The emergence of penicillinase-producing strains of *Neisseria gonorrhoeae* in Durban

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**Summary**

Gonorrhoea was diagnosed in 179 (87%) of 206 Black males who presented with urethritis at a sexually transmitted disease clinic at the King Edward VIII Hospital, Durban. Penicillinase-producing strains of *Neisseria gonorrhoeae* were detected in 7 (5%) of 140 gonococcal isolates, and a further 13 strains were relatively resistant to penicillin. Microscopic examination of Gram-stained smears provided a rapid presumptive diagnosis of gonorrhoea in 162 cases. The modified Thayer-Martin medium proved marginally superior to chocolate agar for the isolation of *N. gonorrhoeae* from urethral exudates. The causation and laboratory diagnosis of urethritis in males and the antibiotic susceptibility pattern of the gonococcal isolates are discussed.

Urethritis in males is broadly classified into gonococcal urethritis (GU) and non-gonococcal urethritis (NGU). Although penicillin is still the drug of choice for the treatment of uncomplicated gonorrhoea it is totally ineffective in NGU, for which the drug of choice is tetracycline. A rapid and accurate differential diagnosis of the two conditions is therefore important in determining the appropriate initial treatment of urethritis in males.

Since their emergence in 1976 strains of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) have been reported with increasing frequency from many parts of the world. PPNG strains were first detected in South Africa in 1977 when isolated cases of infection were reported from Johannesburg and Durban. Since then no further isolates have been reported from this country. Epidemiologically, PPNG strains have been linked with either the Far East or West Africa. They have also been detected in West Africa, Zambia and Zimbabwe, but the extent of the problem in most African countries is not yet known.

The rising incidence of gonorrhoea and non-gonococcal infections has created a greater demand for simple, rapid and reliable diagnostic techniques. The Gram stain plays an important role in the immediate diagnosis of gonorrhoea and will detect up to 90% of urethral infections in men.

In this article we discuss our findings from a study of Black males with urethral discharge attending a sexually transmitted disease (STD) clinic at the King Edward VIII Hospital, Durban. We also report on the antimicrobial susceptibility pattern of gonococci isolated during this study.

**Patients and methods**

**Patients**

Black males complaining of urethral discharge were examined at the STD clinic at the King Edward VIII Hospital. New patients who attended the STD clinic between 27 June 1983 and 5 July 1983 and who had not received antibiotics during the previous 2 weeks were entered into the study.

**Collection of specimens**

Intra-urethral secretions were collected using a calcium alginate-tipped swab (Calgiswab) and smears were prepared on sterile glass slides for direct microscopy and Gram staining. The swabs were then inoculated in random order onto modified Thayer-Martin medium and chocolate agar. The plates were immediately placed in a candle extinction jar and incubated at 37°C. Gram-stained smears were examined by an experienced technologist for the presence of leucocytes and intracellular Gram-negative diplococci. The number of leucocytes per high-power field (i.e. x 1,000 oil-immersion high-power field) was recorded. Urethral exudates were also examined by direct microscopy for yeasts and *Trichomonas vaginalis*.

**Isolation and identification of *N. gonorrhoeae***

After 48 hours' incubation the plates were examined and the identification of *N. gonorrhoeae* was confirmed by the morphological features of the colonies, a positive oxidase test, characteristic appearances of the Gram-stained specimen and a carbohydrate utilization test.

**Penicillinase (β-lactamase) production**

One hundred and forty gonococcal isolates were screened for penicillinase production by the Intra-lactam Strip method (Mast Laboratories) and the chromogenic cephalosporin test.

**Antibiotic susceptibility testing**

Minimal inhibitory concentrations (MICs) of penicillin for *N. gonorrhoeae* were determined by the agar dilution method using Diagnostic Sensitivity Test (DST) (Oxoid) agar supplemented with 6% lysed horse blood. Plates containing penicillin in suitable concentrations were inoculated by means of a multipoint inoculator which delivered 0,001 ml of broth containing approximately 10⁶ colony-forming units. The Oxford *Staphylococcus*
aureus was included as a control. Plates were read after overnight incubation in a candle extinction jar. The MIC was recorded as the lowest concentration of penicillin which completely inhibited the growth of the organism.

Antimicrobial disc susceptibility of the 7 PPNG strains found was determined by the method of Stokes. Filter-paper discs, containing penicillin 2 U, penicillin 10 U, ampicillin 10 μg, tetracycline 10 μg, erythromycin 15 μg, co-trimoxazole 25 μg, cefalothin 30 μg, cephamandole 30 μg, cefuroxime 30 μg, cefoxitin 30 μg, cefotaxime 30 μg, kanamycin 30 μg, tobramycin 10 μg, gentamicin 10 μg and spectinomycin 50 μg, were placed on DST agar supplemented with horse blood. Plates were incubated at 37°C in a candle extinction jar. After 24 hours' incubation the zone sizes of the test strains were compared with those of an Oxford Staph. aureus control and the strains were graded as sensitive, intermediate-resistant or resistant.

Results

The age distribution of the 206 patients studied was as follows: < 20 years — 36 patients (17%); 20 - 24 years — 74 patients (36%); 25 - 29 years — 43 patients (21%); 30 - 34 years — 23 patients (11%); 35 - 39 years — 14 patients (7%); and > 40 years — 16 patients (8%). The mean age was 25,9 years and 75% of the patients were aged between 20 and 40 years.

Microbiological findings

None of the 206 urethral exudates were found to contain T. vaginalis or yeasts on microscopic examination. A diagnosis of GU was established by microscopy and/or culture of urethral swabs in 179 cases (87%). Using the criteria of Alani et al.,11 NGU was diagnosed in the remaining 27 cases (13%). The findings on examination of Gram-stained smears and the results of cultures of urethral swabs from the 179 patients with gonorrhoea are given in Table I. N. gonorrhoeae was detected by microscopy and culture in 123 of the 179 cases of gonorrhoea (68,7%). Microscopic examination detected 90,5% (162 of 179) of cases of gonorrhoea and culture on modified Thayer-Martin medium and chocolate agar 78,2% (140 of 179). Of the 140 positive gonococcal cultures, 139 (99%) were isolated on modified Thayer-Martin medium and 133 (95%) on chocolate agar. Penicillinase-producing strains of N. gonorrhoeae were detected in 7 (5%) of the 140 isolates.

Antibiotic sensitivity

The MICs of penicillin for 100 gonococcal strains are shown in Table II. Eighty per cent of strains were fully sensitive to penicillin, with MICs of 0,06 μg/ml or less. Fourteen strains, including 1 strain of PPNG, showed intermediate resistance to penicillin (MICs 0,125 - 0,5 μg/ml). Six other strains which were all penicillinase-producing were resistant to penicillin, with MICs of 1 μg/ml or more.

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<tr>
<th>Strain No.</th>
<th>Penicillin G 2 U</th>
<th>Penicillin G 10 U</th>
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<th>Tetracycline 10 μg</th>
<th>Erythromycin 15 μg</th>
<th>Co-trimoxazole 25 μg</th>
<th>Cephaprole 30 μg</th>
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<th>Cefuroxime 30 μg</th>
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S = sensitive; I = intermediate-resistant; R = resistant.

Disc susceptibility of the 7 strains of PPNG to 14 antimicrobial agents is shown in Table III. Five strains were completely resistant to penicillin 2 U, while 2 showed intermediate resistance. All 7 were sensitive to tetracycline 10 μg, erythromycin 15 μg, cephaprole 30 μg, cephamandole 30 μg, cefuroxime 30 μg, cefoxitin 30 μg, cefotaxime 30 μg, gentamicin 10 μg and spectinomycin 50 μg. Four strains were sensitive to kanamycin and tobramycin, but only 1 was sensitive to co-trimoxazole.
Discussion

Major advances in diagnostic techniques and potent chemotherapeutic agents have failed to curb the world-wide increase in the incidence of STDs. Reliable statistics on the incidence of these diseases in South Africa are not readily available. In the UK gonorrhoea and nonspecific urethritis are two of the commonest STDs. Control measures against gonorrhoea have been hampered by many factors, including the absence of a vaccine, increased sexual permissiveness, difficulty in finding asymptomatic carriers and the emergence of penicillin-resistant strains. Of the 206 Black male patients with urethritis in this study, 179 (87%) were diagnosed as having GU by means of Gram staining and/or culture. Using the diagnostic criteria of Alani et al., NGU was diagnosed in 27 cases (13%). These findings are in agreement with previous reports from the UK and the USA and with a recent report from this country that GU is more common than NGU in Black male patients. This is in contrast to the findings in White males with urethritis, for whom the reverse holds true. Because laboratory facilities were not available, concomitant infections with *Chlamydia trachomatis* and *Ureaplasma urealyticum* were not sought in cases of GU, nor could we determine the role of these organisms in NGU.

Microscopic examination of Gram-stained smears detected 90,5% (162 of 179) of all cases of gonorrhoea. In comparison, positive cultures on both the modified Thayer-Martin medium and chocolate agar detected only 78,2% of the 179 cases. These findings confirm previous observations by others that in most cases of symptomatic urethritis in males examination of a carefully prepared Gram-stained slide by an experienced technologist provides a simple, inexpensive and rapid diagnosis of gonorrhoea. However, the Gram stain will not detect all cases of gonorrhoea. This was the case in 17 of our patients, for whom a positive result was obtained on culture only. Ideally, when the necessary facilities are available urethral cultures should be performed routinely. The Thayer-Martin selective medium is widely used in many laboratories and has proved particularly useful in isolating *N. gonorrhoeae* from contaminated sites such as the rectum, pharynx and cervix. In our study culture on the modified Thayer-Martin medium detected 99% of the 140 cases of culture-positive gonorrhoea, compared with 95% detected by culture on chocolate agar. Furthermore, the modified Thayer-Martin medium facilitated the detection of gonococci in urethral cultures which were contaminated with staphylococci and coliform organisms. At present we are evaluating the modified New York City medium, which is reported to give results superior to those provided by the Thayer-Martin medium in the cultural isolation of *N. gonorrhoeae*.

The rapid detection of β-lactamase production in gonococcal isolates is desirable so that appropriate measures can be taken to limit the spread of PPNG in the community. In our study the commercially available Intraclactam Strip provided a simple and rapid method for the detection of β-lactamase production. Penicillinase-producing strains of *N. gonorrhoeae* were detected in 5% of 140 gonococci isolated from Black patients with urethritis. It has been reported that in certain areas of the Philippines 30-40% of gonococcal infections are caused by PPNG. In a previous study in Zimbabwe we found that 10% of gonococcal infections are caused by these strains. Since their recognition in the 1950s strains of *N. gonorrhoeae* which are relatively resistant to penicillin have been reported from several countries around the world. We found that 14% of 93 non-β-lactamase-producing strains had intermediate resistance to penicillin (MICs between 0,125 and 0,5 μg/ml). Liebowitz et al. reported that 23% of strains isolated in Johannesburg were of intermediate resistance. Eighty percent of our isolates were fully sensitive to penicillin (MIC ≤ 0,06 μg/ml). Gonococcal strains of intermediate resistance (but not PPNG) also remain susceptible to high-dose penicillin therapy (procaine penicillin G 4,8 million U and probenecid 1 g). At present, penicillin should therefore remain the drug of choice for the treatment of uncomplicated gonorrhoea. However, patients with gonorrhoea should be actively followed up so that treatment failures due to PPNG can be identified and appropriate measures taken to prevent dissemination of these strains in the community. Furthermore, we would like to emphasize the need for continued monitoring of the situation. If PPNG becomes more widespread a reappraisal of treatment regimens will clearly be needed. Spectinomycin, cefoximox, cefoxitin and cefotaxime have been shown to be highly effective against both penicillin-sensitive and penicillin-resistant gonococci. Recommended doses of these antibiotics which would be effective in the treatment of both PPNG and non-PPNG are as follows: intramuscular spectinomycin 2 g in a single injection; intramuscular cefotaxime 2 g in a single injection plus oral probenecid 1 g; intramuscular cefotaxime 1-1,5 g with oral probenecid 1 g; and intramuscular cefotaxime 1 g in a single injection. However, it should be noted that spectinomycin and cefoximox are ineffective in pharyngeal infections, for which the recommended drug is trimethoprim-sulphamethoxazole, a single daily dose of 9 tablets for 5 days. Our strains of PPNG were shown to be sensitive to most of the antimicrobial agents tested, including spectinomycin, tetracycline, for which the recommended dosage is 500 mg orally 4 times a day for 7 days, and the newer cephalosporins.

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REFERENCES