<td>6</td>
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<td>67</td>
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<tr>
<td>46</td>
<td>99</td>
</tr>
<tr>
<td>48</td>
<td>107</td>
</tr>
<tr>
<td>46/48</td>
<td>3</td>
</tr>
<tr>
<td>62</td>
<td>17</td>
</tr>
<tr>
<td>80</td>
<td>12</td>
</tr>
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</table>
Capita-G tests were also negative (index 0.69) and although there was no history of a previous infection the TPHA gave a titre of 160 and the FTA-abs a weak positive reaction. Four weeks later a repeat specimen from the patient was positive in all tests: Western blot (bands at 14/15, 17, 39, 40, 46 and 48 kDa), RPR (titre of 8), TPHA (titre of 320), FTA-abs (strong positive reaction), Capita-G (index 2.2). The patient with an indeterminate Western blot (reactive with 48 kDa antigen only) was a case of treated late stage infection: the RPR was negative, the TPHA equivocal, the FTA-abs weak positive and the Capita-G positive with an index of 1.25. The remaining 9 specimens negative in the RPR included 3 treated late latent and 6 untreated cases (2 primary and 4 late latent).

None of the 17 non-treponemal sera were reactive with multiple bands: 2 sera reactive on screening with the TmpA (44 kDa antigen) EIA gave a weak reaction with a single band (one with the 46 kDa band and the other with the combined 46/48 kDa band).

### DISCUSSION

The Western blot test gave a sensitivity of 99.1% (113/114) and a specificity of 88.2% (15/17) when indeterminate reactions were scored positive and 98.2% (112/114) and 100% (17/17) when indeterminate reactions were scored negative. Sensitivity was high in both treated and untreated infection (Table 4). Corresponding sensitivities for the TPHA and FTA-abs when equivocal reactions were scored negative were 97.5% (111/114) and 99.1% (113/114). The high sensitivity of the FTA-abs in this study is probably due to the large number of untreated primary infections. Our results with the Western blot confirm earlier studies using 'in-house' test systems\textsuperscript{14,15} and support a role for a commercial Western blot test in the confirmatory diagnosis of syphilis.

Direct comparison with other studies regarding the percentage reactivity with bands of specific molecular weight is difficult as minor differences in the molecular masses of certain antigens have been described by different investigators: for example an antigen with a consensus mass of 15.5 kDa has been reported as 12–14 kDa as well as 15.5 kDa\textsuperscript{18}.

Dettori et al\textsuperscript{14} using an anti-treponemal IgG conjugate showed that antibodies against a single antigen of 15.5 kDa were present in 97% of 110 cases of syphilis of known stage and 99% of 294 cases of serologically diagnosed syphilis of undetermined stage: reactivity was 100% in patients with secondary or early latent syphilis, both untreated and treated, 98.3% in those with late latent treated syphilis and 100% in patients with neurosyphilis. Reactivity to the 14/15 kDa antigen was lower (87.7%) in our study and reactivity to this antigen alone would make the test insensitive. The difference in overall reactivity of 97% to the 15.5 kDa antigen of Dettori and colleagues\textsuperscript{14} and 87.7% to the 14/15 kDa antigen in our study may be related to our much higher proportion of primary cases. In the former study there were only 3 cases of untreated primary infection and 2 were negative. Although no data were given it was stated that the sensitivity of the test for primary syphilis was improved by using anti-human IgM conjugate rather than anti-IgG.

Others\textsuperscript{15} have based a positive immunoblot test result on reactivity to at least 3 of 4 major antigens of 15.5, 17, 44.5, and 47 kDa. Based on this definition their assay had a sensitivity of 91.7% and a specificity of 100% for clinically defined samples. A positive result was obtained in 93% (37/40) of cases of primary syphilis giving 97.5% agreement with the FTA-abs test. The high reactivity in primary infection probably results from the use of conjugate containing anti-human IgG plus IgM. These criteria\textsuperscript{15} would seem too stringent as 19 of our sera were reactive with only 2 bands (Table 2) and although reactivity in the low and high molecular weight regions was most important, on a few occasions the second band was in the 33 to 37 kDa range.

A positive result based on reactivity to 2 distinct bands would not compromise specificity as none of the 17 non-treponemal sera were reactive with multiple bands: 2 sera reactive on screening with the TmpA EIA gave a weak reaction with a single band (one with 46 kDa band and the other with the combined 46/48 kDa band).

### Table 4. Comparative sensitivity of Western blot and other serological tests for syphilis

<table>
<thead>
<tr>
<th>Test</th>
<th>Number and (%) reactive in relation to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated (n = 93)</td>
</tr>
<tr>
<td>RPR</td>
<td>86 (92.5)</td>
</tr>
<tr>
<td>TPHA</td>
<td>93 (100)</td>
</tr>
<tr>
<td>Capita-G</td>
<td>92 (98.9)</td>
</tr>
<tr>
<td>FTA-abs</td>
<td>93 (100)</td>
</tr>
<tr>
<td>Western blot</td>
<td>92 (98.9)</td>
</tr>
</tbody>
</table>

Comparison of the number, pattern and intensity of band reactivity may be of value in diagnosing re-infection, particularly when the response to specific anti-treponemal IgM tests may be deficient\textsuperscript{19}. Immunoblotting using anti-human IgM...
Conjugate has considerable potential in the diagnosis of congenital infection. Western blot analysis of serum from a patient with secondary syphilis and HIV showed antibodies to far fewer antigens than are usually present in secondary sera. Loss of serological markers of syphilis after patients become infected with HIV also suggests that other confirmatory tests, in addition to the FTA-abs test, may be helpful in determining the treponemal status of HIV-infected patients. In HIV-infected patients neurological complications of syphilis may occur more frequently and at an earlier stage and may increase the risk of treatment failure. These interactions between HIV and syphilis are likely to lead to widespread use of treponemal antigen tests for screening thereby decreasing the utility of the FTA-abs as a confirmatory test. Although the prototype Western blot test kit requires some refinement we consider that such a test would be a useful adjunct to the confirmatory diagnosis of syphilis, particularly when treponemal tests are used for screening. This conclusion supports the view of Byrne and colleagues that Western blotting, because of its high sensitivity and specificity, simplicity and objectivity, was a good confirmatory test. The advent of a commercially available Western blot system will allow a much larger number of laboratories to benefit from this technology. Further evaluation is required, however, in order to confirm that the criteria for designating a positive result will ensure optimum sensitivity and specificity. Such studies should include larger numbers of patients with untreated and treated late stage infection as well as larger numbers of non-treponemal cases. The use of a combined or additional mono-specific anti-IgM conjugate also merits further investigation.

References
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(Accepted 11 May 1994)
Original article

A sociological and serological study of attenders of family planning clinics in Addis Ababa

M Elizabeth Duncan¹, Gerard Tibaus, Andrée Pelzer, Letebirhan Mehari, Peter L Perine, John Peutherer, Hugh Young, Yasmin Jamil, Sohrab Darougar, Inga Lind, Karin Reimann, Peter Piot, Erwin Roggen

Abstract: A study of 542 women attending family planning clinics (FPC) and 1568 women attending obstetric and gynaecologic clinics in Addis Ababa showed utilisation of FPC was highest in those with a family income of 100-500 EB per month (36%), in women who were: Tigrawi (33%) or Amara (31%), aged 20-34 years (30%), age 16 or older at first marriage/coitus (28%), parity of >_2 children (35%), > 5 lifetime husbands/sexual partners (39%), or were bargirls (73%) or prostitutes (43%). FPC attendance was lowest among the nulliparous (2.3%), women from rural areas (10%), the Guragie (10%) and Oromo women (19%), Moslem women 14%, those of subsistence income (<10EB per month) (14%). The seroprevalence rates indicative of exposure to STD pathogens were high as was the prevalence of essentially asymptomatic pelvic inflammatory disease (PID). Only 4% of FPC attenders had no serological evidence of STD: 64% had 3 or more different STD. Specific present or active STD infection prevalence for syphilis (VDRL) 28%, Neisseria gonorrhoea 31%, genital chlamydia 46% and HSV-2 21% was higher in FPC attenders than among women attending other clinics. Clinical evidence of PID was also more common in the FPC attenders (54%), 37% having evidence of salpingitis. Thus FPCs provide a useful setting for screening women particularly at risk. Because of lack of symptoms, these women are unlikely to attend either an STDs clinic or a hospital for routine check up, and as such are not treated and represent a population from which STDs can spread into the population. Measures to screen, treat and educate FPC attenders, their partners and their clients, are recommended in an attempt to control STDs and ultimately HIV in the community. [Ethiop. J. Health Dev. 1995;9(1):19-30]

Introduction

Ethiopian women have practised traditional methods of contraception for protection against pregnancy. However, family planning is not generally discussed among women, not even among the young and educated. Many men refuse to allow their wives to use contraceptives either because they wish to have more children, or have misconceptions about birth control. They refuse using condoms for fear of spoiling their sexual enjoyment.

The Family Guidance Association of Ethiopia (FGAE) provides clinical and educational services to the community as well as training services in family planning for various categories of health workers. Family planning clinics (FPC) have been provided by hospitals and Maternal and Child Health Clinics (MCHC) run by community nurse/midwives, initially under medical
supervision and with regular consultant referral clinics.

The MCHCs are within easy reach of the community they serve and play a key role in women's health by providing primary health care, antenatal, intrapartum and postnatal care, family planning advice and child welfare/vaccination as well as treatment for minor ailments within the same compound. Moreover, because of the overcrowding at busy hospitals, many women prefer to attend the MCHCs for gynaecological advice where they can be seen more quickly. A further bonus for women unaccustomed to gynaecological examination is that they are seen by a female health attendant. MCHC community nurse/midwives with training in family planning became expert at diagnosing common gynaecological problems and treating pelvic inflammatory disease (PID), the most common consequence of sexually transmitted diseases (STDs).

According to a literature review from 1976-1992 (2-8) there are no reports of STDs prevalence rates among FPC attenders in Ethiopia and very few in the rest of Africa. These few publications report knowledge of the fertile period (6), degree of sexual activity among adolescents (6,7), knowledge of contraception (6,7), AIDS and spread of HIV (8). Despite an increasing volume of literature regarding sexual activity among adolescents (9-14), knowledge and use of contraception (13-18), AIDS and spread of HIV (9-12,14-17,19-21), there is serious ignorance about STDs in general (22) and amongst men in particular (23). At the same time there is a dearth of information coming from Africa on prevalence of STDs, the socioeconomic aspects of their transmission, and of their sequelae. Most reports are from old surveys of STD clinic attenders which are not representative of the population, or screening of antenatal clinic attenders (J Wilson Carswell, personal communication). Cervical cancer (CC), STDs and pelvic sepsis have been major causes of morbidity and mortality among Ethiopian women (24,25).

The aim of this study was (i) to determine the prevalence of selected STDs, PID and CC in women attending FPC and (ii) to draw a socioeconomic profile of FPC attenders in order that services could be targeted more effectively. The data on the women studied are taken from a larger investigation of sociodemographic factors associated with STDs, PID and CC in Addis Ababa. The study was carried out with the permission of the Ministry of Health.

Methods

Two thousand one hundred eleven women were included in this cross-sectional study without pre-selection: 542 were attenders at regular FPCs; 1121 were attending gynaecological outpatient departments (GOPD), 342 and 106 attended antenatal (ANC) and postnatal (PNC) clinics, respectively. The selection of two teaching hospitals in Addis Ababa, (the Black Lion and St. Paul's Hospitals) and Lidetta MCHC for this study was because (i) it was anticipated that the patients seen would be representative of the hospital population of Addis Ababa; (ii) the staff of the obstetric and gynaecologic units are accustomed to collaborating in research projects; (iii) one of the investigators (Dr. Duncan) was a consultant at those centres and thus the investigation could be carried out without disruption of ongoing clinical work and teaching; (iv) by using the teaching hospitals and the Lidetta MCHC, medical students could be exposed to research methodology and concepts during their training. For women attending the FPC, ANC and PNC inclusion criteria were (i) they were the first to be registered that day, thus possibly being kept longer at the MCHC than otherwise would have been the case. For women attending the GOPD of the two hospitals their selection criteria were that they were first-visit attenders (i) who would not be likely to have received antibiotics recently and (ii) who as first attenders would be more representative of the GOPD clientele, than those returning for repeated investigation and treatment. Verbal
informed consent was obtained from all participating women who were informed that a blood sample would be taken from them and a free-of-charge test for syphilis would be carried out and treatment given. No woman refused consent for either the questionnaire, the blood taking or the clinical examination. The VDRL results were taken back to the clinics within 48-72 hours where standard penicillin (PAM) treatment was prescribed for syphilis if indicated and if other appropriate antibiotic treatment had not already been given. Other gynaecological conditions were also handled similarly.

The number of women in the study was determined by the results of preliminary studies regarding (i) the prevalence of N.gonorrhoea, trichomoniassis and the clinical evidence of PID in 100 GOPD attenders; (ii) the prevalence of N.gonorrhoea in 200 parturient women; (iii) the aetiology and treatment of 134 women with pelvic and puerperal sepsis, and pelvic abscess; all these studies had been carried out in St Paul's Hospital. A minimum of 200 patients was considered to be a suitable number to allow for meaningful statistical analysis of the results. The breakdown of the study group into approximately 50% symptomatic GOPD attenders and 50% asymptomatic attenders divided into half each of FPC and obstetric patients was selected for the same reason. The collection of data, cytology slides and sera, and the VDRL test was made during an eight month period in 1975 and 1976 while ethnic and socioeconomic factors could be assessed independently of population migration that occurred during the revolution. Analysis was done abroad but the data and specimens could not, however, be despatched from the country until 1977 and 1978. Further serologic testing using micro-methods in five European laboratories was carried out from 1983-1990 as appropriate tests and funding became available.

*Personal details were obtained by means of a questionnaire completed, in private, initially by two senior Ethiopian nurse midwives

| Table 1: Socioeconomic factors of women using family planning clinics in Addis Ababa |
|-----------------------------------------|----------------|----------------|
| n FPC/n of women studied (%) | p value |
| **Ethnic group** |
| Amhara 373 / 1223 | 31 |
| Oromo 61 / 317 | 19 |
| Gurage 32 / 318 | 10 |
| Tigray 58 / 176 | 33 |
| Other " | 18 / 72 | 25 |
| **Religion** |
| Orthodox 494 / 1856 | 27 |
| Moslem 29 / 208 | 14 |
| **Origin** |
| Urban 494 / 1718 | 29 |
| Rural 36 / 352 | 10 |
| **Monthly Income Ethiopian Birr (EB)*** |
| 1 - 10 | 51 / 367 | 14 |
| 10 - 50 | 213 / 669 | 32 |
| 50 - 100 | 60 / 118 | 32 |
| 100 - 500 | 114 / 317 | 36 |
| > 500 | 25 / 79 | 32 |
| **Age** |
| < 20 | 31 / 181 | 17 |
| 20 - 34 | 428 / 1421 | 30 |
| 35 - 49 | 76 / 443 | 18 |
| > 50 | 0 / 53 | <0.001 |
| **Parity** |
| 0 | 9 / 392 | 2 |
| 1 | 67 / 392 | 17 |
| 2 | 106 / 353 | 30 |
| 3-5 | 238 / 636 | 37 |
| > 6-10 | 114 / 304 | 38 |
| > 10 | 8 / 25 | 32 |
| **Age at first marriage** |
| < 13 | 106 / 462 | 23 |
| 13-15 | 211 / 839 | 25 |
| 16-18 | 143 / 507 | 28 |
| > 18 | 53 / 199 | 27 |
| **Number of husband/sexual partners** |
| 1 | 242 / 910 | 27 |
| 2-5 | 143 / 720 | 20 |
| > 5 | 119 / 304 | 39 |
| **Unknown status/profession** |
| Single | 4 / 20 | 20 |
| Married | 286 / 1244 | 23 |
| Divorced | 89 / 345 | 26 |
| Widowed | 11 / 46 | 24 |
| Maid | 37 / 160 | 23 |
| Tall Seller | 35 / 132 | 27 |
| Prostitute | 45 / 105 | 43 |
| Bargin | 30 / 41 | 73 |

* n = number. Because the data were not recorded for some variables, the number analysed does not add up to the total number of women in the study.

**Fifty percent (36/72) were Moslem

*** Many of the women did not know that their husbands income was, they only handled the "housekeeping money".

# Cochrane-Mantel-Haenzel no-zero correlation
by microscopy Papanicalaou sent to smear and upper and involving the urethra (U), gynaecological examination was prepared specially by Wilson. The who also made the reason or menarche, at age (born), pregnancies, parity (number of viable partners, number of sexual life number divorcee, housemaid, talla seller, status/profession (single, married, age, born),) were: present Tigringa by (usually a other languages, not speak Amharic, either interpretation from was Amharic the clinical completion by Dr. Duncan and the Ethiopian trained in the hospital/clinic. The who worked with Dr. Duncan and the Sisters who also checked the findings prior to the clinical examination. The language used was Amharic with, for those women who did not speak Amharic, either interpretation from other languages, or the questionnaire was completed by a senior clinic or hospital nurse (usually a sister) who spoke Orominga or Tigringa as a first language. Data included were: present age, ethnic group, religion, residence, family monthly income, marital status/profession (single, married, widow, divorcee, housemaid, talla seller, prostitute, bargirl), number of lifetime husbands/sexual partners, number of years married, duration of sexual life (years), total number of pregnancies, parity (number of viable children born), age at menarche, age at first marriage, age at first coitus, and relation of first coitus to the menarche, self-history of STDs (syphilis or gonorrhoea) and whether treated or not, use of contraceptives and type, and present complaint or reason for attending the hospital/clinic.

Clinical data were obtained by Dr. Duncan, who also made the cytological smears and collected the blood samples, assisted by Dr. Wilson. The procedure for collection of clinical data was standardised and recorded on specially prepared sheets.

Gynaecological examination: Full abdominal and gynaecological examination was carried out using a good light source (daylight or angle poise lamp). Particular attention was paid to the state of the cervix and for evidence of upper and lower genital tract infection involving the urethra (U), salpinges (S) and Bartholin glands (B) which were inspected (U,B) and palpated (U,S,B) (26).

Cervical cytology: A cervical cytological smear preparation was made. The slides were sent to Liège, were stained using the Papaniclaou stain and examined by light microscopy by Dr Pelzer for evidence of cancer cells, excessive numbers of polymorphonuclear (PMN) cells (evidence of cervical infection), and for presence of trichomonas and monilial hyphae (evidence of heavy vaginal infection involving the cervix).

Serological tests: Ten ml of whole blood was obtained by the clinic doctor from each woman using disposable needles and vacutainer tubes prelabelled with name and study number. The serum was prepared the same day and stored at -20°C. Serological tests for syphilis were carried out on all sera using VDRL. The sera were then frozen and stored at -20°C. Sera were transported frozen to the United Kingdom and kept at -20°C until further testing could be carried out: in Edinburgh for syphilis using TPHA (27) and hepatitis B virus (HBV) (28); in London for herpes simplex virus (HSV2) (29) and Chlamydia trachomatis D-K (CTD-K) and Lymphogranuloma venereum 1-3 (LGV) (30); in Copenhagen for N. gonorrhoea (gonococcal antibody test (GAT) (31); and in Antwerp for Haemophilus ducreyi (32,33).

Statistical analysis was made using the Chi-square and Cochran-Mantel-Haenzel General Association Statistic (34).
Results

There were 2111 women enrolled for the study of whom 542 (25.7\%) attended FPC clinics.

Utilisation of FPC and contraceptives was highest among women who were Tigraawi (33\%), Amara (31\%), Ethiopian Orthodox (27\%), urban dwellers (29\%), had a family income of 100-500 EB per month (36\%), were aged 20-34 years (30\%), had two or more children (35\%), were age 16 or older at first marriage (28\%), had more than five lifetime husbands sexual partners (39\%), and were by profession (bagirls 73\%, prostitutes 43\%) (Table 1). Regarding the last factor, it should be noted that the categories of last marital status/profession were mutually exclusive: eg. a divorcee who became a prostitute was recorded as the latter.

FPC utilisation was lowest among women who were nulliparous (2.3\%), came from rural areas (10\%), were Guragie (10\%) or Oromo (19\%), or Moslem (14\%) and those of subsistence income (<10 EB per month) (14\%).

Most common complaints among clinic attenders were abdominal or pelvic pain, menstrual disorder, vaginal discharge and urinary symptoms. Women attending FPC had few symptoms compared with those attending other clinics such as GOPD, ANC, PNC (Table 2).

The prevalence rates for serological evidence of exposure to all six STDs were higher in the FPA group compared with women attending other clinics, but only statistically significantly increased for gonorrhoea, and high titre genital chlamydiae (Table 3). Only 4\% of FPC attenders had no serological evidence of STD (Table 4) and 64\% had serologic evidence for three or more STDs. Clinical evidence of PID, past or present, was more common among the FPC attenders chiefly because they had more salpingitis. In contrast, cervical cancer, dysplasia, cervical infection and trichomoniasis (the latter two being diagnosed cytologically) were all significantly less common among FPC attenders. Only eight women (1.5\%) attending FPC were found to be pregnant on clinical examination: whether these women were pregnant as a consequence of failed contraception/forgetting to take the combined pill, or came in hopes of having an IUCD inserted (as an abortifacient), we could speculate.

More FPC attenders required treatment for salpingitis and urethritis than non-FPC attenders. Cervical ectropion (erosion) was more common among FPC attenders (Table 5).

Discussion

The analysis of social and economic factors associated with acceptance of family planning and contraception is complex.

The higher utilisation of FPC among Tigraawi and Amara women was associated with higher income and, possibly, with education. Family monthly income may be an unreliable measure, but should be included in any further study of FPC attenders. The impression was that there were more Tigraawi women amongst the professional group (nurses, teachers, secretaries) who also were in the higher income group and, by virtue of their job, better educated, and thus better motivated to achieve a smaller family size. Thus the reason for the association of FPC utilisation with income may have been ethnic and economic rather than religious. Indeed, there was a twofold difference of FPC attendance between the Orthodox and Moslem women. But from cross variable analysis (details not shown) there were proportionately more Orthodox Christian women than Moslems in the higher income bracket (100-500 EB per month). Furthermore, significantly fewer attenders at FPC belonged to the poorest (<10 EB per month) group. The economic aspect is enigmatic as the service was entirely free at MCHC clinics and at one of the teaching hospitals. There must be other reasons such as education, tradition, or possibly better survival of children, which promotes family planning acceptance among upper income women.
Table 3: Prevalence of sexually transmitted diseases among women attending family planning and other clinics in Addis Ababa.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number tested</th>
<th>Number positive</th>
<th>OR</th>
<th>95% CI</th>
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<tr>
<td></td>
<td>FPC</td>
<td>Other</td>
<td></td>
<td></td>
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<tr>
<td>Syphilis (TPHA)</td>
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<td>180</td>
<td>39</td>
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<td>1362</td>
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<td>382</td>
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<td>1370</td>
<td>266</td>
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<tr>
<td>Gonorrhoea</td>
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</tr>
<tr>
<td>C. trachomatis (D-K &amp; LGV1-3)</td>
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<td></td>
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<tr>
<td>Hepatitis B virus</td>
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<td></td>
</tr>
<tr>
<td>H. ducreyi</td>
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<tr>
<td>Cervical cytological diagnosis of infection</td>
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</tr>
<tr>
<td>Trichomoniasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID (BUS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bartholinitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urethritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salpingitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cancer (all diagnoses)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical cytological diagnosis of Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n - number; OR - Odds ratio; FPC - Family planning clinic attenders; NS - non significant; Other - Women attending other clinics (gynaecological or Obstetric); TPHA - Treponema pallidum haemagglutination assay; CAT - Gonococcal antibody test; HSV-2 - Herpes simplex virus 2; * - High titre indicates present or active infection; ** - Negative statistical correlation with family planning attenders compared with the attenders at other clinics.
The greatest demand for FPC services was from women with more than 5 lifetime husbands or sexual partners, the majority of whom were employed as commercial sex workers. For social reasons the demand for FPC services was lower for women having 2-5 lifetime sexual partners/husbands but not involved in prostitution. Possibly of significance is the observation from analysis of the infertility data that the complaint of infertility was highest in those with two or three lifetime sexual partners or husbands.

In this study, the main differences among a prostitute, bargirl or talla seller were age, age at first marriage and duration of marriage. As reported by the women, 95% of each group had a monthly family income of <50 EB. The classification of women involved in prostitution into these three groups was based on how the women initially described themselves. On detailed cross variable analysis it was found that the three classifications did in fact fall into separate groups. Bargirls were the youngest, had been married for the shortest time (presumably employed to serve as waitresses in bars for the physical needs of their customers) and were paid a monthly wage irrespective of their work load. The professional prostitutes (setinga adari) were self-employed and put a red coloured light in the windows of their houses. The youngest often stand (to solicit) in front of their doorways or in the streets, while the older women sit at home and wait for their clients. Tall sellers worked from home, many sitting outside their houses selling talla to passers-by. These women, frequently widows or divorcees, are among the oldest and poorest involved in prostitution. Not all tall sellers were involved in prostitution: 5% of those we studied reported having become tall sellers without explicitly stating that they were separated from their husbands. Professional prostitutes and talla sellers were first married very young. In general, prostitutes and bargirls maintained their jobs and livelihood, by virtue of not becoming pregnant. Both groups had higher income than talla sellers who, on the other hand, were frequently widowed or divorced, and would not lose their jobs should they become pregnant.

Detailed analysis shows increased usage of FPC according to age at first marriage. Attendance was lowest (20%) amongst those married before the age of 10 years, and highest (38%) among those married after the age of 25 years. Age at first marriage/coitus had a further indirect influence of FPA acceptance. Detailed analysis showed that parity was significantly (P<0.001) associated with age at first coitus: those with sexual debut at age 13-18 had the largest number of children. Those married youngest also had the lowest family income (whether as cause or effect cannot be determined by our data), and age at first marriage/coitus increased with higher family income and, as stated above, possibly with education.

The effect of these factors on FP acceptance is thus related to the following: (i) very young age at first coitus leads to increased risk of STD especially gonorrhea and genital chlamydial infection (35,36) with resultant PID and infertility; such infertile women would not require FP advice/contraception, but would attend FPC for infertility advice; (ii) age of sexual debut at 13-18 is associated with higher parity and could prompt a demand for FP advice; (iii) older age at first marriage is associated with higher income, better education, decrease in infant and perinatal mortality, and hence, lower parity, and is likely to lead to more FP acceptance, as has been shown elsewhere (37).

Women attending the FPC had remarkably few symptoms/complaints compared with other clinic attenders, although they had more clinical evidence of past/present PID, and more active infection requiring immediate treatment. They also had more serological evidence of exposure to STDs, and higher titres which were indicative of active/present infection. Less than 3% complained of vaginal discharge although 9% had T. vaginalis in their cervical cytology smear which would
have been even contraception have of laboratory test STDs. The contraceptive. Thus certain of STDs and PID, and old/earlier women are prostitution. Studies IUCD the using the contraceptive showed of Oral contraceptives epithelium being latter increase in PID, and STDs (19) increasing may reflect a very severe infection involving the cervix.

The increased STDs and subsequent PID may be explained by oral contraception and the use of IUCD. Some of the combined pills (oral contraceptives) cause cervical ectropion, as was observed in our study, with the transformation zone and the endocervical epithelium being exposed to bacterial and viral agents. Oral contraceptives are per se a factor increasing transmission/acquisition of STD. Detailed analysis of PID prevalence and type of contraceptive showed 4% increase in PID in women using the contraceptive pill, and 12% increase in PID in those using an IUCD, the latter despite our deliberate policy not to insert the IUCD in women with clinical evidence of old/earlier PID, and women engaged in prostitution. Studies elsewhere have shown that women are quick to blame ill health or symptoms of STDs and PID on a contraceptive. Thus certain effective methods of contraception have fallen into disrepute and even led to the failure of FP programmes (3).

The tables show an apparent discrepancy in the number of serological tests for specific STDs. The first 100 women had their VDRL test carried out in St Paul's Hospital. The laboratory was unwilling to continue the routine free testing of the study patients (especially as some women would normally have been able to pay for the test), nor to store the sera. Thus from patient study number 101 onwards the VDRL testing and storage of sera was done by NAMRU-5. For consistency, the VDRL results of the first 100 patients were discounted, although the patient's socioeconomic, clinical and cytological data were used in the analysis of serum. Thus the earlier tests VDRL were carried out on 1955 sera, and the latest (for antibody to H. ducreyi) on 1831 sera.

The seroprevalence rates for exposure to STD pathogen among Ethiopian FPC attenders is high. In particular there were 31% with GAT titres ≥1/3209, 45% with antibody to present active or recent gonococcal, genital chlamydial or herpetic infections, respectively. These prevalence rates are higher than the few published, available data from FPC elsewhere in Africa: gonorrhoea 3% in Zaire (2) and 5% in Ibadan (38) Nigeria, 10% in South Africa (3), and 17.5% in Kenya (39); genital chlamydiae 16%, culture positive in South Africa (5), and 35% seropositive in Nigeria (40); or syphilis (TPHA) 18% in Nigeria (2). Swaziland had 15% trichomoniasis among FPC attenders (4). We could not find published records of FPC prevalence rates for either genital herpes or chancroid in Africa. It could be argued that the data from this study are rather old and hence irrelevant to Ethiopia in the mid 1990s. This study however is useful as a seroepidemiological baseline and should prompt another study as a matter of urgency particularly as there are indications that teenage sexual activity, previously virtually unknown, and STD prevalence are both on the increase (14).

**Significance of marital status/profession:**
A contributing factor to the high STD prevalence rate among FPC attenders reported in this study may have been the proportion of women in prostitution attending the FPC (20%) which is twice times higher than the number of women in prostitution attending other clinics (11%). Detailed analysis of association of the classical STDs (syphilis and gonorrhoea) with marital status/profession has shown the lowest prevalence to be among

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>FPC attenders</th>
<th>Other clinic attenders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
</tr>
</tbody>
</table>
those still with their first husband/sexual partner. Women who were married to their second or subsequent husbands, widows, divorcees or maids formed an intermediate group between those with a stable first marriage and those involved in prostitution. Among the commercial sex workers, prevalence rates for classical STDs increased from being lowest among *talla* sellers to highest among bargirls (42). However detailed analysis of these three groups (i) women with stable first marriage, (ii) women in prostitution, and (iii) all other women (single, widowed, divorced, maids, married to second or subsequent husband) showed that there was no difference in the proportion of group (i) women attending FPC or other clinics, and there were rather fewer (45%) compared with 55% of the group (ii) women attending FPC. Hence, it is unlikely that this group was responsible for the increase in STDs amongst FPC attenders. We have combined marital status and profession, as according to the information the women gave us, the two were mutually exclusive. "I was divorced, now I am a prostitute", "I was married now I am a bargirl", "My husband died now I am a *talla* Seller". To exclude those who are now in prostitution from the analysis would be to exclude 14% of the women.

As most gonococcal and genital chlamydial infections in women are silent (26,36) women with these infections form a reservoir of infection, infecting and being infected by promiscuous men who transfer the infection to others (43,44), whether the transfer be from women in prostitution to the wife or vice versa. Thus as women across the social and marital spectrum attend FPC, a new responsibility for family planning services is the detection, treatment and prevention of reproductive tract infections (44), although as Hopcraft et al observed, "In family planning

Table 5: Gynaecological conditions requiring immediate treatment in family planning and other women's clinics in Addis Ababa

<table>
<thead>
<tr>
<th>Condition</th>
<th>Attenders at FPC</th>
<th>Attenders at other clinics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Total</td>
</tr>
<tr>
<td>Number of women seen</td>
<td></td>
<td>542</td>
</tr>
<tr>
<td>Pelvic inflammatory disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PID/parametritis</td>
<td>20</td>
<td>106</td>
</tr>
<tr>
<td>Pelvic Abscess</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>124</td>
</tr>
<tr>
<td>Salpingitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute/chronic</td>
<td>141</td>
<td>222</td>
</tr>
<tr>
<td>Pyosalpinx</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>239</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper &quot;UTI&quot;</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Urethritis</td>
<td>101</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>38</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>Noted but not treated</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Cervical erosion</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number; FPC = family planning clinic; * required emergency admission
programmes in most developing countries the question of diagnosing and treating STDs appears to be almost completely ignored, probably because of fear of calling attention to a possible association between contraception and promiscuity, and partly because of implications for extension of staff training and clinic services" (39). Indeed at the time of this study, the MCHC’s were designed to run at low cost and thus only did basic investigations. Bacteriological and cervical cytology examinations were done in referral hospitals, and then were only available to the very few who could afford to pay for these tests. However as we have shown elsewhere there is increased PID among those who have been infected by N. gonorrhoeae or genital chlamydiae (26,36). The diagnosis of PID by detecting palpably thickened Bartholin glands, urethra or salpinges (26) can be made easily by trained community nurse/midwives. Subsequent treatment with a two week course of tetracyclines should clear both infections as well as treating co-existent syphilis.

FPCs, especially those in MCHCs which have a good rapport with the surrounding community, are then in a strong position to carry out discrete tracing and treatment of contacts of infected women. Ideally interim microbiological surveys of FPC attenders at clinics in different locations would monitor the STDs prevalence and the effectiveness of intervention strategy. These tests are expensive and can only be carried out in specialised centres. The advent of serological tests by micromethods while not always diagnostic, none-the-less are a valuable sero-epidemiological tool, and used along with clinical and sociological data in the more readily available statistical programs are valuable in detecting populations at risk.

In conclusion, a high seroprevalence of STDs and PID among FPC attenders is reported. The FPC provides a useful setting for screening and treating women particularly at risk, who for lack of symptoms, will not attend either an STD clinic nor a hospital for routine investigation. These untreated women represent a population from which STDs are introduced in the community. We recommend that FPCs should adequately screen, treat and educate FPC attenders, their partners, and their clients, in an attempt to control STDs in the community. It is imperative to treat the male sexual partner(s) concurrently to avoid reinfections. This is particularly important because of the risk of HIV spreading rapidly through the community (46), which can be anticipated as it has been observed that gonorrhoea, a marker of sexual activity (47), is highly prevalent among the FPC attenders studied. Moreover, STDs, especially those causing genital ulceration, facilitate the transmission of HIV. Further surveys should be implemented to monitor STDs prevalence and the effectiveness of intervention programs.

Acknowledgments

We thank the staff and patients of St. Paul and the Black Lion Hospitals and Lidetta Clinic for their co-operation and Dr. Philippa Wilson for her assistance in collecting and compiling data; NAMRU-5 for storing the sera and for syphilis testing using VDRL. We thank the Family Guidance Association of Ethiopia for financial assistance with the salary of a part-time Ethiopian clerical assistant who collected the socioeconomic data from the women studied.

We acknowledge with thanks financial assistance from Allied Medical Group for serological testing, the Swedish Agency for Research Cooperation with Developing Countries (SAREC: Grant SPE-AIDS-HN-03-AV) for support of pp and ER, and The Welcome Trust for travel and secretarial expenses for MED. We thank Mrs M Pearce for secretarial help.

References


27. Young H, Henrichsen C and Robertson DHH.
Prevalence and significance of sexually transmitted diseases among Ethiopian women attending antenatal clinics in Addis Ababa

M Elizabeth Duncan¹, Gerard Tibaus, Andrée Pelzer, Letebirhan Mehari, John Peutherer, Hugh Young, Yasmin Jamil, Sohrab Darougar, Inga Lind, Karin Reimann, Peter Piot, Erwin Roggen, Peter L Perine

Abstract: To determine the prevalence of sexually transmitted diseases (STDs) and the risk for (i) the mother regarding pregnancy wastage and puerperal sepsis and (ii) the child with regard to congenital and neonatal infection, 342 routine antenatal clinic (ANC) attenders were investigated. The prevalence of antibodies showing exposure to specific STD pathogens in pregnant women attending ANC was: syphilis (TPHA) 27%, (VDR:) 28%, gonorrhoea 43%, genital chlamydiae 54%, HBV 37%, HSV-2 35%, H ducreyi 10%. High titre seropositivity suggestive of active infection was: gonorrhoea 10%, genital chlamydiae 31%, HSV2 19%; with HBV SAg 5% - all of which are likely to be transmitted to the foetus in utero or during delivery. Only 10% of ANC attenders had no serological evidence of any STD: 72% had serological evidence for two or more STDs. Among conditions requiring treatment vaginitis was the most important, 20% having a severe trichomonal infection. Despite the frequency of this condition it was noted that few women (4%) complained of vaginal discharge. Thus women attending the ANC revealed a high prevalence of STD. Consequently the foetus and neonate are put at risk because of intrauterine or intrapartum transmission of infection. The high prevalence among ANC attenders also reflects the relative prevalence of STDs in the community. Measures such as screening at ANC and information and education regarding prevention are required to reduce STDs in pregnant women and their sexual partners. Prophylaxis for the neonate can be considered until this goal is achieved. [Ethiop. J. Health Dev. 1995;9(1):31-40]

Introduction

With the decline of endocarditis and pyelonephritis in pregnant women, the problems of serious infections in obstetric practice have shifted from the pregnant or parturient woman to her unborn or infant child (1). The foetus and the premature newborn, physiologically disadvantaged by immaturity, are prey to endogenous micro-organisms harboured in the birth canal. The likely etiological causes of congenital infection are grouped together in the unlovely word STORCH (syphilis, toxoplasmosis, other (viruses), rubella, cytomegalovirus and herpes viruses) each of which may cause clinical manifestations in the newborn (1). Gonococcal ophthalmia, once a scourge in industrialised countries, is still extremely important in developing countries with highly prevalent STDs (2). Maternal Chlamydia trachomatis infections cause premature rupture of the membranes and low birth weight as well as neonatal ophthalmia and pneumonia (3). Maternal syphilis has long been recognised as

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a health hazard for the child, while more recently hepatitis B (4,5) and HSV2 have been shown to be vertically transmitted, the latter being passed from mother to child from infected genital lesions during vaginal delivery (1).

Ethiopia is a country where STDs are highly prevalent (6). The prevalence of STDs among ANC attenders has not been quantified but for two notable exceptions. Screening for syphilis at a mother and child health clinic showed that 15%-17.6% (7,8) of the mothers were sero-positive. The prevalence of Neisseria gonorrhoeae was 9% in 200 isolates from asymptomatic parturient women (9). N. gonorrhoeae were also isolated from 28% of women with puerperal sepsis (9).

Routine antenatal screening for STDs has been, and still is for many African countries, only by serological tests for syphilis, usually using the Venereal Diseases Reference Laboratory (VDRL) or Rapid Plasma Reagin (RPR) tests. Gonorrhoea has been identified by the presence of intracellular gram negative diplococci in Gram-stained smears of endocervical or vaginal discharge. Only recently have serological tests using microtechniques become available for an increasing number of STDs.

To quantify the problem in terms of prevalence of STDs and the related risk for the mother developing puerperal sepsis, and of the child with regard to congenital and neonatal infection, the pregnant women attending ANC were investigated. The data are part of a larger investigation of socioeconomic factors associated with the prevalence and transmission of STDs and cervical cancer among 2111 Ethiopian women conducted in 1975. Permission to carry out the study and to send patient data, sera and cytological slide preparations to Europe for processing was given by the Ministry of Health of Ethiopia.

Methods

A total of 2111 Ethiopian women attending clinics in two teaching hospitals and an MCH clinic in Addis Ababa were enrolled in this cross-sectional study (Table 1), after giving informed verbal consent. Pregnant women attending the ANC regularly were entered in the study on the basis of first come first to register for the clinic without other criteria for pre-selection. None of those invited to take part refused to do so.

Details regarding the study number and patient criteria, collection of data, serum storage and timing of testing are recorded elsewhere (10). The collection of data, cytology slides and sera, and the VDRL test were made during an eight month period in 1975 and 1976 while ethnic and socioeconomic factors could be assessed independently of population migration as occurred during the revolution. In brief:

Patient socioeconomic data was collected by means of questionnaire completed, in private, by an Ethiopian female assistant, in Amharic with translation into other languages if required.

Clinical data including the cytological smears and blood samples, were obtained by two physicians. Particular attention was paid to the state of the cervix and for evidence of upper and lower genital tract infection, involving the urethra (U), salpinges (S) and Bartholin glands (B) which were inspected (U,B) and palpated (U,S,B) (11).

Cervical cytological data for cancer cells, excessive numbers of polymorphonuclear (PMN) cells (evidence of cervical infection), and for presence of trichomonas and monilial hyphae (evidence of heavy vaginal infection involving the cervix) were obtained from Papanicalaou stained smears examined in Liège by a physician.

Serological tests for syphilis were carried

<table>
<thead>
<tr>
<th>Clinic</th>
<th>No. seen</th>
<th>No. pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>Postnatal</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td>Family Planning</td>
<td>542</td>
<td>8</td>
</tr>
<tr>
<td>Gynecological</td>
<td>1121</td>
<td>184</td>
</tr>
<tr>
<td>Total</td>
<td>2111</td>
<td>534</td>
</tr>
</tbody>
</table>

No. refers only to the women attending the ANC

Table 1: Distribution of pregnant women by clinic
out in Addis Ababa using VDRL; in Edinburgh for syphilis using TPHA (12) and hepatitis B virus (HBV) (13); in London for herpes simplex virus (HSV2) (14) and Chlamydia trachomatis D-K (CTD-K) and Lymphogranuloma venereum 1-3 (LGV) (15); in Copenhagen for N. gonorrhoeae (gonococcal antibody test (GAT) (16); and in Antwerp for Haemophilus ducreyi (17,18).

Statistical methods: Data were stored and computed in Liège by G.Tibaux. Statistical analysis was made using the Chi-square and Cochran-Mantel- Haenzel General Association Statistic (19).

Results
Sociodemographic data of women attending ANC are shown in Table 2.

The prevalence of antibodies showing exposure to specific STD pathogens in pregnant women attending ANC is shown in Table 3 (syphilis (TPHA) 27%, (VDRL) 28%; gonorrhoea (GAT) 43%; genital chlamydiae 54%; HBV 37%; HSV-2 35%; H ducreyi 10%). Of particular interest is the prevalence of high titre seropositivity suggestive of active infection: gonorrhoea 10%, genital chlamydiae 31%, HSV-2 19%; also HBV SAg 5% and HBBeAg 4%. All these infections are likely to be transmitted to the foetus in utero or during delivery.

Palpable thickening of urethra, salpinges and Bartholin glands with/without tenderness was regarded as evidence of present/past genital infection (11). Assessment of Fallopian tubes by palpation was only possible up to 14-16 weeks gestation. Clinical evidence of PID was 23% overall, chiefly due to urethritis, although five women with a normal pregnancy had acute salpingitis, and 11 (3%) had Bartholinitis (Table 3).

Cervical cytology revealed no cervical cancer among ANC attenders, but 36% had grossly infected slides indicative of cervicitis, 20% had T. vaginalis in the smear indicating presence of a severe vaginal infection.

### Table 2: Sociodemographic status of ANC attenders

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>No</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amhara</td>
<td>188</td>
<td>(55)</td>
</tr>
<tr>
<td>Oromo</td>
<td>41</td>
<td>(12)</td>
</tr>
<tr>
<td>Gurage</td>
<td>69</td>
<td>(20)</td>
</tr>
<tr>
<td>Tigre</td>
<td>28</td>
<td>(8)</td>
</tr>
<tr>
<td>Other</td>
<td>14</td>
<td>(4)</td>
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<tr>
<td>Total</td>
<td>340</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Religion</th>
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<tbody>
<tr>
<td>Ethiopian</td>
<td>301</td>
<td>(89)</td>
</tr>
<tr>
<td>Moslem</td>
<td>36</td>
<td>(11)</td>
</tr>
<tr>
<td>Origin</td>
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<tr>
<td>Urban</td>
<td>312</td>
<td>(92)</td>
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<tr>
<td>Rural</td>
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<td>(8)</td>
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<table>
<thead>
<tr>
<th>Age</th>
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<th>(%)</th>
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<tbody>
<tr>
<td>&lt;20</td>
<td>49</td>
<td>(15)</td>
</tr>
<tr>
<td>20-24</td>
<td>106</td>
<td>(31)</td>
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<tr>
<td>25-29</td>
<td>95</td>
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<td>30-34</td>
<td>57</td>
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<td>40-49</td>
<td>7</td>
<td>(2)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>0</td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Parity</th>
<th>No</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>(26)</td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td>(19)</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>(16)</td>
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<tr>
<td>3-5</td>
<td>84</td>
<td>(25)</td>
</tr>
<tr>
<td>6-10</td>
<td>45</td>
<td>(13)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>2</td>
<td>(1)</td>
</tr>
</tbody>
</table>

50% (36/72) were Moslem

NB. Of women attending ANC, only 27 (8%) had used any form of contraception: 25 pill, 2 IUCD

Only 10% of ANC attenders had no serological evidence for any STD: 72% had serological evidence of two or more STDs. (Table 4).

The chief complaints among ANC attenders were due to the discomfort of late pregnancy, in three cases to onset of labour in the former group and to abortion in the second group (Table 5). The majority of ANC attenders were seen in the third trimester of pregnancy. Among conditions requiring treatment (Table 6) vaginitis was the most important, chiefly due to trichomonal or monilial infection. Despite the frequency of this condition it was noted that few ANC attenders (4%) complained of vaginal discharge.
Discussion
Epidemiological surveys in selected population groups yield useful data on STDs prevalence in the community in which antenatal patients are considered as 'normal risk' group (20) of healthy women of reproductive age group. Hence the high exposure to STDs detected among ANC attenders must be of concern, as they reflect the relative prevalence of STDs in the community. The higher seroprevalence of STDs in pregnant women attending GOPD may reflect the socioeconomic profile of these women. Of particular concern to obstetricians and neonatologists is the risk to the mother of puerperal sepsis (2,21), and vertical transmission to the foetus in utero or during parturition.

Prevalence rates for syphilis among African pregnant women are high (22,31). The high prevalence of reactive serological tests for syphilis in this study indicates not only that the prevalence in the community is high, but also that antenatal screening and treatment for this disease is essential. Syphilitic infection in the mother may result in abortion, intrauterine death, intrauterine growth retardation (IUGR) and congenital syphilis. With highly endemic maternal syphilis, congenital syphilis causes foetal and perinatal death in up to 40% of the infants affected. Rates for congenital syphilis are 850/100,000 live births in Lusaka and 3,200/100,000 in Addis Ababa (32,8).

Treatment of syphilis in pregnancy is normally based on positive specific serology. If, however, facilities are not available to confirm or disprove the positive non-specific test (VDRL) treatment is normally given without confirming the diagnosis. In areas of high prevalence, particularly where there is past history of abortion or unexplained perinatal death (including macerated foetuses), it is suggested that routine penicillin prophylaxis is not only justifiable (33) but that mass treatment with a single dose regimen of penicillin of all clinic attenders would be cost effective, leaving clinic staff free to investigate the high risk groups (22). Without simultaneous treatment of infected sexual partners, reinfection would be likely and high prevalence (25%) of congenital syphilis may still be found (30).

In Ethiopia, 21% of children of seropositive mothers developed signs of syphilis, while the stillbirth and abortion rate of infected women was almost double that of the normal clinic population (7). A contemporaneous study to this one found that syphilis was the fourth most common cause of perinatal death and accounted for 10% of the 70 perinatal deaths per 1000 births and almost 5% of all postneonatal deaths (34).

Table 3: Sexually transmitted diseases and gynaecological conditions in ANC attenders

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No tested</th>
<th>No positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syphilis (TPHA)</td>
<td>307</td>
<td>83</td>
<td>27</td>
</tr>
<tr>
<td>Syphilis (VDRL)</td>
<td>314</td>
<td>84</td>
<td>27</td>
</tr>
<tr>
<td>N. gonorrhoeae (GAT)</td>
<td>308</td>
<td>131</td>
<td>43</td>
</tr>
<tr>
<td>'Titre $\geq$ 1/320</td>
<td>308</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>C. trachomatis (D-K &amp; LGV1-3)</td>
<td>306</td>
<td>164</td>
<td>54</td>
</tr>
<tr>
<td>'Titre $\geq$ 1/64</td>
<td>306</td>
<td>96</td>
<td>31</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>309</td>
<td>113</td>
<td>37</td>
</tr>
<tr>
<td>HBV SAg positive</td>
<td>309</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td>HSV 2</td>
<td>306</td>
<td>108</td>
<td>35</td>
</tr>
<tr>
<td>'Titre $\geq$ 1/128</td>
<td>306</td>
<td>59</td>
<td>19</td>
</tr>
<tr>
<td>H. ducreyi</td>
<td>306</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>#Seronegative</td>
<td>342</td>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>Cervical cytology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>298</td>
<td>107</td>
<td>36</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>298</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>PID (BUS)</td>
<td>339</td>
<td>79</td>
<td>23</td>
</tr>
<tr>
<td>Bartholinitis</td>
<td>339</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Urethritis</td>
<td>339</td>
<td>73</td>
<td>22</td>
</tr>
<tr>
<td>&quot;Salpingitis&quot;</td>
<td>339</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

No = number; TPHA = Treponema pallidum haemagglutination assay; GAT = Gonococcal antibody test; HSV 2 = Herpes simplex virus 2; PID = Pelvic inflammatory diseases; B = Bartholinitis; U = Urethritis, S = Salpingitis; #Seronegative = Women with no serological evidence for any of the STDS shown above.

* Salpingitis could only be assessed on those with pregnancy <14-16 weeks.
In Zambia congenital syphilis/syphilitic infection is implicated in 20-30% of the total perinatal mortality which is 50 per 1000 births (35). Additionally 19% of miscarriages in Zambia are attributed to syphilis (36), while there is a 28-fold increased risk for stillbirths among women with RPR seroreactivity at titre > 1/23 (24). Almost 9% of the infants under three months of age admitted to hospital and 7.5% of neonates admitted to intensive care units had congenital syphilis (28).

The 43% GAT seropositivity in normal risk Ethiopian women indicates a high level of gonococcal infection in the community. The 10% seropositivity at titre ≥1/320 indicative of active infection is comparable to 9% culture positivity found in parturient women (9,11), and to the prevalence reported from many sub-Saharan countries (35,37,40).

Gonococcal infection in the mother, usually asymptomatic, is found in the endocervix, urethra and Bartholin glands. Acute gonococcal salpingitis may occur in the first trimester and simulate the signs and symptoms of ectopic pregnancy. The significance of a high level of infection in parturient women is the subsequent development of puerperal sepsis. *N.gonorrhoeae* is an important primary pathogen isolated from 18-34% of women with puerperal sepsis (9,37,40,41).

Untreated antenatal maternal gonorrhoea is also important because of risk of contamination of the neonate during its passage down the birth canal. Gonococcal ophthalmia is an emergency condition requiring immediate treatment because of risk of corneal ulceration. Silver nitrate drops into each eye at birth is effective but may cause chemical conjunctivitis. Alternatives are 10% sulphacetamide drops, penicillin, chloramphenicol or tetracyclines eye ointment, the latter also being effective against ocular but not pharyngeal *C.trachomatis* infection. In some developing countries, eye prophylaxis reduced the incidence of neonatal ophthalmia by 83% and 93% when using silver nitrate and tetracycline ointment, respectively (42).

Chlamydial serovars A-C, D-K and LGV are all sexually transmissible. The prevalence of antibody to genital chlamydiae (54%) in this study was very high: 31% had IgG titres ≥1/64 or IgM titres ≥1/8 indicating present/active infection (43). The role of genital chlamydial pathogens in the aetiology of pelvic infections is well established in industrialised countries, but a similar situation in developing countries has only recently been appreciated (44). For lack of reference, prevalence rates for *C.trachomatis* culture positive infections in pregnant African women (39,45-48) are similar to those for *N.gonorrhoeae*. The prevalence rates of 27-71% diagnosed by serology without giving high titre seropositivity (49,50,25), or Chlamidiazyme tests (29) cannot be compared with the culture rates. Although the role of *C.trachomatis* as a pathogen in puerperal sepsis has not yet been established, suggestive evidence for this is that *C.trachomatis* has been isolated from 6-7% of healthy postnatal African women (40,38) but 8-20% with puerperal sepsis (51,41,40). *C.trachomatis* does however cause post-abortion sepsis (52). *C.trachomatis* has replaced *N.gonorrhoeae* as the most important single aetiological agent of neonatal infections, worldwide, causing up to 32% to all cases (2). The transmission rate

---

**Table 4: Seroprevalence of STDs among Antenatal Clinic attenders in Addis Ababa**

<table>
<thead>
<tr>
<th>Seroprevalence</th>
<th>ANC attenders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
</tr>
<tr>
<td>Negative (All STD)</td>
<td>33 (10)</td>
</tr>
<tr>
<td>Positive 1 STD</td>
<td>60 (18)</td>
</tr>
<tr>
<td>2 STDs</td>
<td>74 (22)</td>
</tr>
<tr>
<td>3 STDs</td>
<td>73 (21)</td>
</tr>
<tr>
<td>4 STDs</td>
<td>49 (14)</td>
</tr>
<tr>
<td>5 STDs</td>
<td>18 (5)</td>
</tr>
<tr>
<td>6 STDs</td>
<td>35 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>342</td>
</tr>
</tbody>
</table>
from an infected mother to her newborn is 30-45% for *N. gonorrhoea* and 30% for *C. trachomatis* (37,2).

Inclusion conjunctivitis, typically, develops within the second week of life; nasopharyngitis, otitis media or afebrile pneumonia may occur 2-3 months after birth, associated with cough and marked tachypnoea. *C. trachomatis* has recently been reported to be the most common cause of ophthalmia neonatorum in The Gambia (33%), compared with *N. gonorrhoeae* (25%) (53). Prevention of infection of the newborn is based on the identification and treatment of the mother during pregnancy. Where the diagnostic methods/facilities are lacking, empirical treatment is indicated for pregnant women whose male sexual partners have non-gonococcal urethritis (NGU). The mother should be treated with erythromycin 500mg, twice daily for ten days; tetracyclines, the standard treatment for chlamydial infections in men, should be avoided if possible during pregnancy because of possible adverse effects on the foetus.

<table>
<thead>
<tr>
<th>Table 5: Symptoms of ANC attenders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Number of women seen</td>
</tr>
<tr>
<td>Pain/discomfort</td>
</tr>
<tr>
<td>Abdominal</td>
</tr>
<tr>
<td>Pelvic</td>
</tr>
<tr>
<td>Backache</td>
</tr>
<tr>
<td>Other gynae, pain</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Abnormal bleeding</td>
</tr>
<tr>
<td>Amenorrhoe**</td>
</tr>
<tr>
<td>Continuous bleeding</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Vaginal discharge</td>
</tr>
<tr>
<td>Urinary symptoms</td>
</tr>
<tr>
<td>Dysuria</td>
</tr>
<tr>
<td>$$ Frequency</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* Percentage of total symptoms may be misleading, as some patients had more than one.

** These women did not realise that they were pregnant.

§ Unusually there were no complaints of frequency.

Maternal herpes infection during pregnancy has been associated with spontaneous abortion, congenital malformations of the central nervous system (CNS) and prematurity, while neonatal infections caused by HSV2 transmitted during the second stage of labour affect predominantly the skin and CNS. In industrialised countries, if the virus is known to be present in the genital tract at or near term, elective Caesarean section is recommended (1). Apart from this study the only record of the prevalence of HSV2 in pregnant women in Africa is 53% in the Gambia (54). Congenital infection is rare in some developing countries because HSV1 and HSV2 infections are almost universal in childhood, and women are immune to the viruses by the time they become pregnant.

Hepatitis B is endemic in Ethiopia, with regional variations (55). The prevalence among ANC attenders (HBV 37% (all markers), 5% HBsAg and 4% HBeAg) is at the lower end of the population ranges reported for both Ethiopia (56) and elsewhere (24,25,45,57). Where risk of vertical transmission is most important, vaccination of babies born to HBsAg positive mothers should be adequate. However, where horizontal transmission may present an equally significant risk, ideally, all babies should receive active vaccination at birth. The cost of such a programme makes it a practical proposition only for a very few who are rich. (58). In Ethiopia, where hepatitis is the most important non-obstetric cause of maternal mortality (59), there were 93 cases with 31 maternal deaths (3.9/1000 deliveries) in 1973-74 (60): the mortality rate in 1973 rose to 43% during the third trimester while the known foetal wastage was 60% for those delivering in hospitals (58).

The risk to the mother of *H. ducreyi* infection is of developing genital ulcerative disease which would predispose to infection with HIV. In turn HIV could be transmitted to the foetus in utero. Apart form this, other possible risks to the neonate from maternal infection are at present unknown, and require further investigation.
Trichomoniasis, although common among Ethiopian pregnant women, the prevalence being in the middle of the reported range from other African countries (14-49%) (41,45,53,61,62), caused little symptomatic vaginitis. However as trichomonas can also be found in the urethra and Skene's glands, the symptoms may be confused with those of bacterial urinary tract infection. Probably the most important effect of the infection is the alteration of the vaginal pH, thus interfering with defence mechanisms in the vagina (33). The 2 g stat dose of metronidazole administered in the ANC (63) has revolutionised the treatment of what can be a troublesome and distressing condition.

This study has shown that women attending ANC in Addis Ababa in 1975/76 had a high prevalence of STDs. STDs can affect the mother, as well as putting the foetus and neonate at risk because of intrapartum or intrapartum transmission of infection. The high prevalence among ANC attenders also reflects the relative prevalence of STDs in the community. It has taken the emergence of HIV/AIDS, a fatal STD, to highlight the problems of STDs across Africa and the need to understand the epidemiology of these conditions, and to control transmission.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women seen</td>
<td>342</td>
</tr>
<tr>
<td>In labour</td>
<td>3</td>
</tr>
<tr>
<td>With medical, gynaecological or obstetric complication</td>
<td>27</td>
</tr>
<tr>
<td>UTI (upper)</td>
<td>5</td>
</tr>
<tr>
<td>Urethritis</td>
<td>15</td>
</tr>
<tr>
<td>Vaginitis</td>
<td>71</td>
</tr>
<tr>
<td>Cervicitis(acute)</td>
<td>3</td>
</tr>
<tr>
<td>Salpingitis</td>
<td>4</td>
</tr>
<tr>
<td>Acute/chronic</td>
<td>4</td>
</tr>
<tr>
<td>Bartholin</td>
<td>1</td>
</tr>
<tr>
<td>cys/abscess/infection</td>
<td>3</td>
</tr>
</tbody>
</table>

by all means that are socially and culturally acceptable. Because of the mothers' concern for a healthy baby, screening and treating pregnant women and their husbands/sexual partners is recommended as a first step prevention of STDs among ANC attenders and must be a high priority for health care providers and administrators. Awareness of SAD as a problem, and understanding of the mode of transmission is essential. Education for STD prevention requires sensitivity as well as cultural attitudes and beliefs. In some African languages STDs are translated as women's diseases. Withholding details of the diagnosis in the mother or child until the male partner/father has been tested, to inform him of his (positive) result before his wife is given her result has been found to be an effective way of dealing with this misconception. Ultimately the best prevention is fidelity in stable closed heterosexual relationships (6-4) with barrier methods for those involved in prostitution for economic survival. Such methods will result in the control of SAD, HIV/AIDS in particular.

Acknowledgements

We thank the staff and patients of St Paul and the Black Lion Hospitals as well as Lidetta Clinic for their co-operation; Dr Philippa Wilson for her assistance in collecting data and the staff of NAMRU-3 for syphilis testing using VDRL. We acknowledge with thanks financial assistance received for serological testing from Allied Medical Group (TPHA, HBV, HSV and C.trachomatis); the Swedish Agency for Research Cooperation with Developing Countries (SAREC: Grant SFRG-03-AY) for support of pp and ER; The Wellcome Trust for travel and secretarial expenses for NED and Mrs M.Pearce for secretarial help.

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course of chronic hepatitis D in our cohort of HIV-immunosuppressed patients, and we postulate that high replication of the delta virus and the presence of HCV co-infection, in conjunction, could explain this worse outcome.

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Accepted for publication 31 October 1994

Spontaneous loss of PPNG resistance plasmids

The Scottish Neisseria gonorrhoeae Reference Laboratory Annual Report for 1992 highlighted one of the points made in the study on gonococcal epidemiological data from Stockholm, Sweden.

A small cluster of penicillinase-producing Neisseria gonorrhoeae (PPNG) infection in Central Region, Scotland, demonstrated spontaneous loss of plasmid encoding during the process of clinical assessment, screening and treatment. Patient 1 who attended on 17 August 1992 with minor vulvodynia (and who had had a hysterectomy in 1986) was found to have a gonococcal infection with a PPNG isolate of serovar IB-1/Bopst. Her partner, who was contact traced on 2 September 1992 and reported a casual contact in Tenerife, was also shown to be infected with a IB-1/Bopst PPNG isolate. It is of interest that the casual contact originated from a Scottish Health Board Area (Fife) adjacent to Central Region. Patient 3 (no connection with nos 1 & 2) attended with urinary symptoms on 10 September 1992 with positive microscopy and a IB-1/Bopst non-PPNG isolate was reported. His partner was contact traced on 11 September 1992, had complained of cystitis over a five month period, and a IB-1/Bopst PPNG (showing a weak reaction in the chromogenic cephalosporin test) was isolated; the culture was later shown to contain both penicillin sensitive and penicillin resistant IB-1 isolates. A repeat culture from the same patient received one week later was found to be IB-1/Bopst non-PPNG. All of the PPNG isolates carried 2.6, 3.05 and 24.5 Mda plasmids, were non-requiring (NR) on auxotyping and had a ciprofloxacin MIC of 0.06 mg/L. The non-ppnccilin isolates were also auxotype NR with a ciprofloxacin MIC of 0.06 mg/L. There were no other IB-1/Bopst strains isolated in Scotland during 1992.

As all patients were contact traced, the cluster of infection was contained with the added bonus of demonstrable spontaneous loss of β-lactamase plasmid during surveillance. This report also highlights the importance of national surveillance. It is unlikely that the probable source of infection in Tenerife, who originated from Fife, has returned to Scotland with an infection as this would have been detected through the Scottish Neisseria gonorrhoeae Reference Laboratory.

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Accepted for publication 25 October 1994

Disseminated infection due to penicillin resistant gonococci—is it still rare?

Penicillin resistant gonococci have only rarely been implicated in disseminated gonococcal infection. Two reports attributed two separate cases of gonococcal arthritis to penicillinase producing organisms but these cases were not well documented. In neither case were the organism cultured directly from a disseminated site and the relation of the arthritis to the gonococcal infection was presumptive, being based on positive throat or urethral cultures. However, five cases of gonococcal arthritis due to penicillinase producing organisms that were cultured directly from infected joints have been reported. As an addition to these cases, we describe a case of gonococcal arthritis due to penicillinase producing organisms, based on culture from the infected joint.

A 25 year old West Indian woman was admitted to the orthopaedic department in September 1993 with a history that following return from Jamaica, she was suffering from flitting joint pain affecting particularly her
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Storage and repeated recovery of *Neisseria gonorrhoeae* using cryovials

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(Accepted 25 August 1994)

Abstract: One hundred gonococcal isolates, representing eight protein IA serovars and 14 protein IB serovars, were stored at −70°C using the Pro-Lab Microbank™ cryovial storage system. At monthly intervals a bead from each cryovial was removed and cultured on modified New York City medium. The overall recovery rate was 98.6% (2365/2400), the 35 failures representing 13 separate isolates. There was a small but significant decrease in recovery in the last 12 months of the trial (97.8%) compared with the first 12 months (99.8%), which may have been due to a sampling problem rather than a temporal phenomenon. Failures were significantly associated with minor serovars, suggesting that the transmissibility/viability of minor serovars may be lower than that of common serovars and could be a significant factor in the epidemiology of gonococcal infection. We conclude that Pro-Lab Microbank™ cryovials provide a highly effective and convenient system for storage of *Neisseria gonorrhoeae*, particularly when multiple retrieval is required, and the system offers many advantages over conventional lyophilisation.

Key words: Cryopreservation. *Neisseria gonorrhoeae*. Serotyping.

Introduction

Due to the fastidious nature of *Neisseria gonorrhoeae* a simple, inexpensive and efficient system for the storage and recovery of clinical isolates and quality control strains is required for good clinical laboratory practice, in research and for epidemiological studies. Various methods such as use of cooked meat broth, lyophilisation or freezing in liquid nitrogen are available for the storage of bacteria but none is ideal, particularly for gonococci. The limitations associated with these methods are the variable recovery of bacteria, the time and inconvenience involved in the preparation and maintenance of cultures, and the financial cost in the purchase and maintenance of expensive equipment.

The technique of storing organisms at −70°C described by Nagel and Lawrence in 1971 has given rise to simple and convenient commercial storage systems such as the Pro-Lab Microbank™, which uses coloured beads in a 'cryovial' containing cryopreservative fluid. After inoculation and storage a single bead can be removed to inoculate culture media.

We examined the Microbank system with a view to its overall convenience of use for storage and recovery. The recovery rate after medium-term storage and repeated sampling was analysed and an evaluation made of the efficacy of recovery in relation to the spectrum of antigenic types (serovars) of gonococci that occur in nature.

Materials and methods

**Bacterial strains.** One hundred clinical isolates of *N. gonorrhoeae*, including 30 penicillinase-producing *N. gonorrhoeae* were included in the study. These strains represented the wide variety of antigenic types of gonococci encountered in natural infection and comprised 26 serogroup IA strains covering eight different serovars, 61 serogroup IB strains covering 14 different serovars, and 13 serogroup IB strains which were non-typeable with the standard monoclonal antibody serotyping panel.

**Preservation and storage of strains.** Using a sterile cotton bud, gonococcal colonies were harvested from an
18–24 hour culture on modified New York City medium and a suspension made in the cryopreservative fluid of the cryovial approximately equivalent to a McFarland No. 4 standard. The inoculated vial was closed and the contents inverted 4–5 times to coat the beads with bacteria. Excess cryopreservative fluid was removed with a sterile pastette, the vial closed and immediately placed in a −70°C freezer.

Retrieval of bacteria. All 100 gonococcal isolates were sampled each month for 24 consecutive months. Twenty cryovials at a time were removed from the −70°C freezer, using an aluminium transfer block to retain a low temperature. Using sterile forceps a single bead was removed from the cryovial, placed onto the surface of a culture plate containing modified New York City medium, and allowed to thaw. A sterile loop was used to streak out the area around the bead to obtain separate colonies and the culture plates incubated for 48 h in a carbon dioxide-enriched atmosphere.

Serotyping. Serotyping of the gonococcal strains was performed using the Genetic Systems panel of monoclonal antibodies.

Statistical analysis. The chi-squared test was used for all statistical analysis.

Results

As shown in Table 1, the overall recovery rate from 2400 retrievals for the 24 months of the trial was 98.6% (2365/2400), and all strains were recovered in eight of the 24 months (100% recovery). The recovery rate for the remaining months ranged from 99% to 96%: 99% in five of the months, 98% in five of the months, 97% in four of the months, and 96% in two of the months. Although the recovery rate was extremely good there were 0.8% (9/1191) failures in the first 12 months compared with 2.2% (26/1174) in the last 12 months—a significant difference ($\chi^2 = 8.4; P < 0.01$).

Table 1. Monthly recovery rate for 100 gonococcal isolates

<table>
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<th>No. of failures</th>
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<td>24</td>
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<tr>
<td>Total</td>
<td>2365 (98.6%)</td>
<td>35 (1.5%)</td>
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<table>
<thead>
<tr>
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<td>IB15</td>
<td>13,14,15,20,23,24</td>
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<td>IB29</td>
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<td>11,14,16,20,22</td>
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IB00 (Non-typeable strain)

Thirty-five isolates comprising serovars IA02, IA06, IB01, IB02, IB03, classified as major serovars from continuous prevalence studies in our geographical area, accounted for only 2.9% (1/35) of the failures. The remaining 65 isolates, classified as minor serovars, accounted for 18.3% (12/65) of the failures—a significant difference ($\chi^2 = 4.9; P < 0.05$).

Discussion

Lyophilisation has long been accepted as the ‘gold standard’ method for the preservation of microorganisms, but the high cost in equipment and processing time precludes its use in many routine laboratories. Modern technology has made −70°C facilities readily available and the small capacity
required to store large numbers of isolates makes cryovial storage systems extremely convenient for the clinical laboratory which requires easy access to strains. Concerns over refrigeration failure and the subsequent loss of valuable strains can be alleviated by fitting carbon dioxide back-up systems, designed to activate at a pre-set temperature to the freezer.9

Nagel and Lawrence2 first described a method for the preservation of multiple replicate units of bacteria using sterile glass beads and a mixture of equal parts of broth culture and horse blood allowing storage of at least 200 beads in a plastic tube at −70°C. In a subsequent study Feltham et al.10 used different concentrations of cryoprotectants in the storage media used to make the bacterial suspensions, and stored the beads at −76°C. They observed a reduction in the number of viable bacteria with nutrient broth lacking cryoprotectant and with nutrient broth containing 15% dimethyl sulphoxide. Nutrient broth supplemented with either 10% dimethyl sulphoxide, 10% glycerol or 15% glycerol showed no such reductions. In a further study White and Sand11 demonstrated the viability of organisms after storage at −76°C for two years, using glass beads and brain-heart infusion broth containing 10% glycerol as the emulsifying fluid.

In this study we have shown that the Microbank system offers a simple commercially available system for medium-term storage and multiple recovery of N. gonorrhoeae. The overall recovery rate of 98.6% is extremely good and, together with the ability to sample up to 25 times, represents substantial cost benefits. The failure of four isolates to grow at least five times each may be associated with the strains, or may be a simple physical problem of insufficient primary inoculum in these vials. The overall recovery rate could possibly be improved with the use of special recovery medium. Morton and Smith12 advocated the use of a solution of 20% sucrose in phosphate-buffered saline for the recovery of fastidious organisms such as Neisseria spp., though clearly this is not essential for the vast majority of gonococcal isolates.

There was a small but significant decrease in recovery in the last 12 months of the trial, which may reflect a sampling problem rather than a temporal phenomenon, and further long-term studies are underway to differentiate between these possibilities. The finding that failures were significantly associated with minor serovars suggests that the transmission/viability of minor serovars may be lower than that of common serovars, and could be a significant factor in the overall epidemiology of gonococcal infection. The selective loss of minor serovars on storage could also lead to a bias in epidemiological studies based on isolates that have been stored for some time.

Table 3. Distribution of 13 failures in relation to individual serovars

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<tr>
<td>IB00</td>
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</tbody>
</table>

IB00 (Non-typeable strain)

References
3 Pro-Lab Diagnostics, Unit 7, Westwood Court, Clayhill Industrial Estate, Neston, Wirral L64 3UH, England, UK.
6 Not available commercially. Antibodies supplied for this study by Dr Cathy Ison, St Mary’s Hospital, Paddington, London, England, UK.
9 Denley Instruments Limited, Natts Lane, Billingshurst, Sussex RH14 9EY, England, UK.
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Gonococcal serovar patterns in Glasgow: 1990–1992

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*Department of Genitourinary Medicine, Lauriston Building, Edinburgh Royal Infirmary, Edinburgh EH3 9YW, Scotland, UK; †Department of Genitourinary Medicine, Glasgow Royal Infirmary, Scotland, UK; and §Department of Medical Microbiology, University of Edinburgh, Teviot Place, Edinburgh, Scotland, UK

(Accepted 25 October 1994)

Abstract: Using monoclonal antibodies directed against protein 1 (major outer membrane protein) in the cell wall of Neisseria gonorrhoeae it is possible to serotype the gonococcus into different sub-groups. This study was designed to analyse the distribution of such serovars in Glasgow, Scotland, and report associations between serovars and clinical features of infection. N. gonorrhoeae isolated from all patients with a diagnosis of gonorrhoea attending genitourinary medicine clinics in Glasgow were serotyped between January 1990 and December 1992. The results were then correlated with sexual orientation of patients, penicillin sensitivity, site of infection, location of acquisition of infection and presence of symptoms. Six hundred and four episodes of gonococcal infection were analysed and an association between certain serovars with sexual orientation, penicillin sensitivity and asymptomatic infection was found. No association between serovar type and locality of acquisition of infection was apparent. Although there was a decreasing trend in the incidence of gonorrhoea overall, infections in homosexual men increased over the three-year study period. The associations between serovars and other features of gonococcal infection are discussed. The observed increase in homosexually-acquired infection has implications with regard to the spread of human immunodeficiency virus infection in this area, and suggests that attempts to promote safer sex in this group are failing.

Key words: Epidemiology. Homosexuality. Neisseria gonorrhoeae. Serology.

Introduction

Using monoclonal antibodies directed against epitopes on the protein 1 molecule present in the outer membrane of Neisseria gonorrhoeae, it is possible to serotype the gonococcus. Isolation of such serovars has been correlated with antibiotic sensitivity, sexual orientation of patients, site of infection, presence of symptoms, dissemination of infection and geographical origin. Given the integral role of protein 1 in the pathogenesis and immune response to gonococcal infection some of these correlations are not surprising. However, although epidemiological associations can be demonstrated, individual serovars are not totally homogeneous in their characteristics—i.e. gonococci of the same serotype may exhibit different properties.

In this prospective study the pattern of gonococcal serovars isolated over a three-year period in Glasgow is described with particular reference to the relation of individual serovars with penicillin resistance, the ability to produce asymptomatic infections, and association with anatomical site of infection.

Methods

All patients attending the Departments of Genitourinary Medicine at Glasgow Royal Infirmary and the Southern General Hospital, Glasgow, between January 1990 and December 1992 with a diagnosis of gonorrhoea were included in the analysis. The diagnosis of gonorrhoea was made on the basis of culture of N. gonorrhoeae on selective and non-selective media from the urethra, rectum, endocervix and/or throat. All male patients had a single urethral swab taken while female patients had urethral and endocervical swabs cultured.
on two separate occasions to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea, and when the history indicated that this site had been placed at risk. Rectal and throat cultures were taken routinely from men who gave a history of homosexual contact.

All gonococcal isolates were forwarded to the Scottish Gonococcal Reference Laboratory for antibiotic sensitivity testing, and serotyping with the American panel of monoclonal antibodies (as has been described previously). Minimum inhibitory concentrations (MICs) of penicillin were determined by an agar plate dilution method using a series of plates incorporating 0.015, 0.06, 0.12, 0.5 and 1.0 mg/L of penicillin. MICs <0.12 mg/L were classed as sensitive, and isolates with MICs ≥0.5 mg/L as reduced susceptibility.

Information was obtained from the case notes of patients on sex, sexual orientation, site of infection, location from which the infection had been acquired, and age and presence of symptoms. Serovar data were then analysed with respect to the clinical data.

The data were entered into a dBase database programme and statistical analysis performed using chi-square or Fisher's exact test (as appropriate) on the EpiInfo statistical package.

Results

A total of 604 episodes of gonorrhoea were diagnosed in 595 patients over the three-year study period. The demographic characteristics of the study group are shown in Table 1. 527 patients were seen at Glasgow Royal Infirmary and 77 at the Southern General Hospital, Glasgow. Serovar data were available for 532 isolates (88%) and penicillin susceptibility data on 530 (88%).

Twenty-five different serovars were isolated (Table 2) and these were sub-divided into major serovars (isolated on over 10 occasions) and minor serovars (isolated on 10 or less occasions), with major serovars accounting for 469 (88%) of typed isolates. Analysis was performed separately for major serovars and collectively for minor serovars. The overall quarterly prevalence of gonorrhoea remained stable over the three-year period, but sub-analysis of heterosexually and homosexually acquired infections demonstrated an increase in infections in gay men while heterosexual infections declined (Fig. 1). There was an increase in male homosexually acquired rectal infections from four in 1990 to 11 in 1993. Twenty-two different serovars were isolated in heterosexual patients, with a decline in the incidence of infections due to 1A-2, 1B-2 and 1B-3 over the study period (Table 3). Eleven different serovars were isolated from gay men with no marked variation in individual serovar isolation with time (Table 3).

No significant difference was found between serovars with respect to penicillin sensitivity (in non-penicillinase-producing N. gonorrhoeae [PPNG] strains) with two exceptions - serogroup 1A infections were more sensitive than 1B infections (P<0.001), and serovar 1B-7 had reduced sensitivity compared to other 1B serovars (reduced susceptibility in 18/21 1B-7 isolates cf. 132/356 for all 1B serovars, P<0.001). Comparisons were made between the penicillin sensitivities of individual serovars in heterosexual and homosexual patients (Table 4; this table excludes isolates for which serovar or penicillin susceptibilities were not available). Serovar 1B-2 was found to be less susceptible to penicillin when isolated in heterosexual patients compared to homosexuals (relative risk for reduced susceptibility in heterosexual patients 8.25, 95% confidence interval 2.5–35), with the reverse true for serovar 1B-3 which was less susceptible when isolated in gay patients. Comparisons of penicillin sensitivity by serogroup and sexual orientation revealed that 1A strains isolated in gay men were less sensitive than those found in heterosexuals (for serogroup 1A 6/85 heterosexual men with reduced penicillin sensitivity cf. 2/3 homosexual men, P=0.02).

Table 1. Characteristics of patients with gonorrhoea

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Br J Biomed Sci 1995; 52
Table 2. Distribution of serovars by sexual orientation in Glasgow 1990–1992

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<tr>
<td>1B-00**</td>
<td>3</td>
<td>2</td>
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<td>0</td>
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<td>1</td>
<td>5</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
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<tr>
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<td>1</td>
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<td>0</td>
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<td>1B-15</td>
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<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
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<tr>
<td>1B-16</td>
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<td>0</td>
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<tr>
<td>1B-31</td>
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<td>1</td>
<td>6</td>
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<td>58</td>
<td>11</td>
<td>1</td>
<td>2</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>440</td>
<td>97</td>
<td>16</td>
<td>51</td>
<td>604</td>
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</table>

*P<0.001; **Untypable strains.

Table 3. Temporal changes in serovars in heterosexual and homosexual patients

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Heterosexual</th>
<th>Homosexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A-2</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>1A-21</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>1B-1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1B-2</td>
<td>38</td>
<td>22</td>
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<tr>
<td>1B-3</td>
<td>30</td>
<td>14</td>
</tr>
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<td>1B-6</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>1B-7</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>1B-17</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Unknown</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>179</td>
<td>150</td>
</tr>
</tbody>
</table>

There were no significant differences in the penicillin sensitivity of isolates acquired locally (within Glasgow) compared to those acquired further afield (reduced susceptibility in 68/289 local isolates cf. 42/128 non-local isolates, P=0.06).

Thirteen PPNG infections were isolated from seven different serovars (1A-2, 4 isolates; 1A-4, 1 isolate;

Br J Biomed Sci 1995; 52
Table 4. Penicillin sensitivity of serovars in heterosexual and homosexual patients (non-PPNG infections where sexual orientation is known)

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Heterosexual</th>
<th>Homosexual</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A-2</td>
<td>1/110</td>
<td>-</td>
</tr>
<tr>
<td>1A-21</td>
<td>0/29</td>
<td>-</td>
</tr>
<tr>
<td>1B-1</td>
<td>8/19</td>
<td>1/6</td>
</tr>
<tr>
<td>1B-2</td>
<td>32/64*</td>
<td>4/37</td>
</tr>
<tr>
<td>1B-3</td>
<td>6/52</td>
<td>5/8**</td>
</tr>
<tr>
<td>1B-6</td>
<td>14/20</td>
<td>6/19</td>
</tr>
<tr>
<td>1B-7</td>
<td>10/12</td>
<td>6/6</td>
</tr>
<tr>
<td>1B-17</td>
<td>2/25</td>
<td>-</td>
</tr>
<tr>
<td>Minor serovars</td>
<td>13/42</td>
<td>5/8</td>
</tr>
<tr>
<td>Total</td>
<td>86/373</td>
<td>27/84</td>
</tr>
</tbody>
</table>

*Significantly less susceptible than homosexually acquired infections, \( P<0.001 \).
**Significantly less susceptible than heterosexually acquired infections, \( P=0.003 \).

Table 5. Asymptomatic infections in heterosexual men with urethral gonorrhoea

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Symptomatic</th>
<th>Asymptomatic</th>
<th>Symptoms unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A-1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1A-2</td>
<td>52</td>
<td>5</td>
<td>2</td>
<td>59</td>
</tr>
<tr>
<td>1A-4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1A-6</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1A-16</td>
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<td>0</td>
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</tr>
<tr>
<td>1A-20</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1A-21</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>14</td>
</tr>
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<td>1A-25</td>
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</tr>
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<td>1B-00*</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1B-17</td>
<td>6</td>
<td>4**</td>
<td>0</td>
<td>10</td>
</tr>
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</tr>
<tr>
<td>1B-29</td>
<td>0</td>
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<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1B-31</td>
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<tr>
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</tr>
<tr>
<td>Total</td>
<td>222</td>
<td>19</td>
<td>7</td>
<td>248</td>
</tr>
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</table>

*Not typable; **\( P=0.004 \).

1A-6, 1 isolate; IB-1, 1 isolate; 1B-2, 1 isolate; 1B-5, 2 isolates; 1B-7, 1 isolate and 2 isolates from serogroup 1B which were non-reactive with the monoclonal antibody panel). The origin of infection was known in 11 of these 13, with eight originating locally. Six of the eight locally acquired infections were isolated within the second and third quarter of 1990.

A total of 248 heterosexual men had urethral infections with Neisseria gonorrhoeae. The presence or absence of symptoms in this group is shown in Table 5. Infections with serovar 1B-17 were significantly more likely to be asymptomatic than average (odds ratio 7.1, 95% CI 1.3—33). The 10 infections produced by serovar 1B-17 occurred over the whole study period, but the four asymptomatic infections all occurred within the five-month period December 1991 to April 1992. The frequency of asymptomatic infections in all men with urethral gonorrhoea was 20/338 (6%).

Discussion

The total incidence of gonorrhoea in Glasgow over the three-year study period varied according to sexual orientation—heterosexual infections decreased but homosexually acquired infections increased (Fig. 1). A fall in the incidence of gonorrhoea has been reported from many parts of the world over the past decade, reflecting increased awareness of the risks of infection with the human immunodeficiency virus.12–15 More recently an increase in rectal gonorrhoea has been noted in England,16 which suggests that safer sexual practices are being ignored and our data support this worrying trend. The decrease in heterosexual infections was associated with a fall in isolates of 1A-2, 1B-2 and 1B-3 strains. It has been reported previously that changes in the incidence of individual serovars may not mirror the total incidence of gonorrhoea, with some strains remaining at a constant low level within the community while others fluctuate.5 It is of interest to note that although heterosexual infections with serovar 1B-2 declined, this serovar still remained the commonest homosexually acquired strain and increased slightly between 1990 and 1992. Significant associations were noted between serovars 1A-2 and 1A-21 with heterosexual infections and 1B-2 and 1B-6 with homosexually acquired infections. We and others have also demonstrated the association of certain serovar classes with sexual orientation, although such associations may alter with time.3,4

The geographical variation of serovars is well known,17 with areas of even close proximity often having different spectra of serovars isolated.6 Comparison of these data with those collected from two geographically close cities (Edinburgh and Newcastle) over the same time period4 shows that most of the major serovars isolated in Glasgow were also found in the other two cities, although at different prevalences. However, infections with two serovars (1A-21 and 1B-17), which combined accounted for
10% of infections in Glasgow, were not isolated in either Edinburgh or Newcastle in 1992.

Many investigators have reported an association between serogroup and antibiotic sensitivity, with strains of the 1A serogroup generally being more sensitive than 1B strains.2,6,18-20 Particular serovars, such as 1B-7, are also associated with reduced penicillin sensitivity,1,2 as was the case in this study. Comparisons with serovars isolated in Edinburgh and Newcastle (using the same methodology) reveal that for at least one serovar the sensitivity pattern differs from that seen in Glasgow. Serovar 1B-2 was isolated frequently in both studies, but although fully sensitive in 60/72 (83%) isolates in Edinburgh and Newcastle this proportion was 80/124 (65%) for the present study. Of relevance may be the higher proportion of gay men in the Edinburgh/Newcastle study (85/211, 40%) compared to the present study (16% gay men). Resistance to antibiotics tends to be greater in infections acquired through homosexual contact, but whether this reflects adaptation to a local environment (such as the rectum21) or some other factor(s) associated with homosexual infection22 is not known. The reported association between cell membrane phenotype and serogroup in gay men may be relevant, although this might not be applicable in heterosexually acquired infections.22

Homosexually acquired infections are also associated with the 1B serogroup, and our data and those of others suggest that even the few 1A infections that occur in this group are due to serovars uncommon in heterosexuals, and appear to have a reduced susceptibility to penicillin compared to those in heterosexuals.23 Within the 1B serogroup our data show significantly reduced penicillin sensitivity in homosexually acquired infections caused by serovar 1B-3 but, interestingly, serovar 1B-2 infections were more susceptible in homosexuals. By using transformation experiments a genetic linkage can be demonstrated between protein 1B outer membrane protein and antibiotic resistance.24

An association between asymptomatic infections and different strains of Neisseria gonorrhoeae has previously been demonstrated, with AHU+ auxotypes and 1A-2 serovar infections reported to be more often asymptomatic than average.2,25 In the present study the only serovar associated with a lack of symptoms in male heterosexuals with urethral infection was 1B-17. While 1A-2 was one of the most successful serovars in this population, an association with asymptomatic infection was not found. Most cases of male urethritis are asymptomatic, but many serovars occasionally produced asymptomatic infections, suggesting that other factors apart from the serovar class per se may be relevant. The grouping of asymptomatic 1B-17 infections into one five-month period suggests that the factor(s) responsible for asymptomatic infection may be transmissible and reside with the strain rather than the host. It is also possible that dynamic changes in these (as yet unrecognised) factors may occur, which can alter the presentation of gonorrhoea.26

We found no differences in the penicillin sensitivity of isolates acquired within Glasgow and those acquired outside the city. Ison et al.,27 in a study based in Africa, reported that imported infections had a higher level of antibiotic resistance than endemic infections but this will depend on the areas from which Neisseria gonorrhoeae is imported. This indicates that importation of resistant strains was not a problem in the present study population, and provides a baseline for future comparisons. The overall level of PPNG observed in Glasgow was low, accounting for only 2% of isolates. The variety of serovars associated with PPNG was less than seen in non-PPNG infections, as has been reported by others.27-29

The pattern of gonococcal serovars isolated in Glasgow differs from that seen in neighbouring cities, although some similarities are evident. An association between serovars with sexual orientation, penicillin sensitivity and asymptomatic infections was demonstrated, with a lack of association noted for locality of acquisition of infection. PPNG infections remain a minor problem in this area at present, but the increase in homosexually acquired infections gives cause for concern.

Thanks are extended to Dr Grace Gallagher (Department of Bacteriology, Southern General Hospital), Dr John Hood (Department of Bacteriology, Glasgow Royal Infirmary) and the technical staff in both laboratories for their support in sending isolates to the Scottish Gonococcal Reference Laboratory.

References


Borland Software. dBase IV version 1.5.


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Temporal changes in the sensitivity of Neisseria gonorrhoeae to penicillin in Edinburgh, Scotland

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Department of Genitourinary Medicine, ¹Edinburgh Royal Infirmary, and ²Department of Medical Microbiology, University of Edinburgh, Teviot Place, Edinburgh, UK

Summary: Increased resistance of Neisseria gonorrhoeae to penicillin has been reported from many centres around the world since the introduction of antibiotic therapy in the 1940s. This study reports the temporal changes in gonococcal penicillin sensitivity over a 3-year period. All patients with a diagnosis of gonorrhoea in the city of Edinburgh, Scotland between 1990 and 1992 were included in the study. Penicillin sensitivity of isolates was analysed in relation to the sex and sexual orientation of the patient and the antibiotic therapy given.

Four hundred and twenty-seven new patient episodes of infection occurred of which penicillin sensitivities were available in 426. Eleven episodes of PPNG infection occurred. Chromosomally mediated penicillin resistance did not increase over the study period. No differences were evident in the sensitivity of isolates from homosexual and heterosexual patients.

Unlike many other areas the relative resistance of N. gonorrhoeae is not increasing in Edinburgh at present. This may relate to local antibiotic policies combined with intrinsic characteristics of N. gonorrhoeae itself. Unlike previous reports there did not appear to be any increased resistance to penicillin in isolates from gay men.

Keywords: Gonorrhoea, antibiotics, homosexuality, treatment

INTRODUCTION

Shortly after the introduction of antibiotic therapy for the treatment of Neisseria gonorrhoeae in the 1940s, in the form of sulphonamides, resistance rapidly developed which led to reduced clinical efficacy¹-². Penicillin then superseded sulphonamide therapy and initially all strains of the gonococcus were sensitive to even low doses of this antibiotic³ but in the 1950s and 60s low level chromosomally-mediated resistance developed⁴ necessitating an increase in the treatment dose⁵-⁷. More recently penicillinase producing N. gonorrhoeae (PPNG) have appeared yielding high level resistance⁸,⁹ and high level chromosomal resistance (CMRNG) has led to treatment failures with penicillin¹⁰,¹¹. Within different areas the extent of N. gonorrhoeae resistance to penicillin varies as does the velocity of change in resistance. This study describes the changes in penicillin resistance in the Lothian Region of Scotland in relation to sexual orientation, antibiotic usage and treatment failure and discusses possible causes for the differences observed between different areas.

METHODS

All patients with a positive culture for N. gonorrhoeae isolated by the laboratories in the city of Edinburgh between January 1990 and December 1992 were analysed. The diagnosis of gonorrhoea was made on the basis of culture of N. gonorrhoeae on modified New York culture medium from the urethra, rectum, endocervix and/or throat. All male patients had a single urethral swab taken whilst female patients had urethral and endocervical swabs cultured on 2 separate occasions to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea and when the history indicated that this site had been placed at risk. Rectal cultures were taken routinely from men who gave a history of homosexual contact and in all women attending.

Gonococcal isolates were identified on the basis of biochemical and immunological tests as has been described previously¹². Minimum inhibitory
concentration (MICs) of penicillin were determined by an agar plate dilution method using a series of plates incorporating 0.015, 0.06, 0.12, 0.5 and 1.0 mg/l of penicillin: MICs less than or equal to 0.06 mg/l were classed as sensitive, MICs greater than 0.06 mg/l but less than 1 mg/l as intermediate, and isolates with MICs greater than or equal to 1 mg/l as resistant. PPNG were detected by the breakdown of a chromogenic cephalosporin.

Information was obtained from the casenotes of patients with a diagnosis of gonorrhoea on sex, sexual orientation, date of diagnosis, antibiotic given and test of cure result. Patients were offered a review appointment one week after treatment at which time a repeat swab was taken as a test of cure (TOC). Male patients who became asymptomatic after therapy did not have a routine TOC performed.

The data were entered into the dBase (Borland Software) database programme and statistical analysis was performed using chi-square on the Epinfo statistical package (WHO public domain software).

RESULTS

Four hundred and twenty-seven new patient episodes of infection occurred over the 3-year period, of which penicillin sensitivities were available in 426. The patient characteristics and sites of infection are shown in Table 1. The proportion of homosexually-acquired cases increased over the study period—32% (56/176) in 1990, 35% (56/160) in 1991 and 38% (35/91) in 1992.

A total of 23 infections (5%) were resistant to penicillin. Eleven PPNG infections were identified over the study period with no increase observed over time—no PPNG were isolated in 4 separate quarters, one isolated in 6 quarters, 2 isolated in one quarter and 3 isolated in one quarter. Of the 12 CMRNG no significant increase in resistance was seen over time (Table 2). Despite this there was a decline in the use of beta-lactam antibiotics with an increase in the relative use of ciprofloxacin (Figure 1). There were very few treatment failures as assessed by positive tests of cure and no increase over the 3-year period (2 in 1990, 5 in 1991 and 2 in 1992) although the total number of gonococcal infections declined (Figure 2). Of the 9 treatment failures 6 occurred in men, all of whom were homosexual, and 3 in women. In 2 of the 9 the gonococcal isolates were resistant to penicillin (2 PPNG and 1 CMRNG) and amoxicillin was the antibiotic that had been initially prescribed. Four of the 6 men who had positive tests of cure had infection of either the rectum (n = 1) and/or pharynx (n = 4).

No significant differences were evident in the sensitivity of N. gonorrhoeae to penicillin between heterosexually and homosexually acquired infections: comparing isolates from heterosexual patients and homosexual patients isolates were sensitive in 56% (147/260) cf. 54% (80/147, P = 0.68), intermediate in 39% (101/260) cf. 40% (59/147, P = 0.80) and resistant in 4.2% (11/260) cf. 5.4% (8/147, P = 0.58).

DISCUSSION

Over the 3-year study period there has been no significant rise in penicillin resistance within the Lothian region of Scotland and unlike many other regions the level of clinically resistant N. gonorrhoeae remains low. PPNG were first isolated in the United States and the United Kingdom in 1976 and by the early 1980s plasmid-mediated resistance was widespread within many African countries with rates as high as 81% of isolates in
Ibadan in Nigeria\textsuperscript{14}. Widespread geographical differences were evident, with a PPNG isolation rate of 23\% in Dakar in Senegal\textsuperscript{15} and a decline in PPNG noted in Gabon in the mid 1980s\textsuperscript{15}. More recently, imported PPNG has been seen increasingly from the Caribbean region rather than from Africa and SE Asia\textsuperscript{16}. In the Western world contact tracing at first appeared effective but in some areas PPNG became endemic\textsuperscript{17-20}. Although in the UK PPNG has been declining since the mid 1980s\textsuperscript{16,21-23} it continues to be an increasing problem in the US\textsuperscript{21} whilst in Sweden numbers remain static\textsuperscript{21}. In common with others we have experienced small clusters of PPNG infections which are short lived and may bias a short term study of gonococcal resistance\textsuperscript{24}.

Unlike PPNG, chromosomal resistance tends to produce a slow stepwise decline in penicillin sensitivity until clinically significant levels are reached (CMRNG—MIC greater or equal to 1 mg/l). By the mid 1980s CMRNG was present in the US\textsuperscript{25} although chromosomal resistance was of less clinical relevance in the UK at this time. The method of measurement of different levels of penicillin resistance may vary in different laboratories and caution has been advised in directly comparing MICs\textsuperscript{26}.

Variation in the prevalence of penicillin resistance may be accounted for by a number of mechanisms. Where the MIC of the organism is close to that of the therapeutic level of antibiotic even a moderate decrease in sensitivity may give a particular strain of \textit{N. gonorrhoeae} a selective advantage. The widespread availability of penicillin within a community and potential for sub-therapeutic dosage regimens may encourage the development of resistance\textsuperscript{3} as may the prophylactic use of penicillin by prostitutes\textsuperscript{27}. PPNG in particular has the potential to spread rapidly within a community increasing in Nigeria, for example, from 0\% of isolates to 70\% over a 5-year period\textsuperscript{14}.

Although resistance to penicillin may be perceived as an advantageous property for a particular strain of \textit{N. gonorrhoeae} decreased sensitivity does not appear to correlate well with the overall prevalence of infection\textsuperscript{3}. This implies that increasing antibiotic resistance also confers some disadvantages for the organism such as changes in membrane permeability which may limit not just the action of antibiotics but also the uptake of nutrients. Thus antibiotic resistance will be only one variable with regard to survival of a particular strain of \textit{N. gonorrhoeae} within a community although its importance may increase in populations where exposure of the organism to sub therapeutic levels of antibiotics is high.

Sexual behaviour patterns are an important determinant of the prevalence of gonorrhoea and over the past decade gonococcal infection has declined in Lothian\textsuperscript{28}. This reduction in the number of infections may be expected to increase the selective pressures on \textit{N. gonorrhoeae}. Since there has been no change in penicillin resistance over a 3-year period this suggests that resistance is not a major factor in determining the prevalence of infection in this area at present. Alternatively, the small number of resistant strains may account for the decline in overall prevalence but this is less likely given the similar fall in gonorrhoea seen in many other countries following widespread media coverage of the risks of HIV infection which follow unprotected intercourse\textsuperscript{29}.

Despite the low level of penicillin resistance observed and low level of treatment failure the use of beta lactam antibiotics declined relatively over the study period. The antibiotic policy within the unit remained the same over this time with a stat dose of 2 g of amoxicillin with 1 g of probenecid recommended for uncomplicated urethral or endocervical gonorrhoea and ciprofloxacin 500 mg for rectal infections and a one-week course of amoxicillin for pharyngeal infections. The policy also permitted the use of ciprofloxacin for patients with uncomplicated infections where there was thought to be a risk of resistance to penicillin or where rectal or pharyngeal infection was suspected. A consequence of this policy and the increased proportion of isolates from homosexual men may therefore have led to the observed increased use of ciprofloxacin.

In our population there was no difference in antibiotic susceptibility in homosexually and heterosexualy acquired strains of \textit{N. gonorrhoeae}. This conflicts with previous reports which suggested
that infections in gay men tend to be with more resistant strains\(^{30,31}\) as was the case in our own area in the mid 1980s\(^2\). This suggests a change in the dominant gonococcal strains, and associated resistance, seen in this group and is in keeping with the exchange of serovars that may occur between homosexual and heterosexual populations\(^{29,33}\).

In the Lothian region of Scotland there has been no significant change in the sensitivity of *N. gonorrhoeae* to penicillin over the 3-year period 1990–1992 with a low level of reduced sensitivity present and PPNG infections uncommon. Despite this the use of non-beta-lactam antibiotics has increased. No difference in the sensitivity of *N. gonorrhoeae* to penicillin was found between heterosexual and homosexual patients.

References


(Accepted 21 July 1994)
Letters to the Editor

Referral of cases of gonococcal infection

Sir: We read with interest Rodgers et al.'s audit of referral of cases of gonococcal infection to a genitourinary medicine clinic1. In Edinburgh our experience differs somewhat to that in Chester.

Between January 1990 and December 1993 508 cases of gonorrhoea were diagnosed by the 3 microbiological laboratories in Edinburgh and passed on to the Scottish Gonococcal Reference Laboratory. Of these 464 (91%) were seen at the GU Medicine clinic in the city. The source of referral for those seen at the GUM clinic is shown in Table 1. Of the 44 remaining patients who did not attend the GUM clinic the referral source is shown in Table 2. As can be seen from the tables although GPs referred a quarter of all cases subsequently diagnosed as gonorrhoea, they also account for almost half of the cases which were not referred. The gynaecology department accounted for 18% of the cases which were not referred despite being on the same hospital site as GUM. Data was not available to assess how many patients had been given an appointment to attend GUM and had failed to so but a more formal referral procedure may be required to ensure adequate follow-up of these patients.

The 44 patients who had not attended GUM were compared to the 464 who had. Patients who had not attended GUM were more likely to be women than men (112/464 [24%] female GUM patients of 25/44 [57%] female non-GUM patients, P<0.001) and were also more likely to be under the age of 20 (78/464 [17%] for GUM attenders of 13/44 [30%] for non-GUM attenders, P=0.035). This may reflect opportunistic screening of women by GPs taking routine cervical smears or the greater reluctance of some women to attend a GUM clinic.

Ten of the 44 non-GUM attenders with gonorrhoea were diagnosed in the first quarter of 1991 whilst the rest of the cases were evenly distributed over the 4 year period. Of the 10 cases 6 were from GPs and 4 from gynaecology. The reason for this sudden and transient increase in non-referred cases is unclear.

Table 1. Referral source for patients with gonorrhoea attending Edinburgh GUM Clinic

<table>
<thead>
<tr>
<th>Referral source</th>
<th>No. of patients</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self referred</td>
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<tr>
<td>General practitioner</td>
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<td>Contact tracing</td>
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<tr>
<td>Total</td>
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</table>

Table 2. Source for patients with gonorrhoea NOT attending Edinburgh GUM Clinic

<table>
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<th>%age</th>
</tr>
</thead>
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<tr>
<td>Other inpatients</td>
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</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>100%</td>
</tr>
</tbody>
</table>

There was no significant difference in the serovar pattern of the isolates in those referred and those who were not (P=0.12) nor in the serogroup pattern (GA : 1B ratio 134 : 328 for GUM referrals cf. 14 : 30 for non-GUM referrals [2 GUM referrals were not serogrouped], P=0.84). The pattern of penicillin sensitivity likewise did not differ (P=0.55). This would imply that the gonococcal strain pattern as measured in patients attending GUM clinic is representative of the community as a whole.

Since most STD statistics are based on GUM clinic returns the assessment of the number of cases of gonorrhoea diagnosed outwith GUM clinics is of considerable importance. Further research is required both to quantify the number of STD patients who are diagnosed and treated by their GP without referral to GUM and also those treated empirically without bacteriological confirmation of infection.

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Reference


Falling prevalence of Chlamydia trachomatis infection—no time for complacency

Sir: The reported prevalence of genital Chlamydia trachomatis infection depends on the population screened and the identification procedure used. In 1974 Oriel reported a prevalence of C. trachomatis cervical infection in 18% of the women attending an inner London genitourinary medicine (GUM) Clinic. In 1989 two GUM clinics3,4 reported a fall in prevalence of this cervical infection to levels similar to others reported in lower risk populations such as women attending for termination of pregnancy, family planning and cervical cytology (5-12%).

To establish the current prevalence of C. trachomatis in an inner city clinic, consecutive female patients attending the Department of GUM at University College Hospital London from June to September 1990 were screened for genital infections including diagnosis of bacterial vaginosis using Amsele's criteria5, culture of vaginal secretions for Trichomonas vaginalis and Candida spp. Cervical cultures for Neisseria gonorrhoeae and C. trachomatis were taken (13 patients were tested for C. trachomatis by enzyme-linked immunosorbent assay (ELA)). Case notes were reviewed to extract data about symptoms, subjective cervicitis (cervical erythema and mucopus), objective cervicitis (>10 polymorphonuclear cells per high power field), contraceptive use, number of sexual partners in the preceding month and reason for attendance.

Specimens for C. trachomatis culture from 12 of 405 women were toxic. These women were excluded from further analysis. Fifty-four women were culture positive and one was positive by ELA (confirmed by direct
Serovar specific immunity to Neisseria gonorrhoeae: does it exist?

J D C Ross, A Moyes, H Young

Abstract

Objective—To determine whether the host immune response to gonorrhoea provides limited serovar specific protection from reinfection.

Subjects—508 episodes of gonorrhoea diagnosed at a city centre genitourinary medicine clinic including 22 patients with multiple infections over a 4 year period.

Methods—Patients with recurrent gonococcal infection were analysed with respect to the initial and subsequent serovars isolated.

Results—No significant difference was seen in the prevalence of serovars isolated following a repeat infection compared with those without repeat infections. The site of the initial infection did not appear to influence the subsequent serovar isolated.

Conclusion—We found no evidence of serovar specific immunity in our population. It remains possible that populations with a higher prevalence of gonorrhoea and more frequent infections may have a quantitatively greater immune response.

(Genitourin Med 1995;71:367-369)

Keywords: gonorrhoea, immunity, serotyping, sexual orientation

Introduction

The host immune response to gonococcal infection is primarily humoral in nature and, although a variety of antibodies can be detected both in serum and in genital secretions,1-4 individuals with gonorrhoea do not appear to be protected from reinfection with N gonorrhoeae as occurs with many other bacterial infections. This lack of protective immunity gives rise to the “core group” theory of infection, where a small core group of individuals with multiple partners produce the majority of infections within a community.5 However, it has been suggested that limited serovar specific immunity may occur and reinfection of an individual with the same serovar of N gonorrhoeae may be less likely than with a different strain.6 In this context it is of interest that immunoglobulin in vaginal fluid reacts comparatively more with Protein I, the serotyping antigen, than does immunoglobulin in serum.7

This study was designed to examine recurrent gonococcal infections in patients attending a Department of Genitourinary Medicine with respect to their initial and subsequent serovar as a surrogate marker of serovar specific immunity.

Methods

All patients presenting to the Department of Genitourinary Medicine at Edinburgh Royal Infirmary between January 1990 and December 1993 were analysed. The diagnosis of gonorrhoea was made on the basis of culture of N gonorrhoeae on modified New York culture medium from the urethra, rectum, endocervix and/or throat. All male patients had a single urethral swab taken whilst female patients had urethral and endocervical swabs cultured on two separate occasions to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea and when the history indicated that this site had been placed at risk. Rectal cultures were taken routinely from men who gave a history of homosexual contact and from all women.

Gonococcal isolates were identified on the basis of biochemical and immunological tests and serotyping was performed using the American panel of monoclonal antibodies as has been described previously.8 Information was obtained from the casenotes of patients with a diagnosis of gonorrhoea on age, sex, sexual orientation and date of diagnosis. The data on serovar prevalence was then analysed with respect to the clinical information.

The data were entered into the dBase (Borland Software) database program and statistical analysis was performed using chi square on the Epinfo statistical package (WHO public domain software).

Results

Five hundred and eight patient episodes of gonococcal infection occurred over the 4 year study period. Twenty one patients were infected with gonorrhoea on two separate occasions over the four year study period and one patient on three occasions (total of 45 infectious episodes). The sex, age, sexual orientation, time between infections, serovars isolated and serovars of sexual contacts are shown in the table. Eighteen of the patients were homosexual and four heterosexual (one male and three female). Six homosexual patients were initially infected with serovar 1B-2 and three of these were subsequently reinfected with the same serovar (50%). The
The prevalence of 1B-2 infections in all gay men over the same period was not significantly different at 48% (87/182 [p = 0.76]). Seven gay men had 1B-7 infections initially with one subsequently becoming reinfected with the same serovar (15%). The overall prevalence of 1B-7 infections in gay men was similar at 12% (22/182 [p = 0.68]).

Six homosexual patients initially presented with infection at multiple sites, two of whom had rectal infections. In total six of the gay men with repeat infections initially presented with rectal infections, two of whom were subsequently reinfected with the same serovar. Of eight gay men initially with pharyngeal infections three re-presented with an infection of the same serovar and of 10 urethral infections the same serovar was subsequently isolated in one.

Discussion

Protein I forms the basis for serotyping and the immune response to Protein I depends on a variety of factors including previous immune status, site of infection and duration of infection. Following gonococcal infection antibodies to Protein I can sometimes be detected in serum particularly following local or systemic spread of infection. Although Protein I is less immunogenic than pilus or Protein II when serum antibody is measured there is a better antibody response to Protein I in vaginal secretions. In serum, Protein I interacts with antibody and complement to produce a bactericidal reaction that can kill N gonorrhoeae but the importance of this interaction in the mucosa is unclear. N gonorrhoeae expressing Protein IB are more readily killed by this interaction than Protein IA gonococci. Both Protein IA and Protein IB antibodies can activate complement via the classical pathway and act as opsonins. A study of prostitutes in Africa found that women infected with one Protein I gonococcal strain are less likely to become reinfected with the same strain subsequently. Our data do not support the presence of such immunity with no difference observed in the reinfection rates for patients previously infected. Unlike the African study the reinfections that we observed occurred primarily in homosexual men who may differ from women in the magnitude of their immune response to infection. The serum antibody response is greater in women than men, possibly reflecting a greater infective burden in the female genital tract or a more chronic infectious process. Infection at multiple sites might also be expected to invoke a greater immune response. The site of initial infection did not appear to influence the serovar subsequently isolated although the numbers involved are small and a significant difference cannot be ruled out.

There are other important differences between the two studies. The time period for the African study was 16 months during which period the women had an average of four gonococcal infections. Our study took place over four years during which period patients had on average only 1.04 infections although this was higher in gay men (1.11 infections). Nevertheless the time interval between infections in our study averaged 14.7 months (range 1–35) allowing more time for an antibody response, particularly a local mucosal response, to wane. Therefore the conclusion from the African study that infection with a specific gonococcal serovar results in specific but incomplete protection against subsequent infection with the homologous serovar only holds for a highly active population with frequent exposure after a short time interval.

It is also possible that some of those re-presenting with isolates of the same serotype had been inadequately treated or non compliant with therapy rather than reinfected. This may account for some episodes which re-presented after a short time interval but is unlikely to be a major factor for those with a longer interval between infections. Although one case of presumed re-infection in the study occurred after one month the remainder had a time interval of at least four months making it unlikely that this would compromise the study's conclusions.

The results of our study do not support the concept of serovar specific immunity to gonococcal infections occurring in homosexual men. Because of the low level of gonorrhoea in this population and the limited number of infections immunity does not appear to be a major variable in determining the prevalence of infection.

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Serovar specific immunity to Neisseria gonorrhoeae: does it exist?

Ciprofloxacin resistant Neisseria gonorrhoeae

Treatment failure with ciprofloxacin has been reported for isolates with MIC's \( \geq 0.05 \) mg/l. The mechanism of quinolone resistance in gonococci has recently been shown to be due to mutations in the gyrA gene analagous to the mutations that occur in Escherichia coli and Staphylococcus aureus suggesting that there is potential for very high levels of ciprofloxacin resistance in gonococci. We describe two unrelated cases of ciprofloxacin resistant gonococcal infection occurring in the first six months of 1995: the first case was associated with the development of symptoms following treatment with ciprofloxacin; and the second exhibited extremely high level ciprofloxacin resistance (MIC 16 mg/l) similar to the levels of resistance found in Enterobacteriaceae.

A 41 year old caucasian man presented to the genitourinary medicine (GUM) department complaining of spots on his penis. He denied any symptoms of urethral discharge or dysuria. Ten days previously he had had unprotected intercourse with a casual sexual partner in Brazil. On examination, there were two erythematous macules on the glans penis and no evidence of urethral discharge. Routine microscopy of a urethral smear demonstrated the presence of greater than 20 pus cells per high power field and gram negative diplococci, both intra and extra cellularly. A urine specimen was clear on inspection. A presumptive diagnosis of gonorrhoea was made and he was treated with ciprofloxacin 250 mg orally as a stat dose, followed by oxytetracycline 250 mg four times daily for seven days. The diagnosis was subsequently confirmed by urethral and throat culture; urethral chlamydial ELISA, VDRL and TPHA tests and Hepatitis B Ag were all negative. After counselling, he elected to have an HIV test deferred for three months.

When reviewed 14 days later, he had four days previously developed a purulent urethral discharge, although no associated dysuria. He stated that he had completed the course of oxytetracycline, and had not had any intercourse. On examination a profuse urethral discharge was noted and a 2-glass urine test demonstrated a turbid first aliquot with a clear second. Microscopy and culture were again positive for \( N. gonorhoeae \). He was treated with spectinomycin 2 g im and the symptoms resolved within one day of treatment, with clinical and microbiological cure at review one and two weeks later. The pre- and post-treatment isolates were forwarded to the Scottish Neisseria gonorrhoeae Reference Laboratory (SNGRL) for typing and susceptibility testing. Both isolates were microbiologically similar: they were non-penicillinase producers of serovar IB3, auxotype PA, resistant to ciprofloxacin (MIC 0.125 mg/l) and tetracycline (MIC 2.0 mg/l), of intermediate sensitivity to penicillin (MIC 0.50 mg/l) and cefuroxime (MIC 1.0 mg/l), but fully sensitive to erythromycin (MIC 0.05 mg/l) and spectinomycin (MIC < 16 mg/l). No other IB3/PA strains have been isolated in Scotland this year supporting the epidemiological data which suggests that the infection was acquired abroad (Brazil).

The development of an asymptomatic infection following unsuccessful treatment of an asymptomatic infection may have resulted from the growth of large numbers of ciprofloxacin resistant bacteria from a mixed pre-treatment population which comprised both susceptible and resistant variants.

The second case involved a 21 year old Caucasian woman who presented to the GUM department with a contact slip from her husband who had been diagnosed as having gonorrhoea at a military hospital in England. She denied any other contacts in the previous three years. She had noticed an increase in vaginal discharge over the previous two weeks but had attributed this to the fact that she was 27 weeks pregnant. On examination, a thick mucopurulent vaginal discharge was noted. Chlamydia trachomatis culture were not noted on microscopy of vaginal, cervical or urethral smears, but epidemiological treatment with a seven day course of amoxycillin was instituted at her initial attendance. \( N. gonorhoeae \) was cultured from the urethra, endocervix and rectum and we were notified that the strain was penicilllinase producing prior to full antibiotic sensitivities being available. By liaising with the referring clinic it was ascertained that her husband's gonococcal strain was also resistant to ciprofloxacin. In view of the multiple antibiotic resistance, and the patient's pregnancy, she was treated with spectinomycin 2 g im. Repeat gonococcal cultures one and two weeks later were all negative.

The isolate was forwarded to the SNGRL and confirmed as a PPNG isolate of serovar IB1, auxotype non requiring, resistant to ciprofloxacin (MIC 16 mg/l), of intermediate sensitivity to cefuroxime (MIC 0.50 mg/l), tetracycline (MIC 1.0 mg/l), erythromycin (MIC 2.0 mg/l), but fully sensitive to spectinomycin (MIC < 16 mg/l). The isolate carried a 3.05 MDa resistance plasmid as well as the 24.5 MDa transfer and 2.6 MDa cryptic plasmids.

Overall the prevalence of ciprofloxacin resistance (defined by an MIC > 0.05 mg/l) is low in Scotland and was found in only 1.3% (25/1960) of all isolates tested by the SNGRL between 1991 and 1994. The prevalence of ciprofloxacin resistance for the serovars described in this report was 1.6% (2/123) for IB3 (both non-PPNG isolates) and 8.5% (11/130) for IB1 (7 PPNG isolates). The actual level of ciprofloxacin resistance also tended to be low: the MIC of the 25 strains were 0.064 mg/l (4 strains); 0.125 mg/l (6 strains); 0.25 mg/l (7 strains); 0.50 mg/l (7
strains), and 2.0 mg/l (1 strain). The level of ciprofloxacin resistance (16 mg/l) in Case 2 is exceptionally high although the same level of resistance was previously found in a serovar IB3, auxotype PA, PPNG strain isolated in Liverpool from a patient who had acquired his infection in Spain.3 These cases highlight the importance of importation of ciprofloxacin resistant strains which should be taken into account in selection of therapy for patients who may have acquired their infections outwith the UK or in areas with a high level of penicillin resistant gonococci. In Japan, where fluoroquinolones have been widely used as first-line therapy for gonorrhoea for several years the decrease in the susceptibility of gonococci to quinolones has been so rapid that fluoroquinolone resistance in gonorrhoea may be a new worldwide problem complicating the treatment of gonococcal infections.4

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Prevalence of antibodies to HIV-1 and HIV-2 in women attending a sexually transmitted disease clinic in Luanda, Angola

North and East Angola border on countries such as Congo, Zaire and Zambia that show a high incidence of AIDS. In these regions, as in most African countries, the spread of HIV is mainly via the heterosexual route and is more prevalent in urban areas than in the rural ones. At present few data are available on HIV viruses spread in Angola.1,2 In order to depict the diffusion of HIV-1 and HIV-2 infections in an urban area of Angola, we performed a retrospective survey on 400 females attending the outpatient clinic of Maternidade Lucrecia Paim in Luanda during July and August 1992 and presenting with symptoms of sexually transmitted diseases. Patients were submitted to gynaecological examination and blood sample collection. The presence of vaginal or cervical ulcers was found in 51 out of 400 women (12.75%). Sera from blood samples were submitted to HIV-1 and HIV-2 ELISA (Murex). Nineteen out of 400 (4.75%) showed a positive or equivocal result. These sera were further assayed by Western blot (Diagnostic Biotechnology) kit to detect the pattern of antibodies against HIV-1 and HIV-2 (table). Eight out of 19 sera showed a typical HIV-1 pattern, the others were negative (3/19) or indeterminate (8/19). None out of eight HIV-1 positive samples met the criteria for HIV-2 positivity (presence of 2 anti env antibodies) when assayed by HIV-2 Western blot, but most of them were positive for the HIV-2 core proteins. Among the three sera negative for HIV-1 antibodies, two evidenced antibodies against both p26 and gp41 and one against p26 HIV-2 proteins. The eight indeterminate sera showing a single antibody against core (g6/8) or pol (1/8) or env (1/8) HIV-1 proteins, were also indeterminate for HIV-2 tests showing the presence of p26 (g6/8) or both p26 and gp41 (2/8) HIV-2 proteins. The presence of at least one antibody against HIV-2 in all sera submitted to Western blot remains to be clarified since the anomaly in testing African sera has been reported.5 All indeterminate sera were further analysed by Western blot for antibodies against HTLV-1 and HTLV-2. A slight reactivity to core proteins were highlighted in three sera only.

Our data indicate that four out of eight (50%) HIV-1 positive women showed vaginal or cervical ulcers, whereas among the indeterminate ones only four out of 14 (28%) showed vaginal or cervical ulcers. These ulcer prevalences are much higher than that found in the

Western blot (WB) assay on ELISA HIV-1 and 2 positive sera, and presence of vaginal and cervical ulcers in a study population of 400 women in Luanda, Angola

<table>
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<th>HIV-1 WB</th>
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ORIGINAL ARTICLE

Markers of past syphilis in HIV infection comparing Captia Syphilis G anti-treponemal IgG enzyme immunoassay with other treponemal antigen tests

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Summary: The sensitivity of the Captia Syphilis G anti-treponemal IgG enzyme immunoassay (EIA-IgG) was compared with the Treponema pallidum haemagglutination assay (TPHA) and the Fluorescent Treponemal Antibody Absorbed (FTA-Abs) test as a marker for past syphilis in 28 HIV-infected and 31 HIV-negative patients with a past history of syphilis. The specificity of EIA-IgG was compared in 89 patients without a history of syphilis who were known to be HIV antibody positive with a control group of 89 patients who had tested HIV negative. In patients with a past history of syphilis each treponemal test (EIA-IgG, TPHA and FTA-Abs) gave a lower sensitivity (82%, 86%, 79%) in the HIV-positive group than in the HIV-negative group (97%) but the difference was significant only in the case of the FTA-Abs test (P<0.05). In the HIV-positive patients 11% (3/28) were negative in all 3 treponemal tests while 25% (7/28) were negative in at least one treponemal test. In patients without a past history of syphilis the EIA-IgG antibody index in the HIV-positive group (0.436) was significantly higher than in the HIV-negative group (0.378): the specificity, however, was similar in the HIV-positive (100%) and HIV-negative groups (99%). We conclude that the Captia Syphilis G anti-treponemal IgG enzyme immunoassay is of similar specificity in HIV-positive and HIV-negative patients and is of similar sensitivity to the TPHA and FTA-Abs as a marker of past syphilis in HIV-infected patients. Because HIV-infected patients may lose markers to one or more treponemal antibody tests the exclusion of past treponemal infection is more reliable if sera from such patients are tested using several different tests.

Keywords: Syphilis, syphilis serodiagnosis, HIV, enzyme immunoassay

INTRODUCTION

Over the past few years many interactions between syphilis and infection with the human immunodeficiency virus (HIV) have been reported1-3. With regard to syphilis serology these include: delay in producing an anti-treponemal antibody response4,5; the possibility that polyclonal B-cell activation might interfere with the reliability of treatment monitoring by quantitative cardiolipin tests6; the disappearance of serological evidence of treated syphilis after patients become infected with HIV9,10. Johnson and colleagues10 found that specific antibody, as measured by the Treponema pallidum haemagglutination assay (TPHA) and Fluorescent Treponemal Antibody Absorbed (FTA-Abs) test was undetectable in up to 10% of AIDS patients who had previously given positive treponemal tests whereas antibody remained detectable in 100% of control patients. Haas et al.9 also found that none of a group of HIV-negative individuals lost reactivity to a treponemal test whereas 7% of HIV-positive asymptomatic individuals and 38% of those with symptomatic HIV infection had lost reactivity to either the TPHA or FTA-Abs test. These findings suggest that there is a reduction in levels of specific anti-treponemal antibody to T. pallidum in some HIV-infected individuals as they progress through their illness. This means that negative specific serology
does not necessarily exclude a past syphilis infection in patients with AIDS; the significance of this in relation to differential diagnosis in patients with neurological problems is not yet known. Clearly it is important that new serological tests for syphilis are evaluated for sensitivity and specificity against HIV-infected patients. Although there are a few general evaluations of the Capita Syphilis G anti-treponemal IgG enzyme immunoassay (EIA-IgG)\textsuperscript{12,13} there are no reports which deal specifically with HIV-infected patients. The aim of this study is to ensure that the anti-treponemal EIA-IgG: (i) is as sensitive as other treponemal tests as a marker for past syphilis in HIV-infected patients and (ii) specificity is not compromised in HIV-infected patients without syphilis as a result of polyclonal B-cell activation\textsuperscript{14}.

METHODS
To determine the sensitivity of serological markers for syphilis all patients with a past history of syphilis and who were known to be HIV antibody positive attending the Department of Genitourinary Medicine at Edinburgh Royal Infirmary, Scotland were compared with a group of patients who also attended the clinic and had a past history of syphilis but were known to be HIV-negative. Both groups were tested using the Venerale Research Laboratory (VDRL) test, Treponema pallidum haemagglutination assay (TPHA), Fluorescent Treponemal Antibody Absorbed (FTA-Abs) test and the Capita Syphilis G anti-treponemal IgG enzyme immunoassay (EIA-IgG)\textsuperscript{15}. The results of the EIA-IgG test were expressed as an antibody index and interpreted as follows: $<0.9$ Negative; $0.9-1.09$ Equivocal; and $\geq1.1$ Positive\textsuperscript{15}; reactivity in the initial serum dilution (1 in 40) tested in the TPHA was scored equivocal while reactivity at higher doubling dilutions were scored as titres ranging from 80 to 5120\textsuperscript{15}; the VDRL test was titrated to the end point\textsuperscript{15} while categories of reactivity in the FTA-Abs test were as described previously\textsuperscript{16}. The clinical stage of syphilis at treatment was noted for each group and the mean time from syphilis treatment to serological testing for syphilis was measured where the treatment date was known. Seronegative primary infections were excluded from the analysis. Within the HIV-positive group comparisons were made between patients with positive and negative syphilis serology with respect to T4 lymphocyte count and time from syphilis treatment to testing.

To determine if HIV infection gives rise to false positive reactions we compared EIA-IgG results in patients without a history of syphilis who were known to be HIV antibody-positive with a control group who had tested HIV-negative: the control patients had all attended the Department of Genitourinary Medicine and had been tested during the same time period as the HIV-positive patients.

Statistical analysis was performed on the Minitab Statistical Software Package using Mann-Whitney, Two sample-T and Chi Square tests as appropriate.

RESULTS
Fifty-nine patients with a past history of positive syphilis serology were analysed, 28 of whom had a positive HIV antibody test and 31 of whom had tested negative for HIV antibody. In the HIV positive group 3 patients had been diagnosed as having primary syphilis, 13 secondary syphilis, 4 early latent syphilis, 2 neurosyphilis, 2 late latent syphilis and 4 stage unknown. In the HIV-negative group 6 patients had had primary syphilis, 9 secondary syphilis, 3 early latent syphilis, 2 neurosyphilis, 5 late latent syphilis and 6 stage unknown. The distribution of clinical stage for syphilis did not differ significantly between the 2 groups ($P>0.05$). The date of syphilis treatment was known in 24/28 (86%) of the HIV-positive patients and 27/31 (87%) of the HIV-negative patients. The stage of HIV infection according to the CDC classification system\textsuperscript{17} was known for 26 of the patients: stage II (3), stage III (8) and stage IV (15). The mean number of months from treatment of syphilis to the last repeat testing of syphilis serology was 101.3 for HIV-positive patients (range 2–265) and 126.9 for HIV-negative patients (range 2–288) which was not significantly different using the Mann-Whitney test. The sensitivity of the various serological tests for syphilis are shown in Table 1. The VDRL test was significantly less sensitive ($P<0.001$) than either the TPHA, FTA-Abs or EIA-IgG in both HIV-positive and HIV-negative patients. Each treponemal test gave a lower sensitivity in the HIV-positive group than in the HIV-negative group but the difference was significant only in the case of the FTA-Abs test ($P<0.05$). The mean antibody index for the EIA-IgG was 1.81 for the HIV-positive patients and 1.99 for the HIV-negative patients: this difference is not significant ($P=0.37$). Likewise the mean TPHA titre in the HIV-positive group (901) was not significantly different from that in the HIV-negative group (1072). Details of the individual cases negative in at least one treponemal test are given in Table 2. The 2 HIV-negative patients had been tested on one occasion only while one of the patients (15828/91) had an immunodeficiency syndrome. Only 2 of the HIV-positive patients (18617/91 and 16254/89) had been tested on only one occasion. All tests had been negative 36 months earlier for patient 18976/91 and 30 months earlier for patient 12334/90. In patient 2128/92 all tests had been negative on 3 occasions during the preceding 2 years. Patient 17670/91 had given the same results 6 months earlier while the EIA-IgG had also been positive 12 months before. The FTA and TPHA had been negative in patient 5082/93 one month earlier while all treponemal tests had been positive 18 months previously. The 7 HIV-positive patients who had a negative serological test result in at least one treponemal antigen test were compared with those with positive syphilis serology: no statistically significant differences were demonstrated between T4 lymphocyte counts (mean 194.6 for the negative syphilis serology group and 161 for
Table 1. Syphilis serology results in HIV-positive and HIV-negative patients

<table>
<thead>
<tr>
<th>Test</th>
<th>HIV-positive (n=28)</th>
<th>HIV-negative (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Equiv</td>
</tr>
<tr>
<td>VDRL(^b)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>TPHA</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>FTA-Abs</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>EIA-IgG</td>
<td>23</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Equivocal scored positive
\(^b\) 2 HIV-positive sera insufficient volume for test

the positive syphilis serology group) or time in months from treatment to syphilis testing (101.6 for the negative group and 101.1 for the positive group).

There were no false positive EIA-IgG reactions from the 89 HIV-positive patients with no history of syphilis and only one false positive reaction in the 89 HIV-negative control patients. The mean antibody index for the HIV-positive patients (0.436) was significantly higher (\(P=0.0006\)) than for the controls (0.358).

### DISCUSSION

Our results confirm that the relatively new anti-treponemal EIA-IgG is of similar sensitivity to the TPHA and the FTA-Abs as a marker for past syphilis: there was no significant difference in the sensitivity of these tests when evaluated in both HIV-positive and HIV-negative patients. Although each of the treponemal tests gave lower sensitivity in the HIV-positive than in the HIV-negative group the difference was only significant with regard to the FTA-Abs, 79% versus 97% (\(P<0.05\)), suggesting that the FTA-Abs test is of slightly lower sensitivity. Lower sensitivity in the FTA-Abs than in other treponemal tests is in keeping with previous findings.\(^6\)

The EIA-IgG antibody index in the HIV-positive group (0.436) was significantly higher than in the HIV-negative group (0.378). The specificity, however, is similar in HIV-positive (100%) and HIV-negative groups (99%), suggesting that the increase in antibody index, which could be due to polyclonal B-cell activation\(^4\), has no effect in the region of the cut-off value (antibody index = 0.9). This finding is reassuring as there is some evidence that nontreponemal cardiolipin antigen tests may yield high titres (\(>16\)) in HIV-infected patients without syphilis.\(^18\)

Our results also confirm previous findings\(^9,10\) that serological evidence of past syphilis is more likely to be absent in HIV-positive than in HIV-negative patients. None of the HIV-negative patients were negative in all 3 treponemal antibody tests while 6.4\% (2/31) were negative in at least one of the treponemal antibody tests. In contrast 11\% (3/28) of the HIV-positive patients were negative in all 3 treponemal tests and 25\% (7/28) were negative in at least one treponemal test: the period since treatment for syphilis ranged from 2 months to approximately 22 years (average approximately 8 years). These values are similar to those of Johnson and colleagues\(^10\) who found that when samples were looked at 3 years apart 10\% (3/29) of AIDS patients were negative in both the TPHA and FTA-Abs tests: the time period between treatment for syphilis and serological testing was not given. Haas et al.\(^9\) tested sera by either the TPHA or the FTA-Abs test and found that 7\% (5/69) of HIV asymptomatic patients and 38\% (8/21) symptomatic HIV patients had lost reactivity to either the TPHA (7 patients) or the FTA-Abs (6 patients): the mean duration since the last documented episode of syphilis was 52 months for the seronegative patients and 62.9 for the seropositive patients. Although our sample is much smaller we did not find a correlation between loss of markers with stage of HIV infection: one or more treponemal tests were negative in 33\% (1/3) of patients with stage II, 38\% (3/8) with stage III, and 20\% (3/15) with stage IV infection. We also found

Table 2. Summary of patients with a history of syphilis giving a negative reaction in a treponemal test

<table>
<thead>
<tr>
<th>Patient</th>
<th>Syphilis stage</th>
<th>Months since treated</th>
<th>EIA-IgG</th>
<th>TPHA</th>
<th>FTA</th>
<th>HIV*</th>
<th>WBC (10(^6)/l)</th>
<th>T4 (10(^6)/l)</th>
<th>T4/T8</th>
</tr>
</thead>
<tbody>
<tr>
<td>15828/91 Unknown</td>
<td>Unknown</td>
<td>0.82</td>
<td>80</td>
<td>Neg</td>
<td>No</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7450/91 Secondary</td>
<td>110</td>
<td>1.23</td>
<td>80</td>
<td>Neg</td>
<td>Equiv</td>
<td>No</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>18617/91 Secondary</td>
<td>127</td>
<td>0.58</td>
<td>80</td>
<td>Neg</td>
<td>Neg</td>
<td>IV</td>
<td>5.2</td>
<td>300</td>
<td>0.4</td>
</tr>
<tr>
<td>18976/91 Secondary</td>
<td>126</td>
<td>0.5</td>
<td>80</td>
<td>Neg</td>
<td>Neg</td>
<td>III</td>
<td>3.6</td>
<td>210</td>
<td>0.2</td>
</tr>
<tr>
<td>12333/90 Secondary</td>
<td>89</td>
<td>0.75</td>
<td>80</td>
<td>Neg</td>
<td>Neg</td>
<td>III</td>
<td>4.5</td>
<td>180</td>
<td>0.1</td>
</tr>
<tr>
<td>2128/92 Primary</td>
<td>122</td>
<td>0.68</td>
<td>80</td>
<td>Neg</td>
<td>Neg</td>
<td>III</td>
<td>6.9</td>
<td>234</td>
<td>0.2</td>
</tr>
<tr>
<td>17670/91 Early latent</td>
<td>114</td>
<td>0.81</td>
<td>80</td>
<td>Neg</td>
<td>II</td>
<td>4.5</td>
<td>245</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>16254/89 Primary</td>
<td>78</td>
<td>1.1</td>
<td>40</td>
<td>Neg</td>
<td>IVC</td>
<td>2.6</td>
<td>43</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>5082/93 Secondary</td>
<td>55</td>
<td>1.23</td>
<td>80</td>
<td>Neg</td>
<td>Equiv</td>
<td>IVC</td>
<td>4.1</td>
<td>150</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Stage of HIV infection\(^17\)  
ND = Not done
no difference in mean T4 lymphocyte counts between the group who had lost serological markers for syphilis (194.6 × 10^6/l) and the group that retained markers (161 × 10^6/l). These findings are different from those of Haas and colleagues who reported that loss of markers increased as T4 lymphocyte counts declined and was most marked at counts of <200 × 10^6/l: the mean T4 lymphocyte count was 368 × 10^6/l for the group that had lost markers compared with 968.5 × 10^6/l for the group that had retained markers. The lack of correlation between loss of markers and T4 lymphocyte counts suggest that factors other than the extent of immune dysfunction contribute to loss of markers. For example, selective loss of serological markers of syphilis has been reported in the absence of serious immune deficit: in this case loss of treponemal antibody occurred within 2 years of treatment of secondary syphilis and before the onset of AIDS while IgG responses to other infectious agents such as rubella, cytomegalovirus, haemolytic streptococci, and staphylococci remained detectable. This observation combined with our results and those of Haas et al. that the loss of treponemal antibody reactivity did not increase with time since the last episode of syphilis suggests that certain unknown critical interactions between HIV infection and the immune system are involved in the specific loss of treponemal antibody.

We conclude that the Captia Syphilis G antitreponemal IgG enzyme immunoassay is of similar specificity in HIV-positive and HIV-negative patients and is of similar sensitivity to the TPHA and FTA-Abs as a marker of past syphilis in HIV-infected patients. Because HIV-infected patients may lose markers to one or more treponemal antibody tests the exclusion of past treponemal infection is more reliable if sera from such patients are tested using several different tests.

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(Accepted 19 August 1994)
14
Neisseria, Moraxella, Acinetobacter

R. J. Fallon  H. Young

The medically important aerobic Gram-negative diplococci are members of the genus Neisseria and of the sub-genus of Moraxella, Moraxella (syn. Branhamella) catarrhalis, although the Gram-negative rods of Acinetobacter spp may sometimes appear as diplococci in stationary phase culture.

The distinguishing features of these are shown in Table 14.1. The meningococcus, N. meningitidis, and the gonococcus, N. gonorrhoeae, are the important pathogens in the genus Neisseria; other Neisseria species are commonly found as commensals in the upper respiratory tract. N. meningitidis gives rise to septicemia and meningitis with or without septicaemia; it is normally carried in the throat but may be found in other sites such as the genital tract, where its presence is not usually of pathological significance. N. gonorrhoeae causes gonorrhoea, a sexually transmitted infection of the genitourinary tract, but may occasionally be found in the throat. Sometimes systemic spread gives rise to disseminated gonococcal infection, characterized by arthritis with or without skin lesions.

Moraxella catarrhalis is the only important member of the sub-genus Branhamella (Bovre 1984). This organism is normally an upper respiratory tract commensal but occasionally gives rise to respiratory infection, usually as an opportunistic pathogen. M. catarrhalis was formerly classified in the genus Neisseria but differs from Neisseria in DNA base content, fatty acid composition, and inability to produce acid from carbohydrates (Catlin 1990).

Neisseria spp and M. catarrhalis are recognized in a clinical specimen by the appearance of oval Gram-negative diplococci with flattened or concave opposing edges and long axes parallel, either lying free in the specimen or, in the case of N. meningitidis and N. gonorrhoeae, often inside polymorphonuclear leucocytes. The colonies of neisseriae differ according to the species, but all are oxidase positive. Those of the pathogenic N. meningitidis and N. gonorrhoeae species are small and mucoid, whereas those of other species tend to be larger, may be smooth or rough and tend to be sticky so that colonies adhere to a wire loop. In general, gonococci and meningococci may be distinguished from other neisseriae by their ability to grow on appropriate selective media. However N. lactamica, which is commonly found in the throat and resembles N. meningitidis closely on culture, grows well on the selective media, as does N. polysaccharae; some strains of M. catarrhalis may also grow on the selective media.

N. meningitidis and N. gonorrhoeae must be identified accurately both for general medical and for medico-legal purposes; the biochemical reactions that distinguish the species of Neisseria from each other are shown in Table 14.1. Several commercial identification systems based on a variety of biochemical and immunological methods are available for the rapid identification of pathogenic Neisseria (Dillon et al 1988). Four of the most popular commercial systems are the API NH (bioMérieux) and that of Rosco Diagnostica which use a combination of sugar utilization and chromogenic substrates, Gonochek II (Turner) which relies solely on chromogenic substrates, and the Phadebact Monoclonal GC test (Boule) which is based on separate pools of monoclonal antibodies to gonococcal protein I and differentiates between gonococcal and non-gonococcal organisms only. The majority of identification systems have limitations (Young & Reid 1988) and it is prudent not to rely on a single test; isolates giving discrepant results should be forwarded to an appropriate reference laboratory. The AccuProbe culture confirmation test for N. gonorrhoeae (Gen Probe) which utilizes a DNA probe derived from rRNA is highly reliable in differentiating between gonococcal and non-gonococcal organisms (Young & Moyes 1993) and should prove useful in confirming the identity of gonococci in the reference laboratory. Tests that require the preparation of suspensions of suspected N. meningitidis must be done in a microbiological safety cabinet.
<table>
<thead>
<tr>
<th>Species</th>
<th>Pigment</th>
<th>Growth at 22°C</th>
<th>Blood/serum required for growth</th>
<th>Glucose</th>
<th>Maltose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Beta galactosidase*</th>
<th>Lactose</th>
<th>Glutamyl aminopeptidase*</th>
<th>Protease inmini-</th>
<th>Polysaccharase*</th>
<th>Tributyrin DNase*</th>
</tr>
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<tbody>
<tr>
<td>N. gonorrhoeae</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>N. meningitidis</td>
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<tr>
<td>N. lactamica</td>
<td>+/-</td>
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<td>+</td>
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<td>N. pharyngis</td>
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<td>+</td>
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<td>N. subflava</td>
<td>+</td>
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<td>N. flavissima</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>N. perflava</td>
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<td>N. sicca</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>N. cinerue</td>
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<td>N. polysaccharae</td>
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<td>+</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>M. catenatai</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
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<td>+</td>
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</tbody>
</table>

*Test(s) included in various commercial identification systems.
*b N. meningitidis will grow on Mueller–Hinton medium without the addition of blood or serum.
*c Occasionally isolated on selective medium.
(Ch. 3) and the suspensions must be made within the cabinet.

Quality control

Reliable diagnosis or exclusion of gonococcal or meningococcal infection depends upon high microbiological standards. Procedures must be subjected to proper quality control and the efficiency of culture carefully monitored. The percentage of microscopy-positive specimens that fail to yield gonococci on selective medium (possibly because of antibiotic sensitivity) should be determined by correlating the results of microscopy and culture; a figure of 1–2% is acceptable.

NEISSERIA MENINGITIDIS

*N. meningitidis* may be differentiated from commensal neisseriae and *M. catarrhalis* by its requirement for enriched culture media, the beneficial effect of (if not absolute requirement for) added CO₂ in the atmosphere used for primary isolation, and growth within a narrow temperature range (Table 14.1). It is further differentiated from other species by its utilization of both glucose and maltose and, in capsule strains, by agglutination with group-specific antiserum; these are the features of principal importance in diagnostic work.

Morphology and staining

Oval Gram-negative diplococci, with flattened or concave opposing edges and the long axes parallel; about 0.8 μm in diameter; typically seen in large numbers inside polymorphonuclear leucocytes. Films from cultures show more rounded cocci and some pleomorphism with irregular staining. Capsules are not ordinarily evident; non-sporing; non-motile.

Cultural characters

Aerobe, but primary cultures grow better in an atmosphere containing 5–10% CO₂. Temperature range 25–42°C, optimum 35–36°C. Optimum pH 7.0–7.4. Strains will grow on Mueller–Hinton medium without the addition of blood or serum but grow poorly if at all on most unenhanced media. After incubation in 5–10% CO₂ in air for 24 h at 37°C colonies on blood agar are 1–2 mm in diameter, convex, grey and translucent. After 48 h colonies are larger with an opaque raised centre and thin transparent margins which may be crenated. No haemolysis on blood agar. Colonies are slightly larger on heated blood (chocolate) agar than on ordinary blood agar.

Biochemical reactions

Oxidase reaction: quickly positive when the reagent is flooded on to agar cultures. Utilize glucose and maltose but not lactose or sucrose. Occasional strains are found that utilize only glucose or maltose on primary isolation; repeated subculture of these strains may be necessary before they utilize both sugars. Non-malto-utilizing strains of *N. meningitidis* may be mistaken for gonococci. This could be of medicolegal importance but may be resolved by testing for gamma-glutamyl aminopeptidase activity which is found in meningococci but not gonococci (see Table 14.1). If there is any doubt about the identity of the neisseria in this situation it should be forwarded to a reference laboratory for further examination. An organism with the growth and biochemical characteristics of *N. meningitidis* and the serological characteristics of *N. gonorrhoeae* has been isolated from a vaginal swab (Hodge et al 1987). This underlines the importance of seeking expert advice with atypical neisseriae.

Sensitivity to physical and chemical agents

Dies within a few days at room temperature but cultures may be maintained on Dorset's egg medium or heated blood agar slopes in screw-capped bijou bottles for several weeks. Colonies emulsified in peptone water will survive at −70°C or in liquid nitrogen for years, but freeze-drying is preferable for long-term storage (see also Microbank system described under *N. gonorrhoeae* below). Killed at 55°C in 5 min. Readily killed by disinfectants at their correct use-dilution.

Antibiotic sensitivity

*N. meningitidis* is sensitive to a wide variety of antibiotics, of which benzylpenicillin, chloramphenicol and rifampicin are the most important; it is also sensitive to the more recent cephalosporins, e.g. cefuroxime and cefotaxime, and to 4-quinolones, e.g. ciprofloxacin. However strains with a raised MIC of penicillin (=1 mg/litre) occur, as do strains resistant to rifampicin, both naturally or following rifampicin prophylaxis applied to contacts of cases of meningococcal infection. Some 10–20% of strains

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are resistant to sulphonamides, and all strains are resistant to trimethoprim and colistin.

Serogrouping

The serogroups of meningococci of pathological importance whose polysaccharide antigen structure has been determined are: A, B, C, X, Y, Z, Z₁ (29E) and W135. Further serogroups H, I, K and L have also been described but their pathological significance is not yet clear. A group D was described by Branham (1958) but no capsular polysaccharide specific for this group has yet been demonstrated. Meningococci of groups A and C are those principally associated with epidemics; group B meningococci are the common inter-epidemic strains but epidemics due to these organisms have been described in Scandinavia, Cuba and South America. Non-serogroupable strains are commonly found in carriers, but rarely in disease.

Outer membrane protein and lipoprotein serotypes occur within groups A, B, C, Y and W135 and may be identified by reference laboratories for epidemiological purposes. Meningococci are typed on the basis of their Class 2 or 3 outer membrane proteins (OMP) and sub-typed by their class 1 OMP. A detailed identification of a meningococcus by group, type and subtype antigens, e.g. a group B meningococcus of type 15 with the protein subtype antigen P1.16 would be shown as B:15:P1.16 (Frasch et al 1985). Types 2, 15 and recently 4, have been particularly associated with Group B meningococcal disease. Group, type and subtype are phenotypic characteristics; hence, for example, group B meningococci of types 4 or 15 may belong to the same genotype (Caugant et al 1991). The majority of group C meningococci are of type 2 (2a or 2b).

Animal pathogenicity

Intravenous inoculation of viable meningococci into rabbits may be fatal within 24 h but animal tests are not used for diagnosis.

LABORATORY DIAGNOSIS OF MENINGOCOCCAL INFECTION

Specimens may include cerebrospinal fluid (CSF), blood for culture (which may come from a patient with meningitis, a haemorrhagic rash or pyrexia of uncertain origin), aspirate from skin lesions or pus from an infected joint. Throat or nasopharyngeal swabs should always be taken from suspected cases in case CSF or blood are not available or fail to yield growth on culture. In certain circumstances, genital swabs may be collected from suspected carriers. Swabs are plunged into transport medium (e.g. Stuart’s) for forwarding to the laboratory; all specimens where meningococcal infection is suspected must be submitted to the laboratory immediately.

Cerebrospinal fluid

1. Perform a cell count (see Ch. 4); the exudate in meningococcal meningitis is typically polymorphonuclear.
2. Centrifuge the remaining CSF. Make a smear of the centrifuged deposit and stain with Gram stain. Stain a second film with methylene blue to determine the cell type; occasionally, diplococci may be seen more easily with this stain. CSF from a typical case of meningococcal meningitis will show Gram-negative diplococci inside a limited proportion of the pus cells; many are extracellular. If fluorescein isothiocyanate-coupled antiseraum is available, a smear of the deposit may be examined for the direct identification of the meningococcal serogroup responsible for infection (Fallon 1983).
3. Divide the supernatant CSF into two aliquots—one to be kept if necessary for biochemical examination, the other to be examined for the presence of meningococcal polysaccharide antigen by counter-immunoelectrophoresis, latex agglutination* or coagglutination.
4. Plate out the centrifuged deposit on both blood and heated blood agar and incubate at 37°C in 5–10% CO₂ for 24 h. Add Robertson’s cooked meat broth to the remaining deposit, incubate overnight and subculture in the same way. Examine colonies appearing after incubation by Gram stain and oxidase reaction.
5. Set up the rapid carbohydrate utilization test (RCUT)* or inoculate a set of Flynn & Waitkins sugar media* for utilization reactions. In addition to glucose, maltose, lactose and sucrose, inoculate an ONPG tube; the ONPG medium base used is Mueller–Hinton broth. This latter test will identify strains of N. lactamica where lactose utilization on solid media may be slow.

Forward cultures of organisms identified as meningococci by sugar utilization reactions to an appropriate reference laboratory for determination of sulphonamide sensitivity, serogrouping and, where

* Refer to Methods at the end of this chapter.
appropriate for epidemiological purposes, serotyping (PHLS Meningococcal Reference Unit, Public Health Laboratory, Withington Hospital, Manchester M20 8LR; Dr L. E. Smart, Scottish Meningococcus/Pneumococcus Reference Laboratory, Ruchill Hospital, Bilsland Drive, Glasgow G20 9NB).

6. Set up antibiotic sensitivity tests. Strains resistant to penicillin have not yet been described although for a small proportion of strains isolated in Spain (Sáez-Nieto et al 1992) the MIC is as high as 0.8 mg/litre; this antibiotic should be tested along with cefotaxime, chloramphenicol and rifampicin. Appropriate concentrations are benzylpenicillin 1.2 µg, (2 units)/disc, cefotaxime 10 µg, chloramphenicol 10 µg, rifampicin 10 µg. Disc testing for sulphonamide sensitivity is satisfactory provided a 25 µg sulphonamide disc is used in a Stokes technique with a control sensitive meningococcus. Preferably, test by the agar dilution method using Mueller–Hinton agar as described by Fallon (1978). The inoculum should be a 1-in-50 dilution of a shaken overnight broth culture and a suitable range of concentrations of sulphonamide is 0.1, 1, 10, 50 and 100 mg/litre. Strains resistant to 10 mg/l are resistant to sulphonamide therapy; strains resistant to 1 mg/l may not be eradicated from the nasopharynx if sulphonamides are used for chemoprophylaxis.

7. Serogrouping is performed by slide agglutination with hyperimmune sera (Murex). Care must be taken to test a strain against antiserum to each serogroup as fine non-specific agglutination may occur which may be thought to be specific unless the reaction with all antisera has been checked. If difficulty is experienced in serogrouping, the culture may be plated on Mueller–Hinton agar containing about 5% (depending on the strength of the serum) group-specific antiserum. A halo of precipitate appearing in the medium round the colonies after 24–48 h incubation is a positive reaction (Craven & Frasch 1979).

Blood cultures
Subculture to blood agar and heated blood agar. Incubate cultures in 5–10% CO₂ for 24 h and examine oxidase-positive colonies of Gram-negative diplococci as above.

Pus, aspirates and swabs
Examine Gram-stained films and inoculate pus, aspirates and swabs on to selective heated blood agar* or Modified New York City (MNYC) medium* in addition to media normally used for the examination of pus from any source.

Serological diagnosis
Paired sera may be tested for the presence of complement-fixing antibodies (Ross & Stevenson 1962). This test is helpful in cases where no organisms have been isolated or in obscure pyrexias, which may be due to chronic meningococcal septicaemia. Specific antibodies to capsular polysaccharide may be demonstrated by a haemagglutination test (Edwards & Driscoll 1967). ELISA tests are being standardized internationally for the demonstration of antibodies both as evidence of infection as well as of response to immunization or carriage.

NEISSERIA GONORRHOEAE

Morphology and staining of N. gonorrhoeae are identical to those of N. meningitidis (see above). The main character that distinguishes the gonococcus from the meningococcus is the ability to produce acid from glucose but not maltose.

Cultural characters
A delicate organism with exacting nutritional and environmental requirements. Aerobe, but most strains have an absolute requirement for CO₂. Optimum pH 7.0–7.4. Recommended culture media contain a rich nutrient base supplemented with blood, either partially lysed by heat (chocolate agar) or completely lysed by saponin; unlysed blood agar is not recommended for diagnostic cultures. Selective media are valuable in isolating gonococci from heavily contaminated sites such as the rectum or pharynx.

After incubation for 24 h in a moist aerobic environment enriched with 5–10% CO₂ colonies on Modified New York City (MNYC) medium are small (c. 1 mm), grey and convex; after 48 h the colonies are larger (1.5–2.5 mm), sometimes with a crenated margin and an opaque raised centre. Considerable variation in size occurs with gonococcal colonies and on most culture media the colony outline is irregular, unlike the circular colonies of N. meningitidis. On Thayer–Martin medium growth is slower; although colonies are similar to those on MNYC medium they are usually smaller.

Naturally occurring variants with specific requirements for particular amino acids, bases or vitamins
may be detected; this has been exploited (auxotyping) for epidemiological tracing (Cadin 1973).

Biochemical reactions
The gonococcus is oxidase positive and utilizes glucose but not maltose, sucrose, lactose or fructose (see Table 14.1). The rapid carbohydrate utilization test (RCUT; Young 1978a) measures preformed enzymes and provides a quicker and more reliable identification than conventional growth-dependent sugar tests using solid or semi-solid media (Tapsall & Cheng 1981).

Sensitivity to physical and chemical agents
Readily killed by drying, soap and water, and many other cleansing or antiseptic agents at their correct use-dilution. Organisms may remain viable for a day or so in pus contaminating linen or other fabrics. Cultured gonococci die in a few days at room temperature. Survival can be ensured for several months by harvesting an overnight plate culture into 1 ml of tryptone soya broth containing 6% lactose and freezing at -20 or -70°C. Freeze-drying is the most reliable method for long-term storage of gonococci but storage at -70°C or in liquid nitrogen may be more convenient for intermediate storage. The commercial Microbank system (Pro-Lab) is a simplified and reliable method of storing at -70°C; harvest organisms into a cryopreservative fluid, add to a vial containing 20 small porous beads, remove excess fluid and then freeze.

Antibiotic sensitivity
The gonococcus is usually sensitive to many antibiotics including penicillin, cefuroxime, cefotaxime, ceftriaxone, ciprofloxacin, spectinomycin, cotrimoxazole, tetracycline, erythromycin and streptomycin. However, as sensitivity of isolates may vary geographically it is important to base antibiotic policies on the sensitivity of local isolates.

A definite progression towards decreased sensitivity to various antibiotics has occurred over the past three or four decades and has been reviewed elsewhere (Easmon 1985). Resistance in gonococci may be due either to the alteration of the cell envelope by multiple chromosomal mutations or to R plasmids. Resistance to several antibiotics has reached clinically significant levels in a proportion of isolates. Penicillin and tetracycline resistance is now common in many parts of the world while resistance to spectinomycin and the newer quinolones has also been reported (Easmon 1990). A high prevalence of ciprofloxacin-resistant strains has been noted in Japan where fluoro-quinolones have been widely used as first-line therapy for gonorrhoea for some years (Tanaka et al 1994). The prevalence of resistant strains shows marked geographical variation with the greatest resistance problems associated with parts of Africa and the Far East. The main types of resistance are as follows.

Chromosomally resistant Neisseria gonorrhoeae (CMRNG). Mutations at a series of loci on the chromosome result in small additive increases in penicillin resistance. Mutations at certain loci result in increased resistance not only to penicillin but to other antibiotics such as cephalosporins, erythromycin, tetracycline, spectinomycin and aminoglycosides. CMRNG have penicillin MICs ≥ 1.0 mg/l; in contrast, fully sensitive wild strains of gonococci have penicillin MICs of less than 0.06 mg/l. CMRNG are widespread in the United States (Rice et al 1986) and accounted for approximately 10% of non-plasmid-mediated resistant strains surveyed in the mid 1980s at St Mary's Hospital, London (Json & Easmon 1991).

Penicillinase-producing Neisseria gonorrhoeae (PPNG). PPNG were first reported in 1976 and were epidemiologically linked with West Africa (strains with a 3.2 MDa plasmid) and the Far East (strains with a 4.4 MDa plasmid); initially, about half of the strains from the Far East also harboured a 24.5 MDa transfer plasmid. The links between geographical location and plasmid pattern no longer hold true: strains carrying the 3.2 MDa plasmid and the 24.5 MDa plasmid occur in various areas. Several other plasmids are involved, including 2.9 MDa, 3.05 MDa and 4.0 MDa plasmids but these remain relatively uncommon compared to the 3.2 and 4.4 MDa plasmids. PPNG account for approximately 2-5% of strains in the UK but may reach levels of approximately 50% in some developing countries. All plasmids control the production of the same TEM-1 β-lactamase, the smaller plasmids being deletion mutants of the 4.4 MDa plasmid.

Tetracycline-resistant Neisseria gonorrhoeae (TRNG). Tetracycline resistance in gonococci was reviewed by Heritage & Hawkey (1988). Strains with plasmid-mediated high level resistance to tetracycline (MIC ≥ 16 mg/l) were first reported in the United States in 1985. Similar strains have been found in the UK, the Netherlands and parts of Africa. The tetracycline resistance plasmid is 25.2 MDa, self-transmissible and is found in both PPNG and non-PPNG strains. 288
Mutations also occur that increase the permeability of the membrane, making isolates hypersensitive to certain antibiotics; hypersensitivity may include vancomycin (Exner et al 1982) and therefore influence choice of selective media.

Epidemiological typing

Auxotyping and serotyping are now recognized as the standard typing methods for gonococci: the development of gonococcal typing and the epidemiological significance of current typing protocols have been reviewed elsewhere (Sarafian & Knapp 1989).

Auxotyping. Auxotyping characterizes gonococci according to their nutritional requirements determined on a set of defined media, each medium lacking a specific nutrient. The main nutrients tested are arginine, proline, hypoxanthine and uracil; gonococci that have no requirements are designated non-requiring (NR) or prototrophic (Proto), while those that require arginine are designated (Arg), and those requiring proline (Pro), etc. Isolates with multiple requirements such as arginine, hypoxanthine and uracil (AHU) and proline, arginine (citrulline) and uracil (PAU, or PCU) are also found.

Sero . Serotyping characterizes gonococci according to their reaction pattern (serovar) when tested against a panel of monoclonal antibodies reactive with epitopes on protein I (Pr I). A panel of six Pr IA and six Pr IB reagents is normally used and can discriminate up to 25 IA serovars (designated IA-1, IA-2, etc.) and 32 IB serovars (designated IB-1, IB-2, etc.).

Auxotype/serovar (A/S) Classification. Dual classification on the basis of auxotyping and serovar analysis provides greater discrimination than either method alone and has been widely used in epidemiological studies. A total of 107 different A/S classes were initially identified among a world-wide collection of 1400 gonococcal isolates (Knapp et al 1984a). The A/S classification system has been used to study the epidemiology of gonococcal infection including antibiotic-resistant strains, and geographical and temporal variation in serovars, as well as correlation between certain serovars and homosexually acquired infection. In the case of PPNG, A/S can also be combined with analysis of plasmid profiles. As a general rule, IB serovars tend to be more resistant than IA serovars and also tend to be associated with homosexually acquired infection. Certain IB serovars tend to show a stronger correlation than others, e.g. CMRNG are often serovar IB 5/7 (Ison & Easmon 1991); NR/IB-6 correlated strongly with homosexual transmission, although temporal changes may alter the situation (Young et al 1991, Moyes & Young 1993); spectinomycin-resistant isolates in the USA in 1985-86 were mainly Pro/IB-1; approximately 50% of TRNG isolates studied initially also belonged to Pro/IB-1 but TRNG are now found in at least 23 A/S classes showing widespread dissemination of the resistance plasmid (Sarafian & Knapp 1989).

LABORATORY DIAGNOSIS OF GONORRHOEA

The main task of the bacteriologist is to determine whether or not N. gonorrhoeae is present in a specimen and, if present, whether infection with the isolate concerned is likely to be cured by standard treatment regimens. Since the management of gonorrhoea includes the tracing of infected contacts, laboratory diagnosis is best carried out in association with a special department of genitourinary medicine.

The greater the number of sites examined the better will be the chance of detecting gonococcal infection. Details of specimens required for bacteriological diagnosis are given by Robertson et al (1988). In men, urethral samples usually suffice (with rectal cultures in homosexual males), but in women urethral, cervical and rectal specimens should always be examined. Although repeated sampling of multiple sites is ideal, a single well taken endocervical swab will detect approximately 90% of gonococcal infections in women. A high vaginal swab is not suitable and, if this is the only specimen taken, 1 in 3 infected women is likely to be missed.

Throat infection also occurs and should be sought where appropriate. In suspected disseminated gonococcal infection (DGI), specimens may include blood, swabs of skin lesions, or pus aspirated from a joint. Occasionally conjunctival material is examined, particularly in neonatal ophthalmia. Any urine specimen showing Gram-negative diplococci in a Gram stain should be cultured on an appropriate selective medium.

The gonococcus is very fastidious and care is needed in the collection of specimens and their transport to the laboratory. Best results are achieved by the direct inoculation of culture plates with patients' secretions, followed by immediate incubation at 36-37°C in a moist atmosphere containing 5-10% CO₂. When direct plating and immediate incubation is impracticable several transport and culture systems are available. These consist of a selective medium, usually present in a small chamber containing CO₂ or a CO₂-generating system, e.g.
Transgrow or Jembec (Martin & Jackson 1975). The media can be inoculated directly from the patient and transported to the laboratory either before or after incubation. Such systems are expensive and it is more usual to send a swab in Amies transport medium (see Ch. 5) in which case a plain swab is adequate. Dry swabs should not be sent as the gonococcus is very susceptible to drying.

The direct detection of antigen in patient secretions has been used in an attempt to overcome problems of transport. The Gonozyme test (Abbott) uses a polyclonal antibody to detect gonococcal antigen by an enzyme immunoassay. Unfortunately the test lacks specificity and consequently has a poor positive predictive value in populations where the prevalence of gonorrhoea is low. Developments in nucleic acid technology should lead to more sensitive and specific assays. Currently under evaluation are: the Gen Probe PACE 2 assay system which uses a single-stranded DNA probe labelled with an acridinium ester that is complementary to gonococcal rRNA; and the PCR-based AMPLICOR diagnostic test kit (Roche).

As a routine, the laboratory diagnosis of gonorrhoea proceeds as follows.

1. Examine Gram-stained smears of urethral discharge from men, and urethral and cervical secretions from women. The observation of characteristic kidney-shaped Gram-negative diplococci lying within polymorphonuclear leucocytes with a few extracellular organisms is typical of gonococcal infection and the smear is reported as positive. If Gram-negative diplococci are seen only extracellularly, the result of the smear examination is equivocal; a diagnosis should not be made on this basis. If no Gram-negative diplococci are seen, report the smear as negative.

Approximately 95% of infected men and 55–60% of infected women will yield a positive smear; if the smear is examined while the patient is at the clinic, immediate treatment can be given.

2. Plate out the specimen on selective culture media and, in the case of specimens from normally sterile sites, on the same medium lacking antibiotics; incubate immediately in a moist CO₂-enriched aerobic atmosphere at 37°C.

The original selective medium of Thayer and Martin (TM medium) contains the antibiotics vancomycin, colistin and nystatin. Although widely used in many laboratories, TM medium has been criticized because 3–10% of gonococcal strains are inhibited by vancomycin (Mirret et al 1981). A modified TM medium (Martin et al 1974) gives superior results; however, MNYC medium* is preferred because it gives better growth and the use of lincomycin as selective agent avoids the problem of vancomycin sensitivity.

3. Examine plates after 24 h incubation and test suspect colonies by touching with a cotton bud soaked in oxidase reagent; oxidase-positive bacteria turn the contact area of the bud purple within 5–15 seconds. If oxidase-positive, Gram stain an identical colony. Incubation of primary isolation plates is continued for 48 h and cultures are re-examined by the above procedures before any specimen can be reported negative.

A presumptive diagnosis of gonorrhoea made on the basis of oxidase-positive Gram-negative diplococci growing on selective medium is approximately 99% accurate for specimens taken from the male urethra and female urethra, cervix or rectum. A presumptive diagnosis of gonorrhoea is much less reliable in the case of rectal cultures from homosexual males: from 1978 to 1985, 9–17% of rectal Gram-negative diplococci from men were meningococci (Young & Reid 1988). Particular attention must be paid to throat cultures, where gonococcal and meningococcal colonies may coexist; Gram-negative diplococci isolated from the throat are most likely to be meningococci.

4. If there is sufficient growth on the primary isolation plate, set up the rapid carbohydrate utilization test (RCUT),* including a tube to detect β-lactamase production. Otherwise subculture on antibiotic-free medium and incubate overnight to obtain sufficient material for the test.

5. The Phadebact Monoclonal GC test (Boule) which uses monoclonal antibodies reactive with epitopes on P1 may be used in place of the RCUT (Young & Moxey 1989) for identification of an isolate. These reagents, which are 100% specific, do not cross-react with N. lactamica or meningococci and, provided that the test is properly controlled, a positive reaction is a reliable indicator of gonococcal infection at any site. Although the sensitivity is very high (99.7%) it is prudent to confirm the identity of any non-reactive genital isolate by RCUT. Biochemical confirmation is also recommended whenever medicolegal proceedings may be involved.

6. Inoculate a suitable non-selective medium (e.g. the isolation medium lacking antibiotics) with the growth from several colonies and place a 6 μg penicillin disc on the well. If the zone of inhibition is less than 20 mm after overnight incubation, test for β-lactamase by the chromogenic cephalosporin method (Ch. 8). Filter paper acidimetric (Sing et al 1981) and starch paper iodometric (Odugbemi et al
1977) methods are also described. A 10 μg tetracycline disc can be used to screen for high-level tetracycline resistance. Tetracycline-resistant gonococci show no zone of inhibition.

Since the majority of patients with gonococcal infection will have been treated on the basis of a positive smear, antibiotic tests other than those to detect β-lactamase are of little help in the initial management of a patient. However, they are important in planning rational therapy for use in the geographical area concerned. As a result of the decrease in the prevalence of gonorrhoea it is now desirable and practicable to test all isolates. Prepare a turbid suspension of organisms in peptone water for use as inoculum in the agar dilution or disc diffusion sensitivity test. Diffusion tests using discs of several strengths can be made to give acceptable results but an agar dilution method is preferable (Jephcott 1981). The Adatab system, available commercially (Mast), provides tablets containing suitable quantities of a wide variety of antibiotics.

7. In suspected disseminated gonococcal infection, set up blood cultures with a biphasic medium (Jephcott 1981) and incubate in a CO₂ incubator with standard closures replaced by cotton-wool plugs. Immunofluorescence staining may be of value in examining exudate from skin lesions (Tronca et al 1974). Culture on non-selective media may be advisable.

Reference Facilities
Consultation and liaison with a recognized reference laboratory is important. In the UK, the addresses are as follows.

Scotland: Dr H Young, Scottish Neisseria gonorrhoeae Reference Laboratory, Department of Medical Microbiology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG. All gonococci isolated in Scotland should be sent to this laboratory for MIC testing and epidemiological typing; plasmid analysis is also performed on PPNG and TRNG.

England and Wales: Dr A E Jephcott/Dr A Turner, Gonococcus Reference Unit, Public Health Laboratory, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW. Owing to the larger numbers involved, this laboratory normally examines only antibiotic-resistant strains, isolates from medicolegal cases, and isolates with identification problems.

Serological diagnosis
The low sensitivity and specificity of existing serological tests, and the persistence of antibody due to past infection, limit their value in clinical practice. Serological tests are not suitable for screening for gonococcal infection and should not be used in this way to diagnose or exclude gonorrhoea.

Atypical gonococci
Neisseria gonorrhoeae ssp hochii. Mazloum et al (1986) suggested that an unusual neisseria isolated from conjunctival cultures in rural Egypt and originally described by Robert Koch as an atypical gonococcus merits subspecies status. These isolates do not react with the monoclonal antibodies currently used in the serological classification of gonococci.

COMMENSAL NEISSERIAE

These organisms occur on various mucous surfaces of the body; they are regularly found in the throat, nose and mouth and, less frequently, on the genital mucosae. When inflammatory or other pathological conditions affect these mucous membranes, Gram-negative diplococci may constitute a prominent feature of the bacterial flora and may possibly act as secondary infecting agents in such conditions. The commensal neisseriae are much less well characterized than the important human pathogens described above.

Neisseria lactamica. Carriage of this organism occurs more frequently in infants and young children than in adults. Morphology and staining is similar to N. meningitidis but it differs in its relative lack of virulence and ability to utilize lactose. It grows readily on selective media and some strains cross-react with antiserum raised against gonococci and meningococci. Whenever Gram-negative diplococci are isolated from the throat, biochemical tests are required to provide accurate differentiation between N. lactamica and the meningococcus and gonococcus (see Table 14.1).

Neisseria pharyngis. The classical nasopharyngeal commensals, N. subflava, N. flava, N. perflava and N. sicca are included in the umbrella species N. pharyngis; their main characters are given in Table 14.1. Most of these nasopharyngeal commensals produce moist pigmented colonies and utilize glucose, maltose, fructose and sucrose when tested by the RCUT. Although N. sicca utilizes the same sugars, colonies are dry, tough, adherent to the medium and opaque.

Neisseria polysacchareae (which resembles the meningococcus in appearance) grows on selective
media, utilizes glucose and maltose (Riou et al 1983), and is found in the nasopharynx of healthy carriers (Boquete et al 1986). Unlike N. meningitis, it produces polysaccharide when grown on medium containing 5% sucrose and lacks gamma-glutamyl aminopeptidase activity.

*Neisseria cinerea* has been isolated as a commensal, frequently from oropharynx and less commonly from genital sites (Knapp et al 1984b). Occasionally it is isolated on selective medium, when it resembles *N. gonorrhoeae*. Although considered to be asaccharolytic it may utilize glucose in certain biochemical test systems (Dossett et al 1985). *N. cinerea* is non-reactive in the coagglutination test for the gonococcus. Lack of DNase helps to differentiate it from *M. catarrhalis*.

*Neisseria flavescens*. This organism was described in 1930 as the causative pathogen in a group of cases of meningitis in America but has not with certainty been isolated since. It resembled the meningococcus in morphology but on blood agar produced golden-yellow colonies. Initially the isolates did not utilize carbohydrates but later developed the ability to produce acid from glucose, maltose and sucrose. It may be biologically related to *N. pharyngis*.

*Neisseria mucosa* differs from other members of the group in being definitely capsulate and producing mucoid colonies. It has been isolated only sporadically from cases of meningitis, endocarditis and also opportunistic infections (Gini 1987). Its carbohydrate utilization reactions are similar to those of *N. sicca* but it reduces nitrates and, like *N. polysaccharae*, it synthesizes polysaccharide. There is much variation in the characters of the organisms described under this name (Brodie et al 1971, Johnson 1983).

**Rod-shaped Neisseria species**

Two further rod-shaped members of the genus, both pathogenic for man, have recently been described. Both are Gram-negative, non-motile, oxidase-positive, aerobic bacteria and have been speciated on the basis of biochemical and DNA studies.

*Neisseria elongata* subspecies *nitroreducens*, formerly known as CDC group M-6 (Grant et al 1990), is most commonly associated with endocarditis, bacteremia and osteomyelitis (Wong & Janda 1992) but may be present in other sites such as wounds, peritoneal fluid and the respiratory tract. In culture some strains utilize glucose weakly but all reduce nitrate, grow poorly at 22°C and are catalase positive.

*Neisseria wasseri* sp nov is a Gram-negative, non-motile, oxidase- and catalase-positive aerobic bacterium, known as CDC group M-5, associated with dog bite wounds (Anderson et al 1993). DNA relatedness and 16S rRNA sequence studies show greatest homology to *N. animalis*, *N. flavescens*, *N. canis* and *N. elongata*.

### MORAXELLA

The genus *Moraxella* includes *Moraxella lacunata* which causes a form of purulent conjunctivitis classically presenting as an angular blepharconjunctivitis. The moraxellas occur as components of the normal flora of the upper respiratory tract, the conjunctiva, the skin and the genital tract. The organism formerly known as *Branhamella catarrhalis* has been reclassified as *Moraxella catarrhalis* (see below). Moraxellas may be involved in opportunistic infections in compromised patients.

The moraxellas are stout Gram-negative cocci or short stout rods; they typically occur in pairs and may simulate gonococci. They are strict aerobes, non-capsulate, non-motile. *M. lacunata* and *M. atlantae* require serum for growth but some other species are less demanding. Loeffler medium is pitted by colonies of *M. lacunata* and the variant *M. liquefaciens*. *M. lacunata* cannot grow on MacConkey agar but some other species can. The moraxellas are relatively inactive in biochemical tests. They are oxidase positive and usually catalase positive. They do not ferment sugars and do not produce indole or H₂S. *M. lacunata* produces a gelatinase. Sensitivity to penicillin has been regarded by some workers as a feature of the moraxellas that distinguishes them from acinetobacters.

### MORAXELLA CATARRHALIS

The characters of *Moraxella* (syn *Branhamella*) *catarrhalis* that are useful in identification include oxidase positivity, inability to produce acid from sugars, production of DNase, hydrolysis of tributyrin and ability to grow on medium lacking blood. *M. catarrhalis* is antigenically distinct from commensal neisseriae; there are no recognized serogroups, but a method of differentiating strains for epidemiological purposes has been described (Peiris & Heald 1992).

**Morphology and staining**

Oval Gram-negative cocci about 0.8 μm in diameter.
Sometimes organisms are single, but more often in pairs with adjacent sides flattened; occasionally found in groups of four as a result of characteristic division in two successive planes at right angles to one another. On occasion they may be found inside polymorphonuclear leucocytes.

Cultural characters
Aerobe with optimum temperature about 36°C but growth of many strains occurs at 22°C. Although CO₂ may enhance growth there is no absolute requirement. Most strains grow on nutrient agar. After incubation for 24 h, colonies on blood or heated blood agar are 1-2 mm in diameter, non-haemolytic, often friable, white or greyish, convex with an entire margin later becoming irregular. After 48 h colonies are larger, more elevated with a raised opaque centre. Most strains do not grow on media selective for pathogenic neisseriae.

Biochemical reactions
Oxidase positive; does not produce acid from glucose, maltose, sucrose, lactose or fructose; reduces nitrate to nitrite; hydrolyses tributyrin (see Table 14.1).

Sensitivity to physical and chemical agents
Appears to be more resistant than the meningococcus or gonococcus. Cultures may remain viable for several months at 20°C if prevented from drying. May survive in sputum for 3-4 weeks.
Susceptible to a wide range of antibiotics but many strains produce β-lactamase and are resistant to penicillin and ampicillin. Sensitivity tests can be done by disc or agar dilution methods as for meningococci and gonococci.

LAbORATORY DIAGNOSIS OF MORAXELLA CATARRHALIS

M. catarrhalis is normally considered to be a harmless commensal of the upper respiratory tract and is most often encountered when examining throat swabs and specimens of sputum. The finding of a few colonies of M. catarrhalis in a mixed culture containing other upper respiratory tract commensal organisms is probably of little or no significance. However, in patients with compromised lung function, M. catarrhalis may be a pathogen of the lower respiratory tract. In these patients a relatively pure growth of M. catarrhalis is often obtained from sputum and other specimens such as transtracheal aspirates.

Specimens should be cultured on blood agar and a selective medium. After overnight incubation in 5-10% CO₂ in air, cultures are examined by the oxidase test and, if positive, Gram stained. Oxidase-positive Gram-negative diplococci are then tested by the RCUT for their ability to utilize sugars and to produce β-lactamase. If the isolate grows well on selective medium it should also be shown to be immunologically distinct from N. gonorrhoeae and N. meningitidis by the tests described previously. It has been reported (Doern & Morse 1980) that clinically significant isolates of M. catarrhalis grow well on modified TM medium, produce β-lactamase and do not grow on nutrient agar at 22°C. The extent of the correlation between pathogenicity, β-lactamase production and ability to grow on selective media remains to be elucidated (see Catlin 1990).

OLIGELLA

Oligella has recently been delineated as a new genus separate from Moraxella on the basis of DNA:rRNA hybridization and serological data (Rossau et al 1987). Oligella urethralis, the type species, formerly Moraxella urethralis, is a rare cause of septic arthritis but its importance lies in possible mis-identification as N. gonorrhoeae as it is a non-motile, Gram-negative diplococcus, both oxidase and catalase positive which will grow on Thayer-Martin medium. However, Oligella will grow on MacConkey agar (Mesnard et al 1992).

KINGELLA

These are Gram-negative rods and the genus contains three species (K. kingae, K. indologenes and K. denitrificans) that differ from Moraxella in being saccharolytic and catalase negative. They are of low pathogenicity but, as they grow on Thayer Martin medium and are oxidase positive, they could be mistaken for pathogenic neisseriae.

Kingella species are included in the so-called HACEK group of oral bacteria (Haemophilus spp, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens and Kingella spp). These
organisms are known to colonize endovascular tissue and produce vegetations on heart valves.

**ACINETOBACTER**

This genus contains strictly aerobic short, stout, often capsulate, non-motile Gram-negative (or Gram variable) bacilli or cocobacilli (often diplococcal bacilli) that grow well on simple media. They are usually free-living saprophytes in soil and water. The genus contains only one species, Acinetobacter calcoaceticus, which embraces two variants: A. calcoaceticus var. anitratus produces acid oxidatively from glucose whereas A. calcoaceticus var. lwoffii does not. This terminology supersedes earlier terms such as Herellea vagincola and A. anitratum which correspond to the anitratus variant, and Moraxella lwoffii which correspond to the lwoffii variant. In the past, Achromobacter species names were also assigned to these variants.

Acinetobacter organisms occur frequently as components of the commensal flora of man and animals and are therefore regular contaminants of the hospital environment. They are increasingly recognized as opportunistic pathogens associated with infections that range from bronchopneumonia to septicemia in compromised patients. Predisposing factors include the presence of a prosthesis, endotracheal intubation, intravenous catheters, and prior antibiotic therapy in a seriously ill patient in hospital.

_A. calcoaceticus_ is oxidase negative, catalase positive, and indole negative. Some strains produce urease. Acinetobacter organisms do not reduce nitrites and do not ferment sugars. The _anitratus_ variant produces acid from glucose and other sugars oxidatively but the _lwoffii_ variant does not. Colonies are white or cream coloured, smooth, circular with an entire edge, sometimes raised and opaque, and may show surface spreading. Some strains are haemolytic on blood agar. Some strains liquefy gelatin slowly.

All strains are penicillin resistant. Hospital strains of _Acinetobacter_ are often resistant to many other antibiotics. Most strains are resistant to sulphonamides, penicillins including ampicillin, the cephalosporins, erythromycin, the tetracyclines and chloramphenicol. They are often resistant to gentamicin and other aminoglycosides (see Bergogne-Berezin & Joly-Guillou 1985). It is essential to guide antimicrobial management by antimicrobial sensitivity tests.

Hospital strains can be traced by a combination of biotyping, antiogramms, serotyping, bacteriocin typing and immunofluorescence tests, but these approaches to tracing have limitations at present (see Stone & Das 1986).

**METHODS**

**Modified New York City (MNYC) medium**

*(Young 1978b)*

**Preparation of yeast dialysate.** Mix 908 g baker's yeast to a smooth paste with 2.5 litres distilled water. Autoclave at 110°C for 10 min and dialyse against 2 litres distilled water for 48 h at 4°C. Dispense the dialysate (material outside sac) into 25 ml amounts and autoclave at 121°C for 15 min. Store at -20°C.

**Ingredients**

- GC Medium Base (Difco) 36 g
- Yeast dialysate 25 ml
- Human or horse blood (100 ml), lysed with 5 ml of 10% saponin 105 ml
- Glucose (10%) sterilized at 115°C for 10 min 10 ml
- Colistin (6 mg/litre) 1 ml
- Lincomycin (1 mg/l) 1 ml
- Trimethoprim (5 mg/l) 1 ml
- Amphotericin B (1 mg/l) 1 ml

**Method.** Dissolve the GC agar base in 856 ml distilled water and autoclave at 121°C for 15 min. Allow to cool and hold at 50°C. Add lysed blood, glucose, yeast dialysate and the antibiotics. Mix and pour plates.

Set up quality control cultures by inoculating plates from each batch of medium with the following cultures: _N. gonorrhoeae_ (a recent clinical isolate); _N. meningitidis; N. pharyngis; Staphylococcus aureus; Escherichia coli; Proteus mirabilis; Candida albicans_. Incubate plates in a CO₂ incubator as for gonococcal cultures. Release a batch of plates for routine use only if the medium supports good growth of the pathogenic neisseriae while inhibiting completely the growth of _N. pharyngis_ and the other test organisms.

Products for the preparation of MNYC medium are available commercially (GC Agar Base, CM367; Yeast Autolysate Supplement, SR105; LCAT Antibiotic Supplement, SR95; Oxoid, Unipath).

**Selective heated blood agar**

Heated blood (chocolate) agar may be made selective for the pathogenic neisseriae by the addition of vancomycin 3 mg/litre, colistin 7.5 mg/l and nystatin 12 500 units/l (or use Oxoid VCN Antibiotic Supple-
Rapid carbohydrate utilization test (RCUT) and penicillinase test (Young 1978a)

In the RCUT, preformed enzyme is measured by adding a suspension of the overnight growth of the test organism to a buffered (non-nutrient) solution containing the sugar to be tested and a pH indicator. β-lactamase can be detected by substituting ampicillin for sugar. Acid production resulting from sugar utilization or from the splitting of ampicillin to penicilloic acid is detected by a colour change of the pH indicator from red to yellow.

Buffer-salt solution (BSS). Prepare by mixing the following solutions: 40 ml 0.1 mol/litre K₂HPO₄ 12 ml 0.1 mol/litre KH₂PO₄; 100 ml 8% (w/v) KCl; 10 ml 1% (w/v) aqueous phenol red; and 838 ml sterile distilled water. Check pH and if necessary adjust to 7.10–7.15. Dispense in 20 ml amounts in screw-cap bottles and store at −20°C.

Sugar solutions. Prepare 100 ml of 10% (w/v) solutions in distilled water of glucose, maltose, sucrose, fructose and lactose; filter sterilize; dispense in 4 ml amounts. Note: Some batches of maltose contain excessive amounts of glucose and give false-positive results.

Ampicillin solution. Dissolve the contents of a 500 mg vial of sodium ampicillin in 2 ml BSS to give a concentration of 250 mg/ml. Both sugar and ampicillin solutions can be used to prepare microtitre trays immediately; otherwise store at −20°C.

Preparation of microtitre trays. Add 25 µl of BSS to each well in columns 1, 3, 5, 7 and 9 of a microtitre tray. Add 25 µl of glucose to each well in column 1. Add 25 µl maltose to column 3, 25 µl sucrose to column 5, 25 µl lactose to column 7, 25 µl fructose to column 9, and 25 µl ampicillin to column 11. Store the trays in sealed polythene bags at −20°C.

Method. Four controls – a PPNG, N. meningitidis, N. lactamica, and N. perflava – should be tested daily.

1. Remove a microtitre tray from the freezer and allow to thaw.
2. Add 400 µl of RCUT buffer to a plastic tube for each test.
3. With a dry cotton bud, remove sufficient growth from a 16–24 h culture to make a heavy suspension of the test organism. This procedure should be carried out in a safety cabinet to give protection from splashing and aerosols.

4. Add 25 µl of the test suspension to each of the six wells (glucose, maltose, sucrose, lactose, fructose and ampicillin) in a row across the plate; tap to mix and place in 37°C water-bath. As a row of the microtitre plate is required for each test, up to four test isolates can be tested on each tray along with the four control organisms.

5. After 3 h examine for sugar utilization, or breakdown of ampicillin, as evidenced by a colour change from red to yellow (or yellow/orange); record a colour change as positive and no change as negative.

Serum-free agar sugars (Flynn & Waitkins 1972)

Supplement. Solution A: dissolve 1 g of L-glutamine in 90 ml distilled water. Solution B: dissolve ferric nitrate 0.05 g in 10 ml distilled water. Prepare the supplement by adding 90 ml of solution A to 10 ml of solution B.

Sugars. 10% solutions of glucose, sucrose or maltose, sterilized by filtration.

Method. Boil 36 g of GC Medium Base (Difco) in 970 ml distilled water and when clear add 20 ml of the supplement and 10 ml of phenol red (0.2% stock solution). Adjust to pH 7.6 with NaOH (1 mol/litre), and distribute in 90 ml volumes in screw-cap bottles. Autoclave at 121°C for 10 min. Cool to 50°C. Add 10 ml of the appropriate sugar solution (aseptically) to 90 ml of medium to give a final concentration of 1% sugar. Dispense 3 ml amounts into sterile 5 ml screw-cap bottles and allow to set as slopes.

For use, slopes should be inoculated heavily and incubated at 37°C in an atmosphere containing 5–10% CO₂, the screw caps of the containers being loosened. A positive result (colour change to yellow) should be obtained after overnight incubation, although cultures should routinely be kept for 48 h. With very small inocula a longer period of incubation may be necessary.

Coagglutination test

This is a rapid (10 min) coagglutination slide test which uses protein-A-containing staphylococci with murine monoclonal antibodies bound to the Fc portion to the protein A. The test uses two reagents, WI and WII/III, composed of separate pools of monoclonal antibodies reactive with PriA and PriB respectively. When a test sample containing gonococci, usually a primary culture of an isolate, is mixed with the reagents, the specific monoclonal antibodies react with the appropriate Pri antigen. A coagglutination lattice is
formed which is visible to the naked eye. Kits are available from Boule Diagnostics. The test should be performed, according to the manufacturer’s instructions, with a light suspension of the organism boiled in 0.9% saline. The unit volume of reagents may be reduced to 15 μl for economy.

Polysaccharide antigen of *N. meningitidis* Groups A, B, C, Y and W135 can be detected in CSF (treated to remove non-specific reactions) by coagglutination with a kit available from Boule.

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**Latex agglutination**

The polysaccharide antigens of meningococci can be detected in CSF, urine (concentrated if necessary) or serum, by agglutination of antibody-coated latex particles. Body fluids must be heated or centrifuged to remove non-specific reactive material. The group B reagent will cross-react with *Escherichia coli* K1 antigen which may be found in neonatal meningitis. Kits are available from Murex.
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Determinants of the Gonococcal Serovar Pattern in Edinburgh, Scotland: A multivariate analysis

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keywords: gonorrhoea, serotyping, multivariate analysis

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Abstract

The geographical and temporal variety of gonococcal serovar patterns are well described but it remains uncertain what characteristics possessed by the organism, or sexual behaviour pattern in the patients, determine the particular serovar pattern in a given area at a given time. This study was designed to assess the relative contribution of various demographic and clinical features of infection to the observed pattern of serovars in Edinburgh between 1990 and 1993. 508 isolates were included in a multivariate analysis model to control for potential interactions between variables. Associations were noted between certain serovars and an asymptomatic clinical presentation, method of acquisition and site of infection. Certain physical characteristics of \textit{N. gonorrhoeae} in conjunction with the sexual behaviour patterns of patients are partially responsible for observed serovar patterns but more detailed analysis requires further sub-classification of serovars using molecular techniques.
Introduction

The study of gonococcal serovar patterns has demonstrated differences in the strains of *Neisseria gonorrhoeae* isolated from patients in different geographical areas and, within the same area, at different times 1-4. Various associations between particular serovars and the individuals infected and the clinical features of infection are also well recognised. Thus serovars of the IB group tend to be more common in gay men and less sensitive to penicillin 5,6 whilst 1A serovars are associated with disseminated infection and may produce less symptoms 7,8. It is unlikely that factors responsible for serovar prevalence are independent however - for example, gay men are infected at different anatomical sites, tend to be older and have more sexual partners compared to heterosexuals. The aim of this study was to use a multivariate model to identify demographic and clinical variables which were independently associated with infection caused by individual serovars.

Methods

All patients diagnosed as having gonorrhoea in Edinburgh between January 1990 and December 1993 on the basis of a positive culture on modified New York culture medium from the urethra, rectum, endocervix and/or throat were included in the analysis. At first attendance with a new complaint all male patients attending the Department of GU Medicine were screened for gonorrhoea with a single urethral swab whilst female patients had urethral and endocervical swabs cultured on modified New York City culture media on two separate occasions to diagnose or exclude gonorrhoea. Throat cultures were performed in all partners of patients with gonorrhoea who attended the clinic, when the history indicated that this site had been placed at risk and in those patients with gonorrhoea at another anatomical site. Rectal cultures were taken routinely from men who gave a history of homosexual contact and from all women.

Gonococcal isolates were identified on the basis of biochemical and immunological tests and serotyping was performed using the American panel of monoclonal antibodies as has been described previously 9. Minimum inhibitory concentrations (MICs) of penicillin were
determined by an agar plate dilution method using a series of plates incorporating 0.015, 0.06, 0.12, 0.5 and 1.0mg/l of penicillin: MICs less than or equal to 0.12mg/l were classed as fully sensitive and isolates with MICs greater than or equal to 0.5 as reduced susceptibility.

Information was obtained from the case notes of patients and health visitor records on area of acquisition of infection, sex, sexual orientation, date of diagnosis and presence of symptoms, age and number of named contacts within the preceding 3 months.

The data was entered into the dBase (Borland Software) database programme and statistical analysis was performed on the SPSS® (SPSS Inc.) software package. A separate multivariate logistic regression analysis was performed for each of the major serovars and for the combined group of minor serovars. In order to separate out the significant variables for each serovar forward conditional analysis was used.

Results

Twenty four thousand one hundred and twenty four new patient episodes (defined as a patient presenting with a new complaint) were seen in the GU Medicine clinic over the 4 year study period of which 17838 (74%) were screened for gonorrhoea. Of the 7381 women screened, 7347 (99.6%) had rectal cultures performed and of the 1390 gay men screened, 1107 (80%) had rectal cultures taken and 1292 (93%) had pharyngeal cultures taken.

508 episodes of infection were diagnosed in 463 patients. Four hundred and sixty four (91%) patient episodes of gonorrhoea were seen at the GU Medicine clinic at Edinburgh Royal Infirmary with the remainder largely diagnosed in general practice. The availability of data for each of the measured variables is shown in Table 1. Over the study period the annual number of cases of gonorrhoea declined from 176 to 81 and the proportion of
Infections acquired through homosexual contact increased from 31% (54/176) to 41% (33/81).

Four 'major' serovars accounted for 71% (363) of all infections - 1A-2 (97 isolates), 1B-1 (42 isolates), 1B-2 (186 isolates) and 1B-3 (38 isolates). The 'minor' serovars were designated as those isolated on less than 10 occasions over the four year study period and included 15 different serovars accounting for 55 isolates. The remaining 90 isolates comprised infections with 1B-6 (31 isolates), 1A-6 (23 isolates), 1B-7 (22 isolates), 1A-16 (12 isolates) and 2 untyped isolates.

The relationship between serovar and age of patient, presence of symptoms, number of named sexual contacts, sexual orientation, location of acquisition, penicillin sensitivity, sex of patient and site of infection is shown in Table 2. Marked differences were evident between the serovars with respect to the absence of symptoms, sexual orientation of patients, penicillin sensitivity and site of infection.

The association between serovars and individual variables as assessed by logistic regression is shown as an odds ratio in Table 3. Asymptomatic infection was more likely to occur in infection with 1B-1 and less likely for infections caused by minor serovars. Homosexual acquisition of infection was associated with 1B-2 infections and significantly less likely in 1A-2 infections. Minor serovars were less likely to cause infections in men and 1B-3 infections were less likely to affect the endocervix.

Discussion

It is evident from a number of studies that the strains of *N. gonorrhoeae*, as assessed by serotyping or auxotyping, that dominate within a population vary between different areas. Some of the variation may be explained by the natural selection of those strains which possess particular advantageous properties within a given environment e.g. penicillin resistance may be an important trait in areas where penicillin is widely
available 14,15. In other areas, even those in close geographical proximity, the reasons for differences are less obvious 10,12,16 and it remains unclear whether strain heterogeneity is a result of intrinsic properties of the gonococcus and/or geographical variations in sexual behaviour.

In describing the association between gonococcal serovars and either the characteristics of patients or the physical properties of *N. gonorrhoeae* it is likely that some relevant factors are not independent predictors of disease but act as confounding variables. Thus rectal infections, which have been associated with hydrophobic serovars suited to survival in the rectum 17, may be less likely to produce symptoms than urethral infections by virtue of the site of infection rather than intrinsic properties of the serovar. To control for these interactions it is possible to perform simple analyses comparing a single factor (e.g. presence of symptoms) in subgroups of the population (e.g. heterosexual men with urethral infections). Although equally valid this approach reduces the power of the study considerably, potentially leading to a type II (false negative) error 18. It still remains possible, however, that relevant co-factors may not be included in the analytical model which could lead to inaccuracy. For these reasons we chose to perform the analysis utilising a multivariate logistic regression technique with forward conditional analysis. This technique involves entering the variables into the statistical model and then sequentially dropping the variable which has the least effect to distil out those factors of greatest predictive value.

It was interesting to note that although the descriptive statistics showed marked differences between serovars in their penicillin sensitivity, in particular the high level of penicillin sensitivity for 1A-2 infections, there were no significant differences on multivariate analysis. This suggests that another factor, such as sexual orientation, has the potential to act as a confounding variable since there is a strong negative association between full penicillin sensitivity and homosexual acquisition for this serovar on univariate analysis (Table 2). Previous studies have suggested that homosexually acquired infections
are more resistant to penicillin than those acquired through heterosexual contact. Individual serovars do not represent genetic clones however and exhibit heterogeneous characteristics including different penicillin sensitivities and different nutritional requirements. In addition typing with a second panel of monoclonal antibodies suggests that isolates of the same serovar differ between homosexual and heterosexual patients. Recent molecular analysis of the gonococcal genome using restriction fragment analyses also suggest that individual serovars encompass a number of different related clonal groups; however, these techniques have not yet been applied to analyse differences between isolates of the same serovar for homosexual and heterosexual patients.

The observation that antibiotic sensitivity was not found to be a significant factor suggests that antibiotic pressure is not a major determinant of serovar prevalence in Edinburgh. This may be because changes in outer membrane structure of N. gonorrhoeae associated with chromosomally mediated penicillin resistance, including increased cross linking of peptidoglycan, impair the transfer of nutrients into the bacterium. There has been little change in the antibiotic resistance patterns in Lothian over the past few years suggesting that antibiotic pressure has remained stable.

The absence of symptoms associated with particular gonococcal serovars such as 1B-1 will prolong the infection since such individuals are presumably less likely to present for treatment. It is of interest that the 'minor' serovars, representing those infections which had failed to become established in the community, were only about a third as likely to be asymptomatic as average suggesting that this may be a major variable in determining the successful integration of a newly introduced serovar. We have previously noted an association between serovar 1A-2 and asymptomatic infection in heterosexual men with urethritis using a univariate analysis and others have noted a lack of symptoms in auxotype AHU- strains of infection which are often of the 1A serogroup. It has been postulated that gonorrhoea only remains endemic within the community due to the presence of a
group of high frequency transmitters of infection comprising a 'core group' 28. The maintenance of this core group would depend upon a high level of infection and re-infection of its members suggesting that absence of symptoms may be an important factor.

Unexpectedly minor serovars were found to be less commonly associated with infection in men. This may represent a chance finding or be due to the exclusion of some unknown confounding variable. It has been postulated that the minor serovars are present largely due to constant re-introduction of strains of infection from other areas rather than being truly endemic within a community 29. If this were the case their persistence would depend upon individuals who travel outside the district contracting infection and bringing it back with them. It seems probable that men would be equally or possibly more likely to exhibit this type of sexual behaviour than women which suggests that there may be some intrinsic property of the minor serovars which may select against infection of men. Since minor serovars were also associated with less asymptomatic infections it is possible that the natural selection disadvantage that this causes is partially offset by the lower incidence of symptoms in women generally.

A negative association was found between endocervical infection and the 1B-3 serovar. Previous reports have presented conflicting evidence for serovar site specificity with some suggesting that serogroup WII infections are better adapted for survival in the rectum than others 17. There has also been one other study which also reported a negative association between serovar 1B-3/1B-6 and endocervical infection 30. The relevance of this association in the present study is unclear, particularly since although it achieves significance at the 5% level it fails to do so at the 1% level.

We have demonstrated that certain characteristics related to sexual behaviour and the physical structure of N. gonorrhoeae including the presence of symptoms, sexual orientation of patient, sex of patient and site of infection are independently associated with different serovars of infection suggesting that these factors may contribute to reported
serovar patterns in different geographical areas. Further study at the molecular level will be required to look for similar associations between bacterial clones or related clone groups.
Table 1  Availability of Data for Analysis for Patients with Gonorrhoea

<table>
<thead>
<tr>
<th>Variable</th>
<th>Variable Positive</th>
<th>Variable Negative</th>
<th>Variable Unknown</th>
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<tbody>
<tr>
<td>Aged under 25</td>
<td>275</td>
<td>225</td>
<td>8</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>118</td>
<td>341</td>
<td>49</td>
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<tr>
<td>More than 2 named sexual contacts</td>
<td>15</td>
<td>444</td>
<td>49</td>
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<tr>
<td>Homosexually acquired infection</td>
<td>182</td>
<td>301</td>
<td>25</td>
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<tr>
<td>Infection acquired within local region</td>
<td>326</td>
<td>94</td>
<td>88</td>
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<tr>
<td>Fully penicillin sensitive</td>
<td>283</td>
<td>222</td>
<td>3</td>
</tr>
<tr>
<td>Male infection</td>
<td>368</td>
<td>137</td>
<td>3</td>
</tr>
<tr>
<td>Endocervical infection</td>
<td>126</td>
<td>9</td>
<td>0*</td>
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<tr>
<td>Rectal infection</td>
<td>96</td>
<td>153</td>
<td>70**</td>
</tr>
<tr>
<td>Throat infection</td>
<td>103</td>
<td>274</td>
<td>131</td>
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<tr>
<td>Urethral infection</td>
<td>335</td>
<td>116</td>
<td>57</td>
</tr>
</tbody>
</table>

* Denominator for calculation of unknown data for endocervical infection was total number of women with gonorrhoea (n=137)
** Denominator for calculation of unknown data for rectal infection was total number of women plus number of gay men with gonorrhoea (n=319)
<table>
<thead>
<tr>
<th>No. of Infections for each serovar</th>
<th>IB-1</th>
<th>IB-2</th>
<th>IB-3</th>
<th>IB-3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors associated with Infections due to Different Serovars</strong></td>
<td>55</td>
<td>38</td>
<td>186</td>
<td>42</td>
</tr>
<tr>
<td>Aged under 25</td>
<td>67 (69%*)</td>
<td>19 (45%</td>
<td>97 (52%)</td>
<td>18 (47%)</td>
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<tr>
<td>Asymptomatic</td>
<td>31 (32%)</td>
<td>15 (36%</td>
<td>36 (19%)</td>
<td>8 (21%)</td>
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<tr>
<td>More than 2 named sexual contacts</td>
<td>31 (56%)</td>
<td>18 (47%</td>
<td>97 (52%)</td>
<td>19 (45%</td>
</tr>
<tr>
<td>Homosexually acquired infection</td>
<td>37 (18%)</td>
<td>9 (21%)</td>
<td>41 (22%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Male infection</td>
<td>146 (78%)</td>
<td>32 (76%)</td>
<td>146 (78%)</td>
<td>27 (71%)</td>
</tr>
<tr>
<td>Urethral infection</td>
<td>121 (65%)</td>
<td>4 (10%)</td>
<td>121 (65%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Endocervical infection</td>
<td>109 (59%)</td>
<td>29 (69%)</td>
<td>109 (59%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Throat infection</td>
<td>121 (65%)</td>
<td>4 (10%)</td>
<td>121 (65%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Rectal infection</td>
<td>121 (65%)</td>
<td>4 (10%)</td>
<td>121 (65%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Injection acquired within local region</td>
<td>109 (59%)</td>
<td>29 (69%)</td>
<td>109 (59%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Penicillin sensitive</td>
<td>121 (65%)</td>
<td>4 (10%)</td>
<td>121 (65%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>Percentage refer to the proportion of the variable within each serovar and may be compared along each row **</td>
<td>121 (65%)</td>
<td>4 (10%)</td>
<td>121 (65%)</td>
<td>28 (74%)</td>
</tr>
<tr>
<td>The number of infections for each serovar is less than the sum of the individual sites of infection as a result of some patients having multiple sites of infection</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
<table>
<thead>
<tr>
<th>Minor Serovars</th>
<th>1B-3</th>
<th>1B-2</th>
<th>1B-1</th>
<th>1A-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 (0.2-0.98)</td>
<td>1.2 (0.6-2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.3 (0.1-0.5)</td>
<td>0.4 (0.2-0.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.4 (0.1-0.8)</td>
<td>1.6 (1.1-2.4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>2.3 (0.9-2.5)</td>
<td>2.3 (0.9-2.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Homosexually acquired infection</td>
<td>1.5 (0.9-2.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Male infection</td>
<td>-</td>
<td>0.2 (0.1-0.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Endocervical infection</td>
<td>-</td>
<td>0.1 (0.3-0.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>More than 2 named sexual contacts</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Logistic Regression Multivariable Analysis of Factors Associated with Infections due to Different Serovars
References


21. Ross JDC, Young H. Emergence of a serogroup 1A strain of Neisseria gonorrhoeae in gay men with reduced sensitivity to penicillin. Genitourin Med 1994; 70:425


Treponema: serological tests for syphilis

H. Young

The general term spirochaete is often used to embrace *Treponema* species and organisms of similar spiral morphology belonging to the genera *Borrelia* and *Leptospira* (see Ch. 34).

Syphilis is an infectious venereal disease caused by *Treponema pallidum* subspecies *pallidum* (*T. pallidum*). A primary lesion or chancre tends to appear on the genitalia at the site of entry of treponemes. The disease is systemic from the onset and the natural course of infection may span several decades (Robertson et al. 1988a). *T. pallidum* is the only pathogenic treponeme indigenous to Britain. Other morphologically indistinguishable treponemes that are pathogenic for man include *T. pallidum* subspecies *pertenue*, the cause of yaws, a non-venereal but communicable disease found in tropical countries, and *T. carateum*, the cause of pinta, a mild contagious disease similar to yaws but confined to Central and South America.

These pathogenic treponemes cannot be cultured *in vitro*, either alone or with mammalian cells of various types. In contrast commensal treponemes can be cultured successfully in artificial media. Many commensal species occur in the mouth (e.g. *T. macrodentium* and *T. microdentium*) and on the mucous surfaces of the genitalia (e.g. *T. calligyrum*, *T. genitalis*) where their differentiation from *T. pallidum* is of importance in the diagnosis of primary syphilis.

Cultivable spirochaete-like organisms have frequently been observed in large numbers in the lower intestinal tract of humans, where their role in disease is controversial. The observation of spirochaetes in the bowel of as many as 30–36% of homosexual men compared with only 2–8% of heterosexual men is striking and may provide clues as to their role, if any, in health and disease. A major obstacle has been the lack of adequate taxonomic data, although it seems likely that the term 'intestinal spirochaetes' refers to a heterogeneous group of organisms (Ruane et al. 1989).

**TREPONEMA PALLIDUM**

**Morphology and staining**

A very delicate, spiral filament 6–14 μm (average 10 μm) by 0.2 μm, with 6–12 coils which are comparatively small, sharp and regular; the length of the coils is about 1 μm and the depth 1–1.5 μm; the ends are pointed and tapering. A capsular or slime layer has been observed occasionally on the surface of *T. pallidum* and may explain the lack of serological reactivity of organisms freshly isolated from animal tissues. A multilayered membrane, referred to as the outer envelope or outer membrane, encloses the cell; the viability of spirochaetes is dependent on an intact outer envelope.

Usually three, but occasionally four, endoflagella lie inside the outer membrane and are inserted at the tapering portion at each end of the cell. The endoflagella are more than half the length of the organism and run along the axial aspect of the spiral body (Penn & Pritchard 1990). They have a form typical of bacterial flagella, with a basal body with disc- or collar-like structures which interact with layers in the cytoplasmic membrane and cell wall. The endoflagella are presumed to be responsible for motility although there is no direct evidence for this. Spirochaetes show rotary corkscrew-like motility and also movements of flexion; angulation, with the organism bending almost to 90° near its centre, is highly characteristic of *T. pallidum*. Its progression is relatively slow compared to that of many motile bacteria.

*T. pallidum* is feebly refractile, and darkground illumination is normally used to visualize the organism. It cannot be seen by ordinary staining methods; special techniques, such as silver impregnation, may be used to demonstrate the organism, particularly in tissue, but this tends to alter the morphology.
Immunofluorescence methods can now be used to detect treponemes in tissues and body fluids.

Cultivation

The Nichols strain of *T. pallidum* was isolated in 1913 from the CSF of a patient with neurosyphilis; it is still virulent for man and is maintained in rabbits by intratesticular inoculation and weekly passage. It divides by binary fission approximately once every 30 h when environmental conditions are favourable. It is generally agreed that pathogenic *T. pallidum* has not been cultivated in artificial media, embryonated eggs or tissue cultures. However, there is now a better understanding of the conditions that permit prolonged survival of treponemes in vitro, with retention of their pathogenicity for animals. Pathogenic treponemes (unlike cultivable non-pathogens) attach themselves to mammalian cells in culture. The discovery that *T. pallidum* is microaerophilic rather than a strict anaerobe has aided the prolonged survival of these microorganisms in vitro (Fieldsteel et al 1982, Norris & Edmonson 1986).

The genetic relationships between *T. pallidum* and cultivable treponemes have been studied by reassociation assays with 125I-labelled treponemal DNA (Miao & Fieldsteel 1978). Three groups were distinguished: virulent Nichols strain of *T. pallidum*, *T. phagedenis* and its biotype Reiter; and *T. raingrens*. Features such as the diameter and amplitude of the spiral, the number of axial filaments and the presence of intracytoplasmic microtubules can be used to differentiate between the pathogenic non-cultivable treponemes, which have similar if not identical morphology, and the cultivable treponemes (Hovind-Hougen 1976).

Sensitivity to physical and chemical agents

*T. pallidum* is so feebly viable outside its host that syphilis is ordinarily acquired only by sexual intercourse. The organism dies rapidly in water and is very sensitive to drying. However, it can remain viable and maintain its virulence in necropsy material for some time at room temperature, and in serum kept in sealed capillary tubes it remains motile for several days. It is readily killed by heat, e.g. 41.5°C for 1 h. When infected blood is stored at 5°C in citrate anticoagulant, infectivity is lost in 120 h or less. Treponemes survive for only a few days or weeks at -10 to -20°C but remain viable for extended periods at -45°C and for an indefinite period when stored at -78°C. Freezing followed by desiccation (freeze-drying) kills the organism.

Antibiotic sensitivity

Although a method has been described for determining MICs and MBCs of antimicrobial agents against *T. pallidum* (Norris & Edmonson 1988) the procedure is outside the range of all but highly specialized laboratories. Susceptibility testing may be of value in assessing new antimicrobial agents and monitoring possible changes in susceptibility of *T. pallidum* to existing agents but it does not currently apply to individual therapy. Penicillin remains the drug of choice in treating syphilis. Therapeutic regimens for early syphilis should aim to maintain a minimum serum penicillin concentration of 0.03 units/ml for a period of 10–15 days. Acceptable alternatives in penicillin-allergic patients include ceftriaxone or doxycycline (Goldmeier & Hay 1993).

Although *T. pallidum* is extremely sensitive to penicillin (healing of lesions occurs rapidly and treponemes disappear from early stage lesions), biological cure (i.e. eradication of treponemes) is difficult to prove since *T. pallidum* cannot be cultured in vitro. In a few patients who have been adequately treated with penicillin, residual *T. pallidum* has been detected in CSF, lymph nodes, etc., by electron microscopy; in a very few cases some of these treponemes were inoculated into rabbits and produced typical lesions. In such cases the surviving treponemes remained penicillin-sensitive and these patients may be considered to indicate treatment failure, probably due to abnormal penicillin metabolism in the patients ('quick penicillin secretors'), rather than acquired drug resistance.

Animal pathogenicity

Intratesticular injection leads to a syphilitic orchitis in rabbits; intradermal inoculation also produces lesions. Experimentally infected rabbits have been widely used to test various antisyphilitic drugs and to study the immune response to *T. pallidum*. Monkeys and anthropoid apes can also be infected experimentally. At present the only source of *T. pallidum* for preparing antigens and for experimental work is from the testes of infected rabbits. The production of specific *T. pallidum* protein antigens in *Escherichia coli* is ethically preferable to animal culture and would overcome the technical problems of producing large quantities of pure antigen from rabbit testes. Details of production and a list of the 25 or so *T. pallidum*
recombinant antigens produced so far are given by Norris et al (1993).

**Laboratory diagnosis of syphilis**

In recent years there have been several developments in relation to syphilis and its diagnosis (Young 1992) and these must be taken into account in our current diagnostic approaches. The epidemiology of syphilis has changed considerably, probably because of behavioural changes resulting from fears concerning HIV and AIDS. In the UK and many parts of Europe there has been a marked decline in the incidence of syphilis: the incidence in the UK is now in the region of 1-2 cases per 100,000. Syphilis however remains an important infection in many parts of the world; in the United States, after an initial overall decline in the mid 1980s, which was due largely to fewer infections in homosexual men, there was a marked increase in heterosexually acquired infection. The incidence of early infection reached a peak in 1990 with 18 cases per 100,000 and subsequently fell to 13 cases per 100,000 by 1992 (Centers for Disease Control 1993). There was a concomitant increase in congenital infection which also peaked around 1990-91 at a rate of almost 20 cases per 100,000 live births.

The many interactions between syphilis and HIV demand that every patient with positive syphilis serology should be tested for HIV (with the patient’s informed consent) and that every HIV-positive patient should be tested for syphilis (Anonymous 1989). Control of genital ulcer disease such as syphilis which disrupts the integrity of the mucosal epithelium thus increasing susceptibility to HIV is therefore important in the control of HIV infection. Other important considerations are the findings reviewed by Ruhi (1989) and Young (1992) that: (1) HIV-infected patients who acquire syphilis may fail to produce anti-treponemal antibodies. This has extremely serious implications for the diagnosis and control of syphilis, although some of the patients described as seronegative are more accurately classified as showing ‘delayed seropositivity’. (2) Serological evidence of treated syphilis may disappear after patients become infected with HIV. Accordingly, negative specific serology does not necessarily exclude a past syphilis infection in patients with AIDS. (3) The natural course of syphilis is altered in HIV-infected patients. Syphilis runs a more severe course and lesions may be more aggressive in both early and late stage disease. Neurological complications of secondary syphilis occur more frequently and at an earlier stage and may increase the risk of treatment failure.

The above changes and interactions are important with regard to the role of the laboratory in screening as well as confirming a clinical diagnosis of syphilis by (a) demonstrating *T. pallidum* in the exudates from the lesions, or (b) demonstrating antibodies in the serum.

**Demonstration of T. pallidum in lesion exudates**

**Darkground microscopy**

The infectious stages of treponemal infections can usually be diagnosed most quickly and effectively by the demonstration of motile treponemes in wet preparations of serous exudate expressed from suspected primary and secondary lesions. Where topical antibiotics have been used, examination of material obtained by lymph gland puncture may prove useful.

As there is a serious risk of infection it is important to use gloves when obtaining material for darkground microscopy. After cleansing the surface of the lesion with a swab soaked in sterile saline, serum is squeezed by gentle pressure from the depth of the lesion. This serum may be collected directly on a glass cover-slip or, if this is difficult, in a glass capillary tube. One end of the capillary is heated to expel fluid neatly onto the centre of a cover-slip, which is then positioned on a slide. After firmly pressing the cover-slip and slide between pieces of filter paper, the preparation can be examined by darkground illumination using the oil-immersion objective.

*T. pallidum* is recognized by its slender structure, characteristic slow movements and angulation. It must be carefully distinguished from other treponemes that may occur in genital ulcers, but these tend to be surface organisms and are not found in the depth of the lesions. Nevertheless, because of interference from commensal spirochaetes, darkground microscopy may not be reliable in the case of rectal and non-penile genital lesions. If the initial test is negative the procedure should be repeated daily for at least 3 days; antibiotics should be withheld during this period although sulphanilamide and local saline lavage may be used to reduce local sepsis. Organisms are not easily found in skin lesions of secondary syphilis except those in moist skin areas. Other drawbacks to darkground microscopy in this context (Lukehart & Baker-Zander 1988) include the need to examine potentially infectious specimens that may also contain HIV (unfixed material must be examined within 10-30 min to maintain treponemal motility). Accurate interpretation of darkground microscopy demands much experience but, with the
The decrease in primary syphilis in Europe, opportunities for gaining the necessary experience in Europe are limited.

**Immunofluorescent staining**

The direct fluorescent-antibody staining for *T. pallidum* (DFA-Tp) test whereby a smear of exudate is made on a slide, fixed in acetone, and sent to the laboratory, overcame the problem of examining potentially infectious material but did not solve the problem of specificity. Originally, smears were stained with a conjugated syphilitic serum made specific for *T. pallidum* by absorption with cultivable treponemes (Daniels & Ferneyhough 1977). In spite of the absorption stage the DFA-Tp test gave non-specific results and was less reliable than darkfield microscopy (Luger 1981). The use of a DFA-Tp test based on a fluorescein-labelled pathogen-specific monoclonal antibody (H9-1) specific for a 47-48 kDa antigen on *T. pallidum* subspecies (Lukehart et al 1985) results in 100% specificity (Hook et al 1985). If the monoclonal antibody DFA-Tp test becomes widely available commercially it may make darkfield microscopy redundant.

**Enzyme immunoassay (EIA) and the polymerase chain reaction (PCR)**

The Visuwell Syphilis Antigen EIA is a recent commercial attempt (ADI Diagnostics) to develop an alternative to microscopy for detecting *T. pallidum* in early lesions. Antigen extracted from lesion material collected on a swab is captured by a pathogen-specific monoclonal antibody reactive with a 47 kDa protein and detected by rabbit polyclonal antibody against *T. pallidum*. The presence of captured antigen is demonstrated by a goat anti-rabbit urease conjugate. Although EIA may be comparable to darkground microscopy with regard to sensitivity, lack of specificity is a major problem. While PCR is highly specific with regard to a wide range of organisms (Hay et al 1990, Burstain et al 1991), the tests used so far will not differentiate between *T. pallidum* subspecies *pallidum* and the closely related *T. pallidum* subspecies *pertenue* and are of no help in differentiating between yaws and syphilis. PCR remains a research method although its suitability for detecting DNA in various specimens in early syphilis has been investigated in experimentally infected rabbits (Wicher et al 1992).

**Identification of *T. pallidum* in tissues**

The report that the diagnosis of secondary syphilis in an HIV-infected man with Kaposi sarcoma required biopsy of a skin lesion with silver staining to show spirochaetes (Hicks et al 1987) re-awakened interest in this form of diagnosis. The Centers for Disease Control (CDC), Atlanta, recommend that when clinical findings suggest syphilis, but serological tests are negative, biopsy tissue should be examined by immunofluorescence or silver staining (Anonymous 1989). These procedures are not part of routine diagnosis and should be performed only by highly-specialized laboratories.

**Serological diagnosis of syphilis**

There is no demonstrable immunological difference between the treponemes responsible for syphilis, yaws or pinta. Although this should not often give rise to problems in the UK, the possibility should be borne in mind with patients from areas where these diseases are endemic.

The various methods used to measure antibody responses in treponemal infection can be divided into two major categories: (1) tests to measure antibodies produced against non-specific treponemal antigens, i.e. the cardiolipin or lipoidal antigen tests, formerly referred to as ‘reagin’ tests; and (2) tests to measure antibodies against antigens specific for pathogenic treponemes, i.e. the *T. pallidum* haemagglutination assay (TPHA), enzyme immunoassay (EIA) and the fluorescent antibody absorbed test (FTA-ABS).

**Cardiolipin antigen tests**

Cardiolipin, a complex diphospholipid, is widespread in nature and can be isolated from many mammalian tissues as well as from treponemes. Cardiolipin for use as antigen is traditionally prepared from mammalian tissues such as beef heart. Only a few of the numerous tests for detection of antibodies to cardiolipin antigen are still widely used.

The classical Wasserman complement fixation test has been almost completely replaced by the Venereal Diseases Research Laboratory (VDRL) test or one of its modifications (Young & Penn 1990); these tests are cheaper, more rapid and simpler to perform and control. In its original form the VDRL test is performed by mixing heat-inactivated patient’s serum with a freshly prepared suspension of cardiolipin—lecithin—cholesterol antigen and reading the resulting flocculation (aggregation of antigen–antibody complexes in suspension) microscopically with a low-power objective. Most laboratories now use a rapid plasma reagin (RPR) test which employs a
stabilized VDRL carbon antigen (e.g. Murex); this antigen comprises VDRL antigen with choline chloride (to block inhibitors in serum, eliminate the need for heat-inactivation and allow testing of plasma), EDTA (to stabilize the antigen and allow it to be used for up to 6 months when stored at 4-10°C), and finely divided carbon particles (to enable the result to be read by eye instead of microscopically). A fingerprick blood sample can be tested on plastic or paper cards, making the RPR test useful in field studies in developing countries. A modification of the RPR, the Toluidine red unheated serum test (TRUST), may have an additional advantage in hot climates as the antigen is more stable than RPR antigen on storage at room temperatures of 26-31°C (Parham et al 1984). The sensitivity and specificity of the VDRL, RPR and TRUST tests are similar (Luger 1988). Tests such as the RPR can also be automated for use in centres where large numbers of specimens must be tested.

Because of their simplicity and accuracy, the cardiolipin antigen tests are used as screening or first-line procedures for both routine diagnosis and mass screening programmes. These tests usually become positive 10-14 days after the appearance of the chancre, the titre gradually increasing. The titre diminishes and the test tends to become negative after treatment. In late or latent syphilis the cardiolipin antigen tests are often negative. A more serious limitation of these tests is the occurrence of false-negative reactions resulting from an excess of antibody which prevents the formation of antigen-antibody complexes – the prozone phenomenon; a positive reaction is usually obtained on diluting the serum. Attention has been drawn to the importance of the prozone phenomenon in routine screening (Young et al 1992, Young 1992), in detecting syphilis in patients with HIV (Jurado et al 1993) and in ante-natal screening: four cases of false-negative serological tests due to the prozone phenomenon were encountered in women who gave birth to infants with congenital infection (Berkwitz et al 1990). The prozone phenomenon was also considered to be responsible for a false-negative VDRL reaction with cerebrospinal fluid (CSF) from an HIV-positive patient with neurosyphilis (Feraru et al 1990).

Since cardiolipin antigen tests detect antibodies against a non-specific antigen shared by treponemes and mammalian tissues, a positive result is sometimes obtained with sera from healthy individuals or patients without clinical evidence of syphilis; these reactions are termed Biological False Positives (BFP). Tests using specific T. pallidum antigen are required to distinguish between positive cardiolipin antigen tests resulting from BFP reactions and those due to treponemal infection.

### T. pallidum haemagglutination assay

The TPHA (e.g. Fujirebio) is very simple to perform and was the first of the specific tests suitable for routine screening. It is often negative in untreated primary syphilis (possibly owing to variability in the IgM-binding capacity of the TPHA reagent). In general, the TPHA is more sensitive and more specific than the FTA-ABS test (Luger 1988). Occasionally, false-positive haemagglutination may result from heterophile antibody in the serum of patients with infectious mononucleosis. (This occurs only if the control cells fail to agglutinate, otherwise a non-specific agglutination reaction would be recorded.) In certain tropical countries a small percentage of BFP reactors have also given apparent false positive TPHA results; because of the sensitivity of the test, these could represent the residue of previous infection with endemic treponematoses.

### Enzyme immunoassay

A major advantage of EIA is its suitability for automation, including electronic generation of reports. The development of EIA in syphilis diagnosis, since its first use in 1975 (Veldkamp & Visser 1975) to the development of commercial tests, has been reviewed elsewhere (Young & Penn 1990). The most widely used commercial EIA is Captia Syphilis G (Centocor), in which antibody bound to T. pallidum antigen coated on to a plastic well is detected by a tracer complex comprising biotinylated monoclonal antibody against human IgG and streptavidin-horseradish peroxidase conjugate. The overall sensitivity of Captia G is almost identical to that of the TPHA and although the specificity may be slightly lower it is ideal as a screening test (Young et al 1992). In spite of using an anti-treponemal IgG conjugate, sensitivity in primary syphilis is high as has been found in a number of reports using both 'in-house' and commercial EIA systems (Young 1992).

### Fluorescent antibody absorbed test

In the FTA-ABS test (e.g. Mast), binding of specific antibody by T. pallidum is demonstrated by the indirect immunofluorescence technique. The FTA-ABS is an accepted reference test and is highly specific and sensitive at all stages of syphilitic infection although a small percentage of false positive reactions occurs.
e.g. in patients with systemic lupus erythematosus and other connective tissue diseases.

Other serological tests

The *T. pallidum* immobilization (TPI) test (Nelson & Mayer 1949) was the first to use specific treponemal antigen but it has been superseded by the TPHA and FTA-ABS tests (Rein et al 1980, Sprott et al 1982). Because the TPI test employs live treponemes it is time-consuming, expensive and technically demanding. A few reference laboratories still perform the TPI test on selected sera for research purposes.

The *Reiter protein complement fixation* (RPCF) test detects antibodies produced against a group-specific treponemal antigen shared by pathogenic and commensal treponemes. Since the Reiter treponeme can be grown in relatively simple media, sufficient antigen can readily be obtained for large-scale screening. The RPCF test has now been superseded by the TPHA.


METHODS

As almost all serological tests for syphilis are now performed with commercial kits it is essential that tests are performed according to the manufacturer’s recommended instructions unless there are good reasons for deviating from these. For example, in the Captia Syphilis G EIA (Centocor), when using manual pipetting rather than an automated system, the author recommends that sera are pre-diluted 1:19 (20 µl + 380 µl) and 100 µl transferred to the microtitre strip well rather than making an in-well dilution of 1:20 (5 µl + 100 µl). Guidance regarding procedures for the performance of the VDRL, TPHA and FTA-ABS test can be found in the previous edition of this book. Alternatively readers may wish to consult the CDC Manual of Tests for Syphilis 1990, edited by Sandra Larsen, Elizabeth Hunter and Stephen Kraus. Whichever test kits and strategies are used it is essential that laboratories participate in external quality assurance programmes (e.g. NEQAS in the UK) as well as maintaining a high level of internal quality control (see Ch. 1).

Serological screening

The continuous serological screening of pregnant women, blood donors and ‘at risk’ groups is helpful in the detection and control of syphilis. When used together, the VDRL and TPHA tests provide a highly efficient screen for the detection or exclusion of treponemal infection; both are simple to perform and can be readily quantified. Their activity is complementary; the VDRL test is more sensitive than the TPHA in the detection of very early syphilis while the TPHA is more sensitive than the VDRL in the detection of latent and late infection.

The Captia Syphilis G EIA gives an overall performance equivalent to that of the VDRL and TPHA combination and can be used as a single readily automated screening test (Young et al 1992). Although this test detects only anti-treponemal IgG, by the time clinical signs develop most patients have both IgG and IgM antibody (Baker-Zander et al 1985). Therefore, provided that clinicians are aware of the ‘seronegative window’ that may exist for 1–2 weeks during the early primary infection (usually before symptoms appear), maintain a high index of clinical suspicion and have the facility to request additional tests (e.g. specific IgM or FTA-ABS) in cases of suspect primary infection, then detection of early infection will not be compromised by using anti-treponemal IgG EIA as a single screening test. The FTA-ABS is not suitable for screening large numbers of sera and is reserved as a confirmatory test when one of the screening tests is positive. Unfortunately there is a correlation between false reactivity in the FTA-ABS test and false reactivity on screening with EIA (Chronas et al 1992). The TPHA is the most specific test and, with the possible exception of very early primary syphilis, provides a highly reliable confirmation of the treponemal status of sera positive on screening with EIA.

Interpretation of serological tests

A negative result in either (1) the EIA, or (2) the VDRL and TPHA screening schedule has a high negative predictive value for excluding current or previous treponemal infection and further tests are not normally necessary. Note however that some 10–30% of patients with previous syphilis who acquire HIV may lose serological markers for syphilis as the HIV infection progresses. The pattern of results obtained following reactivity on screening and subsequent confirmatory testing by the FTA-ABS test may give valuable information as to the stage of infection. Table 33.1 provides a guide, but it is important to remember that each case must be interpreted individually in the light of available clinical and epidemiological data. Because of the serious social and medical implications, a diagnosis of syphilis should never be made from the results of a single blood specimen.

Quantitative test results may also prove helpful.
Table 33.1. Pattern of results of serological tests in different stages of acquired syphilis.

<table>
<thead>
<tr>
<th>EIA</th>
<th>VDRL</th>
<th>TPHA</th>
<th>FTA-ABS</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>False-positive reaction; repeat to exclude primary infection</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>False-positive EIA (and possibly FTA-ABS); repeat to exclude primary infection</td>
</tr>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>Primary infection; dark-ground investigation of lesion may be positive</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Untreated (or recently treated); probably beyond the primary stage</td>
</tr>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>History of treated syphilis</td>
</tr>
</tbody>
</table>

Note: In endemic treponemal diseases such as yaws, bejel, or pinta, patterns are similar to those of syphilis.

As there is now extensive experience with VDRL and TPHA over many years, it is valuable to do these quantitative tests on specimens that are positive on EIA screening. During the primary stage of infection the VDRL titre rises to 8 or 16. VDRL tests with titres of 16–128 are commonly found in secondary syphilis and active cardiovascular or neurosyphilis. After the secondary stage the VDRL titre declines and eventually becomes negative in c. 30% of untreated latent and late cases.

The TPHA test is often negative in early primary syphilis but may become positive at low titre (80–320) towards the end of the primary stage. Titres rise sharply during the secondary stage and commonly reach 5120 or greater. The TPHA titre declines during the latent stage but invariably remains positive at a relatively lower level (80–640).

Response to treatment. The cardiolipin antigen tests primarily reflect disease activity. These tests tend to become negative after treatment, particularly in early syphilis. Serial quantitative VDRL testing provides the best means of measuring response to treatment in most stages of treponemal infection.

Differentiation of treated, partially treated and untreated syphilis. The anti-treponemal IgG EIA, TPHA and FTA-ABS tests usually remain positive for life, even in those who have been fully treated with adequate doses of penicillin. On the premise that detection of specific anti-treponemal IgM will denote active syphilis, several methods for demonstrating specific IgM antibodies have been investigated.

One of the most widely used methods relies on the detection of anti-treponemal IgM with monospecific fluorescent-labelled anti-human immunoglobulin in the FTA-ABS test. The performance of this test with unfractionated serum (the IgM-FTA-ABS test) is unsatisfactory as false positive and false negative results are common. Reliable results are obtained when 19S (IgM) antibodies are separated from those of the 7S (IgG) class by gel filtration before performing the test; this test is known as the 19S-IgM-FTA. However this is a time-consuming and technically demanding procedure and is normally restricted to research and reference laboratories. Simpler methods of detecting specific IgM by haemagglutination and enzyme-linked immunosorbent assays are described by Luger (1988).

The Captia Syphilis M EIA test (Centocor) detects anti-treponemal IgM by μ-chain capture; specific IgM is detected by a tracer complex of T. pallidum antigen linked to biotinylated monoclonal antibody against T. pallidum and streptavidin-conjugated horseradish peroxidase. Because of the use of the tracer complex, false positive reactions are not likely to result from rheumatoid factor (Ijsselmuiden et al 1989). The Captia Syphilis M test has high sensitivity, comparable to the 19S-IgM-FTA, in early infection (Ijsselmuiden et al 1989, Lefevre et al 1990). However, sensitivity is poor in both tests in late infection (Lefevre et al 1990). In patients with positive serological tests who lack a history of recent adequate treatment, a reactive anti-treponemal IgM test supports the need for treatment. Occasionally, however, the sera of patients who have been adequately treated will contain BS IgM that may react in assays where immunoglobulins are not fractionated prior to testing (Muller & Lindenschmidt 1982, Tanaka et al 1984). Untreated or inadequately treated infections, particularly beyond the early stages, as well as re-infections, cannot be excluded reliably on the basis of a negative anti-treponemal IgM test, and due consideration must be given to clinical findings, the history of the patient, and quantitative VDRL and TPHA tests.

Congenital syphilis

This is a condition caused by infection in utero with T. pallidum. Although rare in the UK, in the USA there was a very marked increase in congenital infection from the late 1960s through to 1991, highlighting the need for appropriate surveillance and diagnostic tests.
Centers for Disease Control 1990. A wide spectrum of severity exists, and only severe cases are clinically apparent at birth. The serological diagnosis of congenital syphilis is complicated because many of the standard serological tests depend on IgG and IgM antibody responses: the IgG found in the serum of neonates is largely passively acquired through the placenta. The demonstration of specific anti- treponemal IgM by the 19S- IgM-FTA strongly supports a diagnosis of congenital infection. The simpler Capita Syphilis M EIA also correlates well with congenital infection (Ijsselmuiden et al 1989) while Western Blotting may also prove a useful diagnostic method (Meyer et al 1994). Serial examinations are recommended because specific anti- treponemal IgM may not be detectable for several weeks after birth, possibly due to the suppression of neonatal IgM synthesis by high levels of circulating maternal IgG. It is helpful to compare maternal and neonatal sera in parallel. A significantly higher titre in the neonate than in the mother suggests infection. Also, antibody titres will rise in a baby that has been infected, whereas in the absence of infection, e.g. when the mother has been treated during pregnancy, passively transferred antibody detected by the VDRL will decrease in titre and the test will become negative in approximately 3 months; owing to their greater sensitivity treponemal antigen tests usually take slightly longer to become negative.

Diagnosis of neurosyphilis: examination of CSF

The use of CSF for routine screening tests in patients in whom there is no clinical suspicion of syphilis is unjustified; a negative TPHA test on the blood will virtually exclude active neurosyphilis and is a better screen for the detection of all forms of late syphilis.

However, in cases selected on clinical grounds backed by a positive TPHA test on blood, investigations should be carried out on the CSF to detect early invasion of the central nervous system (CNS). A total volume of 6–10 ml is usually sufficient to carry out the necessary tests; note that contamination of the CSF specimen with even a small amount of blood can give misleading results.

Investigation of the CSF should include a cell count, estimation of total protein and estimation of IgG and IgM. Cell counts exceeding 5 cells/mm³ (5 x 10⁹/litre) and total protein values above 40 mg/100 ml are signs of inflammation but are non-specific as indicators of syphilitic involvement of the nervous system. Specific tests such as the VDRL, TPHA and FTA-ABS should also be performed.

The VDRL test alone is not a reliable indicator of CNS involvement since it is non-reactive in 30–60% of patients with active neurosyphilis. However, a negative TPHA test in CSF excludes neurosyphilis. A positive TPHA or FTA-ABS test in CSF does not necessarily indicate active disease, since reactivity may be caused by transudation of immunoglobulins from the serum into the CSF. The TPHA index, which relates CSF TPHA titre to the albumin quotient (CSF albumin concentration x 10⁹/serum albumin concentration), should help exclude errors associated with disturbed function of the blood–brain barrier. The TPHA index and methods for the demonstration of specific IgM antibodies in CSF as indicators of active neurosyphilis have been discussed by Luger (1988) and by Young & Penn (1990).

Thus, although active neurosyphilis can be excluded reliably and simply by a negative TPHA test result on the CSF, unequivocal serological evidence of CNS involvement is essentially a procedure for a specialized laboratory.

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Diagnostic Microbiology

An evaluation of pre-poured selective media for the isolation of Neisseria gonorrhoeae

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Fourteen commercial media supplied as pre-poured plates were compared with an 'in-house' selective medium for their ability to support the growth of 105 gonococcal isolates (representing a wide variety of serovars encountered in natural infection), 25 meningococcal and 20 Neisseria lactamica isolates, and to inhibit the growth of 71 isolates of non-pathogenic neisseriae and miscellaneous organisms. Only two of the pre-poured plate media and the in-house selective medium yielded growth of duplicate cultures of all 105 gonococcal isolates after incubation for 24 h: one other medium provided growth of all the isolates after incubation for 48 h. The ability of the various media to suppress the growth of the 71 isolates of non-pathogenic neisseriae and miscellaneous organisms ranged from 97.2 to 71.8% of isolates inhibited. Of the four media that enabled growth of all the gonococcal strains, inhibition was 94.4% for the in-house medium, 85.9% and 80.3% for the two media on which all gonococci grew after 24 h and 71.8% for the medium on which all the gonococci grew after 48 h. Failure of growth of gonococci was associated with: serogroup IA isolates (p < 0.001), AHU auxotype (p < 0.001) and the presence of vancomycin rather than lincomycin in the selective medium (p < 0.02). The use of 10% blood and a highly nutritious medium based on the original New York City (NYC) or modified New York City (MNYC) formulation were also important in supporting growth of gonococci. One of the main problems in lack of selectivity was a failure to inhibit the growth of yeasts. As effective inhibition of yeasts was obtained with other media containing the same concentration of amphotericin, failure may be due to batch variation of supplement, media preparation, or reduced shelf life of the media. None of the commercially available pre-poured media performed as well as the in-house medium despite the fact that some of the media were prepared to a very similar formula.

Introduction

The definitive diagnosis of gonococcal infection relies on culture, usually on selective media, and identification of Neisseria gonorrhoeae [1]. Most selective media contain a rich nutrient base supplemented with blood, partially lysed by heat (chocolate agar) or completely lysed by saponin, an antimicrobial cocktail is added to inhibit micro-organisms other than pathogenic neisseriae. Details of the main selective media, Thayer Martin (TM) medium and New York City (NYC) medium and their modifications have been reviewed elsewhere [2]. In this laboratory, primary culture for gonococci is performed on modified New York City (MNYC) medium containing lysed horse blood 10% and lincomycin 1 mg/L [3]. Various pre-poured selective media for culture of gonococci are available commercially but surprisingly few comparative data have been published. Fourteen pre-poured selective media available commercially were evaluated. Their ability to support growth of gonococci, meningococci and N. lactamica isolates, and to inhibit growth of 71 isolates of non-pathogenic neisseriae and other miscellaneous organisms was compared with 'in-house' selective and non-selective media.

Materials and methods

Media

Fourteen gonococcal selective media were obtained from four manufacturers of pre-poured media (Table 1). All plates were within their expiry date at the time of evaluation. For comparison, an in-house selective
medium based on the formula for MNYC medium was prepared as described previously [3]; the same medium without antibiotics was included as a control. Each medium was labelled with an identification code to help reduce observer bias (Table 1).

**Organisms**

*N. gonorrhoeae* (105) isolates tested included the five WHO reference strains recommended for antibiotic susceptibility testing and 100 clinical isolates (30 penicillinase-producing) representing a wide variety of serovars encountered in natural infection (Table 2). Other organisms tested included *N. meningitidis* (25, mainly clinical isolates including some of known serogroup); *N. lactamica* (20, all clinical isolates); *Moraxella catarrhalis* (20, all clinical isolates including 19 penicillinase-producing strains); *Gemella haemolytica* NCTC 10243; *N. animalis* NCTC 10212; *N. canis* NCTC 10296; *N. caviae* NCTC 10296; *N. cinerea* NCTC 10294; *N. cuniculi* NCTC 10297; *N. dentificans* NCTC 10295; *N. elongata* NCTC 10660; *N. elongata* subsp. *glycyltica* NCTC 11050; *N. flavescens* NCTC 8263; *N. ovis* NCTC 11018; *N. pharyngis* NCTC 4590; *N. mucosa* NCTC 10777; *N. mucosa var mucosa* NCTC 10774; *Neisseria* sp. NCTC 11049; *Escherichia coli* (6, including NCTC 11560 and 10418); *Proteus mirabilis* (6); *Pseudomonas aeruginosa* (6, including NCTC 10662); *Staphylococcus aureus* (6, including NCTC 6571 and S113); *Streptococcus agalactiae* (6); *Candida albicans* (4); *C. glabrata* (1) and *C. kefyr* (1).

**Test protocol**

Saline suspensions equivalent to a McFarland 0.5 standard, c. 10⁵ cfu/ml were prepared from an overnight culture on in-house non-selective medium. Various dilutions of stock suspensions were used to inoculate in-house selective and non-selective media and the 14 commercial pre-poured media. The entire series of plates was tested with the same suspension dilutions of each organism, inoculated in alphabetical order (Table 1), the in-house non-selective medium being inoculated last and acting as a control. A multi-point inoculator with a pin volume of 1 μl was used for inoculation. Cultures were incubated at 37°C in a CO₂-enriched atmosphere and examined after incubation for 16–24 h and 40–48 h; the number of colonies was calculated from the appropriate dilution and counts were expressed as cfu/ml. All counts were performed in duplicate. Although some spreading of *P. mirabilis* was evident on certain media, the number of discrete colonies could be determined from plates that had only a few colonies present. For organisms expected to grow on selective media (gonococci, meningococci and *N. lactamica*), test inocula were 10⁵, 10⁴ and 10³ cfu/ml; thus 'no growth' represented <100 cfu. For organisms expected

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**Table 1. Gonococcal selective media included in the evaluation**

<table>
<thead>
<tr>
<th>Code</th>
<th>Medium</th>
<th>Manufacture/</th>
<th>Antibiotics*</th>
<th>Blood/supplements</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Modified New York City</td>
<td>In-house</td>
<td>V L C A T N</td>
<td>Lysed horse blood 10%; yeast dialysate 2.5%; glucose 0.1%</td>
</tr>
<tr>
<td>B</td>
<td>GC Selective VCNT (chocolate)</td>
<td>Unipath PO136A</td>
<td>3 7.5</td>
<td>36500</td>
</tr>
<tr>
<td>C</td>
<td>GC Selective VCNT (lysed)</td>
<td>Unipath PO135A</td>
<td>3 7.5</td>
<td>36500</td>
</tr>
<tr>
<td>D</td>
<td>GC Selective VCAT (lysed)</td>
<td>Unipath PO137A</td>
<td>2 7.5</td>
<td>36500</td>
</tr>
<tr>
<td>E</td>
<td>GC Selective LCAT (lysed)</td>
<td>Unipath PO226A</td>
<td>4 7.5</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>NYC plus LCAT</td>
<td>bioMérieux</td>
<td>1 6 1 6.5</td>
<td>Lysed horse blood 10%; polyvitex</td>
</tr>
<tr>
<td>G</td>
<td>Selective chocolate agar</td>
<td>bioMérieux 43241</td>
<td>3 7.5</td>
<td>5</td>
</tr>
<tr>
<td>H</td>
<td>NYC plus VCAT</td>
<td>bioMérieux</td>
<td>2 7.5</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>GC selective medium with lysed blood</td>
<td>Difco 9224-30</td>
<td>3 7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>J</td>
<td>Neisseria isolation medium</td>
<td>Difco 9188-30</td>
<td>3 7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>K</td>
<td>GC selective medium with haemoglobin</td>
<td>Difco 9160-30</td>
<td>3 7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>L</td>
<td>GC selective medium (chocolate)</td>
<td>Difco 9223-30</td>
<td>3 7.5</td>
<td>0.9</td>
</tr>
<tr>
<td>M</td>
<td>New York City</td>
<td>Difco 9387-30</td>
<td>2 7.5</td>
<td>3</td>
</tr>
<tr>
<td>N</td>
<td>Modified New York City</td>
<td>Becton Dickinson</td>
<td>2 7.5</td>
<td>3</td>
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<tr>
<td>O</td>
<td>GC Lect</td>
<td>Becton Dickinson</td>
<td>1 2 7.5</td>
<td>3</td>
</tr>
<tr>
<td>P</td>
<td>Non-selective modified New York City</td>
<td>In-house</td>
<td>None</td>
<td>Lysed horse blood 10%; yeast dialysate 2.5%; glucose 0.1%</td>
</tr>
</tbody>
</table>

V, vancomycin; L, lincomycin; C, colistin; A, amphotericin; T, trimethoprim; N, nystatin.

*Antibiotic concentration in mg/L except nystatin which is given in units/L.
to be inhibited by selective media (non-pathogenic neisseriae and non-neisseriae), test inocula ranged from 10^0 to 10^3 cfu/ml; thus 'no growth' represented complete inhibition of 10^3 cfu.

Statistical analysis was by the χ^2 test with the Minitab PC software package.

**Results**

**Gonococci**

The performance of media in culturing 105 gonococcal isolates is summarised in Table 3. The only media to grow all 105 isolates in duplicate after incubation for 24 h were the in-house selective medium A, commercial media F and N, and the in-house non-selective control medium, P; medium L supported growth of all isolates but only after incubation for 48 h. After incubation for 24 h the vast majority of duplicate cultures gave identical results while after incubation for 48 h there was total concordance. Overall performance ranged from growth of 78.1% of strains (medium J) to 100% (media A, F and N) after incubation for 24 h and 88.6% (medium J) to 100% (media A, F, N and L) after incubation for 48 h. Mean counts after 48-h culture on selective media ranged from 1.28 × 10^8 cfu/ml (medium B) to 2.02 × 10^8 cfu/ml (medium N). The in-house selective medium (A) gave a count of 1.91 × 10^8 cfu/ml compared to 2.21 × 10^8 cfu/ml on non-selective control medium (P) indicating that the cut-off for failure to grow on the selective media was c. 200 cfu.

<table>
<thead>
<tr>
<th>Table 2. Serotype and auxotype of 100 clinical gonococcal isolates included in the evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serovar</strong></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>IA-2</td>
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<tr>
<td>IA-4</td>
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<td>IA-5</td>
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<td>IA-6</td>
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<td>IA-7</td>
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<tr>
<td>IA-16</td>
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<td>IA-21</td>
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<td>IA-25</td>
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<td>IB-1</td>
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<td>IB-2</td>
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<td>IB-3</td>
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<td>IB-5</td>
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<td>IB-8</td>
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<td>IB-15</td>
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<td>IB-17</td>
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<td>IB-19</td>
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<td>IB-25</td>
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<td>IB-26</td>
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<td>IB-29</td>
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<tr>
<td>IB-31</td>
</tr>
<tr>
<td>IB-00</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* Requirement for: A, arginine; F, proline; H, hypoxanthine; U, uracil; NR, non-requiring.
^One IA-5 isolate and the IA-7 isolate failed to grow on any of the auxotyping media.
^Non-typable strains.

<table>
<thead>
<tr>
<th>Table 3. Sensitivity of gonococcal selective media*</th>
</tr>
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<tbody>
<tr>
<td><strong>Medium</strong></td>
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<tr>
<td>---------------------------------------------------------------</td>
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<td>A</td>
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<td>B</td>
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<td>C</td>
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<td>J</td>
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<td>K</td>
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<tr>
<td>L</td>
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<td>M</td>
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<tr>
<td>N</td>
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<tr>
<td>O</td>
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<tr>
<td>P</td>
</tr>
</tbody>
</table>

*One hundred and five isolates tested in duplicate (n = 210).
E (lincomycin) and medium M (vancomycin) failed to support growth of one IA-16/AHU and one IA-4/NR isolate, respectively; medium I (vancomycin) and medium K (vancomycin) both failed with one non-typable IB/NR isolate and with two IA-4/NR and three IA-2/AHU isolates; medium C (vancomycin) failed with three IA-2/AHU isolates, two IA-4/NR 2 isolates, one IA-5/AH isolate, three IA-21/AHU isolates and one non-typable IB/NR isolate; medium J (vancomycin) failed with one IA-2/AHU isolate, one IA-4/NR isolate, one IA-5/AH, three IA-21/AHU isolates, one IB-1/NR isolate and five non-typable IB/NR isolates.

Vancomycin-containing media accounted for 93.2% (59 of 64) of all growth failures and the overall failure rate in relation to the total number of challenges was 1.7% (5 of 300) for media containing lincomycin and 4.9% (59 of 1200) for media containing vancomycin ($\chi^2 = 6.2$; p < 0.02). All five failures on lincomycin-containing medium occurred with the medium containing 4 mg/L (F) and none on the two media containing 1 mg/L (A and F).

The majority of growth failures, 76.6% (49 of 64), were with strains of the AHU auxotype and the remainder were with non-requiring strains (Table 4). Failure rates were 21.8% (49 of 225) for AHU strains, 2% (15 of 750) for non-requiring strains and 0% (0 of 525) for strains of other auxotypes (AHU strains versus non-requiring strains, $\chi^2 = 110.4$; p < 0.001; non-requiring strains versus isolates of other auxotypes, $\chi^2 = 10.63$; p < 0.01).

Although serovar IA and the AHU auxotype were associated with failure to grow, there was a considerable variation between serovar IA isolates: serovars IA-2 (5 of 6 AHU), IA-7 (non-auxotypable), and IA-25 (2 of 2 AHU) showing similar failure rates (p < 0.05), serovars IA-5 (1 of 2 AHU) and IA-21 (3 of 3 AHU) significantly higher failure rates (p < 0.05), and serovars IA-6 (0 of 5 AHU) and IA-16 (4 of 4 AHU) significantly lower failure rates (p < 0.05) when compared with all serovar IA isolates.

**Meningococci**

After incubation for 24 h the performance of media in supporting growth of meningococci was 92% (23 of 25) with media D and J; 96% (24 of 25) with media B, C, E, G, H, I, K, L, M, N and O; and 100% with media A, F and P. After incubation for 48 h results were identical except for media D and E on each of which a further isolate grew. Mean counts after culture for 48 h on selective media ranged from $3.13 \times 10^8$ cfu/ml (medium B) to $4.53 \times 10^8$ cfu/ml (medium 0). The in-house selective medium A gave a count of $4.36 \times 10^8$ cfu/ml while the non-selective control medium P gave a count of $4.40 \times 10^8$ cfu/ml. These results suggest that the cutoff for failure to grow on the selective media was c. 450 cfu. The same meningococcal strain, a non-groupable type 15 strain sensitive to penicillin and sulphadiazine, was responsible for all growth failures.

**N. lactamica**

All 20 strains of *N. lactamica* grew on all of the media after incubation for 24 h. Mean counts after 48 h ranged from $1.28 \times 10^8$ cfu/ml (medium B) to $2.14 \times 10^8$ cfu/ml (medium N). The in-house selective medium A gave a count of $1.88 \times 10^8$ cfu/ml while the non-selective control medium P gave a count of $2.08 \times 10^8$ cfu/ml.

**Non-pathogenic neisseriae and miscellaneous organisms**

The results of testing the 71 non-pathogenic neisseriae and miscellaneous organisms in duplicate are summarised in Table 5. None of the selective media inhibited all of the organisms after incubation for 48 h although medium G gave 100% inhibition after incubation for 24 h. After incubation for 48 h, inhibition ranged from 72.5% (medium L) to 97.9% (medium G): their performance in supporting the growth of gonococci after 48 h was 100% and 96.2%, respectively. Details of the organisms that grew on the various selective media are summarised in Table 6. Generally, results of duplicate cultures were concordant, apart from *N. cinerea* and one of the strains of *N. mucosa* which often grew on only one of the duplicate plates. Although results in Table 6 are based on ‘growth’ or ‘no growth’ the extent of growth was often substantially reduced compared with the non-selective control medium P.
Table 5. Specificity of gonococcal selective media; non-pathogenic neisseriae and miscellaneous organisms tested in duplicate

<table>
<thead>
<tr>
<th>Medium</th>
<th>Number of isolates (%) inhibited at 24 h</th>
<th>Number of isolates (%) inhibited at 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>Number of isolates (%) inhibited at 24 h</td>
<td>Number of isolates (%) inhibited at 48 h</td>
</tr>
<tr>
<td>A</td>
<td>140 (98.6)</td>
<td>135 (95.1)</td>
</tr>
<tr>
<td>B</td>
<td>138 (97.2)</td>
<td>124 (87.3)</td>
</tr>
<tr>
<td>C</td>
<td>128 (90.1)</td>
<td>126 (88.7)</td>
</tr>
<tr>
<td>D</td>
<td>138 (97.2)</td>
<td>132 (93)</td>
</tr>
<tr>
<td>E</td>
<td>138 (97.2)</td>
<td>135 (95.1)</td>
</tr>
<tr>
<td>F</td>
<td>128 (90.1)</td>
<td>123 (86.6)</td>
</tr>
<tr>
<td>G</td>
<td>142 (100)</td>
<td>139 (97.9)</td>
</tr>
<tr>
<td>H</td>
<td>134 (94.4)</td>
<td>125 (88)</td>
</tr>
<tr>
<td>I</td>
<td>134 (94.4)</td>
<td>131 (92.3)</td>
</tr>
<tr>
<td>J</td>
<td>126 (88.7)</td>
<td>132 (86.6)</td>
</tr>
<tr>
<td>K</td>
<td>138 (97.2)</td>
<td>135 (95.1)</td>
</tr>
<tr>
<td>L</td>
<td>116 (81.7)</td>
<td>103 (72.5)</td>
</tr>
<tr>
<td>M</td>
<td>138 (97.2)</td>
<td>136 (95.8)</td>
</tr>
<tr>
<td>N</td>
<td>118 (83.1)</td>
<td>115 (81)</td>
</tr>
<tr>
<td>O</td>
<td>138 (97.2)</td>
<td>135 (95.1)</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*n = 142.

Discussion

Selective media improve the cultural diagnosis of gonorrhoea by preventing the growth of other bacteria which may mask the presence of gonococcal colonies or inhibit the growth of gonococci directly. Many organisms present in the normal endocervical flora inhibit gonococci [4–9]. In assessing the suitability of pre-poured selective media the most important factor is the ability to support the growth of all gonococcal isolates, followed by the effective inhibition of a wide range of other bacteria. As gonococcal infection can occur at levels as low as 10^2 cfu/ml, and other organisms may often be present at levels well in excess of 10^6 cfu/ml [10] cut-off values of c. 10^2 cfu/ml for growth of gonococci and 10^5–10^6 cfu/ml for inhibition of non-pathogenic neisseriae and miscellaneous organisms were chosen to represent a meaningful test of the selective media in clinical practice. The inclusion of AHU strains is important in comparing lincomycin and vancomycin as selective agents; Reichart et al. [11] included only 2.7% of such isolates in their study comparing GC-Lect and modified Thayer Martin (MTM) medium but stressed that future studies should include more AHU isolates. In the present study 15% of isolates were AHU strains.

In addition to the in-house selective media only two of the commercial pre-poured selective media (F and N) supported growth of all 105 isolates of gonococci in duplicate after incubation for 24 h (Table 3); although medium L yielded growth of all 105 isolates after incubation for 48 h it failed to support the growth of three isolates in duplicate and three isolates in one of the duplicate cultures after 24 h. When gonococci grew on a particular medium the counts were generally similar to all other media. A non-pregroupable type 15 isolate of meningococcus failed to grow on media L and N and these media also allowed an unacceptable percentage of non-gonococcal neisseriae or miscellaneous organisms to grow (28.2% and 19.7% respectively). Although medium F was the only commercial selective media to support the growth of all gonococci and meningococci, it allowed 14.1% of non-gonococcal neisseriae or miscellaneous organisms to grow compared with 5.6% for the in-house medium; this difference in inhibition was due to the complete failure of medium F to inhibit growth of yeasts, a serious problem, as certain strains of Candida albicans produce a substance inhibitory to gonococci [8,9]. The other organisms growing on medium F and the in-house selective medium A were identical and included G. haemolsyans, N. cinerea, N. mucosa var mucosa and a methicillin-resistant staphylococcal strain. Clearly, none of the commercial media in this trial performed as well overall as the in-house selective medium.

In general, media that contained 10% blood (A, B, F and H) gave significantly better results for supporting the growth of gonococci after incubation for 48 h (mean

Table 6. Growth of 71 non-pathogenic neisseriae and miscellaneous organisms cultured in duplicate on gonococcal selective media for 48 h

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of isolates</th>
<th>Number (%) of isolates growing on corresponding medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida spp.</td>
<td>6*</td>
<td>A 0 B 4 C 5 D 0 E 6 F 5 G 6 H 5 I 5 J 5 K 5 L 5 M 5 N 5 O 0</td>
</tr>
<tr>
<td>Neisseria spp.</td>
<td>14*</td>
<td>A 2 B 2 C 2 D 2 E 2 F 2 G 2 H 2 I 2 J 2 K 2 L 2 M 2 N 2 O 2</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>20</td>
<td>A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0 I 0 J 0 K 0 L 0 M 0 N 0 O 0</td>
</tr>
<tr>
<td>G. haemolsyans</td>
<td>1</td>
<td>A 1 B 1 C 1 D 1 E 1 F 1 G 1 H 1 I 1 J 1 K 1 L 1 M 1 N 1 O 1</td>
</tr>
<tr>
<td>E. coli</td>
<td>6</td>
<td>A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0 I 0 J 0 K 0 L 0 M 0 N 0 O 0</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>6</td>
<td>A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0 I 0 J 0 K 0 L 0 M 0 N 0 O 0</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>6</td>
<td>A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0 I 0 J 0 K 0 L 0 M 0 N 0 O 0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>6</td>
<td>A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0 I 0 J 0 K 0 L 0 M 0 N 0 O 0</td>
</tr>
<tr>
<td>St. pyogenes</td>
<td>6</td>
<td>A 0 B 0 C 0 D 0 E 0 F 0 G 0 H 0 I 0 J 0 K 0 L 0 M 0 N 0 O 0</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>A 4 B 9 C 6 D 4 E 10 F 2 G 9 H 6 I 10 J 4 K 20 L 3 M 14 N 4</td>
</tr>
</tbody>
</table>

Note: media were tested in duplicate and in most cases growth occurred on both duplicates (see text).

1 N. animalis (1), N. canis (1), N. cativae (1), N. cinerea (1), N. cuniculi (1), N. denitrificans (1), N. elongata (1), N. elongata subsp. glycolytica (1), N. flavescens (1), N. mucosa (1), N. mucosa var mucosa (1), N. oviss (1), N. pharyngis (1), Neisseria sp. NCTC 11091 (1).

* and **one isolate included as growth was inhibited on one of the duplicate cultures.
98.8%) than media that contained 5% blood (C, D, E, I and M; mean 93.5%; p < 0.001) or haemoglobin (G, K and O; mean 95.2%; p < 0.01). Media containing lincomycin performed significantly better than those containing vancomycin; both the media (A and F) that enabled the growth of all gonococcal and meningococcal strains contained lincomycin (1 mg/L), whilst all of the gonococci grew on only two (L and N) of 11 vancomycin-containing media. All growth failures with lincomycin occurred with AHU auxotype strains on the medium containing 4 mg/L but not with the media containing 1 mg/L suggesting that a concentration of 4 mg/L is inhibitory to certain strains of gonococci. All the media containing vancomycin failed to support the growth of the same non-groupable type 15 meningococcal isolate which was known to be sensitive to penicillin and sulphanilazine and may also be sensitive to vancomycin. Vancomycin in selective media is known to have an inhibitory effect on gonococci [12, 13] and is associated with the AHU auxotype [14, 15] which in turn shows a correlation with serogroup IA isolates [16–18]. In 1978 the antibiotic formulation of the original NYC medium [19] was modified by reducing the vancomycin from 3 to 2 mg/L [20] because 3 mg/L was inhibitory to the growth of as many as 10% of gonococcal strains [13, 21]. However, these results suggest that the four media containing vancomycin 2 mg/L performed significantly less well than the two media containing lincomycin 1 mg/L after incubation for 48 h the percentages of gonococci growing were 96.2, 97.1, 93.3 and 100% for the vancomycin media compared with 100% for the two lincomycin-containing media (p < 0.01). Interestingly, the medium containing vancomycin 2 mg/L that yielded growth of all gonococcal isolates was the second least selective medium allowing growth of 19-7% of non-pathogenic neisseriae and miscellaneous organisms. Although an increased growth of unwanted organisms on rectal and pharyngeal cultures has been associated with media containing lincomycin 1 mg/L [22] this can be reduced by decreasing the level of glucose in the media [23]. Furthermore, as most contaminants appear after incubation for >24 h this is not a major problem when plates are examined on day 1. The NYC and MNYC formulation supports luxuriant growth which means that gonococcal colonies are clearly visible by 24 h in the majority of primary cultures [3, 24]. GC-Lect medium (medium O) contains both vancomycin 2 mg/L and lincomycin 1 mg/L but performed no better than media containing lincomycin or vancomycin alone. Reichert et al. found GC-Lect medium performed better than MTM medium containing vancomycin 3 mg/L; however, the isolates missed by the MTM medium were not susceptible to vancomycin, all having vancomycin MICs > 4 [11]. This supports earlier findings comparing MNYC medium containing lincomycin 1 mg/L with Thayer Martin medium containing vancomycin 4 mg/L [3]; none of the strains isolated only on MNYC medium was sensitive to vancomycin at the concentration present in the Thayer Martin medium. The improved performance with lincomycin-containing media may be related to the greater safety margin with lincomycin (MIC usually 16 times the concentration present in the medium) than with vancomycin (MIC usually 2-4-fold greater than the corresponding medium concentration). This increased safety margin combined with the better nutritional value of MNYC medium may be particularly important in the case of small inocula [3]. Although Martin-Lewis medium was not included in this evaluation it was shown recently that NYC medium containing vancomycin 2 mg/L markedly enhanced the recovery of gonococci from clinical specimens when compared with Martin-Lewis medium which contains vancomycin 4 mg/L [24].

Overall, the 15 selective media were significantly poorer (p < 0.001) at supporting the growth of the 26 serogroup IA clinical isolates (86%) than the 74 IB clinical isolates (99.2%), supporting the view that the failure of certain selective media to support the growth of gonococci is due to a combination of nutritional inadequacy – possibly related to a low concentration of blood – and an inhibitory effect of vancomycin. Of the IA clinical isolates included in this evaluation 57.7% (15 of 26) were of the AHU auxotype. AHU strains may be even more prevalent in certain non-selected populations; serovar IA-2 has been shown to account for the majority of serovar IA infections and also to account for as much as 96.8% [18] and 98% [16] of AHU strains. In contrast there is a correlation between serogroup IB isolates and the non-requiring (NR) auxotype which accounted for 58.1% (43 of 74) of IB isolates in the test strains and 64.5% [18] and 63.2% of IB isolates in non-selected populations [25]. Although isolates of serovar IA and the AHU auxotype were associated with failure to grow there was a considerable serovar variation, with isolates of certain minor serovars such as IA-5 and IA-21 showing the strongest correlation. This has important implications for the detection and control of these minor serovars within the community and could be significant in the overall epidemiology of gonococcal infection.

Failure to inhibit yeasts was a significant factor in the lack of selectivity of several media. Only two media (B and C) contained nystatin, which showed extremely poor inhibition of yeasts. Amphotericin has been shown to be superior to nystatin in suppressing yeast contaminants in selective media [26]. In the present study, most media containing amphotericin ≥ 0.9 mg/L inhibited yeasts completely. However, media F, H, J, L and N which contain similar concentrations of amphotericin failed to inhibit yeasts; this may be due to batch variation of supplement, media preparation, or reduced shelf-life of the media. Interestingly, manufacturers of media that failed to inhibit yeasts also produced media containing the same concentration of amphotericin that gave complete inhibition.

Colistin was generally effective in inhibiting non-
gonococcal neisseriae and gram-negative bacilli, although medium L (colistin 7.5 mg/L) allowed the growth of two *Moraxella*, five *Pseudomonas* and six *Proteus* strains. All the media, apart from medium N, allowed the growth of *N. cinerea* in at least one of the duplicate cultures and most of the media also allowed *N. mucosa* var *mucosa* to grow. In practice the latter organism is rarely isolated [27] and can be distinguished readily from *N. gonorrhoeae* by sugar utilisation tests. *N. cinerea* is also a relatively rare isolate but is of much greater significance as, not only is it able to grow on gonococcal selective media [28], but it may cause problems in identification [29, 30] and result in the misdiagnosis of gonorrhoea. Medium N, the only medium to inhibit *N. cinerea*, was poor at inhibiting *P. mirabilis* gram-positive bacteria and yeasts.

Only three media (A, F and G) inhibited all six strains of *P. mirabilis*, suggesting that the failure of the other media, which contained similar concentrations of trimethoprim, must be due to problems in media preparation or stability. The same reasons may explain the failure of media N and O to inhibit certain strains of streptococci and staphylococci. Media (A, E and F) containing lincomycin failed to inhibit the MRSA isolate, in keeping with the poorer inhibition of gram-positive organisms by lincomycin in comparison to vancomycin [21] but this is much less important than the inhibitory effect of vancomycin on gonococci.

These results support previous findings that the improved nutritional status of *Neisseria* based on the original NYC medium [19, 26] or the much simpler MNYC formulation [3] provides superior performance both in terms of the number of positive clinical cultures and the rapidity and quality of growth when compared with conventional media [3, 22-24, 31]. In addition, the results support earlier reports of the superior performance of media containing lincomycin in place of vancomycin in both chocolate agar [21] and MNYC medium [22, 23].

In conclusion, none of the commercially available pre-poured media performed as well as the in-house medium in spite of the fact that some of the media were prepared to a very similar formula. We consider that manufacturers are providing too great a selection of media, some of which perform inadequately, probably due to a combination of factors including the level of nutrition, inappropriate choice of antibiotic supplements, and too long a shelf-life. The results of this evaluation and a review of the literature suggest that the use of 10% blood, a highly nutritious medium (based on the original NYC or MNYC formulation), and lincomycin in place of vancomycin, are crucial to the production of an effective selective medium. We urge manufacturers to take note of these points. A rational restriction in the variety of selective media is likely to decrease production costs, allow a shorter shelf-life and improve the overall performance.

We thank the following companies who supplied plates: Becton Dickinson UK Ltd, Cowley, Oxford OX4 3LY; bioMeireux UK Ltd, Basingstoke, Hampshire RG22 6HY; Difco Laboratories Ltd, West Molesey, Surrey KT8 2SE and Unipath Ltd, Basingstoke, Hampshire, RG24 8PW.

References


Gonococcal Infections in Scotland, 1994

H Young & A Moyes

Isolates from a total of 326 episodes of infection were examined: the distribution of these isolates and corresponding incidence of gonococcal infection by Health Board is shown in Table 1. This represents 70% of the 464 isolates examined in 1993 and is in keeping with a 62% decrease in the number of gonococcal infections reported by Genitourinary Medicine Clinics between 1993 (442) and 1994 (274) rather than a decrease in the proportion of isolates being forwarded to the reference laboratory (Information and Statistics Division, Scottish Health Service Common Services Agency). The incidence of infection in Grampian (13.13/100,000) was again the highest in Scotland and although there was a considerable decrease from the 1993 level (22.87/100,000) it remained more than twice that of the whole country (6.38/100,000). In 1994 the level reported by GUM Clinics in England was 37/100,000 population aged 15-641.

Grampian is also different from the other major areas of infection in that 76% of infections were due to serogroup IA isolates (Figure 1) compared with 42% for the whole country: the corresponding values for 1993 were 73% and 50%. This year we have introduced the dual typing system based on serotyping and auxotyping to increase the degree of discrimination2. The number and distribution of protein IA serovar/auxotypes are given in Table 2. Seven different serovars were found and IA-2 again predominated accounting for 84% of the IA isolates compared with 92% in 1993: this is a significant decrease in the proportion of IA-2 isolates. The combination of serotyping and auxotyping detected 14 serovar/auxotype classes with IA-2/AHU strains accounting for 76% of the IA isolates. In spite of the high number (49) of IA isolates in Grampian they were extremely homogeneous: there were only four serovar/auxotype classes and 86% of isolates were IA-2/AHU suggesting endemic transmission of a successful "core strain" rather than frequent importation of new strains from other localities - by comparison in Greater Glasgow there were only 15 IA isolates but these were represented by 9 different serovar/auxotype classes with IA-2/AHU accounting for only 40%. All three isolates of serovar/auxotype IA-6/P in Lothian were from homosexually acquired infections: the two similar isolates from Glasgow were also from males, one from a rectal culture.

Thirteen protein IB serovars were found among the serogroup IB isolates in 1994 (Table 3) compared with 8 serovars in 1993. Serovars IB-4, IB-9, IB-10, IB-15, IB-16, IB-18 and IB-20 were absent in 1993 but accounted for 8.5% of IB isolates in 1994. Serovars IB-19 and IB-32 which accounted for 3% of IB isolates in 1993 were not found during 1994. As in previous years IB-2 was the most common isolate accounting for 51.3% of isolates in 1994 compared with 59.2% in 1993. Serovar IB-1 and IB-3 isolates which each accounted for 1.7% of isolates in 1993 increased to 6.3% and 8.9% respectively. Serovar IB-6 accounted for 16.3% of isolates which was similar to the 17.2% found in 1993. The combination of serotyping and auxotyping detected 34 serovar/auxotype classes with IB-2/NR strains accounting for 31.6% of the IB isolates. Within Lothian IB-2/NR strains tended to be homosexually acquired whereas IB-2/PAU strains were heterosexually acquired. IB-6/NR isolates also tended to be homosexually acquired whereas IB-6/PAU strains were heterosexually acquired. The finer discrimination resulting from serovar/auxotype classification allows the recognition of geographical associations within serovars, eg although there were eleven IB-8 strains these represented three different foci of infection with IB-8/PAR found only in Forth Valley, IB-8/NR in Glasgow and IB-8/P in Lothian.

Again there was a highly significant difference in the penicillin susceptibility of IA and IB isolates (Tables 4 and 5): excluding penicillinase-producing Neisseria gonorrhoeae (PPNG) 89.5% of IA isolates had a minimum inhibitory concentration (MIC) to penicillin < 0.015 mg/L compared with 11.3% of IB isolates (P<0.001). The corresponding values for 1993 were 89.4% and 15.4%. A total of 27 (15.3%) non-PPNG IB isolates were chromosomally resistant Neisseria gonorrhoeae (CMRNG) as defined by a penicillin MIC > 1.0 mg/L: corresponding values for previous years were 8.6% (1993), 7.2% (1992), 3.4% (1991) and 1.3% (1990). The increase in CMRNG between 1993 and 1994 is statistically significant (P<0.05).

The susceptibility of isolates to various antibiotics according to categories of susceptibility34 is given in Table 6. In general, the categories susceptible, intermediate and resistant are related to predictive values for clinical cure using recommended dosages for uncomplicated urogenital infection: susceptible (>95% efficacy); intermediate (>90% efficacy); and resistant (<85 - 90% efficacy). As shown in Table 6 the vast majority of isolates are within the susceptible and intermediate categories. The percentage of isolates resistant to each antibiotic during 1994 and 1993 was: penicillin 6% vs 4%; cefuroxime 2% vs 0%; ciprofloxacin 1.5% vs 2%; tetracycline 17% vs 9%; erythromycin 0% in 1994 and 1993; spectinomycin 0% vs 0.2%. The increase in tetracycline resistance is highly significant (P<0.001): tetracycline resistant isolates were confirmed by E test. In total 59 isolates (18%) demonstrated resistance to one or more antibiotics. The actual pattern of resistance for these isolates is shown in Table 7. Excluding the 37 isolates which were resistant to tetracycline only (33 chromosomal and 4 TRNG) the level
of resistance to one or more antibiotics was 6.7%. As shown in Table 8 penicillin resistance was strongly associated with PPNG isolates - 3.4% of isolates were PPNG and 0.9% were both PPNG and TRNG: the corresponding values for 1993 were 2.2% and 1.1%. TRNG accounted for 1.2% of isolates compared with 1.1% in 1993. Of the 5 ciprofloxacin resistant isolates 4 were seroivar IB-1 and 1 was seroivar IB-3: as the total number of IB-1 and IB-3 isolates tested was 12 and 17 respectively a level of ciprofloxacin resistance of 33% and 6% for these serovars.

Details of the 18 isolates with plasmid mediated resistance to either PPNG and/or TRNG are given in Table 8. The combination of serotype, auxotype and plasmid profile shows a highly heterogeneous group of 12 different isolates suggesting transient importation with limited endemic spread. Local spread is supported by the small cluster of IB-1/P strains in Grampian and IB-7/11R strains in Lothian. Detailed typing is valuable in contact tracing infection with PPNG as isolates may occasionally lose the beta lactamase plasmid. Control and prevention of subsequent spread of PPNG strains is important as they tend to be acquired in areas (parts of Africa and the Far East) where HIV is also common and could be acquired at the same time.

Eleven ano-genital isolates of Gram negative diplococci (GNDC) isolated from patients proved to be non-gonococcal neisseriae (NGN). These comprised: 9 isolates of N. meningitidis including four male rectal isolates (serogroup W135: type 21: subtype PI.5: serogroup B: non-typable: subtype PI.16: serogroup B: non-typable: subtype PI.2:5: serogroup B: type 2b: subtype PI.10: one female rectal isolate (serogroup Z1: non-typable: subtype PI.16:), and four male genital isolates (serogroup B: type 2a: subtype PI.2:5: non-typable: type 21: sub-type PI.6 - two isolates: non-typable: type 2:1:1-5:1-15:1; one female genital isolate of Moraxella catarrhalis and one female genital isolate of Neisseria cinerea. The levels of ano-genital NGN stress the importance of reliable diagnostic methods as the predictive value of identification methods decreases as the prevalence of NGN increases. N. cinerea, in particular can cause problems in identification1 and has been mis-diagnosed as gonococcal infection4.

References


Acknowledgements

We thank our numerous bacteriological and clinical colleagues for their on-going help and co-operation in submitting isolates to the Scottish Neisseria gonorrhoeae Reference Laboratory and to Dr Les Smart of the Scottish Meningococcal and Pneumococcal Reference Laboratory for typing the ano-genital meningococci.

Special thanks are extended to Mrs Joan McEllinney for careful record keeping and assistance with the preparation of the manuscript.

Appendix

Health Board Area and Laboratories submitting specimens.

**Argyll and Clyde**

AC/DUN:ARL Vale of Leven Hospital, Alexandria, Dunbartonshire
AC/GR/E:CLY Inverclyde Royal Hospital, Greenock
AC/PA/RAB Dept of Bacteriology. Royal Alexandria Hospital, Paisley
Argyll and Arran
AA/YR:ARL Dept of Microbiology. Crosshouse Hospital, Kilmarnock, Ayrshire

**Dumfries and Galloway**

DG/DM:ROY Dumfries and Galloway Royal Infirmary, Dumfries Fife

FF/FI/ARL Fife Area Laboratory, Hayfield Road, Kirkcaldy. Fife

**Forth Valley**

FV/FAL:ROY Falkirk Royal Infirmary, Falkirk
FV/ST:ROY Stirling Royal Infirmary, Stirling

**Greater Glasgow**

GG/GLA:ROY Glasgow Royal Infirmary, Glasgow
GG/GLA/SOU Glasgow Southern General Hospital, Glasgow
GG/VI:VIC Victoria Infirmary, Glasgow
GG/GLA/STO Stobhill NHS Trust. Balornock Road. Glasgow G12 1UW

**Glasgow**

GG/GLA/SCH Royal Hospital for Sick Children & Queen Mothers Hospital. Yorkhill

**Grampian**

GR/ABD:GHB University of Aberdeen. Foresterhill. Aberdeen

**Highland**

HG/IN:RAI Raigmore Hospital. Inverness

**Lothian**

LN/LAN:LYA Lothian Infirmary. Edinburgh

**Lanarkshire**

LN/LAN:WAL Law Hospital, Carluke
LN/LAN:MON Dept of Microbiology. Monklands Hospital, Airdrie

**Lothian**

LO/ED/CIT City Hospital. Greenbank Drive, Edinburgh

**Edinburgh**

LO/ED/CML Central Microbiological Laboratories. Western General Hospital, Edinburgh

**Royale**

LO/ED/ROY Royal Infirmary, Edinburgh

**Lothian**

LO/ED/STD Gentoo-urinary Medicine Clinic. Edinburgh Royal Infirmary, Edinburgh

**Tayside**

TY/TA:STR Strachatho Hospital, Brechin

**TY/DEE:UNI** Ninewells Hospital & Medical School, Dundee

**TY/PER:ROY** Perth Royal Infirmary, Perth
Table 1: Distribution of gonococcal infection by Health Board area: Scotland, 1994
(Based on submission of isolates to the SNGRL)

<table>
<thead>
<tr>
<th>Health Board (Code)</th>
<th>Population</th>
<th>No of cases</th>
<th>Cases/100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argyll &amp; Clyde (AC)</td>
<td>443,118</td>
<td>9</td>
<td>2.03</td>
</tr>
<tr>
<td>Ayrshire &amp; Arran (AA)</td>
<td>374,752</td>
<td>12</td>
<td>3.2</td>
</tr>
<tr>
<td>Borders (BR)</td>
<td>102,141</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dumfries &amp; Galloway (DG)</td>
<td>147,036</td>
<td>13</td>
<td>8.84</td>
</tr>
<tr>
<td>Fife (FF)</td>
<td>344,550</td>
<td>36</td>
<td>10.45</td>
</tr>
<tr>
<td>Forth Valley (FV)</td>
<td>272,077</td>
<td>20</td>
<td>7.35</td>
</tr>
<tr>
<td>Grampian (GR)</td>
<td>502,863</td>
<td>66</td>
<td>13.13</td>
</tr>
<tr>
<td>Greater Glasgow (GG)</td>
<td>951,219</td>
<td>70</td>
<td>7.36</td>
</tr>
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<td>200,628</td>
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<td>3.49</td>
</tr>
<tr>
<td>Lanarkshire (LN)</td>
<td>563,448</td>
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<td>1.63</td>
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<tr>
<td>Lothian (LO)</td>
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<td>7.8</td>
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<td>19,338</td>
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</tr>
<tr>
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<td>224,290</td>
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<td>0</td>
</tr>
<tr>
<td>Tayside (TY)</td>
<td>393,702</td>
<td>26</td>
<td>6.6</td>
</tr>
<tr>
<td>Western Isles (WI)</td>
<td>310,460</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5,112,159</td>
<td>326</td>
<td>6.38</td>
</tr>
</tbody>
</table>

Table 2: Serovar/Auxotype of IA serovars of N. gonorrhoeae: Scotland, 1994

<table>
<thead>
<tr>
<th>Serovar/Auxotype</th>
<th>AC</th>
<th>AA</th>
<th>DG</th>
<th>FF</th>
<th>FV</th>
<th>GR</th>
<th>GG</th>
<th>HG</th>
<th>LN</th>
<th>LO</th>
<th>TY</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-2/AHU</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>104</td>
</tr>
<tr>
<td>IA-2/AHU</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>IA-2/PAHU</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>IA-4/NR</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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(a) IA-2 isolate (GR) not available for auxotyping
*A = Arginine requiring; H = Hypoxanthine requiring; P = Proline requiring; AHU = Arginine, Hypoxanthine and Uric acid requiring; NR = Non-requiring

Table 3: Serovar/Auxotype of IB serovars of N. gonorrhoeae: Scotland, 1994

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Table 4: Penicillin susceptibility of IA Serovars of *N. gonorrhoeae*: Scotland, 1994

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(a) PPNG and TRNG

(b) [x4 PPNG: IA-6 isolate chromosomally resistant (MIC = 2 mg/L)]

Table 5: Penicillin susceptibility of IB Serovars of *N. gonorrhoeae*: Scotland, 1994

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<th>0.12</th>
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(a) 13/18 PPNG: one IB-1, two IB-2, one IB-3 and one IB-7 isolate chromosomally resistant (MIC = 2 mg/L)

(b) 12/14 PPNG: two IB-1 isolates chromosomally resistant (MIC = 2 mg/L)

Table 6: Categories of antibiotic susceptibility for 326 gonococcal isolates, Scotland, 1994

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<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
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<td>&lt;0.06 mg/L</td>
<td>0.12 - 1.0 mg/L</td>
<td>&gt;1.0 mg/L</td>
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<td>Ciprofloxacin*</td>
<td>&lt;0.008 mg/L</td>
<td>0.025 - 0.05 mg/L</td>
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<td>&lt;0.25 mg/L</td>
<td>0.5 - 1.0 mg/L</td>
<td>&gt;1.0 mg/L</td>
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<td>Erythromycin</td>
<td>&lt;0.5 mg/L</td>
<td>1.0 - 4.0 mg/L</td>
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<td>Spectinomycin</td>
<td>&lt;32 mg/L</td>
<td>32 - 64 mg/L</td>
<td>&gt;64 mg/L</td>
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</table>

(a) 14/19 PPNG

(b) 2/5 PPNG and 2/5 CMRNG

(c) 7/56 with MICs consistent with TRNG (including 3 also PPNG)

* Note: Categories are arbitrary with regard to ciprofloxacin. A resistant category for ciprofloxacin has not yet been defined but clinical failures have been associated with MICs >0.05 mg/L.