In-vitro antimicrobial susceptibility of *Neisseria gonorrhoeae* in New Zealand

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SUMMARY Four hundred and forty-two isolates of *Neisseria gonorrhoeae* were tested by an agar dilution method for their susceptibility to penicillin, ampicillin, tetracycline, cephaloridine, and spectinomycin. Of these isolates, 295 were tested for their susceptibility to sulphamethoxazole and to trimethoprim by the same method, using Oxoid diagnostic sensitivity test agar plus 7.5% laked horse blood instead of Proteose No. 3 agar plus 1% IsoVitaleX and 1% haemoglobin. One hundred (22.6%) of the isolates were found to be relatively resistant to penicillin (minimum inhibitory concentration (MIC) >0.1 iu/ml), but only 1.1% had a MIC of 1 iu/ml or higher. Ampicillin was slightly more active than penicillin in that all isolates were inhibited by 0.5 μ g/ml or less. For 3.7% of isolates the MIC of tetracycline was 2 μ g/ml or higher. All isolates were sensitive to spectinomycin. By calculating the Spearman rank correlation coefficient (r_s), a high correlation ($r_s > 0.5$) was found between susceptibility to penicillin and susceptibility to ampicillin, tetracycline, and cephaloridine. Low correlation ($r_s < 0.2$) was found between susceptibility to penicillin and susceptibility to spectinomycin, sulphamethoxazole, and trimethoprim.

Introduction

For years trends of the susceptibility of *Neisseria gonorrhoeae* to antimicrobials, especially to penicillin, have been studied in many countries (Sparling, 1972). Treatment failure associated with a decrease in the susceptibility to penicillin, and to other potentially useful antimicrobials, has been noted with concern. Recent work indicates that this trend to relative resistance may have reached a plateau in some countries (Robson and Salit, 1972; Givan and Keyl, 1974; Stolz *et al.*, 1975; Jaffe *et al.*, 1976). However, with the discovery of penicillinase-producing gonococci in several countries (World Health Organisation, 1976) and reports of *N. gonorrhoeae* strains resistant to spectinomycin (Stolz *et al.*, 1975; United States Center for Disease Control, 1977), surveillance of susceptibility seems to be even more important.

In New Zealand there has been no report of a quantitative study of the susceptibility of *N. gonorrhoeae* isolates to penicillin and other antimicrobials. This study was undertaken to provide a baseline for future surveillance.

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Materials and methods

ISOLATION AND CULTURE OF *N. GONORRHOEAE*

In 1976, 442 unselected isolates of *N. gonorrhoeae* were received from hospital and private medical laboratories throughout New Zealand. Of these, 251 isolates came from 20 different laboratories and the remaining 191 from a single laboratory in the South Island. The isolates, which were received in half-strength dextrose starch agar stabs overlaid with sterile oil, were kept at 35°C until required for antimicrobial susceptibility testing. Each isolate was then plated from the dextrose starch agar stab on to Thayer-Martin medium—GC medium base (Difco) enriched with 1% haemoglobin (Difco), 1% IsoVitaleX (Baltimore Biological Laboratories), and VCN inhibitor (Baltimore Biological Laboratories)—and incubated for 48 hours at 35°C in a candle extinction jar. Isolates were then subcultured on to the same medium without VCN inhibitor and incubated overnight as previously.

SUSCEPTIBILITY TO ANTIMICROBIALS

Susceptibility to penicillin, ampicillin, tetracycline, spectinomycin, and cephaloridine was determined by the agar dilution method of Jaffe *et al.* (1976).

The susceptibility to sulphamethoxazole and trimethoprim of 295 of the 442 isolates was determined by the same method using Oxoid DST agar and 7.5% laked horse blood (DST medium). Of the 295 isolates, 119 were tested simultaneously using the medium of Jaffe *et al.* (1976) with the addition of 5% laked horse blood (Proteose medium) and DST medium. The two sets of plates were prepared from the one series of antimicrobial dilutions and were inoculated from the one set of diluted cultures.

Antimicrobial powders were obtained from: Glaxo Laboratories (NZ) Ltd (penicillin and cephaloridine); Upjohn (Australia) Pty. Ltd (tetracycline and spectinomycin); Beecham Research Laboratories (ampicillin); and Roche Products Ltd (sulphamethoxazole and trimethoprim).

Doubling dilutions over the following range of concentrations were used: penicillin 0.002–1.0 iu/ml; ampicillin 0.004–0.5 µg/ml; tetracycline 0.06–4 µg/ml; cephaloridine 0.25–8 µg/ml; spectinomycin 2–32 µg/ml; sulphamethoxazole 0.25–128 µg/ml on DST medium and 4–128 µg/ml on Proteose medium; and trimethoprim 2–64 µg/ml on DST medium and 8–256 µg/ml on Proteose medium.

Two plates containing no antimicrobials were inoculated before and after each series of antimicrobial plates and included with them in the candle extinction jars. Three control strains of *N. gonorrhoeae* with a range of known MICs for the antimicrobials tested were included with each test. These strains (F14, F18, and F29) were supplied by the US Center for Disease Control, Atlanta.

CORRELATION COEFFICIENT TEST

Spearman's rank correlation coefficient test was used to determine the correlation when MIC values of penicillin were paired with those of the other six antimicrobials tested.

Results

MIC determinations were reproducible within a two-fold limit. Figures 1, 2, 3, 4, and 5 show the distribution of the MICs of penicillin, ampicillin, tetracycline, cephaloridine, and spectinomycin for the 442 isolates of *N. gonorrhoeae*. The MICs of penicillin and ampicillin had a bimodal distribution. One hundred (22.6%) of the isolates were found to be relatively resistant to penicillin (MIC >0.1 iu/ml), and for 1.1% the MIC was 1 iu/ml or higher. The incidence of relative resistance in the 191 isolates from the single laboratory was 20.4%. All isolates were inhibited by 0.5 µg/ml or less of ampicillin.

The MICs of tetracycline, cephaloridine, and spectinomycin were not bimodal in distribution, but some isolates had decreased susceptibility to tetracycline and cephaloridine. For 3.7% of the isolates the MIC of tetracycline was 2 µg/ml or greater, and for 7.7% the MIC of cephaloridine was 8 µg/ml or greater. For all isolates the MIC of spectinomycin was 16 µg/ml or less.

Fig. 1 MIC of penicillin for 442 isolates of *N. gonorrhoeae*

Fig. 2 MIC of ampicillin for 442 isolates of *N. gonorrhoeae*

Table 1 shows the Spearman rank correlation coefficient (r_s) for MIC values of penicillin when paired with those of ampicillin, tetracycline, cephaloridine, and spectinomycin. There is a high correlation ($r_s > 0.5$) between penicillin-ampicillin,

penicillin-tetracycline, and penicillin-cephaloridine susceptibilities. There is a low correlation ($r_s < 0.2$) between penicillin-spectinomycin susceptibilities.

Figures 6 and 7 show the distribution of MICs of sulphamethoxazole and trimethoprim for 295 isolates of *N. gonorrhoeae*. The distribution was not bimodal for either antimicrobial. The MICs of sulphamethoxazole were distributed over a wide range of concentrations, 4.4% of the isolates having a MIC of ≤ 0.25 $\mu\text{g/ml}$ and 5.0% having a MIC ≥ 32 $\mu\text{g/ml}$.

Table 1 Spearman rank correlation coefficients (r_s) between MIC values for 442 isolates of *N. gonorrhoeae*

Antimicrobials	r_s^*
Penicillin	
plus ampicillin	0.85
plus tetracycline	0.54
plus cephaloridine	0.75
plus spectinomycin	0.19
All r_s^* values are statistically significant ($P < 0.001$)

Fig. 3 MIC of tetracycline for 442 isolates of *N. gonorrhoeae*

Fig. 4 MIC of cephaloridine for 442 isolates of *N. gonorrhoeae*

Fig. 5 MIC of spectinomycin for 442 isolates of *N. gonorrhoeae*

Fig. 6 MIC of sulphamethoxazole for 295 isolates of *N. gonorrhoeae*

Fig. 7 MIC of trimethoprim for 295 isolates of *N. gonorrhoeae*

Table 2 shows the Spearman rank correlation coefficient (r_s) when the MIC values of penicillin are paired with the values for sulphamethoxazole and trimethoprim. Low correlation ($r_s \leq 0.2$) is shown between penicillin susceptibility and susceptibility to sulphamethoxazole and trimethoprim.

When 119 isolates of *N. gonorrhoeae* were tested in duplicate on both Proteose and DST media, the range of MICs was approximately eight-fold higher for sulphamethoxazole and four-fold higher for trimethoprim on Proteose medium than on DST medium. The distribution of MICs on both media was similar for both antimicrobials (Figs. 8 and 9).

Fig. 8 MIC of sulphamethoxazole for 119 isolates of *N. gonorrhoeae*

Fig. 9 MIC of trimethoprim for 119 isolates of *N. gonorrhoeae*

Table 2 Spearman rank correlation coefficients (r_s) between MIC values for 295 isolates of *N. gonorrhoeae*

Antimicrobials	r_s
Penicillin plus sulphamethoxazole	0.20*
plus trimethoprim	0.08†

*Statistically significant ($P < 0.001$)

†Not statistically significant ($0.1 < P < 0.2$)

Discussion

The incidence of relative resistance to penicillin among *N. gonorrhoeae* isolates in New Zealand appears to be similar to that in Australia and Britain but considerably lower than that in the USA. Comparisons with Australian and British studies may not be valid because of the use of different methods to test susceptibility. In Australia, Finger and Handke (1977) found 12.3% and Smithhurst (1974) found 31% of isolates had a MIC of penicillin >0.1 iu/ml. In England, Shahidullah and Greaves (1975) found 30% of isolates had a MIC of penicillin >0.06 $\mu\text{g/ml}$ whereas Jaffe *et al.* (1976), in the USA, found 17.4% of isolates had a MIC of penicillin >0.5 $\mu\text{g/ml}$ and a further 51.6% had a MIC of 0.06–0.25 $\mu\text{g/ml}$.

It has been reported (Robson and Salit, 1972; Stolz *et al.*, 1975; Watko and Brownlow, 1975; Meheus *et al.*, 1976; Powell and Bond, 1976) that sensitivity to penicillin is highly correlated with sensitivity to ampicillin, tetracycline, and cephaloridine. The results of this study also show this correlation.

Various workers (Stolz *et al.*, 1975; Meheus *et al.*, 1976; Shtibel, 1976) found no, or a low, correlation between penicillin and spectinomycin susceptibilities, and this work supports that finding also. All isolates were susceptible to spectinomycin. However, in view of recent reports of isolates which are resistant to this antimicrobial (Stolz *et al.*, 1975; US Center for Disease Control, 1977), and its more frequent use in future to treat patients infected with penicillinase-producing gonococci, its useful life may be limited.

Stolz *et al.* (1975) and Meheus *et al.* (1976) found a relatively low correlation between penicillin susceptibility and susceptibility to sulphamethoxazole and trimethoprim. This study also shows a low correlation. Lawrence *et al.* (1973), however, found some correlation between the MIC of penicillin and failure of treatment with a combination of sulphamethoxazole and trimethoprim in a 20 to 1 ratio.

Testing the susceptibility of any organism to sulphonamides and trimethoprim is difficult, as the behaviour of these two antimicrobials is more medium-dependent than that of others. There is the added difficulty that gonococci require a fairly complicated medium to grow well, and growth supplements may interfere with antimicrobial action (Shtibel, 1975).

Most workers have used Oxoid DST agar with 5–10% laked horse blood and no other enrichment for testing the susceptibility of gonococci to sulphamethoxazole and trimethoprim (Phillips *et al.*,

1970; Lawrence *et al.*, 1973; Stolz *et al.*, 1975; Meheus *et al.*, 1976); but growth on this medium was observed to be poorer than on the medium of Jaffe *et al.* (1976).

Yoshikawa *et al.* (1975) found that 95% of gonococcal strains were inhibited by 2.5 $\mu\text{g/ml}$ trimethoprim and 47.5 $\mu\text{g/ml}$ sulphamethoxazole in combination in Oxoid DST agar enriched with 1% IsoVitalX and 5% laked horse blood, whereas only 84% were inhibited by the same concentrations in GC agar with the same enrichment.

The discovery of penicillinase-producing gonococci could lead to a greater evaluation of the clinical use of sulphamethoxazole combined with trimethoprim in uncomplicated gonorrhoea. It is important that a standardised method for testing susceptibility to these antimicrobials is used, so that a firm base can be provided for establishing correlations between laboratory and clinical results.

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