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Comparison of Microdilution and Agar Dilution Procedures for Testing Antibiotic Susceptibility of *Neisseria gonorrhoeae*

MARTIN A. SHAPIRO, CARL L. HEIFETZ,* AND JOSEPHINE C. SESNIE
Pharmaceutical Research, Warner-Lambert/Parke-Davis, Ann Arbor, Michigan 48105

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Studies were run in parallel to compare the broth microdilution method and the chocolate agar dilution method for testing antibiotic susceptibility of *Neisseria gonorrhoeae*. Six clinically relevant drugs were tested against 23 clinical isolates of *N. gonorrhoeae*, including several penicillinase-producing, as well as multiply resistant, strains. Results showed that the MIC obtained by the two methods were not significantly different. The microdilution method appears to be a more sensitive system for discriminating penicillinase activity. The microdilution system is a more expedient method for screening new antibacterial agents and is more readily adaptable to new automated equipment.

Penicillin-resistant *Neisseria gonorrhoeae* infections are being seen with increasing frequency worldwide (2, 5, 8). In the urgent search for new effective drugs, the necessity for a more expedient antibiotic screening method for rapid evaluation of large numbers of compounds has become apparent. To accomplish this, the method should be highly amenable to the utilization of automated equipment. Currently, the chocolate agar dilution method is being used by the Venereal Disease Research Laboratory, Centers for Disease Control, Atlanta, Ga., for testing the antibiotic susceptibility of *N. gonorrhoeae* (1). With the development of a new liquid medium for gonococcal growth (3, 4), the microdilution system (7) was made feasible. Parallel tests were run to compare the results of the two methods with respect to the susceptibilities of several strains of *N. gonorrhoeae* to six clinically relevant drugs.

The clinical isolates used in this experiment were obtained from the Centers for Disease Control and included those resistant to spectinomycin or penicillin or both, as well as several multiply resistant strains. The penicillin-resistant strains included both penicillinase producers and nonproducers. Penicillinase activity was determined by the use of Cefinase disks (BBL Microbiology Systems).

The microdilution procedure was similar to that described by Marymont and Wentz (7). To prepare the inoculum, overnight growth from a chocolate agar plate (1) was suspended in gonococcal broth. The broth consisted of 15 g of proteose peptone no. 3, 4 g of dipotassium phosphate, 1 g of potassium dihydrogen phosphate, 5 g of sodium chloride, 1 g of soluble starch, and 420 mg of sodium bicarbonate dissolved in 1 liter of distilled water; IsoVitaleX (1%) was added after autoclaving, and the pH was adjusted to 7.2. The culture turbidity was adjusted to 40 to 50% transmittance on a spectrophotometer set at a wavelength of 530 nm. The suspension was diluted 100-fold in gonococcal broth, and the final inoculum size was ca. 10^7 CFU/ml (10^6 CFU per well). This inoculum was shown to be optimum in preliminary experiments: the use of 10^8 CFU/ml produced an initial turbidity resulting in a growth artifact; 10^6 CFU/ml resulted in light growth, making endpoint determinations less definable and unsuitable for automated optical density monitors. The plates were incubated for 24 h at 35°C in a humidified candle jar providing an atmosphere of 3 to 3.5% CO_2. The humidity was provided by a moistened paper towel and was not measured. The MIC was the lowest concentration of drug which prevented macroscopically visible growth (Fig. 1) under test conditions. Each test was performed in duplicate.

The agar dilution procedure used was similar to that described by Biddle et al. (1). Using 10^7-CFU/ml cultures, we applied ca. 10^4 CFU of inoculum to each spot by means of a Steers multiple-inoculator apparatus (9). The MIC was the lowest concentration of drug which resulted in growth of five or less colonies per spot. Each test was performed in duplicate.

The results of this experiment are summarized in Tables 1, 2, and 3. MICs without definite endpoints could not be considered in the overall data analysis (Table 2). Since it appears that the microdilution method gives much higher MICs for penicillinase-producing organisms, the MIC pairs for ampicillin and penicillin are listed separately (Table 3) and are not included in the pair frequency analysis (Table 2). Of the 86 MIC pairs so analyzed, 71 varied by twofold or less (82%), and the remainder only varied by fourfold. In general, it appeared that the microdilution method resulted in slightly lower MICs, as indicated in 47 of the 86 pairs (55%). The MICs in duplicate agar or broth tests did not vary by more than twofold at any point in this experiment. The MICs obtained by both methods were similar to published data (6). An MIC of ≥0.2 μg/ml was used as the resistance breakpoint for *N. gonorrhoeae* strains (J. W. Biddle, Centers for Disease Control, personal communication). In no instance

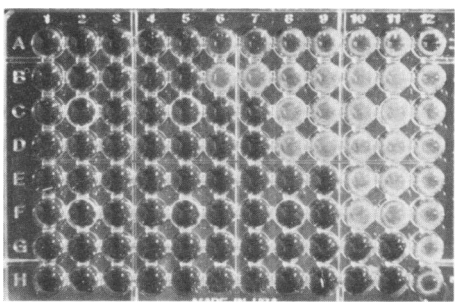

FIG. 1. Growth of *N. gonorrhoeae* in broth on microtiter plate.

* Corresponding author.

TABLE 1. Comparison of MICs by microdilution versus agar dilution procedure

N. gonorrhoeae strain no.	MIC (μg/ml) of[a]: Doxycycline AD	Doxycycline MD	Erythromycin AD	Erythromycin MD	Oxolinic acid AD	Oxolinic acid MD	Spectinomycin AD	Spectinomycin MD
Penicillin resistant, penicillinase negative								
NG-1259	3.1	3.1	0.8	1.6	0.4	0.1	25	25
NG-1260	3.1	0.8	0.8	0.4	0.2	0.1	12.5	12.5
NG-1261	0.8	0.8	0.2	0.2	0.1	0.05	12.5	12.5
NG-1262	3.1	1.6	0.8	0.4	0.2	0.1	6.3	12.5
NG-1263	1.6	0.8	0.8	0.8	0.2	0.1	12.5	12.5
NG-1264	1.6	0.8	0.8	0.8	0.2	0.05	12.5	12.5
NG-1265	3.1	3.1	1.6	1.6	0.2	0.1	12.5	25
NG-1266	3.1	1.6	0.8	1.6	0.2	0.1	25	12.5
NG-1267	3.1	1.6	0.8	0.4	0.4	0.2	25	12.5
Median MIC	1.6	1.6	0.8	0.8	0.2	0.1	12.5	12.5
Penicillin resistant, penicillinase positive								
NG-1271[b]	0.8	0.8	1.6	0.4	0.2	0.05	>100	>100
NG-1272[b]	0.8	0.4	1.6	0.4	0.2	0.05	>100	>100
NG-1273[b]	1.6	1.6	0.4	0.2	0.2	0.1	>100	>100
NG-1274[b]	1.6	1.6	0.4	0.4	0.2	0.1	>100	>100
NG-1276[b]	1.6	1.6	0.4	0.4	0.4	0.2	>100	>100
89[c]	1.6	0.8	0.4	0.2	0.1	0.2	25	12.5
Median MIC	1.6	0.8	0.4	0.4	0.2	0.1	>100	>100
Penicillin susceptible								
NG-1268	0.8	0.2	0.2	0.1	0.1	0.05	12.5	12.5
NG-1269	0.8	0.2	0.2	0.2	0.1	0.1	12.5	12.5
NG-1270[b]	0.8	0.8	0.2	0.2	0.1	0.1	>100	>100
82[c]	0.4	0.1	0.1	0.05	0.1	0.2	12.5	12.5
804[c]	0.4	0.1	0.2	0.05	0.1	0.1	12.5	12.5
P1[c]	0.4	0.1	0.4	0.1	0.1	0.1	12.5	12.5
P954[c]	0.8	0.2	0.4	0.2	0.1	0.1	25	12.5
F-28[b,c]	0.2	0.1	0.1	0.05	0.1	0.1	100	100
Median MIC	0.4	0.2	0.2	0.1	0.1	0.1	25	12.5

[a] AD, Agar dilution; MD, microdilution.
[b] Spectinomycin resistant.
[c] Control strains.

did the difference in MICs between the two methods result in a change in classification of an organism with respect to its drug susceptibility. The data obtained for six control strains (Table 1) was consistent with past results.

The greatest advantage of the microdilution method is that it screens large numbers of drugs at the same time against relatively few selected isolates. This situation presents itself often in new drug evaluation.

In pharmaceutical research laboratories, the microdilution method is advantageous in that microtiter plates containing serially diluted antibiotics can be made, frozen, and stored until several compounds are available for side-by-side tests. With the advent of an automatic dispenser and reader for this method, along with the appropriate interface with computers, it will be possible to perform a large number of susceptibility tests within a relatively short period of time.

These experiments showed that similar results were obtained by the microdilution and agar dilution methods in most cases. The former showed a greater propensity for discriminating between penicillinase-producing and -nonproducing penicillin-resistant strains. The microdilution method may prove to be an extremely useful tool in screening for new effective drugs for treatment of *N. gonorrhoeae* infections.

TABLE 2. MIC pair frequency analysis of microdilution versus agar dilution for doxycycline, erythromycin, oxolinic acid, and spectinomycin

MIC in agar/ MIC in broth	Frequency	% Frequency
0.5	6	7
1	33	38
2	32	37
4	15	18

TABLE 3. Comparison of MICs for microdilution versus agar dilution for penicillin and ampicillin

N. gonorrhoeae strain	MIC (μg/ml)[a] Agar dilution Pen G	Agar dilution Amp	Microdilution Pen G	Microdilution Amp
Penicillin resistant, penicillinase negative				
NG-1259	1.6	0.8	0.8	0.4
NG-1260	1.6	0.8	0.8	0.2
NG-1261	0.8	0.8	0.4	0.4
NG-1262	1.6	0.8	0.8	0.4
NG-1263	0.8	0.8	0.8	0.4
NG-1264	0.8	0.8	0.8	0.2
NG-1265	1.6	0.8	1.6	0.4
NG-1266	1.6	0.8	0.8	0.2
NG-1267	3.1	0.8	1.6	0.8
Median MIC	1.6	0.8	0.8	0.4
Penicillin resistant, penicillinase positive				
NG-1271	12.5	25	>100	>100
NG-1272	6.3	12.5	50	100
NG-1273	12.5	25	100	50
NG-1274	6.3	12.5	50	50
NG-1276	12.5	12.5	>100	50
89	12.5	12.5	>100	50
Median MIC	12.5	12.5	100	50
Penicillin susceptible				
NG-1268	0.1	0.1	0.025	0.05
NG-1269	0.2	0.2	0.2	0.2
NG-1270	0.025	0.1	0.025	0.05
82	0.05	0.1	≤0.013	≤0.013
804	0.025	0.05	≤0.013	≤0.013
P1	0.2	0.05	0.05	0.05
P954	0.2	0.05	0.05	0.05
F-28	≤0.013	0.025	≤0.013	≤0.013
Median MIC	0.1	0.05–0.1	0.025	0.05

[a] Pen G, Penicillin; Amp, ampicillin.

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