Comparison of Broth Microdilution and Agar Dilution for Susceptibility Testing of Neisseria gonorrhoeae

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Broth microdilution was compared with agar dilution to test the susceptibilities of 170 clinical isolates of Neisseria gonorrhoeae to several antimicrobial agents. Percents agreement between the two methods within 1 doubling dilution were 98.8% for enoxacin, 94.3% for penicillin G, 93.5% for spectinomycin, and 98.4% for ceftriaxone.

Isolates of Neisseria gonorrhoeae with chromosomally mediated resistance to penicillin (8), plasmid-mediated resistance to tetracycline and penicillin (1, 5), and spectinomycin resistance (1, 2, 7) have been reported with increasing frequency. Such reports have stimulated active investigation of alternative therapeutic drugs.

The National Committee for Clinical Laboratory Standards (6) recommends the agar dilution procedure for susceptibility testing of N. gonorrhoeae. This method is tedious, time-consuming, and inconvenient for testing large numbers of antimicrobial agents. Shapiro et al. (9) described a broth microdilution method using IsoVitaleX as an enrichment material for susceptibility testing of N. gonorrhoeae and compared results obtained by this method with results obtained by agar dilution using 23 isolates. The present study compares the results from parallel tests using broth microdilution and agar dilution with supplement C as enrichment to test the susceptibilities of 170 clinical isolates of N. gonorrhoeae to enoxacin and to three clinically relevant antibiotics.

All N. gonorrhoeae isolates were recovered from clinical specimens submitted to Henry Ford Hospital, Detroit, Mich., or the Washtenaw County Venereal Disease Clinic, Ann Arbor, Mich. The isolates were identified by oxidase reaction, Ann Arbor, Mich. The isolates were identified by oxidase reaction, Gram stain, and appropriate enzymatic reaction with the Gonochek II system (Du Pont Co., Wilmington, Del.). Penicillinase production was detected by the use of Cefinase disks (BBL Microbiology Systems, Cockeysville, Md.). The fresh isolates were transferred once onto chocolate agar, and the colonies were scraped into a swab into tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing 20% glycerol and were stored at -70°C. All isolates were subcultured twice onto chocolate agar (GC medium base containing 1% IsoVitaleX; BBL) prior to MIC determination.

The antimicrobial agents tested were ceftriaxone (0.002 to 2 μg/ml; Hoffmann-La Roche Inc., Nutley, N.J.), enoxacin (0.008 to 8 μg/ml; Warner Lambert Co., Parke-Davis Div., Ann Arbor, Mich.), penicillin G (0.008 to 8 μg/ml; Pfizer Inc., New York, N.Y.), and spectinomycin (0.128 to 128 μg/ml; The Upjohn Co., Kalamazoo, Mich.). Microdilution trays were prepared by using serial twofold dilutions of antimicrobial agents in gonococcal broth (3) containing 1% supplement C (Difco).

Overnight growth from a chocolate agar plate was suspended in gonococcal broth and adjusted to 40 to 50% transmittance on a Beckman DB spectrophotometer (Beckman Instruments, Inc., Brea, Calif.). Colony counts of the adjusted inoculum indicated that it contained approximately 3 × 10⁶ CFU of organism per ml. The microdilution trays were inoculated with a Dynatech 2000 disposable inoculation device (Dynatech Laboratories, Inc., Alexandria, Va.), resulting in a final inoculum of 10⁶ CFU per well. The trays were incubated for 24 h at 35°C in a humidified atmosphere containing 5% CO₂. The MIC reported for each isolate was the lowest concentration of drug which prevented visible growth in duplicate tests. No isolate differed by more than 1 dilution in duplicate tests.

Agar dilution was performed with GC agar base containing 1% hemoglobin and 1% supplement C. The bacterial inoculum was diluted in gonococcal broth to contain 10⁷ CFU/ml, and the plates were inoculated with a Steers replication device (10), resulting in a final inoculum of ca. 10⁶ CFU per spot. The plates were incubated for 24 h at 35°C in 5% CO₂. The MIC reported for each isolate was the lowest concentration of drug which prevented visible growth in duplicate tests.

Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 29213, and N. gonorrhoeae NG-1271 were run daily as controls. Results were used only if endpoints were the same (±1 dilution) as the control MICs for P. aeruginosa and S. aureus (6) and as that previously published (9) for NG-1271. No test had to be discarded.

The MICs determined by the broth microdilution method correlated well with those determined by the agar dilution method (Tables 1 and 2). Percents agreement between agar and microdilution testing within 1 doubling dilution were 98.8% for enoxacin, 93.5% for spectinomycin, 94.3% for penicillin G (β-lactamase-negative strains), and 98.4% for ceftriaxone (Table 1). In no instance did the difference in

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<tr>
<th>TABLE 1. Agreement of microdilution with agar dilution for susceptibility testing of N. gonorrhoeae</th>
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<tr>
<td><strong>Antimicrobial agent</strong> (no. of isolates)</td>
</tr>
<tr>
<td>-3</td>
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<tr>
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</tr>
<tr>
<td>Enoxacin (170)</td>
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<tr>
<td>Spectinomycin (170)</td>
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<tr>
<td>Penicillin G* (159)</td>
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<tr>
<td>Ceftriaxone (170)</td>
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* Penicillinase-negative strains only. Penicillinase producers were analyzed separately (Table 2).
MICs change the classification of a strain with regard to its drug susceptibility, except for two penicillinase-producing strains, which were classified differently on the basis of MICs of penicillin (data not shown). Penicillinase-producing strains appeared to be more resistant to penicillin when tested by broth microdilution (Table 2). This may be because of the larger inocula used in this method.

Four penicillin-resistant, non-penicillinase-producing strains were examined. Results for all of these strains were in agreement with results obtained by agar dilution (±1 doubling dilution) for all of the agents tested (data not shown).

Our results indicate that the broth microdilution procedure using 1% supplement C as an enrichment material was a reliable method for determining antimicrobial susceptibility of *N. gonorrhoeae*. Results of the broth microdilution method were in complete agreement with those of agar dilution in most instances. The method is easy to perform, and endpoints are easy to distinguish. As a result, this method can readily be adapted to an automatic reader. In contrast to agar dilution testing, which requires fresh preparations of antibiotic-containing media, broth microdilution trays can be prepared in large quantities and stored frozen at −70°C prior to use.

Shapiro et al. (9) used 1% IsoVitaleX as an enrichment material for *N. gonorrhoeae*. The substitution of supplement C for IsoVitaleX may be particularly useful when antimicrobial agents which are rapidly inactivated by the cysteine present in IsoVitaleX are being tested (4). Preliminary MIC determinations for 20 clinical isolates with either 1% IsoVitaleX or 1% supplement C as enrichment yielded similar MICs (±1 doubling dilution) for all four agents tested (data not shown), although the IsoVitaleX supplement did allow somewhat better growth. In addition, our determinations with strain NG-1271 yielded MICs comparable to those published elsewhere (9). Quality control measures must be adequately adhered to, however, particularly when penicillins are being tested, since false penicillin resistance has been reported for *Haemophilus influenzae* when it was tested with agar containing certain production lots of supplement C (11).

Broth microdilution is a simple method for susceptibility testing of *N. gonorrhoeae*. This method may prove useful in the pharmaceutical industry for screening large numbers of potentially effective antimicrobial agents and in the clinical laboratory, should routine susceptibility testing of *N. gonorrhoeae* become a necessity.

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### LITERATURE CITED


