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Microdilution Aminoglycoside Susceptibility Testing of *Pseudomonas aeruginosa* and *Escherichia coli*: Correlation between MICs of Clinical Isolates and Quality Control Organisms

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The monthly variation in geometric mean MICs (GMICs) of amikacin, tobramycin, and gentamicin for *Escherichia coli* and *Pseudomonas aeruginosa* laboratory controls and clinical isolates was followed for 30 months. For 19 months, MICs were determined by using Micro-Media system (MMS; Micro-Media Systems, Inc., Potomac, Md.) microdilution panels, and for the other 11 months, MicroScan (MS; American Scientific Products, McGaw Park, Ill.) MIC test panels were used. The aminoglycoside GMICs for control *E. coli* and *P. aeruginosa* were significantly lower with the MS system than with the MMS system. A significant correlation was observed between the GMICs for controls and clinical isolates more frequently with the MMS system than with the MS system. Differences are believed to be related to the criteria used in the selection of quality control strains.

During an ongoing surveillance program which monitors the susceptibility of microorganisms to antibiotics at the Minneapolis (Minn.) Veterans Administration Medical Center, the monthly geometric mean MICs (GMICs) for clinical isolates were observed to fluctuate (Fig. 1). These variations were dynamic. The purpose of this investigation was (i) to determine whether these fluctuations should have been expected, by looking at the daily performance of reference bacteria, and (ii) to see whether there are any differences in prediction capacity between two commercially available microdilution MIC panels.

MATERIALS AND METHODS

Test strains. Test strains consisted of *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, which were used as daily controls, and clinical isolates of *P. aeruginosa* and *E. coli* submitted to the Clinical Microbiology Laboratory of the Medical Center. Duplicate isolates from individual patients were excluded from data analysis. For 19 consecutive months, the Micro-Media system (MMS; Micro-Media Systems, Inc., Potomac, Md.) was used to determine the MICs for all test strains. During this period, 910 and 1,167 clinical isolates of *P. aeruginosa* and *E. coli*, respectively, were submitted to the laboratory. Then, for the next 11 months, all antibiotic MICs were determined with the MicroScan (MS; American Scientific Products, McGaw Park, Ill.) microdilution method. While the MS system was being used, 715 and 885 clinical isolates of *P. aeruginosa* and *E. coli*, respectively, were submitted.

Susceptibility testing. MMS gram-negative enteric microdilution plates were stored at −20°C until used. Frozen microdilution panels were thawed at room temperature immediately before use. Both the MMS and MS systems were used according to the manufacturers' directions. The MIC was read manually by the technologists and recorded as the lowest concentration of antibiotic which completely inhibited growth of the test organism. For statistical analysis only, test strains with 6-μg/ml MICs of tobramycin and gentamicin were recorded as having MICs of 8 μg/ml, because the MMS MIC test panels did not contain an intermediate well with 6 μg/ml. The MS microdilution method was performed exactly as the MMS method with one exception: the MS MIC test panels were presupplemented with calcium and magnesium rather than supplementing cations with the inoculum, as was the case with the MMS method.

Statistical analysis. Linear regression was determined by the method of least-squares. Tests of significance were performed as described by Colton (3).

RESULTS

The distribution of MICs obtained with the MMS and MS microdilution systems is shown in Table 1. MICs were within one dilution of the model MIC value in more than 95% of the MMS determinations with each antibiotic. The same was true for the MS determinations, except for the amikacin MICs for *E. coli*; 7% of these were out of Veterans Administration Medical Center-accepted control limits. Modal MICs and GMICs of the MMS system were higher than those of the MS system 50% of the time. With one exception, GMIC with the MS system were closer to the modal MICs than with the MMS system. The exception was the amikacin MICs for *E. coli*; the GMIC was midway between two serial dilutions. All GMICs of the MS system were statistically significantly lower than those of the MMS system, except for the gentamicin GMIC for *E. coli*.

The correlation between GMICs for controls and clinical isolates as demonstrated by least-squares regression with these two variables is shown in Table 2. With the MMS system, statistically significant ($P < 0.05$) correlations were found between the GMICs for clinical isolates and controls for all organism-antibiotic combinations except *E. coli*-tobramycin. On the other hand, significant correlation was observed with only one of the organism-antibiotic combinations (*E. coli*-amikacin) with the MS system.

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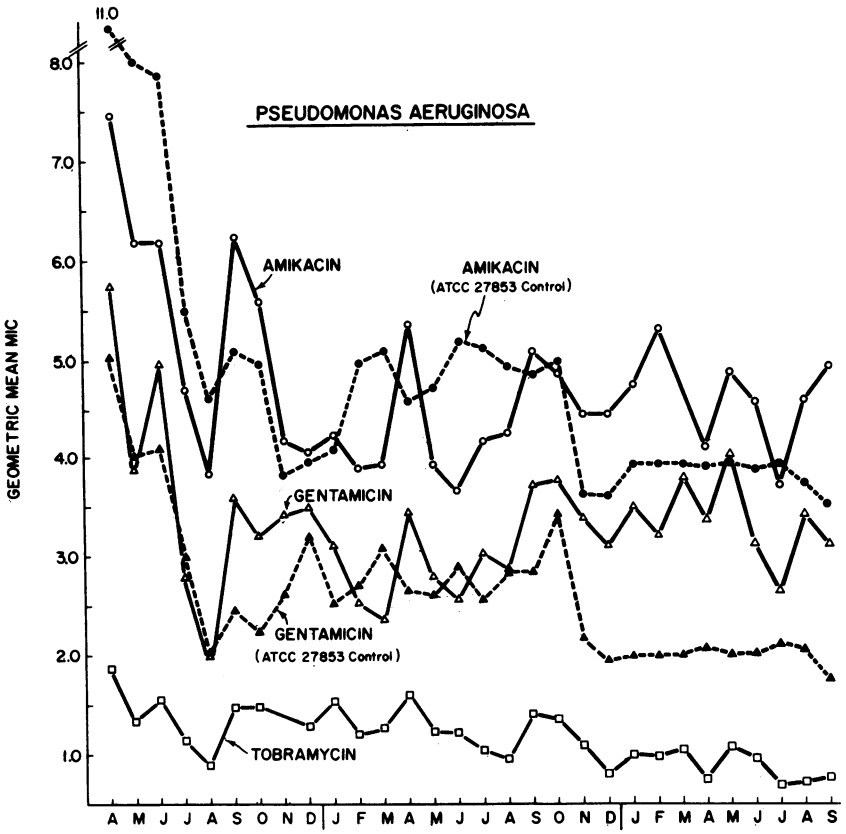

FIG. 1. Monthly variation of amikacin, tobramycin, and gentamicin (open circles, squares, and triangles, respectively) GMICs for clinical isolates and of amikacin and gentamicin (closed circles and triangles, respectively) GMICs for QC organism *P. aeruginosa* ATCC 27853 during 30 months of surveillance.

DISCUSSION

The routine use of commercially available microdilution systems for quantitative MIC determinations has become increasingly common. The attractive qualities of these systems have been reviewed elsewhere (1, 8). A number of reports have shown these systems to be comparable in accuracy and reproducibility (1, 5, 10). Our experience with the MMS and MS systems shows that each method is in compliance with the standards of the National Committee for Clinical Laboratory Standards for quality control (QC) performance (6). Although it is similar to other reports, we believe this study uniquely shows, first, that the GMICs and modal MICs for the QC organisms were significantly different between the MMS and MS systems and, second, that there was a significant correlation between GMICs for QC strains and clinical isolates with a microdilution system (Fig. 1; Table 2).

When changing from the MMS to the MS system, our laboratory compared the two systems on a limited basis and found them similar (unpublished data). However, after extended use of each, we found that the GMICs and modal MICs for reference strains of *E. coli* and *P. aeruginosa* were, with one exception, significantly lower with the MS system. Although the reasons for this difference are purely speculative, several appear to be likely, including medium differences (2, 4, 7, 8), the method of calcium and magnesium supplementation, subtle variations in transfer lids, seed troughs, or panel well shape, and various antibiotic concentrations within the wells. This study was unable to explain the difference in QC modal MICs; however, the important implication is that clinical isolate MICs would be affected in a similar fashion.

Although observed only once with the MS system, there was a strong correlation between QC GMICs and those for clinical isolates with the MMS system (Table 2; Fig. 1). This was observed most often when the GMIC varied 10% or more from the modal MIC, and less than 85% of the determinations were modal. First, we suspect that this may reflect how responsive the reference strains are to fluctuations in the systems used. This is consistent with the observations of Woolfrey et al., who showed that system performance was slightly different with selected strains of *P. aeruginosa* (9, 10). For example, with the MMS system, the QC GMICs were often between serial dilutions, thereby making them very responsive to small changes in the system. The one time this was observed with the MS system, when the GMIC of amikacin for *E. coli* was 1.5 μg/ml, there was also significant correlation (Tables 1 and 2). Also, we suspect that the QC organisms used in this study are not able to show variations in the MS system, because the lowest dilution concentrations may be greater than the actual MICs for the control organisms. The modal MIC (Table 1) in four of six cases was equal to the lowest serial dilution concentration.

In conclusion, we found that QC MICs from the MS and MMS systems were statistically different and that variations in GMICs for clinical isolates are often mimicked by the GMICs for control strains. In our estimation, both observations are at least in part related to the criteria used in the selection of the QC strains. The methodology of one of the

TABLE 1. Variation in QC microdilution MIC determinations

Species, method, and drug	MIC (μg/ml)		% Modal[a]	% At the following no. of dilution(s) from mode:	
	Geometric mean	Modal		One	Two
E. coli ATCC 25992					
MMS[b]					
Gentamicin	0.57	0.5	85	13	2
Tobramycin	0.88	1.0	55	45	0
Amikacin	2.04	2.0	64	33	3
MS[c]					
Gentamicin	0.54	0.5	92	7	1
Tobramycin	0.54[d]	0.5	91	8	1
Amikacin	1.50[d]	1.0	56	37	7
P. aeruginosa ATCC 27853					
MMS[b]					
Gentamicin	2.99	2.0	46	50	4
Tobramycin	0.97	1.0	79	20	1
Amikacin	5.24	4.0	53	46	1
MS[c]					
Gentamicin	1.99[d]	2.0	93	6	1
Tobramycin	0.51[d]	0.5	99	1	0
Amikacin	3.79[d]	4.0	90	10	0

[a] Percentage of MICs that were modal.
[b] Nineteen months of daily control data (n = 484).
[c] Eleven months of daily control data (n = 315).
[d] Significantly different from MMS ($P < 0.05$).

TABLE 2. Regression of monthly GMICs of QC with GMICs of clinical isolates of *E. coli* and *P. aeruginosa*

System and organism	Slope of MIC regression line (P) for:		
	Gentamicin	Tobramycin	Amikacin
MMS			
E. coli	0.91 (0.002)	0.34 (NS)[a]	0.99 (0.0002)
P. aeruginosa	0.70 (0.0008)	1.26 (0.001)	0.62 (0.0009)
MS			
E. coli	−0.32 (NS)	0.98 (NS)	0.55 (0.04)
P. aeruginosa	0.33 (NS)	1.17 (NS)	−0.09 (NS)

[a] NS, No significant correlation.

systems (MMS) used in this study has since been changed. Nevertheless, we recommend caution when comparing epidemiologic data generated by different microdilution systems. QC results should be carefully monitored, preferably with control organisms well within the dilution ranges of the antimicrobial agents under consideration.

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LITERATURE CITED

1. **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.
2. **Cherne, J. E., and L. R. Peterson.** 1980. Microdilution aminoglycoside susceptibility testing of *Pseudomonas aeruginosa* and *Escherichia coli* with a cation-supplemented inoculum. Antimicrob. Agents Chemother. **18**:210–211.
3. **Colton, T.** 1974. Statistics in medicine, p. 185–216. Little, Brown & Co., Boston.
4. **Garrod, L. C., and P. M. Watersworth.** 1969. Effect of medium composition on the apparent sensitivity of *Pseudomonas aeruginosa* to gentamicin. J. Clin. Pathol. **22**:534–538.
5. **Gavan, T. L., R. N. Jones, and A. L. Barry.** 1980. Evaluation of the Sensititre system for quantitative antimicrobial drug susceptibility testing: a collaborative study. Antimicrob. Agents Chemother. **17**:464–469.
6. **National Committee for Clinical Laboratory Standards.** 1983. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, vol. 3, no. 2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Pollock, H. M., B. H. Minshew, M. A. Kenny, and F. D. Schoenknecht.** 1978. Effect of different lots of Mueller-Hinton agar on the interpretation of the gentamicin susceptibility of *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **14**:360–367.
8. **Thornsberry, C.** 1985. Automated procedures for antimicrobial susceptibility tests, p. 1015–1018. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society of Microbiology, Washington, D.C.
9. **Woolfrey, B. F., J. M. K. Fox, R. T. Lally, and C. O. Quall.** 1982. Broth microdilution testing of *Pseudomonas aeruginosa* and aminoglycosides: need for employing dilutions differing by small arithmetic increments. J. Clin. Microbiol. **16**:663–667.
10. **Woolfrey, B. F., R. T. Lally, and C. O. Quall.** 1983. Comparative evaluation of the Micro-Media system, Sceptor, and MIC-2000 microdilution methods for testing *Pseudomonas aeruginosa* against gentamicin, tobramycin, and amikacin. J. Clin. Microbiol. **17**:312–316.