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Microdilution Aminoglycoside Susceptibility Testing of *Pseudomonas aeruginosa* and *Escherichia coli* with a Cation-Supplemented Inoculum

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The use of cation supplementation in aminoglycoside susceptibility testing of *Pseudomonas aeruginosa* by microdilution produced MIC agreement (± one doubling dilution) with agar dilution testing 89% of the time as compared with 35% of the time with unsupplemented controls.

Quantitative antibacterial susceptibility testing can be performed in broth with microdilution trays (1). In a previous paper (5), we demonstrated that the microdilution minimum inhibitory concentration (MIC) results compared well with results by agar dilution for most enteric organisms tested (*Serratia marcescens*, *Klebsiella*, and *Enterobacter*). However, *Pseudomonas aeruginosa* was one to three doubling dilutions more susceptible than shown by agar dilution MICs, and *Escherichia coli* was one to three doubling dilutions more resistant than shown by agar dilution MICs (5). Variation in divalent cation (calcium and magnesium) concentration in agar and broth has been cited as a factor that affects suceptibility results of aminoglycoside testing of *P. aeruginosa* (6). The Micro-Media Systems (MMS) microdilution method used in this and the previous study uses unsupplemented broth. However, the manufacturer currently provides a procedure for incorporating magnesium (Mg^{2+}) and calcium (Ca^{2+}) into the inoculation suspension.

The purpose of this study was to compare the MIC determinations obtained for three aminoglycosides by the MMS method with both Mg^{2+}- and Ca^{2+}-supplemented and unsupplemented inocula with standard agar dilution MICs. A total of 28 isolates of *P. aeruginosa* and 23 isolates of *E. coli* which had been saved by storage at −76°C (3) from patients with multidrug-resistant gram-negative bacillary infections were tested (4). The susceptibility of each of these strains to gentamicin, tobramycin, and amikacin was investigated with cation-supplemented and unsupplemented inocula by the MMS method and compared with the standard agar dilution method.

The methods for performing the unsupplemented MMS and agar dilution MIC tests are described in a previous report (5). The supplemented inoculum was prepared by picking four or five pure colonies from an isolation plate and placing them into a tube containing 0.5 ml of brain heart infusion broth. This broth was then incubated for 4 to 6 h to achieve a stationary growth phase which approximates the turbidity of a no. 5 McFarland turbidity standard. The inoculum was then diluted by placing 0.05 ml of the sample in brain heart infusion broth into a tube containing 25 ml of sterile distilled water supplemented with 0.02% Tween 80, calcium, and magnesium. The cation-supplemented water contained 3.67 g of calcium chloride ($CaCl_2 \cdot 2H_2O$) and 4.18 g of magnesium chloride ($MgCl_2 \cdot 6H_2O$) per liter of water. These amounts of calcium and magnesium provided a final microtiter well concentration of 50 and 25 μg/ml, respectively, for each cation. The seed trough of the MMS tray was then filled with the 25-ml suspension, and the sample wells containing antibiotic were inoculated with the organism by use of the transfer lid. This procedure resulted in a final concentration in each well of approximately 10^5 viable bacteria per ml (2). The transfer lid contained multiple prongs, each of which retains about 5 μl of bacterial suspension after being placed in the seed trough and delivers this inoculum to each antibiotic-containing well in the microdilution tray. The trays were incubated at 35°C for 15 to 18 h and then examined for bacterial growth in each well. The MIC was read as the lowest drug concentration showing no bacterial growth. MIC breakpoints were the same as those previously described (5).

The results are shown in Table 1. The *P. aeruginosa* susceptibility in supplemented broth had an average of 89% agreement (± one doubling dilution) with agar dilutions (gentamicin, 96%; tobramycin, 93%; amikacin, 79%). The unsupplemented *P. aeruginosa* susceptibility tests agreed overall only 35% (gentamicin, 18%; tobramycin, 43% amikacin, 43%). The *E. coli* MICs in the unsupplemented broth media agreed 97% of

TABLE 1. *Comparison of susceptibility results obtained by the MMS method (supplemented and unsupplemented) with those obtained by the agar dilution method*

Species	Drug tested	Total no. of isolates	Agar dilution MIC range (μg/ml)	Correlation (no. of strains) between agar dilution and MMS MICs[a]						
				≤−8	−4	−2	0	+2	+4	≥+8
P. aeruginosa (unsupplemented)	Gentamicin	28	0.5–>128	14	9	3	2	0	0	0
	Tobramycin	28	0.25–>128	4	12	10	2	0	0	0
	Amikacin	28	0.25–32	1	15	10	2	0	0	0
P. aeruginosa (supplemented)	Gentamicin	28	0.5–>128	0	0	2	14	11	0	1
	Tobramycin	28	0.25–>128	0	1	1	11	14	1	0
	Amikacin	28	0.25–32	0	1	1	6	15	5	0
E. coli (unsupplemented)	Gentamicin	23	0.25–16	0	0	6	12	5	0	0
	Tobramycin	23	0.25–32	0	0	1	9	12	1	0
	Amikacin	23	0.5–2	0	0	4	14	4	1	0
E. coli (supplemented)	Gentamicin	23	0.25–16	0	0	3	15	5	0	0
	Tobramycin	23	0.25–32	0	0	2	9	5	6	1
	Amikacin	23	0.5–2	0	0	3	8	8	4	0

[a] 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.

the time as compared with agar dilution, whereas in supplemented broth, the MMS method agreed with the agar dilution method 84% of the time.

The MIC breakpoints for the determination of susceptibility were 4 μg/ml for gentamicin and tobramycin and 16 μg/ml for amikacin (5). The results for *P. aeruginosa* tested in unsupplemented MMS broth media agreed with the agar dilution results in the determination of susceptibility or resistance 89% of the time with gentamicin, 100% of the time with tobramycin, and 93% of the time with amikacin. The results in supplemented broth showed agreement in the determination of susceptibility or resistance 79% of the time with gentamicin, 96% of the time for tobramycin, and 96% of the time for amikacin. Evaluation of the 23 *E. coli* isolates showed complete agreement in susceptibility determination between the MMS method and the agar dilution method for all aminoglycosides tested in both supplemented and unsupplemented broth media. We conclude that the supplementation of MMS broth will improve the comparison of MIC results obtained with this method and with the agar dilution method for *P. aeruginosa*. The procedure recommended by the manufacturer and used in this study was easy to apply and should be employed for testing *P. aeruginosa* isolates. *E. coli*, as well as other gram-negative enteric bacilli, performed satisfactorily in unsupplemented broth (5).

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