

Testing sensitivity of *Neisseria gonorrhoeae* to spectinomycin

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SUMMARY A concentration of 16 mg/l spectinomycin incorporated in agar gave the best discrimination between *Neisseria gonorrhoeae* sensitive and resistant to spectinomycin. This method was compared with spectinomycin sensitivity testing with 25 µg or 100 µg discs. Both methods agreed fully for 197 spectinomycin sensitive and three spectinomycin resistant gonococci. The agar incorporation "breakpoint" concentration technique failed to detect a small spectinomycin resistant population in a fourth isolate, which was detected by disc testing. It may be possible to predict the emergence of spectinomycin resistance among strains of *N gonorrhoeae*.

Introduction

Penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG) were first isolated in 1976,^{1,2} and recent reports indicate that they have now become endemic in Great Britain.^{3,4} McCutchan *et al* suggested that penicillinase resistant antibiotics such as spectinomycin should be used as first line treatment when the prevalence of infections with PPNG strains rises above 5%.⁴ This occurred at this hospital during 1982. Accordingly, from January 1983 spectinomycin was introduced as first line treatment for uncomplicated anogenital gonorrhoea in all patients attending the Praed Street Clinic except homosexual men. Since 1981 we have isolated spectinomycin resistant PPNG strains from 22 patients and spectinomycin resistant non-PPNG strains from 18 patients with gonorrhoea.⁵ It is important to screen all isolates for sensitivity to spectinomycin, but unfortunately there is no simple rapid equivalent to the chromogenic cephalosporin test that is used for penicillinase screening. In view of anecdotal concern about sensitivity testing with spectinomycin discs and the increasing use of agar incorporation breakpoints as an alternative, we have estimated the best spectinomycin concentration breakpoint, have compared this with 25 µg and 100 µg spectinomycin discs, and have investigated the possibility of predicting what gonococcal strains might develop resistance to spectinomycin.

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Materials and methods

BACTERIA

Thirty seven strains of spectinomycin resistant PPNG and non-PPNG strains stored in liquid nitrogen in our culture collection were used to assess sensitivity to spectinomycin. A further 10 spectinomycin sensitive PPNG strains from the same source were used to investigate the possibility of predicting the emergence of spectinomycin resistance. Five were isolates taken before treatment from patients from whom spectinomycin resistant PPNG strains were isolated after treatment. Clinical isolates were identified by Gram stain, a positive oxidase reaction, and by the ability to utilise glucose but not maltose, sucrose, or lactose. Penicillinase production was determined by the chromogenic cephalosporin test.

MEDIA

N gonorrhoeae was isolated on GC (gonococcal) base agar (Difco, East Molesey, Surrey) supplemented with 1% (volume per volume) IsoVitalax (BBL, Paisley, Scotland), which was made selective (where necessary) by the addition of amphotericin B, colistin, trimethoprim, and vancomycin. This medium was used without antibiotic supplement for disc sensitivity testing. The medium for assessing agar dilution breakpoints and minimum inhibitory concentrations (MICs) of spectinomycin was DST (diagnostic sensitivity test) agar (Oxoid, Basingstoke, Hampshire) supplemented with 1% (volume per volume) IsoVitalax and 5% (volume per volume) lysed horse blood.

SENSITIVITY TESTING

We used 25 µg and 100 µg spectinomycin discs (Mast, Bootle, Lancashire) for disc testing. The inoculum was taken from the isolation plate, and sensitivity was defined as any zone of inhibition.

Inocula for MICs and breakpoints were adjusted to give 10^4 - 10^5 colony forming units (cfu) when delivered in a 1 µl volume with a Denley multipoint inoculator. All cultures were incubated overnight at 36°C in an atmosphere of 7% carbon dioxide, and complete inhibition of growth was taken as the end point.

Results

ASSESSMENT OF BREAKPOINT CONCENTRATION

We used three concentrations of spectinomycin: 4 mg/l, 8 mg/l, and 16 mg/l. The ranges of MICs for the 37 gonococcal strains resistant to spectinomycin were 512 mg/l to 1024 mg/l, and for the 13 sensitive strains were 8 mg/l to 16 mg/l. All strains grew at 4 mg/l spectinomycin. At 8 mg/l spectinomycin all the resistant strains grew, but so did three sensitive strains. In contrast, 16 mg/l spectinomycin differentiated between resistant and sensitive *N gonorrhoeae*. Tenfold and hundredfold dilutions of the inoculum were also tested, but there was no inoculum effect. Spectinomycin at 16 mg/l was therefore used in all further experiments.

DISC AND BREAKPOINT TESTING OF CLINICAL ISOLATES

We tested 121 fresh isolates using 25 µg and 100 µg discs, and also several of the reference strains resistant to spectinomycin. No discrepancies were found with either disc strength.

We tested 201 clinical isolates by both breakpoint and disc methods in parallel. Seventeen (8.5%) were PPNG strains, four of which were resistant to spectinomycin. None of the 184 non-PPNG strains were resistant to spectinomycin. Both methods correctly identified all 197 gonococci sensitive to spectinomycin. Disc testing detected the four resistant strains, although one failed to grow at the first attempt, whereas only three of the four resistant strains were identified by breakpoint testing. A subculture taken from the antibiotic free control plate on which the fourth isolate was growing also proved to be sensitive by both disc and MIC testing. Subsequent isolates from the same patient were resistant to spectinomycin by both methods and had an MIC of 512 mg/l.

PREDICTION OF EMERGENCE OF RESISTANCE TO SPECTINOMYCIN

Four methods were used: (1) a heavy inoculum of the

strain to be tested was streaked across the surface of plates containing 4, 16, 64, and 128 mg/l spectinomycin; (2) similar plates were inoculated with a multipoint inoculator; (3) strains were grown for 24 hours in the presence of 4 mg/l spectinomycin before multipoint inoculation; and (4) strains were subcultured daily on plates containing increasing concentrations of spectinomycin. Strains were finally incubated for 96 hours and retested for sensitivity to spectinomycin, and were also identified as described above as being *N gonorrhoeae*.

None of the five spectinomycin sensitive "control" PPNG strains developed resistance to spectinomycin during any of these procedures. Four out of five of the strains sensitive to spectinomycin obtained before treatment grew on plates containing 128 mg/l spectinomycin using method 1. The fifth strain grew on plates containing 64 mg/l spectinomycin. Colonies from these plates were resistant to spectinomycin when tested by the disc method. Direct multipoint inoculation of strains (method 2) had no effect. The same was found for serial subculture on plates containing increasing concentrations of spectinomycin (method 4). Method 3, however, (multipoint inoculation of strains primed by growth in the presence of 4 mg/l spectinomycin) grew two out of the five "test" strains, but none of the five control PPNG strains, in the presence of 128 mg/l spectinomycin.

Discussion

In the Praed Street Clinic spectinomycin resistant gonococci are a worrying but not major clinical problem, with proper surveillance. They were first seen among PPNG strains, but since late in 1983 all resistant isolates have been non-PPNG strains. As all spectinomycin resistant strains that we have tested have a common plasmid type, auxotype, and serovar, we have postulated a common origin and the loss of the 4.4 megadalton penicillinase plasmid late in 1983. In some strains resistance has developed after treatment with spectinomycin, whereas in other cases there is clear evidence of sexual transmission.⁵

Our main problem has been that of screening *N gonorrhoeae* for spectinomycin resistance. Since 1981 we have screened all PPNG strains, and since January 1983 we have screened all isolates of *N gonorrhoeae*, which totals over 3000 isolates a year. We have been using a 100 µg spectinomycin disc for this purpose, taking any zone of inhibition as showing sensitivity, but have received anecdotal reports that this might not be reliable. Our results show that screening in this way with either 25 µg or 100 µg spectinomycin discs is reliable and simple.

We also found incorporation of spectinomycin in agar, at a concentration of 16 mg/l, to be a good way of discriminating between sensitive and resistant gonococci. In the one instance when this method failed to detect resistance we think that the initial population was a mixture of mainly sensitive organisms with a few resistant cells. The relatively small numbers of the latter were missed by the smaller inoculum of the breakpoint test, but detected by the larger inoculum used for disc testing. Subsequent isolates from the same patient had a higher percentage of resistant cells and appeared to be resistant by all methods. Mixed populations of gonococci do occur, and the smaller inoculum used in multipoint inoculation is a potential disadvantage of this method. Laboratories using this method for sensitivity testing, however, should find it satisfactory for gonococci in most cases. With the breakpoint method we found a relatively high contamination rate of 10%, which could simply represent inexperience, as we were not used to this technique.

The use of large inocula and exposure to high concentrations of spectinomycin may permit the prediction of spectinomycin resistance. This needs to be confirmed with large numbers of strains. Our spectinomycin resistant gonococci had a single serovar BACKJ.⁵ With the increased use of monoclonal typing reagents, the prevalence of this serovar can be assessed. We plan to test spectinomycin sensitive strains of this serovar to see

if it could act as a marker for the ability to develop resistance to spectinomycin.

Strains of *N gonorrhoeae* with an MIC of spectinomycin of up to 32 mg/l are considered to be sensitive. In theory the use of a breakpoint concentration of only 16 mg/l could lead to problems with some strains, particularly if low level resistance to spectinomycin built up. When high level resistance emerged we looked carefully at spectinomycin sensitive PPNG and non-PPNG strains to see if there had been a rise in MICs of spectinomycin over the previous two years. There was none. We shall continue to monitor this aspect of spectinomycin susceptibility but at present it does not seem to present any problem.

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