Beta-lactamase-producing isolates of Neisseria gonorrhoeae in Cape Town

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Summary

Isolates of Neisseria gonorrhoeae from patients attending the sexually transmitted disease clinics of the Cape Town Municipal Health Department were examined for β-lactamase (penicillinase) production by a chromogenic cephalosporin test. Of the 208 isolates, 4 (less than 2%) produced β-lactamase. When grown on chocolate blood agar plates, 3 isolates showed no inhibition of growth around a penicillin disc; the 4th had a markedly reduced zone of inhibition. The minimum inhibitory concentrations of penicillin for these 4 strains were from 1 to 4 µg/ml.

Since 1976 penicillin-resistant (β-lactamase)-producing Neisseria gonorrhoeae (PPNG) has been reported from the UK, the USA and more recently from several African countries. These organisms are highly resistant to penicillin, the drug of choice in gonorrhoea, and are responsible for the failure of many patients to respond to therapy. There are two distinct groups of PPNG, based on their resistance phenotypes, plasmid content and geographical origin. The first, epidemiologically linked to the Far East, is resistant to tetracycline while the second originated in West Africa and is sensitive to tetracycline. The isolation of PPNG in the RSA in 1977 emphasized the need for culture and antibiotic sensitivity testing of Neisseria gonorrhoeae.

Since there is no published information about the incidence of PPNG in the Cape Town area, a survey to establish the incidence of β-lactamase-producing Neisseria gonorrhoeae was undertaken.

Patients and methods

Urethral swabs were taken from 276 random male patients presenting with acute purulent urethritis at a sexually transmitted disease (STD) clinic of the Cape Town Municipal Health Department during December 1983 and January 1984. A fine-tipped calcium alginate swab was immediately used in each case to make a smear on a glass slide and to inoculate Thayer-Martin (TM) and chocolate blood agar culture plates. The plates were incubated at 37°C in a CO₂ incubator for 48 hours. The smear was stained by Gram's method and examined for the presence of pus cells and Gram-negative intracellular diplococci. Neisseria gonorrhoeae isolates were identified by colonial morphology and sugar fermentation reactions. Antibiograms were obtained by inoculating 0.5 ml trypticase soy broth (TSB) with several colonies from a chocolate agar plate, streaking this suspension on another chocolate agar plate with a cotton-tipped swab, and placing penicillin and tetracycline discs (1 U and 30 µg respectively) on the inoculum. Zone sizes were measured and interpreted according to the Kirby-Bauer method.

All isolates were checked for β-lactamase production with a chromogenic cephalosporin (Nitrocefin; Glaxo) as the substrate. Minimum inhibitory concentrations (MICs) of penicillin were determined for β-lactamase-producing isolates using a plate dilution method. Reference standard was used to prepare penicillin dilutions ranging from 0.004 to 8 µg/ml in chocolate agar. An overnight broth culture in TSB was adjusted to match 0.5 McFarland turbidity standard (± 1 x 10^8 colony-forming units (CFU)/ml). A further tenfold dilution to an estimated 1 x 10^7 CFU/ml was made. Plates were inoculated using a Denley Multi-point Incubator delivering 0.001 ml, giving an inoculum of 1 x 10^6 CFU/ml. These plates were incubated for 24 hours in a CO₂ incubator at 37°C. The MICs were read as the lowest concentration of the agent that inhibited macroscopic growth of the organisms. In reading end points a barely visible haze of growth or a single colony was disregarded. Two control organisms, Staphylococcus aureus (NCTC 6571) and Micrococcus luteus (NCTC 8340), were included with the PPNG and the MICs obtained were within one dilution of the expected value — 0.03 µg/ml and 0.008 µg/ml respectively.

Results

N. gonorrhoeae was cultured from 208 (75%) of 276 specimens. Of these isolates, 4 were shown to be resistant to penicillin by antibiotic disc susceptibility testing and they were shown to produce β-lactamase, giving an incidence of PPNG of 1.9% (Table I). All the isolates were found to be sensitive to tetracycline (inhibition zone 19 mm or greater using a 30 µg disc) except 1 (a non-PPNG strain) which fell into the intermediate range (17 mm inhibition zone) (Table I).

Although there were only 208 positive cultures, a further 5 specimens had Gram-negative diplococci on direct staining, making a total of 213 patients with a probable diagnosis of gonorrhoea. Of the 213 positive cases, 202 (94.8%) were detected by direct Gram staining and 208 (97.6%) were detected by culture of the organisms on TM and chocolate blood agar plates. There were instances where the organisms failed to grow on one or other of the media; there were 203 (95.3%) positive cultures on TM plates and 207 (97.2%) on chocolate blood agar plates. One isolate grew solely on the TM plate, while 5 isolates grew only on the chocolate blood agar plates.

Discussion

The routine use of penicillin as the first-line treatment for gonorrhoea has been questioned because of the emergence of resistant organisms (PPNG). The incidence of these organisms in some areas has made it necessary to modify the recommended treatment regimens.

In Durban, 2 cases of PPNG were described in 1977. This number has progressively increased from 7 (5%) of 140 isolates in June 1983 to 12% in March 1984 (Y. M. Coovadia — personal communication). In 1980, no PPNG strains were isolated from 257 consecutive N. gonorrhoeae isolates obtained in the Cape Town region (G. J. Coetzee — personal communication). In our study 4 years later we have shown that PPNG
TABLE I. ANTIMICROBIAL SUSCEPTIBILITY OF NEISSERIA GONORRHOEAE ISOLATES

<table>
<thead>
<tr>
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<th>Tetracycline zone (mm)/ disc content 30 µg</th>
<th>MIC of penicillin (µg/ml)</th>
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<tbody>
<tr>
<td>PPNG</td>
<td>27,4 - 34,0</td>
<td>1 - 4</td>
</tr>
<tr>
<td>Non-PPNG</td>
<td>20,4 - 48,8*</td>
<td>Not done</td>
</tr>
</tbody>
</table>

*One isolate had a zone size of 17.0 mm.

was present, but that the incidence of these strains was still below 2%. In a recent study carried out in July and August 1985 the incidence of PPNG strains in Cape Town was found to be 8,6%.[12] The relative prevalence of PPNG strains among STD clinic populations in Johannesburg and Pretoria during the same period was recorded as 12% and 10% respectively.[13] The growth of N. gonorrhoeae on chocolate agar but not on TM plates in our study suggests the presence of vancomycin-sensitive strains in the community. The high sensitivity of culturing the organisms may be due to the immediate inoculation of culture plates in the clinic.

As expected, all the PPNG strains had MICs reflecting resistance to penicillin (Table I). The chromogenic cephalosporin test, a useful laboratory tool in the rapid detection of lactamase-producing organisms, yielded 4 positive results, with an overall incidence rate of 1.9%.

Since all the PPNG isolates were susceptible to tetracycline they may be similar to the West African group of PPNG.

The authors thank Professor A. A. Forder, Head of the Department of Medical Microbiology, University of Cape Town; Dr. L. Steyn and Mrs. K. Pratt, Department of Medical Microbiology, UCT; and Dr A. T. Wilson, Cape Town City Council, for their assistance in this study.

REFERENCES


News and Comment/Nuus en Kommentaar

Chicken soup

It had to happen sooner or later. In their relentless search to find adverse effects to medication with almost anything, American researchers have finally come up with an adverse effect to that traditional sovereign remedy which has reigned supreme in Jewish materia medica since time immemorial — chicken soup (Fujiwara et al., N Engl J Med 1985; 313: 1161). They describe the case of a 75-year-old woman with diabetes mellitus controlled with tolbutamide who was put on hydrochlorothiazide for hypertension. Two days and then 12 hours before admission to hospital, she was found to have a low serum sodium level. She was told to discontinue the diuretic and to drink 'salty broth'. During the next 8-10 hours, she drank 1 - 2 bowls each of three different kinds of salt-supplemented soup, following which she became delirious and was admitted to hospital, where she was found to have a serum sodium level of 162 mmol/l. The hypernatraemia was presumably caused by the relatively rapid intake of salt into a body with diuretic-induced extracellular volume reduction. The authors comment that conscious administration of chicken soup is not without hazard. But really — chicken soup! Is nothing sacred?

Hoeveel aspirien?

Aspirien is bekend as 'n doeltreffende antitrombotiese middel en word algemeen gebruik ter voorkoming van aartekorone omleidingsbronbome, trombose van arteriovenose omleidings in pasiënte wat chroniese hemodialise ondergaan en in die profilaksie van beroertes en koronere trombose. Hoeveel moet egter gegee word? Te veel sal gastrointestinale ongesteldhede soos maagpyn, soorbrand, naarheid en by geleentheid bloeding van die maag en darmkanaal veroorsaak. Te min is ondoeltreffend. Nog 'n kompliserende faktor is dat aspirien in relatief lae dosisse 'n antitrombotiese, maar in hoë dosisse 'n trombogeniese effek het. In 'n onlangs publiseer oorsig oor die onderwerp (Hirsh, Arch Intern Med 1985; 145: 1582) word 'n onderskeid gemaak tussen die gebruik van aspirien in die behandeling van vlietende serebrale isgémie, wanneer 'n dosis van 1 g dag aanbeveel word, en in die behandeling van tromboembolieë afwykings, waarvoor 'n daallike dosis van 325 mg word aanbeveel word. Gastrointestinale newe-effekte kan deur albei dosisse van aspirien gemaak word. Indien dit voorkom, moet die dosis aspirien vermindert of gestaak word.