

## Evaluation of a Commercial Microdilution System for Quantitative Susceptibility Testing of Aminoglycosides Against Multidrug-Resistant, Gram-Negative Bacilli

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Susceptibility of clinical isolates of *Pseudomonas aeruginosa* (29 isolates), *Klebsiella* species (54 isolates), *Escherichia coli* (28 isolates), *Serratia marcescens* (28 isolates), and *Enterobacter* species (29 isolates) to gentamicin, tobramycin, and amikacin was determined by the following three methods: commercial broth microdilution trays, standard agar dilution, and disk diffusion susceptibility. A total of 504 tests were performed by each method, and overall susceptibility or resistance determined by the broth microdilution method agreed with that determined by the agar dilution method in 92.7% of the tests, whereas results from the disk diffusion method agreed with those from the agar dilution method in 91.9% of the tests. The broth microdilution and disk diffusion methods agreed with each other 88.7% of the time. The broth microdilution system results varied from the agar dilution method results by more than one dilution in 121 of 504 determinations (24%); however, this altered susceptibility determinations in only 7.3% of the assays. *E. coli* isolates were found to be quantitatively more resistant to the aminoglycosides with the broth microdilution method than with the agar dilution method. In contrast, the broth microdilution method demonstrated *P. aeruginosa* to be quantitatively more susceptible to the aminoglycosides than when the results were obtained by the agar dilution method. The Micro-Media Systems method is economical, reliable, rapid, and simple to perform and yields quantitative minimum inhibitory concentrations.

Quantitative antibacterial susceptibility testing can be performed in broth media by a microdilution technique (1). The developments in this area have been reviewed recently, and a new commercial system has been evaluated (2). Barry et al. (2) evaluated 16 bacterial isolates and found the Micro-Media Systems (MMS) testing method to be equivalent to standard tube dilution tests with gram-positive cocci. Gram-negative rods tested by the MMS method gave results one doubling dilution lower than the standard tube dilution minimum inhibitory concentration (MIC).

The purpose of the present study was to compare the results for the MIC determinations obtained for three aminoglycosides by the MMS method with the standard agar dilution MIC and the standard disk diffusion susceptibility test when performed on a large number of multidrug-resistant, gram-negative bacilli.

### MATERIALS AND METHODS

**Test strains.** A total of 168 gram-negative bacilli which had been saved by storage at  $-76^{\circ}\text{C}$  (6) from patients with multidrug-resistant, gram-negative rod

infections were tested (7). The organisms tested were a combination of selected isolates which had been previously found to be either susceptible to aminoglycosides or resistant to gentamicin and tobramycin. There were 29 isolates of *Pseudomonas aeruginosa*, 54 of *Klebsiella* (*K. pneumoniae* and *K. oxytoca*), 28 of *Escherichia coli*, 28 of *Serratia marcescens*, and 29 of *Enterobacter* species. Susceptibility of each of these strains to gentamicin, tobramycin, and amikacin was investigated by the MMS method, the standard agar dilution method, and the standard disk diffusion method. Tests were also performed on *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, which were used as reference bacteria.

**Microdilution susceptibility tests.** Sets of commercially available MMS trays were purchased from the nearest distribution center. Susceptibility tests were performed according to the manufacturer's instructions. The inoculum is prepared by picking four to five isolated colonies from an isolation plate and placing them into a tube containing 0.5 ml of brain heart infusion broth. This broth is then incubated for 4 to 6 h to achieve a stationary growth phase. The inoculum is then diluted by placing 0.05 ml of the brain heart infusion broth culture into a tube containing 25 ml of sterile distilled water supplemented with 0.02% Tween 80. The seed trough of the MMS tray is then filled with the 25-ml suspension, and the sample

wells containing antibiotic are inoculated with the organism by use of the transfer lid. This procedure results in a final organism concentration in each well of approximately  $10^5$  viable cells per ml (2). The trays are incubated at 35°C for 15 to 18 h and then examined for bacterial growth in each well. The MIC is read as the lowest drug concentration which completely inhibits bacterial growth. All isolates were considered susceptible to gentamicin or tobramycin if the MIC was less than or equal to 4 µg/ml for these aminoglycosides and less than or equal to 16 µg/ml for amikacin. Organisms with MICs greater than these were considered resistant.

**Agar dilution method.** The International Collaborative Study Group agar dilution method used was the one described by Washington and Barry (5, 12). Antibiotic dilutions were prepared in Mueller-Hinton agar, and an inoculum containing approximately  $2 \times 10^6$  to  $4 \times 10^6$  viable colonies of each organism per ml was used for plating the bacteria with a Steers replicator (11). This process delivers  $10^3$  to  $10^4$  viable organisms to a spot 5 to 8 mm in diameter. The agar plates are incubated at 35°C for 16 to 20 h, and the MIC is read as the lowest concentration of antimicrobial agent which completely inhibits growth of the bacterial isolate (12). All isolates were considered susceptible to gentamicin or tobramycin if the MIC was less than or equal to 4 µg/ml for these aminoglycosides and less than or equal to 16 µg/ml for amikacin. Organisms with MICs greater than these were considered resistant.

**Disk diffusion method.** The disk diffusion tests were performed by the method of Bauer et al. as described by Matsen and Barry (3, 8). The test organism is grown in broth culture and diluted in saline to a turbidity of a one-half no. 1 McFarland standard. A sterile, cotton-tipped applicator is then used to spread the inoculum on Mueller-Hinton agar plates, and the plate is allowed to dry for 3 to 5 min.

The antimicrobial disks containing 10 µg of gentamicin, 10 µg of tobramycin, or 10 µg of amikacin are then placed on the agar plates and incubated in an inverted position for 16 to 18 h at 35°C. The plates are then removed, and zones of growth inhibition surrounding each antibiotic-containing disk are read to the nearest millimeter. Organisms with zone diameters of less than or equal to 12 mm for gentamicin, 11 mm for tobramycin, and 9 mm for amikacin were considered resistant to these drugs, those with zone diameters equal to or greater than 13 mm for gentamicin, 14 mm for tobramycin, and 12 mm for amikacin were considered susceptible, and those with intermediate zones were considered intermediately susceptible (9, 13). A comparison of susceptibility data from this method with the other two methods was made by defining organisms as either susceptible or not susceptible (intermediately susceptible plus resistant bacteria).

## RESULTS

**Microdilution versus agar dilution.** The comparative aminoglycoside MIC determinations found for each group of microorganisms with the two methods are shown in Table 1. By

the MMS method, most isolates of *P. aeruginosa* were found to have an MIC one to three doubling dilutions more susceptible than that determined by the agar dilution technique. Numerous *E. coli* isolates were found to be one to three doubling dilutions more resistant by MIC determinations made with the MMS method as compared with the agar dilution method. These differences were mainly below the susceptibility breakpoints for these organisms and therefore did not affect the actual determination of susceptibility or resistance (Table 2). There were no differences between the two methods in MIC determinations for the other microorganisms. The percent agreement among the three methods for each aminoglycoside and for the total 504 determinations is shown in Table 2.

By the MMS method, *P. aeruginosa* ATCC 27853 organisms were found to be susceptible to gentamicin at 0.5 µg/ml, to tobramycin at  $\leq 0.25$  µg/ml, and to amikacin at  $\leq 1.0$  µg/ml, whereas by the agar dilution method, the MICs were 2.0, 0.25, and 2.0 µg/ml, respectively. In the tests of *E. coli* ATCC 25922 by the MMS method, the MICs were found to be 0.5 µg/ml for gentamicin and tobramycin and  $\leq 1.0$  µg/ml for amikacin. By agar dilution testing, *E. coli* ATCC 25922 organisms were found to be susceptible to 0.5 µg of gentamicin and tobramycin per ml and to 1.0 µg of amikacin per ml.

## DISCUSSION

The routine performance of quantitative MIC determinations is being increasingly seen as both desirable and readily achievable with the development of simplified and automated methods. The MMS method is simple to perform and has been shown to be reproducible and comparable to the International Collaborative Study Group macrodilution broth technique (2). We have compared the MMS method with the agar dilution method and with disk diffusion susceptibility on 168 isolates of gram-negative bacteria isolated from patients in an institution with numerous multidrug-resistant bacteria (7). The system performed well in comparison to standard methods when susceptibility testing of aminoglycosides was evaluated. Overall agreement of the three methods with each other ranged from 88.7 to 92.7% and is comparable to previously published reproducibility between the standard International Collaborative Study Group agar dilution method and disk diffusion results of 90% (4). Quantitative differences between MMS and agar dilution testing are most likely to occur with *P. aeruginosa* and *E. coli*, with *P. aeruginosa* appearing more susceptible and *E. coli* appearing more resistant when tested

TABLE 1. Comparison of susceptibility results obtained by the MMS method with those obtained by the agar dilution method

Species	Total no. of isolates	Agar dilution MIC range ( $\mu\text{g/ml}$ )	Correlation (no. of strains) between agar dilution and MMS MICs <sup>a</sup>						
			$\leq -8$	-4	-2	0	+2	+4	$\geq +8$
<i>P. aeruginosa</i>									
Gentamicin	29	0.5->128	16	7	4	2	0	0	0
Tobramycin	29	<0.25->128	8	11	8	2	0	0	0
Amikacin	29	0.5->128	5	14	8	2	0	0	0
<i>S. marcescens</i>									
Gentamicin	28	<0.5-64	1	3	10	14	0	0	0
Tobramycin	28	0.5->128	0	0	9	19	0	0	0
Amikacin	28	<0.5-128	1	0	7	15	4	0	1
<i>Klebsiella</i>									
Gentamicin	54	<0.5-128	0	5	10	36	2	1	0
Tobramycin	54	0.5-128	1	7	33	13	0	0	0
Amikacin	54	<0.5-4	0	0	9	43	2	0	0
<i>E. coli</i>									
Gentamicin	28	<0.25-16	0	1	4	8	7	1	7
Tobramycin	28	0.25-32	0	2	2	9	7	1	7
Amikacin	28	0.5->128	1	0	7	11	4	1	4
<i>Enterobacter</i>									
Gentamicin	29	0.5-2	0	3	14	9	2	0	1
Tobramycin	29	0.25-2	1	3	13	8	2	1	1
Amikacin	29	1->128	0	5	9	14	1	0	0
Total	504	<0.25->128	34	61	147	205	31	5	21
% of total tests			6.7	12.0	29.2	40.7	6.2	1.0	4.2

<sup>a</sup> Negative numbers indicate MMS MICs less than agar dilution MICs by the stated doubling dilution, and positive numbers indicate MMS MICs greater than agar dilution MICs by the stated doubling dilution.

TABLE 2. Comparison of susceptibility results of gentamicin, tobramycin, and amikacin for all isolates as performed by each method (504 total determinations)

Antibiotic	Method	% Agreement	
		MMS	Agar dilution
Gentamicin	Agar dilution	88.1	100
	Disk diffusion	86.9	89.3
Tobramycin	Agar dilution	93.5	100
	Disk diffusion	89.9	94.6
Amikacin	Agar dilution	96.4	100
	Disk diffusion	89.3	91.7
Total	Agar dilution	92.7	100
	Disk diffusion	88.7	91.9

with the MMS method. The medium contained in the MMS trays is not supplemented with magnesium or calcium, and this has been demonstrated to affect susceptibility testing of *P. aeruginosa* with aminoglycosides (10). The difference in susceptibility of *P. aeruginosa* ATCC 27853 to the aminoglycosides when tested in

either the MMS or agar dilution system demonstrated the change in susceptibility observed when testing is performed in unsupplemented broth. We are uncertain as to the reason for the modest increase in MIC determinations seen in the MMS method when testing *E. coli*. Hospitals treating large numbers of patients infected with *P. aeruginosa* may need to supplement the MMS trays, as suggested by Reller et al. (10). We conclude that the MMS microdilution test of aminoglycosides against gram-negative bacilli performed satisfactorily and was equivalent to other standard susceptibility techniques.

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#### LITERATURE CITED

1. Barry, A. L. 1976. The antimicrobial susceptibility test: principles and practices, p. 95-99. Lea & Febiger, Philadelphia.
2. Barry, A. L., R. N. Jones, and T. L. Gavan. 1978. Evaluation of the Micro-Media System for quantitative antimicrobial drug susceptibility testing: a collaborative study. *Antimicrob. Agents Chemother.* 13:61-69.
3. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M.

- Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *J. Clin. Pathol.* **45**:493-496.
4. Butler, D. A., and T. L. Gavan. 1977. The clinical laboratory as an aid to the chemotherapy of infectious disease, p. 53-77. University Park Press, Baltimore.
  5. Ericsson, H. M., and J. C. Sherris. 1971. Antibiotic sensitivity testing. Report of an International Collaborative Study. *Acta Pathol. Microbiol. Scand. Sect. B Suppl.* 217.
  6. Feltham, R. K. A., A. K. Power, P. A. Pell, and P. H. A. Sneath. 1978. A simple method for storage of bacteria at  $-76^{\circ}\text{C}$ . *J. Appl. Bacteriol.* **44**:313-316.
  7. Gerding, D. N., A. E. Buxton, R. A. Hughes, P. P. Cleary, J. Arbaczawski, and W. E. Stamm. 1979. Nosocomial multiply resistant *Klebsiella pneumoniae*: epidemiology of an outbreak of apparent index case origin. *Antimicrob. Agents Chemother.* **15**:608-615.
  8. Matsen, J. M., and A. L. Barry. 1974. Susceptibility testing: diffusion test procedures, p. 418-427. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
  9. National Committee for Clinical Laboratory Standards. 1976. Performance standards for antimicrobial disk susceptibility tests. National Committee for Clinical Laboratory Standards, Los Angeles.
  10. Reller, L. B., F. D. Schoenknecht, M. A. Kenny, and J. C. Sherris. 1974. Antibiotic susceptibility testing of *Pseudomonas aeruginosa*: selection of a control strain and criteria for magnesium and calcium content in media. *J. Infect. Dis.* **130**:454-463.
  11. Steers, E., E. L. Foltz, B. S. Graves, and J. Riden. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother. (Basel)* **9**:307-311.
  12. Washington, J. A., II, and A. L. Barry. 1974. Dilution test procedures, p. 410-417. In E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
  13. Washington, J. A., II, P. K. W. Yu, T. L. Gavan, F. D. Schoenknecht, and C. Thornsberry. 1979. Interpretation of the disk diffusion susceptibility test for amikacin: report of a collaborative study. *Antimicrob. Agents Chemother.* **15**:400-407.