In Vitro Antibiotic Susceptibilities of Neisseria gonorrhoeae Isolates in the Philippines

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Received 7 May 1991/Accepted 13 November 1991

Vol. 36, No. 2
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Changes in the antibiotic resistance patterns of 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 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 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 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 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.

β-Lactamase testing. β-Lactamase production was tested by use of nitrocefin disks (Cefinetase; BBL) with Haemophilus influenzae ATCC 10211 as a negative control.

<table>
<thead>
<tr>
<th>Antibiotic*</th>
<th>MIC (µg/ml)</th>
<th>90%</th>
<th>50%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G (βL neg)</td>
<td>&gt;64.000</td>
<td>16.000</td>
<td>2.000</td>
<td>0.002–&gt;64.0</td>
</tr>
<tr>
<td>Penicillin G (βL pos)</td>
<td>&gt;64.000</td>
<td>16.000</td>
<td>0.060–&gt;64.0</td>
<td></td>
</tr>
<tr>
<td>Azlocillin (βL neg)</td>
<td>&gt;2.000</td>
<td>0.050</td>
<td>0.030–&gt;2.0</td>
<td></td>
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<tr>
<td>Azlocillin (βL pos)</td>
<td>&gt;2.000</td>
<td>0.030–&gt;2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.000</td>
<td>0.500</td>
<td>0.060–&gt;4.0</td>
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<tr>
<td>Ceftriaxone (βL neg)</td>
<td>&gt;4.000</td>
<td>2.000</td>
<td>0.030–&gt;32.0</td>
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<tr>
<td>Ceftriaxone (βL pos)</td>
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<td>1.000</td>
<td>0.060–&gt;4.0</td>
<td></td>
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<tr>
<td>Spectinomycin</td>
<td>32.000</td>
<td>32.000</td>
<td>16.000–&gt;128.0</td>
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<tr>
<td>Trepomycincin</td>
<td>16.000</td>
<td>8.000</td>
<td>&lt;2.000–&gt;128.0</td>
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<tr>
<td>Trepomycincin</td>
<td>4.000</td>
<td>1.000</td>
<td>0.060–&gt;8.0</td>
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<tr>
<td>Cefoxitin</td>
<td>8.000</td>
<td>8.000</td>
<td>0.060–&gt;8.0</td>
<td></td>
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<tr>
<td>Cefoxitin</td>
<td>8.000</td>
<td>2.000</td>
<td>0.060–&gt;8.0</td>
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<tr>
<td>Cefoxitin</td>
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<td>0.125</td>
<td>0.015–&gt;8.0</td>
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<tr>
<td>Cefoxitin</td>
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<td>0.002–&gt;4.0</td>
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<tr>
<td>Cefoxitin</td>
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<td>0.030</td>
<td>0.002–&gt;8.0</td>
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<tr>
<td>Cefoxitin</td>
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<td>0.060</td>
<td>0.015–&gt;4.0</td>
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<tr>
<td>Cefoxitin</td>
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<td>0.008</td>
<td>&lt;0.001–&gt;4.0</td>
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<td>0.250</td>
<td>0.004</td>
<td>&lt;0.001–&gt;2.0</td>
<td></td>
</tr>
</tbody>
</table>

* Corresponding author.

βL neg, β-lactamase negative; βL pos, β-lactamase positive.

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-
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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 cepfoxime, but the association with β-lactamase production was not significant (Fig. 5 to 9) (8, 9, 13). None of the isolates tested was found to be resistant to cefotaxime, cefpodoxime, cefzai-
dime, ceftriaxone, norfloxacin, or ofloxacin (Fig. 11 to 17) (8, 13).

**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 (MIC₉₀) 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 MIC₉₀ of norfloxacin is now 16-fold higher than the MIC₉₀ 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

![FIG. 9. Distribution of cefuroxime susceptibility.](image)

![FIG. 11. Distribution of cefotaxime susceptibility.](image)

![FIG. 10. Distribution of ciprofloxacin susceptibility.](image)

![FIG. 12. Distribution of cefpodoxime susceptibility.](image)
N. GONORRHOEAE ISOLATES FROM THE PHILIPPINES

contributes significantly to the development and persistence of resistant strains (6). Also of interest is the presence of a β-lactamase plasmid in 55% of all strains and chromosomally mediated resistance to penicillin in 57% of the β-lactamase-negative strains as well. This fact probably reflects the ongoing practice by sexually active persons of continuously taking subtherapeutic doses of oral penicillins. Spectinomycin and the investigational drug trospectinomycin were active against greater than 90% of strains, but the overall level of resistance of susceptible strains is increasing. Spectinomycin (MIC_{90}, 32 μg/ml) is probably useful in the treatment of those for whom ceftriaxone is contraindicated. The prevalence of resistance to spectinomycin has declined from 22% in 1988 to less than 10% in late 1989, but the MIC_{90} and MIC_{50} have continued to rise since the discontinuation of spectinomycin therapy in public health clinics (10). Trospectinomycin (MIC_{90}, 16 μg/ml) was about twice as active as spectinomycin, but class resistance resulting from the abuse of spectinomycin probably limits the application of this drug in the control 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 suscep-

FIG. 13. Distribution of ceftazidime susceptibility.

FIG. 14. Distribution of ceftizoxime susceptibility.

FIG. 15. Distribution of ceftriaxone susceptibility.

FIG. 16. Distribution of norfloxacin susceptibility.
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.

ACKNOWLEDGMENTS
We thank S. T. Bernardo and E. A. Aquino for expert technical assistance in collecting and characterizing the isolates, R. K. Hanson for assistance in carrying out statistical analyses, and E. Donegan for reviewing the manuscript.

REFERENCES