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Evaluation of the AutoMicrobic System for Susceptibility Testing of *Pseudomonas aeruginosa* to Gentamicin, Tobramycin, and Amikacin

BERT F. WOOLFREY,* RICHARD T. LALLY, MARY NEILS EDERER, AND CHARLES O. QUALL

Clinical Microbiology Section, Department of Anatomic and Clinical Pathology, St. Paul-Ramsey Medical Center, St. Paul, Minnesota 55101

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The AutoMicrobic system (AMS; Vitek Systems, Inc., Hazelwood, Mo.) was studied for its ability to produce accurate and precise MIC interpretations for *Pseudomonas aeruginosa* susceptibility to gentamicin, tobramycin, and amikacin. MICs were determined in parallel on 200 selected *P. aeruginosa* isolates by using the AMS discrete-integer MIC program AMS p12.ROB for interpretation of the AMS Gram-Negative General Susceptibility Urinary Card, and a reference small-integer broth microdilution test. Parallel AMS and broth microdilution MICs were also replicated for three selected strains of *P. aeruginosa* for which MICs were representative of the dilution test ranges. For the 200 *P. aeruginosa* isolates, mean AMS MICs were significantly larger than the reference test mean MICs, coefficients of variation were approximately double those of the reference test, and correlation coefficients were unacceptably low for each antimicrobial agent. MIC replication studies for the three selected *P. aeruginosa* strains showed comparable AMS and reference mean MICs in the lower portions of the dilution ranges, significantly higher AMS mean MICs in the upper portions, and mean coefficients of variation of 63 and 9.6%, respectively, for replicated AMS and reference MICs. These results indicate that the AMS, in its present stage of development, does not produce acceptable MIC measurements for *P. aeruginosa* susceptibility to gentamicin, tobramycin, and amikacin.

The AutoMicrobic system (AMS, Vitek Systems, Inc., Hazelwood, Mo.) is a highly automated, commercially available system designed to provide rapid identification and susceptibility testing of most aerobic and facultative bacteria encountered in a clinical microbiology setting. Since its introduction, the AMS has been variously evaluated in the clinical setting and has received generally favorable reports relative to identification and susceptibility testing for *Enterobacteriaceae* (2, 3, 5, 7, 9, 12), nonfermenters (11, 18), and gram-positive cocci (1, 16), and for use as a rapid approach for detection, identification, and susceptibility testing of urinary tract pathogens (4, 8, 10, 14, 17). The present investigation examines the AMS for MIC testing of *Pseudomonas aeruginosa* susceptibility to the aminoglycosides gentamicin, tobramycin, and amikacin; the results of this testing are known to be highly dependent on inoculum size and media characteristics, especially the concentrations and ratios of total, soluble, and ionized calcium and magnesium (5, 6, 13, 15).

MATERIALS AND METHODS

Experimental design. Parallel MIC determinations were made on the susceptibility of 200 stock *P. aeruginosa* clinical isolates to gentamicin, tobramycin, and amikacin, by using the AMS Gram-Negative General Susceptibility Card (GSU) and MIC-2000 (Dynatech Laboratories, Inc., Alexandria, Va.) broth microdilution reference panels. Data for the resultant AMS and on-scale MIC-2000 paired MICs were analyzed by conventional methods for regression and correlation characteristics. Replicability of AMS MICs was evaluated and compared with that of MIC-2000 values by performing paired MIC determinations from 50 separate inoculum preparations for each of three *P. aeruginosa* strains chosen because MICs for them represented the full extent of the reference dilution ranges.

* Corresponding author.

***P. aeruginosa* strains.** A total of 200 previously well-characterized clinical isolates of *P. aeruginosa* were chosen from the St. Paul-Ramsey Medical Center Clinical Microbiology Laboratory (St. Paul, Minn.) stock culture collection for parallel testing with the AMS-GSU card and the MIC-2000 broth microdilution reference panels. Test isolates were selected so that MICs for gentamicin, tobramycin, and amikacin would be distributed throughout the concentration ranges of the reference MIC-2000 broth microdilution tests. Isolates were retrieved from frozen stock culture by sampling with a hot wire loop and streaking on a sheep blood agar plate which was incubated in air at 35°C for 24 h. The center of a well-isolated colony on each stock culture retrieval plate was touched with an inoculating wire and streaked on a second blood agar plate which was also incubated in air at 35°C for 24 h. From each of these subculture plates, several well-isolated colonies were used as a common growth source to initiate the appropriate inoculum suspension required by each system.

Three separate strains of *P. aeruginosa* were chosen for which MIC were representative of the full extent of the MIC-2000 reference dilution ranges. Two of these test strains were obtained from the St. Paul-Ramsey Medical Center Clinical Microbiology Laboratory stock culture collection, and the well-characterized MIC for them were determined in previous comparative studies (20). These two strains, designated SPR-88 and SPR-287, were retrieved from frozen stock culture by the sampling technique described above. The third test strain, *P. aeruginosa* ATCC 27853, was inoculated into 5 ml of tryptic soy broth by using BACTROL disks (Difco Laboratories, Detroit, Mich.). After 4 h incubation in air at 35°C, a loopful of the tryptic soy broth suspension was streaked onto a sheep blood agar plate and incubated in air at 35°C for 24 h. The center of a well-isolated colony was touched with an inoculating wire and streaked on a second blood agar plate, also incubated in air at 35°C for 24 h. Appropriate inoculum suspensions were prepared from these plates as described above. Each of the three strains

TABLE 1. Comparison of MIC characteristics and summary of regression and correlation results for paired MICs determined by the AMS and MIC-2000 system

Antimicrobial agent (no. of isolates)[a]	MIC test system	Mean MIC (μg/ml)	SD	CV[b]	Mean MIC ratio[c]	Slope	r[d]
Gentamicin (122)	AMS	8.6	5.5	63.9	1.4	1.36	0.58
	MIC-2000	6.1	2.3	37.7			
Tobramycin (167)	AMS	4.1	2.5	61.0	1.7	1.2	0.38
	MIC-2000	2.4	0.76	31.7			
Amikacin (178)	AMS	21.8	18.4	84.4	2.1	2.5	0.61
	MIC-2000	10.6	4.4	41.5			

[a] Number of isolates for which both AMS and MIC-2000 MICs were on-scale values.
[b] Coefficient of variation.
[c] $\sqrt{\text{AMS MIC} \div \text{MIC-2000 MIC}}$ for each data pair ÷ total number of on-scale data pairs for each antimicrobial agent.
[d] Correlation coefficient for paired on-scale AMS and MIC-2000 MICs. The probability that the derived correlation coefficient may actually be found for a population exhibiting no correlation was <0.001 for all three antimicrobial agents.

was retrieved from the designated source weekly, and five separate inoculum suspensions were prepared from each secondary subculture plate for the three strains. These were tested daily along with 70 to 90 of the 200 different *P. aeruginosa* isolates described above and served as quality control checks for each system.

AMS Vitek. To facilitate testing of a large range of MICs of gentamicin, tobramycin, and amikacin, the AMS-GSU card was chosen for parallel testing with MIC-2000 panels. AMS-GSU cards contain concentrations of gentamicin and tobramycin at 0.5, 2.0, and 8.0 μg/ml and concentrations of amikacin at 2.0, 8.0, and 32.0 μg/ml. AMS Vitek program AMS P12.ROB was used for MIC determinations with discrete MIC designations made by the instrument with a specific programmable designation to achieve the following values: ≤0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 14, 16, 20, 24, and >24 for gentamicin and tobramycin and ≤2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 24, 28, 32, 40, 48, 56, 64, 80, 96, and >96 for amikacin. The inoculum suspension for each test was prepared by carefully selecting several isolated colonies from the second subculture blood agar plate with a sterile applicator and inoculating 1.8 ml of 0.45% sterile saline to achieve a turbidity equivalent to a 1.0 McFarland standard. The applicator was then transferred to 5 ml of cation-standardized Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) for testing with the MIC-2000 system as described below. Each saline suspension was vortexed briefly, and 50 μl was then transferred with a sterile disposable tip on a transfer pipettor to a sterile tube (12 by 75 mm). Sterile 0.45% saline (1.8 ml) was then dispensed into this tube, after which a transfer tube was attached to an AMS-GSU card and placed into the suspension. A positive oxidase reaction was appropriately designated on each card and the card-transfer tube unit was then processed according to the manufacturer's instructions. Isolates were prepared in groups of 10 to 20 to assure rapid processing of inoculum suspensions.

MIC-2000 small-increment broth microdilution reference test. A single lot of small-increment broth microdilution reference panels was prepared by the St. Paul-Ramsey Medical Center Clinical Microbioloty Laboratory by using the MIC-2000 dispenser system. Details of plate production have been described by us previously (20). Values for gentamicin (Schering Corp., Bloomfield, N.J.) and tobramycin (Eli Lilly & Co., Indianapolis, Ind.) included single-integer increments from 1.0 to 16.0 μg/ml, and values for amikacin (Bristol Laboratories, Syracuse, N.Y.) differed by 2.0 μg/ml and ranged from 2.0 to 32.0 μg/ml. Inoculum suspensions prepared from the second sheep blood agar subculture plate, as described above, were incubated for 3 to 5 h in air at 35°C in 5 ml of Mueller-Hinton broth adjusted by us to provide calcium and magnesium concentrations of 5.5 ± 0.5 and 2.5 ± 0.2 mg/dl, respectively (19). These growth suspensions were adjusted to the density of a 1.0 McFarland standard, with similarly cation-standardized Mueller-Hinton broth, followed by an additional 1:10 dilution. Panels were inoculated with the MIC-2000 inoculating apparatus and incubated at 35°C in air for 18 h before interpretation. The MIC was defined as the minimum concentration of an antimicrobial agent which produced no visual turbidity, no clusters or clumps, and no visual opacity of >1 mm in diameter.

RESULTS

Table 1 lists the MIC characteristics and summarizes the correlation and regression results for paired on-scale AMS and MIC-2000 MICs determined by testing the susceptibilities of 200 isolates of *P. aeruginosa* to gentamicin, tobramycin, and amikacin. Mean AMS MICs of gentamicin, tobramycin, and amikacin for the *P. aeruginosa* sample were, respectively, 1.4, 1.7, and 2.1 times greater than the mean MICs determined by the MIC-2000 reference system. As judged by comparing coefficients of variation, dispersion of AMS MICs was approximately twice that which was observed for the MIC-2000 system. Correlation coefficients for

TABLE 2. Summary of the distribution of AMS MICs with respect to paired reference MIC-2000 MICs for the 200 stock *P. aeruginosa*

ΔMIC[a]	No. (%) of paired MICs differing by various ΔMIC increments		
	Gentamicin	Tobramycin	Amikacin
>3	86 (47.3)	46 (28.4)	109 (58.6)
3	9 (4.9)	25 (15.4)	5 (2.7)
2	18 (9.9)	22 (13.5)	20 (10.7)
1	25 (13.7)	15 (9.3)	13 (7.0)
0	19 (10.4)	10 (6.2)	17 (9.2)
−1	10 (5.6)	5 (3.1)	6 (3.2)
−2	7 (3.8)	27 (16.7)	6 (3.2)
−3	5 (2.8)	1 (0.6)	0
>−3	3 (1.6)	11 (6.8)	10 (5.4)

[a] ΔMIC, Difference in small-increment dilution steps for AMS MICs and paired MIC-2000 reference MICs. Small-increment steps for gentamicin and tobramycin = 1.0 μg/ml. Small-increment steps for amikacin = 2.0 μg/ml.

TABLE 3. Comparison of MICs and MIC dispersion characteristics for replicated on-scale AMS and MIC-2000 gentamicin, tobramycin, and amikacin MICs

Strain	Antimicrobial agent	Mean MIC (μg/ml)		Mean MIC ratio[a]	SD (μg/ml)		CV[b]	
		AMS	MIC-2000		AMS	MIC-2000	AMS	MIC-2000
ATCC-27853	Gentamicin	1.4	2.0	0.7	0.8	0	53.6	0
	Tobramycin	1.3	≤1		3.4		257.0	
	Amikacin	3.4	4.0	0.9	0.92	0	26.6	0
SPR-88	Gentamicin	24.0	7.2	3.4	10.4	0.6	69.2	8.3
	Tobramycin	5.0	2.2	2.3	1.3	0.7	25.1	31.9
	Amikacin	21.1	8.9	2.4	10.7	1.0	50.8	11.3
SPR-287	Gentamicin	20.9	12.9	1.6	3.3	1.3	16.0	9.9
	Tobramycin	5.5	4.9	1.2	1.0	0.6	18.0	12.9
	Amikacin	31.6	25.4	1.2	16.4	3.0	51.9	11.9

[a] $\sqrt{(\text{AMS}) \text{ MIC} \div (\text{MIC-2000}) \text{ MIC}}$ for each data pair ÷ total number of on-scale data pairs for each antimicrobial agent.
[b] CV, Coefficient of variation.

the paired MICs were found to be unacceptably low and were, respectively, 0.58, 0.38, and 0.61 for gentamicin, tobramycin, and amikacin.

Table 2 illustrates the dispersion of AMS MICs in relation to those determined by the MIC-2000 reference test. For gentamicin and amikacin, ca. 50% of the AMS MICs were 4 or more dilution increments greater than the corresponding reference MICs.

Table 3 describes the AMS and MIC-2000 systems in their ability to replicate MICs for three *P. aeruginosa* strains for which known MICs represented low, middle, and upper portions of the test ranges. AMS upper range mean MICs tended to be significantly higher than upper-range MIC-2000 mean values, whereas lower-range AMS mean values more closely matched the MIC-2000 values. Dispersion for the replicated AMS MICs was significantly greater than that for replicated MIC-2000 MICs throughout the test range, with mean coefficients of variation being, respectively, 63.1 and 9.6%.

DISCUSSION

The present study was designed to evaluate the ability of the AMS to produce accurate and precise MICs for *P. aeruginosa* versus three commonly used aminoglycosides. The AMS MIC program is capable of reporting values in integer and fractional units, an approach (20) which we feel provides distinct advantages over twofold dilution schemes when testing antimicrobial agents having low toxic/therapeutic ratios. In the present study, the AMS integer MICs were compared with integer MICs produced in parallel by a microdilution reference test employing small arithmetic increment dilutions. We have previously found (21) MICs for the microdilution test to be equivalent to those produced by agar dilution tests and have found (20) small-increment microdilution MIC precision characteristics to be acceptable for use as a reference test.

The present findings demonstrate that for *P. aeruginosa*, the aminoglycoside MICs produced by AMS are distinctly higher than those for the reference test. Mean MICs for the AMS were frequently double those for the reference test, particularly for middle- and upper-range values. The dispersion of AMS MICs was significantly greater than dispersion for the small-increment reference test and obviates the use of the AMS for effective small-integer MIC reporting. We did not attempt to determine the causes, which may be myriad, for the higher and more disperse AMS MICs; however, this might well be related to inoculum and media variations on which the aminoglycoside MICs for *P. aeruginosa* are known to be highly dependent. It is possible that program changes might be used to adjust MICs downward to match those for reference test values. We conclude that, in the present stage of development, the AMS does not produce acceptable MIC measurements for *P. aeruginosa* susceptibility to gentamicin, tobramycin, and amikacin.

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