In Vitro Antibiotic Susceptibilities of \textit{Neisseria gonorrhoeae} Isolates in the Philippines

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Antibiotic susceptibility surveillance testing was performed on clinical isolates of \textit{Neisseria gonorrhoeae} collected in September 1989 in the Philippines. \textbeta-Lactamase was produced by 77 (55\%) of 140 isolates. In vitro MIC testing revealed significant resistance to penicillin (MIC for 90\% of isolates [MIC\textsubscript{90}], >64 \mu g/ml), tetracycline (MIC\textsubscript{90}, 4 \mu g/ml), and cefmetazole (MIC\textsubscript{90}, 8 \mu g/ml). Spectinomycin resistance was rare (10 of 117), but the MIC\textsubscript{90} was 32 \mu g/ml. Isolates were susceptible to fluoroquinolones and cephap tolazmin at the time of this survey, as evidenced by the MIC\textsubscript{90} of ciprofloxacin (0.25 \mu g/ml), norfloxacin (2.0 \mu g/ml), ofloxacin (0.625 \mu g/ml), cefpodoxime (2.0 \mu g/ml), cefotaxime (1.0 \mu g/ml), ceftazidime (0.25 \mu g/ml), ceftizoxime (0.25 \mu g/ml), and ceftriaxone (0.06 \mu g/ml). To date, ceftriaxone resistance has not emerged, despite the widespread use of this antibiotic in the Philippines.

Changes in the antibiotic resistance patterns of \textit{Neisseria gonorrhoeae} isolates in the Philippines have heralded the emergence of resistant isolates in the United States over the last decade (7, 16). This geographic area continues to serve as a test ground for the emergence of antibiotic-resistant \textit{N. gonorrhoeae} isolates because of antibiotic pressure consequent to the continuous use of inadequately dosed prophylactic antibiotics by those who are repeatedly infected and have high endemic rates of gonococcal infections in prostitutes who live adjacent to military bases.

Penicillin-resistant \textit{N. gonorrhoeae} isolates were first reported in the Philippines in 1965 to 1966 (7, 16). As antibiotic therapy was altered in response to the demonstration of resistance to this agent and later to others, these changes were followed by the emergence of isolates resistant to the newer therapeutic regimens (1, 2, 4–6, 14). Unfortunately, it also appears that once antibiotic resistance develops, it persists in the local reservoir, despite the substitution of unrelated antimicrobial agents in new antibiotic regimens (10).

The present study was undertaken to reassess the antibiotic susceptibility of \textit{N. gonorrhoeae} isolates in the Philippines and, in particular, to examine the possibility of emerging ceftriaxone resistance, since this antibiotic is currently used to treat all gonococcal infections in the area from which these isolates were collected.

**MATERIALS AND METHODS**

Gonococcal isolates. Isolates of \textit{N. gonorrhoeae} were obtained from men attending military sick call with symptoms of a sexually transmitted disease and from female bar hostesses attending public health screening clinics. Initial isolations were made on modified Thayer-Martin agar (BBL Microbiology Systems, Cockeysville, Md.). Suspect colonies were identified by colony morphology, Gram staining, oxidase activity (SpotTest oxidase reagent; Difco Laboratories, Detroit, Mich.), and reaction in the Gonocheck II monoclonal antibody test (Du Pont Co., Wilmington, Del.). Overnight subcultures were placed in cryoprotective medium (Trypticase soy broth [BBL] with 20\% glycerol [Malinckrodt, Inc., Paris, Ky.] and frozen in liquid nitrogen until tested. Thawed specimens were plated on chocolate agar prepared from GC agar base (BBL), 1\% bovine hemoglobin (BBL), and 1\% IsoVitalex (BBL). Pure colonies reisolated on chocolate agar were tested as 18-h growth in second subcultures.

**\textbeta-Lactamase testing.** \textbeta-Lactamase production was tested by use of nitrocefin disks (Cefinase; BBL) with \textit{Haemophilus influenzae} ATCC 10211 as a negative control.

**TABLE 1.** In vitro susceptibilities of \textit{N. gonorrhoeae} isolates in the Philippines

<table>
<thead>
<tr>
<th>Antibiotic*</th>
<th>MIC (\mu g/ml)</th>
<th>90%</th>
<th>50%</th>
<th>Range</th>
<th>No. of isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G (\beta-L neg)</td>
<td>16.000</td>
<td>2.000</td>
<td>0.002–64.0</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Penicillin G (\beta-L pos)</td>
<td>&gt;64.000</td>
<td>16.000</td>
<td>0.060–64.0</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Azlocillin (\beta-L neg)</td>
<td>&gt;2.000</td>
<td>0.500</td>
<td>0.030–2.0</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Azlocillin (\beta-L pos)</td>
<td>&gt;2.000</td>
<td>2.000</td>
<td>0.030–2.0</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.000</td>
<td>0.500</td>
<td>0.060–4.0</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Tetracycline (\beta-L neg)</td>
<td>&gt;4.000</td>
<td>2.000</td>
<td>0.060–32.0</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Tetracycline (\beta-L pos)</td>
<td>4.000</td>
<td>1.000</td>
<td>0.060–4.0</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>32.000</td>
<td>32.000</td>
<td>16.000–128.0</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Trospectinomycin</td>
<td>16.000</td>
<td>8.000</td>
<td>&lt;2.000–128.0</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4.000</td>
<td>1.000</td>
<td>0.060–8.0</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Cefmetazole (\beta-L neg)</td>
<td>&gt;8.000</td>
<td>8.000</td>
<td>0.060–8.0</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>Cefmetazole (\beta-L pos)</td>
<td>8.000</td>
<td>2.000</td>
<td>0.500–8.0</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>2.000</td>
<td>0.125</td>
<td>0.015–8.0</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>2.000</td>
<td>0.030</td>
<td>0.002–4.0</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1.000</td>
<td>0.030</td>
<td>0.002–8.0</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.250</td>
<td>0.060</td>
<td>0.005–10.0</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>0.250</td>
<td>0.060</td>
<td>&lt;0.001–10.0</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.060</td>
<td>0.025</td>
<td>&gt;0.001–4.0</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>0.625</td>
<td>0.038</td>
<td>0.005–5.0</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.250</td>
<td>0.004</td>
<td>&lt;0.001–2.0</td>
<td>135</td>
<td></td>
</tr>
</tbody>
</table>

* \beta-L neg, \beta-lactamase negative; \beta-L pos, \beta-lactamase positive.

* Corresponding author.

Antibiotic stock solutions were prepared in reagent-grade water and frozen at −70°C. Serial twofold dilutions of the antibiotics were prepared in reagent-grade water on the day of use.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was conducted as previously described (15). Quality control organisms inoculated on each plate were Staphylococcus aureus ATCC 29213, S. aureus ATCC 29253, Enterococcus faecalis ATCC 29212, and Escherichia coli ATCC 25922. Subcultures were incubated in a humidified atmosphere of 5% CO₂ for 24 h at 35°C. MICs were read as the lowest concentration of antibiotic that inhibited growth (11).

Statistical analysis. The effect of β-lactamase production on susceptibility to each drug was examined by chi-square analysis with EpilInfo Version 3.00 (Centers for Disease Control Epidemiology Program Office, Atlanta, Ga.). Significance was defined as $P \leq 0.05$. Data are presented sepa-

FIG. 1. Relationship of β-lactamase carriage to in vitro penicillin susceptibility in 61 β-lactamase-negative (■) and 77 β-lactamase-positive (▲) isolates.

FIG. 2. Relationship of β-lactamase carriage to in vitro azlocillin susceptibility in 48 β-lactamase-negative (■) and 60 β-lactamase-positive (▲) isolates.

FIG. 3. Relationship of β-lactamase carriage to in vitro cefmetazole susceptibility in 59 β-lactamase-negative (■) and 75 β-lactamase-positive (▲) isolates.

FIG. 4. Relationship of β-lactamase carriage to in vitro tetracycline susceptibility in 60 β-lactamase-negative (■) and 75 β-lactamase-positive (▲) isolates.
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RESULTS

One hundred forty isolates were confirmed to be N. gonorrhoeae. Although no single drug was evaluated against all isolates (Table 1), β-lactamase was detected in 77 of 140 (55%) isolates and conferred resistance to penicillin (MIC, >2 μg/ml) in 76 of 77 (98%) (13). Thirty-two of 61 (52%) β-lactamase-negative isolates also were penicillin resistant ($P < 0.001$) (Fig. 1). Seventy-one percent (43 of 60) of β-lactamase-positive and 18% (9 of 48) of β-lactamase-negative isolates were resistant to azlocillin (MIC, >2 μg/ml) ($P < 0.001$) (Fig. 2). Sixty-one percent (36 of 59) of β-lactamase-negative and 17.3% (13 of 75) of β-lactamase-positive isolates were resistant to cefmetazole (MIC, >8 μg/ml) ($P < 0.001$) (Fig. 3). Thirty-two percent (24 of 75) of β-lactamase-positive and 66% (40 of 60) of β-lactamase-negative isolates were resistant to tetracycline (MIC, ≥2 μg/ml) ($P < 0.001$) (Fig. 4) (8). A few isolates were resistant to erythromycin, spectinomycin, trospectinomycin, cefoxitin, and cefuroxime, but the association with β-lactamase production was not significant (Fig. 5 to 9) (8, 9, 13). A single isolate was resistant to ciprofloxacin (MIC, ≥4 μg/ml) and was β-lactamase negative (Fig. 10) (13). None of the isolates tested was found to be resistant to cefotaxime, cefpodoxime, ceftria-
DISCUSSION

This survey revealed that established resistance to penicillin and other β-lactamase-susceptible antibiotics, tetracyclines, and erythromycin has continued at a high prevalence. The MICs for 90% of isolates (MIC<sub>90</sub>) of penicillin G (>64 μg/ml), tetracycline (4 μg/ml), and erythromycin (>4 μg/ml) were consistent with those reported in previous studies carried out in the Philippines (1, 2, 4, 10, 14). Azlocillin, a ureidopenicillin, presented no advantage over penicillin G, since it too was susceptible to β-lactamase (12). The β-lactamase-stable broad-spectrum cephalosporins were highly active against all or most of the strains and should each be effective at the usual preferred dose. The fluoroquinolones tested were effective against all or most of the strains in vitro and may represent a viable treatment option in the Philippines. Although no fluoroquinolone antibiotics have been officially recommended in the Philippines since the discontinuation of rosoxacin use by health department clinics several years prior to this study, the MIC<sub>90</sub> of norfloxacin is now 16-fold higher than the MIC<sub>90</sub> reported in 1982 data (3). The uncontrolled availability of antibiotics has previously been cited as a confounding factor in the selection of a therapeutic regimen in and near Subic Bay and probably

dime, ceftizoxime, ceftriaxone, norfloxacin, or ofloxacin (Fig. 11 to 17) (8, 13).
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FIG. 13. Distribution of ceftazidime susceptibility.

Minimum Inhibitory Concentration (ug/ml) n=124

FIG. 14. Distribution of ceftriaxone susceptibility.

Minimum Inhibitory Concentration (ug/ml) n=134

FIG. 15. Distribution of norfloxacin susceptibility.

Minimum Inhibitory Concentration (ug/ml) n=134

FIG. 16. Distribution of cephalosporins and fluoroquinolones remain viable alternatives for the treatment of gonorrhea in the Philippines. Class resistance and lack of Food and Drug Administration approval may preclude the use of trospectinomycin in the clinical setting. While broad-spectrum cephalosporins and fluoroquinolones remain viable alternatives for the treatment of gonorrhea in the Philippines, rising MICs and sporadic resistance suggest increased antibiotic resistance in the future. Continued systematic monitoring of in vitro susceptibilities is necessary to monitor changes in resistance patterns.
tibility patterns coupled with rigorous clinical follow-up of reported treatment failures in gonorrhea cases in the Philippines will be necessary to inhibit the spread of future ceftriaxone-resistant strains from the busy port area of Subic Bay.

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REFERENCES


