

Wide Variability in *Pseudomonas aeruginosa* Aminoglycoside Results among Seven Susceptibility Testing Procedures

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Seven commonly used antimicrobial susceptibility testing methods were used to test the susceptibility of 150 isolates of *Pseudomonas aeruginosa* against gentamicin, tobramycin, amikacin, carbenicillin, and piperacillin. Results were compared with respect to the susceptibility characteristics of the population of isolates as defined by each method. Conventional methods included agar disk diffusion and agar dilution, carried out in accordance with current recommendations of the National Committee for Clinical Laboratory Standards, as well as broth microdilution testing with cation-supplemented Mueller-Hinton broth (CSMHB). Methods in which instrumentation was used for result determination included the Autobac I, Avantage, Sensititre Autoreader (using a breakpoint panel at 18 h of incubation), and Vitek (AMS-240, using the GNS susceptibility card). When necessary for comparison, MIC data were converted to categorical interpretations (susceptible, intermediate, and resistant). With respect to gentamicin, no significant differences were noted among the results of disk diffusion, broth microdilution, Sensititre Auto breakpoint, or Vitek methods which characterized 60 to 67% of isolates as susceptible, 16 to 22% as intermediate, and 13 to 17% as resistant. In contrast, agar dilution, Autobac, and Avantage, although yielding gentamicin results similar to those of one another, were each significantly different in result reporting from the other four methods above for gentamicin results, and they characterized the *Pseudomonas* population largely as susceptible (88 to 97%), with 0 to 6% intermediate and only 3% to 6% resistant. More isolates were characterized as being resistant to gentamicin in the Avantage test if an assay broth supplemented with increased amounts of calcium was used. Cation impregnation of Autobac disks did not appreciably change Autobac results. The geometric mean MIC of gentamicin was 4 µg/ml lower in the agar dilution method than in the CSMHB microdilution method, despite monitoring of the agar for cation content through performance disk diffusion testing with *P. aeruginosa* ATCC 27853. Tobramycin activity was greater than gentamicin activity, and susceptibility to tobramycin ranged from 89 to 97%, with few statistically significant differences noted among the seven methods studied. Differences in MIC distribution and geometric mean MIC between agar dilution and CSMHB microdilution testing were minimal and suggested less of a cation influence on tobramycin than gentamicin results. Although amikacin was also more active than gentamicin (83 to 99% of isolates were susceptible), differences in the amikacin results among methods tended to reflect the same trends in reporting as seen with gentamicin testing, with the exception that results of Avantage testing were similar to those of disk diffusion, CSMHB microdilution, Sensititre, and Vitek. A difference in geometric mean MIC of 5 µg/ml between CSMHB testing and agar dilution testing suggested the influence of divalent cations on amikacin results. Few highly significant differences were noted among methods when isolates were tested against carbenicillin and piperacillin, except that Avantage piperacillin results (66% susceptible) and Autobac piperacillin results (98% susceptible) were noticeably different from the percent piperacillin susceptibility (range, 85 to 92%) measured by the other methods. Method-dependent variability among aminoglycoside susceptibility results, particularly when testing gentamicin, prevents meaningful comparison of *Pseudomonas* susceptibility trends among hospitals when different methods are used and promotes confusion and frustration among clinical microbiologists and clinicians owing to the uncertainties of the clinical meaning of these data.

Successful management of infections caused by *Pseudomonas aeruginosa* requires prompt, aggressive, and appropriate antimicrobial therapy. Detecting resistance of this organism in vitro, particularly to the widely used aminoglycoside group of antibiotics, may exert substantial influence on the selection of therapeutic agents by the physician. To generate these data, a variety of antimicrobial susceptibility testing (AST) methods, both conventional and instrument associated, are available to the clinical microbiologist. Despite differences in technical approach, all methods, ideally, should produce similar results and should demonstrate good performance when compared with a chosen reference method.

However, disagreement among results generated by even conventional susceptibility testing methods (i.e., agar dilution, agar disk diffusion, and microdilution or broth microdilution) when testing *P. aeruginosa* has been the subject of numerous reports (2, 5, 22, 23, 28, 30). Efforts to resolve these disagreements have focused on controlling the factors influencing test results, including inoculum size (25, 26) and the divalent cation concentration of the growth medium (4, 12, 20, 22, 24, 27). Consensus groups, such as the National Committee for Clinical Laboratory Standards (NCCLS), have made specific procedural recommendations for each of these reference AST methods to improve intra- and interlaboratory accuracy and precision (16, 17). However, variation in results observed among different methods is likely to persist owing to the inherent difficulties in medium standard-

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ization and definition of an endpoint of a highly dynamic biological process. Furthermore, the introduction of rapid, nonconventional approaches to susceptibility testing has introduced additional variables, such as altered inoculum size, medium content, growth detection mechanism, and incubation interval, which may account for reported discrepancies between newer methods and reference procedures (M. S. Gradus, C. N. Baker, and C. Thornsberry, *Antimicrob. Newsl.* 2:73-82, 1985).

In one of our laboratories, discrepant results were observed between two technically distinct methods used for determining the susceptibility of *P. aeruginosa* to aminoglycoside antibiotics, suggesting a method-dependent variation in results. To confirm this observation, we examined parallel results generated by a variety of routinely utilized susceptibility testing methods for *P. aeruginosa* tested against commonly used aminoglycosides and selected beta-lactam antimicrobial agents. The intent was not to revalidate the methods studied, since the controversies in the susceptibility-testing literature leave no foundation for the adoption of a definitive standard for *Pseudomonas* testing, or to compare the results of a single isolate when tested by multiple methods. Rather, we sought to compare, in a setting reflecting the testing environment of the clinical laboratory, the characterization of a given population of *P. aeruginosa* isolates among such methods with respect to susceptibility and resistance.

MATERIALS AND METHODS

Organisms. A total of 150 clinical isolates of *P. aeruginosa*, identified by standard criteria, were collected. Each of three area hospital microbiology laboratories (The Christ Hospital, Good Samaritan Hospital, and University Hospital, all in Cincinnati) contributed 50 isolates to the study pool. On the basis of susceptibility results from the method routinely used by that laboratory, isolates were selected within each participating laboratory to reflect a variety of categorical susceptibility patterns and the widest possible range of MICs. The sources of these isolates included blood, sputum, wounds, and body fluids, and care was taken not to include more than one isolate from a single patient, regardless of source, in the collection. Following overnight culture on tryptic soy agar supplemented with 5% defibrinated sheep blood (SBA), 5 to 10 isolated colonies of each strain were suspended in a solution of 2% peptone mixed with an equal part of glycerol. Three aliquots of the suspension were prepared and frozen at -70°C . A frozen aliquot of each isolate was distributed to each participating laboratory for testing by one or more designated AST methods.

Before being tested, organisms were thawed and cultured to SBA. These cultures were incubated at 35°C under 5 to 7% carbon dioxide for 18 to 24 h. Following this incubation, the plates were inspected for contamination, and a subculture was made to SBA by using identical culture conditions. All susceptibility testing was performed from this subculture. To mimic routine testing conditions in a clinical laboratory, each isolate was tested only once by each method; repeat testing of an isolate was carried out only when a procedural error or a system failure was recognized. When possible, a common organism suspension was used in one or more parallel AST methods.

Conventional AST methods. (i) Agar disk diffusion was performed and interpreted as described in NCCLS document M2-A3 (17), with commercially prepared Mueller-Hinton agar (MHA) plates (GIBCO Diagnostics, Madison,

Wis.) and antimicrobial disks (Difco Laboratories, Detroit, Mich.) and yielded qualitative data expressed as susceptible, intermediate, and resistant. A single lot of medium was used during the study.

(ii) Agar dilution was performed as described in NCCLS document M7-A (16), with MHA (Difco) prepared in house and supplemented with twofold-decreasing dilutions of the antimicrobial agents gentamicin (Schering Corp., Syracuse, N.Y.), tobramycin (Dista Products Co., Indianapolis, Ind.), amikacin (Bristol Laboratories, Kenilworth, N.J.), carbenicillin (Beecham Laboratories, Bristol, Tenn.), piperacillin (Lederle Laboratories, Carolina, Puerto Rico). Data were expressed as MICs (in micrograms per milliliter). A single lot of MHA was used throughout the study.

(iii) Broth microdilution was performed by using commercially prepared panels (AP02A; Radiometer/Sensititre, Westlake, Ohio). The assay medium consisted of Mueller-Hinton broth (MHB) supplemented with approximately 50 mg of calcium per liter and 25 mg of magnesium per liter. MIC testing was carried out by following the protocol recommended by the manufacturer, based on NCCLS guidelines (16). A single lot of MHB was used throughout the study.

Instrument-read AST methods. All tests with methods in which endpoints were read by an instrument were performed by following the instructions found in the operating manuals of each system. Consumables used were those routinely available from each manufacturer, except where noted. For each method, a single lot number of consumables was used throughout the study.

(i) **Vitek (AMS-240; Vitek Systems, Hazelwood, Mo.).** Testing with the Vitek method was carried out with the GNS card, the data from which were expressed as MICs with interpretations (susceptible, intermediate, or resistant) being assigned by the instrument. The incubation interval for *P. aeruginosa* averaged approximately 10 h.

(ii) **Avantage (Abbott Diagnostic Laboratories, Abbott Park, Ill.).** The incubation interval for *P. aeruginosa* with the Avantage method was approximately 5 h. In addition to the standard assay broth supplied for the Avantage testing, a separate set of experiments was carried out with a specially formulated assay broth supplemented with increased amounts of calcium by Abbott Laboratories. Data were expressed qualitatively as susceptible, intermediate, or resistant.

(iii) **Autobac I (Organon-Teknika, Durham, N.C.).** The incubation interval for *P. aeruginosa* with the Autobac I method was approximately 5 h. In addition to the standard Autobac procedure, a separate set of experiments was conducted by using Autobac disks (specially prepared by the manufacturer) containing various concentrations of gentamicin with or without supplementation with calcium and magnesium. Data were presented qualitatively as susceptible, intermediate, or resistant.

(iv) **Sensititre Autoreader.** The incubation interval for *P. aeruginosa* with the Sensititre Autoreader method was 18 to 24 h. Qualitative data (susceptible, intermediate, or resistant) were generated through the use of the limited-dilution MIC breakpoint plate, ABP3A (Sensititre AutoBP). The assay broth consisted of buffered cation-supplemented MHB (CSMHB) containing 18-h-format fluorogenic substrates.

Quality control procedures. Control organisms were included with each testing run for all methods and included *Escherichia coli* ATCC 25922 and 29194, *Staphylococcus aureus* ATCC 25923 and 29213, and *P. aeruginosa* ATCC 27853, as recommended in the NCCLS guidelines (16, 17) or

TABLE 1. Susceptibility testing of *P. aeruginosa* (150 isolates) against aminoglycoside and beta-lactam antimicrobial agents

Method	Rounded % of isolates per interpretative category ^a for:														
	Gentamicin			Tobramycin			Amikacin			Carbenicillin		Piperacillin			
	S	I	R	S	I	R	S	I	R	S	R	S	I	R	
Agar disk diffusion	60	22	18	94	3	3	83	8	9	80	20	89	3	8	
Agar dilution ^b	88	6	6	95	2	3	95	4	1	83	17	92	3	5	
Broth microdilution ^b	61	24	15	90	5	5	87	7	6	85	15	86	7	7	
Autobac I	97	0	3	97	0	3	99	0	1	88	12	98	1	1	
Avantage	90	5	5	89	6	5	90	7	3	79	21	66	21	13	
Sensititre AutoBP	65	22	13	93	4	3	90	6	4	88	12	85	7	8	
Vitek (AMS) ^b	67	16	17	92	4	4	90	1	9	79	21	NT ^c	NT	NT	

^a S, Susceptible; I, intermediate; R, resistant.

^b Results were originally expressed as MICs (in micrograms per milliliter). Conversion to interpretative categories was made as follows: susceptible, intermediate, and resistant represent MICs of ≤ 4 , 8, and ≥ 16 $\mu\text{g/ml}$, respectively, for gentamicin and tobramycin; ≤ 16 , 32, and ≥ 64 $\mu\text{g/ml}$ for amikacin; ≤ 16 , 32 to 64, and ≥ 128 $\mu\text{g/ml}$ for piperacillin; and susceptible and resistant represent MICs of ≤ 128 and ≥ 256 $\mu\text{g/ml}$ for carbenicillin.

^c NT, Not tested. Piperacillin was not present on the Vitek GNS card.

as suggested by the system manufacturer. Control values falling within accepted ranges were a prerequisite for a valid run. Broth samples from each system were analyzed for total calcium and magnesium concentration by flame atomic absorption; the manufacturers of agar and broth powder supplied cation analyses for their respective products. For control purposes, in-house preparation of MHA for agar dilution testing was subjected to disk diffusion testing with *P. aeruginosa* ATCC 27853 and a 10- μg gentamicin disk (17).

Interpretative criteria. Interpretative criteria for results, as described in NCCLS documents M7-A (16) and M2-3A (17) or published in the package insert of the system manufacturer, were applied to the data generated during testing. For convenience in comparing qualitative results, the moderately susceptible category was converted to intermediate. Disk diffusion, Autobac I, Avantage, and Sensititre AutoBP yielded qualitative results expressed as susceptible, intermediate, or resistant. Agar dilution, broth microdilution, and Vitek yielded quantitative data as MIC (in micrograms per milliliter). When direct comparison was made between the results of qualitative and quantitative testing methods, categorical interpretations based on MICs were made according to NCCLS guidelines (16).

Calculations and statistical analyses. For comparison of differences among the groups of data generated by each method for each antimicrobial agent, the result for each isolate by a given method was paired with the result for the same isolate by the comparison method. The Statpac statistical analysis package (26) was used to determine the significance of observed differences between two methods by Student's *t* test. A probability of less than 0.05 was considered to represent a statistically significant difference between the two methods compared. Analysis was carried out between all possible pairings of methods for each drug studied. For analysis of qualitative data, each interpretative category was converted to a numerical value (susceptible = 1, intermediate = 2, resistant = 3). To determine the geometric mean MICs when comparing quantitative methods, we converted MIC results to $\log_2 + 9$, averaged them, and reconverted them to MICs (25).

RESULTS

Qualitative results—aminoglycoside antimicrobial agents.

Results generated by each test method are shown in Table 1.

For ease of comparison, the MIC results by agar dilution and broth microdilution were converted to an interpretative category by using current NCCLS guidelines (16). These data reveal a technique-dependent difference among results from the seven methods tested, which was especially noticeable when they were tested for gentamicin.

(i) **Gentamicin.** On the basis of the percentage of isolates called susceptible by each method, two groupings of methods were apparent. Autobac, Avantage, and agar dilution were associated with susceptibility percentages ranging from 88 to 97%, whereas agar disk diffusion, broth microdilution, Vitek, and Sensititre AutoBP showed susceptibility percentages ranging from 60 to 67%. The distribution of isolates into either the intermediate or resistant category within each method examined was roughly equivalent. The number of isolates falling into either of these two categories, however, was larger in the second grouping of methods. Among methods considered conventional, there was no statistically significant difference between the data generated by agar disk diffusion and broth microdilution, whereas differences between either of these two methods and the third method, agar dilution, were highly significant ($P < 0.001$) (Table 2). The qualitative results generated by nonconventional systems (in which test endpoints are determined by instrumentation) could be divided into two groupings based on performance. Autobac and Avantage tended to characterize the 150 isolates largely as susceptible (>90%), whereas Vitek and Sensititre AutoBP characterized the population as less susceptible (<67%). Autobac and Avantage results tended to resemble those of agar dilution, whereas Vitek and Sensititre AutoBP results showed no statistically significant differences from either agar disk diffusion or broth microdilution results.

(ii) **Tobramycin.** Method-dependent variation in results was not pronounced when testing tobramycin. Comparison of qualitative results suggested no obvious trends that would allow a grouping of methods based on performance. In fact, there were no statistically significant differences between methods when testing against tobramycin, with the exception of Autobac versus Avantage and Autobac versus Vitek comparison (Table 2), and these differences were not highly statistically different ($P < 0.05$). The population of *P. aeruginosa* isolates was characterized to be largely susceptible to tobramycin, regardless of method.

TABLE 2. Comparison of qualitative AST results between pairs of various AST methods for each drug tested

Systems compared ^a	Significance of differences for following drugs ^b :				
	Gentamicin	Tobramycin	Amikacin	Carbenicillin	Piperacillin
Autobac I vs D-D	<0.001	NS	<0.001	<0.05	<0.05
Autobac I vs BMD ^c	<0.001	NS	<0.001	NS	0.001
Autobac I vs AutoBP	<0.001	NS	<0.05	NS	0.001
Autobac I vs Avantage	NS	<0.05	<0.001	<0.05	<0.001
Autobac I vs Vitek ^c	0.001	<0.05	0.001	<0.05	NT ^d
Autobac I vs agar dil. ^c	<0.05	NS	NS	NS	<0.05
D-D vs BMD	NS	NS	NS	NS	NS
D-D vs AutoBP	NS	NS	NS	<0.05	NS
D-D vs Avantage	<0.001	NS	NS	NS	<0.001
D-D vs Vitek	NS	NS	NS	NS	NT
D-D vs agar dil.	<0.001	NS	NS	NS	NS
BMD vs AutoBP	NS	NS	0.001	NS	NS
BMD vs Avantage	<0.001	NS	NS	NS	<0.05
BMD vs Vitek	NS	NS	NS	<0.05	NT
BMD vs agar dil.	<0.001	NS	NS	NS	NS
AutoBP vs Avantage	<0.05	NS	<0.001	<0.05	<0.05
AutoBP vs Vitek	NS	NS	NS	<0.05	NT
AutoBP vs agar dil.	0.001	NS	NS	NS	NS
Avantage vs Vitek	0.001	NS	NS	NS	NT
Avantage vs agar dil.	0.001	NS	NS	NS	<0.001
Vitek vs agar dil.	<0.001	NS	<0.001	NS	NS

^a D-D, Agar disk diffusion; BMD, broth microdilution; AutoBP, Radiometer/Sensititre Auto Breakpoint; agar dil., agar dilution.

^b Significance is expressed as *P* values. NS, No significant difference between results (*p* > 0.05).

^c Original MIC data converted to susceptible, intermediate, or resistant by following NCCLS guidelines.

^d NT, not tested. Piperacillin was not present on the Vitek GNS card.

(iii) **Amikacin.** As with gentamicin, technique-dependent differences were seen with the amikacin qualitative data (Table 1), although the differences were much less marked than those observed with gentamicin. Disk diffusion, broth microdilution, Avantage, Sensititre AutoBP, and Vitek results were in good agreement with regard to *P. aeruginosa* susceptibility; the population was characterized as being largely susceptible (range, 83 to 90%). No method among this group was found to be statistically different from any other method within this group. However, agar dilution and Autobac results were similar to one another and were significantly different from those of the other methods tested, in that isolates were more likely to be characterized as susceptible, and only one isolate was considered to be resistant.

Qualitative results—beta-lactam antimicrobial agents. Qualitative results of testing the *P. aeruginosa* isolates against the two beta-lactam antibiotics used in this study (carbenicillin and piperacillin) are given in Table 1.

(i) **Carbenicillin.** The Autobac and the Sensititre AutoBP methods tended to produce more susceptible results than the other methods did. Although these results were statistically different from those of other methods, the significance was not high (*P* < 0.05). The results were similar to those of broth microdilution, although the broth microdilution results were calculated to be significantly different only from the Vitek results (*P* < 0.05) when comparison was made beyond Autobac and Sensititre AutoBP.

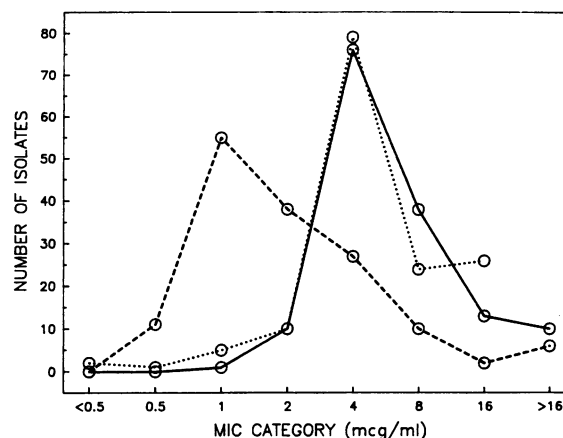


FIG. 1. Comparison of gentamicin results among quantitative AST methods: broth microdilution (—), agar dilution (- - -), and the Vitek system (· · ·). Results are presented as a frequency distribution of MICs for 150 isolates of *P. aeruginosa*. The datum point at 16 μ g/ml for the Vitek system represents isolates for which the MICs are >8 μ g/ml, which is the upper limit of the Vitek GNS card.

(ii) **Piperacillin.** Results with piperacillin showed general agreement among test systems, except for Avantage and Autobac, the results of which were polarized. A high incidence of intermediate and resistant results was reported for the Avantage method (21 and 13%, respectively), whereas Autobac reported only 1% intermediate and 1% resistant results.

Quantitative results—aminoglycoside antimicrobial agents. Among the methods yielding MIC data, differences in the distribution of test results mimicked those noted when comparisons were made between methods based on susceptible, intermediate, and resistant interpretations derived from the MICs. The most striking differences, again, were in the results generated against gentamicin.

(i) **Gentamicin.** A comparison of broth microdilution, agar dilution, and Vitek MIC results for gentamicin, expressed as a frequency distribution of isolates among MICs, is shown in Fig. 1. Agar dilution results showed a fourfold shift downward (toward susceptibility) in the modal MIC for these isolates (from 4 to 1 μ g/ml) when compared with either broth microdilution and Vitek results. The gentamicin geometric mean MIC was accordingly much lower (2 μ g/ml) for agar dilution testing than for either broth microdilution (6.04 μ g/ml) or the Vitek system (5.16 μ g/ml).

(ii) **Tobramycin.** The quantitative data, like the qualitative data, were in fairly close agreement for tobramycin among the three methods (Fig. 2). The modal MIC (1 μ g/ml) was the same for all three methods, and the geometric mean MIC differed by less than 1 dilution (1.41 μ g/ml for agar dilution; 1.65 μ g/ml for broth microdilution; and 1.91 μ g/ml for Vitek).

(iii) **Amikacin.** A comparison of results derived for amikacin by the three quantitative methods is summarized in Fig. 3. The trends were somewhat similar to the gentamicin results with respect to differences among methods. The modal MIC shifted from 2 μ g/ml for agar dilution to 8 μ g/ml for broth microdilution to 16 μ g/ml for the Vitek instrument results. The geometric mean MIC was 1.85 μ g/ml for agar dilution, 7.24 μ g/ml for broth microdilution, and 8.24 μ g/ml for Vitek.

Quantitative results—beta-lactam antimicrobial agents.

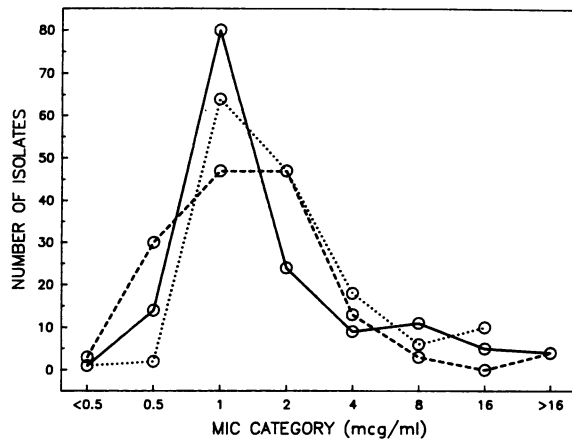


FIG. 2. Comparison of tobramycin results among quantitative AST methods: broth microdilution (—), agar dilution (---), and the Vitek system (···). Results are presented as a frequency distribution of MICs for 150 isolates of *P. aeruginosa*. The datum point at 16 $\mu\text{g}/\text{ml}$ for the Vitek system represents isolates for which the MICs are $>8 \mu\text{g}/\text{ml}$, which is the upper limit of the Vitek GNS card.

Quantitative results for the beta-lactam antimicrobial agents are summarized below.

(i) **Carbenicillin.** The modal MIC with both agar dilution and broth microdilution was 64 $\mu\text{g}/\text{ml}$; the modal MIC with Vitek was 1 dilution lower, at 32 $\mu\text{g}/\text{ml}$, with a somewhat broader distribution in each MIC category than that noted for either of the other two methods. The mean MIC with the three systems differed by less than 1 dilution (agar dilution, 65.9 $\mu\text{g}/\text{ml}$; broth microdilution, 72.3 $\mu\text{g}/\text{ml}$; and Vitek, 80.0 $\mu\text{g}/\text{ml}$). No significant differences were found when the interpretative category results of each system were compared (Table 2).

(ii) **Piperacillin.** Quantitative results for piperacillin testing were available for broth microdilution and agar dilution only. The distribution of results was nearly identical between the two methods, as were modal MICs (4 $\mu\text{g}/\text{ml}$). The geometric mean MIC with broth microdilution (5.48 $\mu\text{g}/\text{ml}$) was only

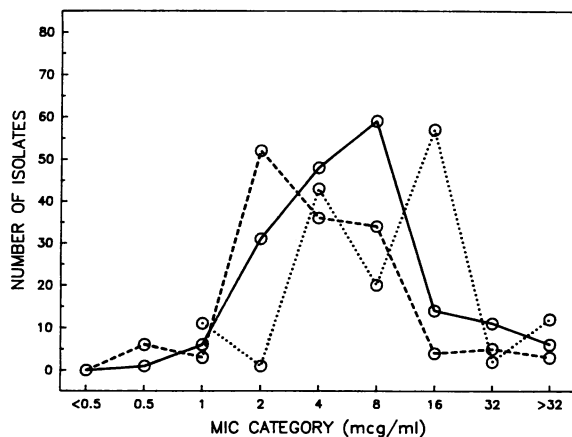


FIG. 3. Comparison of amikacin results among quantitative AST methods: broth microdilution (—), agar dilution (---), and the Vitek system (···). Results are presented as a frequency distribution of MICs for 150 isolates of *P. aeruginosa*. The datum point at 1 $\mu\text{g}/\text{ml}$ for the Vitek system represents isolates for which the MICs are $<2 \mu\text{g}/\text{ml}$, which is the lower limit of the Vitek GNS card.

TABLE 3. Results of *P. aeruginosa* testing on the Autobac I against disks containing various concentrations of gentamicin with and without cation supplementation

Interpretative category	Rounded % of isolates at following disk content (μg):							
	9 ^a	9S ^b	7	7S	5	5S	3	3S
Susceptible	97	97	97	97	96	97	90	89
Intermediate	0	0	0	0	1	0	5	4
Resistant	3	3	3	3	3	3	5	7

^a The standard Autobac I gentamicin disk contains 9 μg of drug with no cation supplementation.

^b S signifies disks of the indicated gentamicin concentration containing a supplement of calcium and magnesium. All disks were supplied by the manufacturer as "experimental."

slightly higher than the mean calculated for agar dilution (5 $\mu\text{g}/\text{ml}$).

Experimental modification of Autobac and Avantage. We compared data generated following technical manipulation of certain components of the Autobac and the Avantage with results generated by using the systems as recommended by the manufacturer.

(i) **Autobac I.** Results were generated with all 150 *P. aeruginosa* isolates by using antimicrobial agent disks (supplied by the manufacturer as experimental disks) containing various concentrations of gentamicin with and without cation supplementation. The distribution of data by interpretative category is shown in Table 3. No differences in performance relative to the standard Autobac disk (9 μg of gentamicin, no cations) were noted until the gentamicin disk concentration was reduced to 3 μg . At this level, there was an increase in the number of isolates characterized as intermediate or resistant. The tendency toward resistant categorization was slightly enhanced when the 3- μg disk contained the cation supplement. Cation supplementation of the Autobac disks, however, had no effect on performance with disk contents of 5, 7, and 9 μg of gentamicin, and no disk examined here gave results comparable to those of agar disk diffusion testing.

(ii) **Avantage.** Results with isolates tested by the Avantage method with an assay broth which contained increased calcium supplementation are shown in Table 4. All three aminoglycosides were tested with this broth. The use of this supplemented broth shifted the category distribution of isolates so that the susceptibility profile of the population when tested against gentamicin and amikacin now more closely resembled the profiles of these drugs when tested by agar disk diffusion. The increased calcium supplementation did not affect tobramycin results relative to those generated in the standard Avantage broth, the results of which approximated those of agar disk diffusion.

Quality control data. Analysis of the calcium and magnesium content of the culture medium component of each method revealed levels in the broths used for Autobac to be 52 and 25 mg/liter, respectively; those for Sensititre AutoBP were 47 and 32 mg/liter, respectively; and those for Sensititre broth microdilution were 46 and 21 mg/liter, respectively. These values approximated those recommended by the NCCLS (50 mg of calcium per liter; 25 mg of magnesium per liter [16]). Although the standard Avantage broth was found to be deficient in calcium as reported by the manufacturer's analysis (20 to 25 mg/liter), magnesium levels were reported to be 20 mg/liter. The specially formulated Avantage assay broth contained increased amounts of calcium (50 mg/liter) only. The agar used in this study had varying concentrations of magnesium and calcium according to the manufacturer's

TABLE 4. Effect of calcium supplementation of broth on susceptibility testing of *P. aeruginosa* (150 isolates) to aminoglycosides by using the Avantage system

Antibiotic and method	Rounded % of isolates in following category:		
	Susceptible	Intermediate	Resistant
Gentamicin			
Agar disk diffusion	60	22	18
Avantage, unsupplemented ^a	90	5	5
Avantage, supplemented ^b	72	10	18
Tobramycin			
Agar disk diffusion	93	4	3
Avantage, unsupplemented ^a	89	6	5
Avantage supplemented ^b	89	5	6
Amikacin			
Agar disk diffusion	83	8	9
Avantage, unsupplemented ^a	90	7	3
Avantage, supplemented ^b	81	7	12

^a Standard broth supplied for the Avantage system.

^b Standard broth to which additional calcium was added by the manufacturer.

analyses. GIBCO agar used for disk diffusion had 19 mg of calcium per liter and 50 mg of magnesium per liter, whereas the Difco agar used for agar dilution had 13 mg of calcium per liter and 73 mg of magnesium per liter. However, despite this variation, the sizes of zones of inhibition produced by the control strain of *P. aeruginosa* when tested on these media against aminoglycoside disks were well within recommended limits. Throughout the study, all control runs of organisms appropriate for each testing method were found to be within published limits (16, 17).

DISCUSSION

Numerous factors besides AST results influence the choice of antimicrobial agents by a physician, including the site and severity of the infection, the clinical and immunological status of the patient, the pharmacology and toxicity of the drug, the clinical experience of the physician in treating certain disease syndromes, and the direct and indirect costs of therapy. However, the AST result remains a prominent and frequently used factor in the selection of a particular antimicrobial agent. Although the microbiologist views the AST as an expression of the in vitro relationship between an organism and a drug tested under a given or standardized set of conditions, there is a tendency among clinicians to assign a predictive therapeutic value to the laboratory results. If in vitro relationships as measured by AST are to be meaningful clinically, a consistent and reproducible laboratory data base is necessary. To that end, the microbiological community has endeavored over the last three decades to emphasize rigid standardization in the testing of bacteria against antimicrobial agents in the clinical laboratory. Despite this effort, the observations of this trial on parallel testing of seven commonly used AST methods, all meeting recommended control criteria, with respect to *P. aeruginosa* and aminoglycoside antimicrobial agents reveal a disconcerting pattern.

In this study, the clinical isolates of *P. aeruginosa* were characterized as largely susceptible to gentamicin by accepted criteria if tested by agar dilution, Autobac, or Avantage. In contrast, when tested by agar disk diffusion, cation-supplemented broth microdilution, Sensititre AutoBP, or

Vitek, the same population appeared to be susceptible in only 60 to 67% of instances, with approximately 20% of isolates falling into an intermediate interpretative category and 13 to 17% of isolates showing resistance. In almost all cases, when the results of any of the first group of methods were compared with the results of any of the second group of methods, statistically significant differences ($P > 0.05$) were calculated (Table 2), suggesting that AST results for gentamicin and *P. aeruginosa* are very much method dependent.

The large number of susceptible characterizations by agar dilution compared with broth microdilution calls attention to the longstanding problem of divalent cation content and its influence on susceptibility testing of aminoglycoside antibiotics against *P. aeruginosa*. The progressive increase in aminoglycoside MIC against *Pseudomonas* spp. as the concentrations of calcium or magnesium, or both, are increased in the test medium has been well documented (6, 10, 11, 14, 31). Broth dilution results for aminoglycosides against *Pseudomonas* spp. generally gave lower MICs than agar dilution testing, presumably because the agar contained more cations than the broth did. These observations led to the recommendation that cation-deficient broth be supplemented with near physiologic levels of calcium and magnesium to bring into better agreement aminoglycoside results of broth and agar methods (15, 22). Although the use of CSMHB has become nearly universal, a recent study by Barry et al. (1) has demonstrated an improved concordance of results between agar dilution and broth dilution methods when testing an aminoglycoside, netilmicin, with a Mueller-Hinton broth having cation levels one-half those currently recommended. Perhaps as a result, the NCCLS has proposed just such a change in cation supplementation for CSMHB in document M7-T2 (19). The impact on results for aminoglycosides against *Pseudomonas* spp. when this altered broth is used may be substantial for both hospital laboratory and industry practices. Shifts are likely to be noted in susceptibility patterns among hospitals testing with the systems based on current CSMHB. Also, formulations of media for commercial microdilution systems and rapid automated methods may require similar alteration and reevaluation to provide results consistent with those obtained by using the newly proposed standard broth.

Assessing the effect of cations in agar on the performance of agar-based methods has been problematic. Pollak and co-workers (12, 20) found that cations may be distributed among insoluble, soluble, and ionized pools in agar, with the biological effect residing probably in the ionized pool, and that the distribution of the added cations among the various pools cannot be easily controlled. Furthermore, Washington et al. (27) have pointed out that the relationship between cation content and MIC seen in broth testing does not necessarily hold when a correlation between the total content of various cations in agar and the performance in agar dilution testing of *Pseudomonas* spp. is attempted. These findings challenge the usefulness of agar cation level analysis as a means of predicting method performance. An alternative approach is to evaluate agar medium based on performance testing with *P. aeruginosa* ATCC 27853. Such a recommendation has been defined in NCCLS document M6-P (18), which encourages standardization of MHA against a national "reference lot," during the manufacturing process by using specified biological performance criteria. The current NCCLS AST recommendations, therefore, do not call for cation supplementation or measurement in agar-based media (16, 17).

In the present study, despite rigid adherence to recommended performance criteria for agar-based media and the presence of physiologic levels of calcium and magnesium in broth-based methods, the results of agar dilution methods differed significantly from those of agar disk diffusion and from that of broth microdilution testing in CSMHB, the last two methods yielding comparable results. Presumably, a cation effect is responsible, indicating that the problem is far from resolved when the current performance criteria approach to medium evaluation is used. Inspection of the frequency distribution of gentamicin MICs between agar dilution and broth microdilution (Fig. 1) clearly shows a shift toward resistance when CSMHB is used. In contrast, such a shift between the two methods was not apparent (data not shown) for carbenicillin or piperacillin MICs, since the testing of beta-lactam antimicrobial agents is largely unaffected by the cation content of media.

The differences in gentamicin results with Autobac or Avantage and those with agar disk diffusion are disquieting, since presumably these rapid turbidometric methods were designed to mimic the performance of standard disk diffusion. Our observations are not surprising, given the findings of Gradus et al. (Antimicrob. Newsl.), who, upon reviewing the literature of studies comparing newer AST methodologies with conventional susceptibility testing, found that the most frequently reported problems were among results of aminoglycoside testing against *Pseudomonas* spp. As with broth dilution, cation supplementation of the standard broth formulation used in the Avantage system caused results, particularly for gentamicin and amikacin testing (Table 4), to shift toward a more resistant characterization of the 150 *P. aeruginosa* isolates. In May 1988, Abbott Laboratories released a newly formulated Avantage assay broth for use with gram-negative organisms, including *P. aeruginosa*. The concentrations of calcium and magnesium in this broth are identical to those in the specially formulated assay broth used in this study. Additional reductions in the inorganic salt concentration of this new gram-negative broth are designed to improve comparative results with beta-lactam antibiotics. The eugonic broth used in the Autobac system was found to have cation concentrations consistent with NCCLS guidelines.

Cation supplementation of Autobac disks had minimal impact on results (Table 3). Only a few isolates were shifted toward resistance, and this occurred only when the gentamicin disk mass was lowered appreciably from the standard 9 to 3 μg . Thus, other mechanisms such as inoculum heterogeneity, described by Mayo et al. (13) as a source of false-susceptible gentamicin results for some isolates with Autobac, may be responsible for this phenomenon. Although no resistant subpopulation was detected among isolates when tested by agar dilution, the phenomenon was specifically not examined in our study. Whatever the mechanism, users of the Autobac method should be aware that estimates of the susceptibility of *P. aeruginosa* to gentamicin are likely to be considerably greater than estimates made by standard disk diffusion or broth microdilution methods using CSMHB.

In contrast, two other instrumentation methods, Sensititre AutoBP and Vitek, produced results comparable to those of the disk diffusion or CSMHB microdilution procedures. In the Sensititre technology, fluorogenic enzyme substrates incorporated into the CSMHB assay medium are hydrolyzed by microbial enzymes produced during growth, releasing a detectable fluorescent product. The test uses overnight incubation of the organism in CSMHB with substrate, and

thus the conditions closely paralleled those of broth microdilution. In a previous report by Dorn et al. (9), a high concordance was found between results of fluorogenic tests and those of manual reading of a parallel series of control breakpoint plates for *P. aeruginosa* testing. Therefore, a similarity of aminoglycoside results from Sensititre AutoBP and conventional microdilution testing in CSMHB was expected. The Vitek GNS card yields results after approximately 10 h of incubation, and these data were comparable to those of disk diffusion or conventional broth microdilution with CSMHB. Because the assay broth used in the Vitek method contains cation supplementation, this agreement is not surprising. Woolfrey et al. (29) have reported that when Vitek results for aminoglycoside activity against *P. aeruginosa* were compared with a small-increment broth microdilution reference, the Vitek results were distinctly higher, but owing to the nature of the present study, this observation could neither be supported nor denied.

The testing of *P. aeruginosa* isolates against tobramycin revealed far less method-to-method variation in results than those observed with gentamicin. Regardless of the method used, the 150 isolates of *P. aeruginosa* were generally categorized as being susceptible to tobramycin. Tobramycin activity against *P. aeruginosa* on a per-unit-weight basis has been shown to be greater than that of gentamicin (3, 21). Since identical MIC breakpoints are used for interpretation of both antimicrobial agents, the generally lower tobramycin MICs are less likely to be subject to interpretation changes due to the influence of cations or other test variables that shift test results toward higher MICs. This would be particularly true for isolates whose resistance is mediated by permeability rather than enzymatic factors, since gentamicin MICs for such isolates tend to be near the interpretation breakpoints. In comparing the distribution of MICs resulting from agar dilution versus CSMHB microdilution testing, differences are far smaller with tobramycin testing (Fig. 2) than with gentamicin testing (Fig. 1). It is tempting to speculate that the cation influence on tobramycin activity is smaller than on gentamicin activity. Earlier work suggesting that the influence of serum in decreasing both bactericidal and inhibitory activity of aminoglycosides as measured in cation-deficient broth is smaller on tobramycin than on gentamicin (7, 8) is consistent with this line of thought. However, Barry et al. (1) recently demonstrated that although the tobramycin MICs for 74 isolates of *P. aeruginosa* were generally two doubling dilutions lower (i.e., more active) than the MICs of gentamicin when tested in cation-deficient MHB, both aminoglycosides (as well as amikacin and netilmicin) showed a definite, proportional shift toward higher MICs when tested in CSMHB. Therefore, although the cation content probably influences the results of testing with all aminoglycosides, the impact with respect to interpretative category changes is greater with gentamicin than with tobramycin, since gentamicin MICs for *P. aeruginosa* generally lie closer to the interpretative breakpoints, regardless of the test medium environment. This fact, plus the small numbers of intermediate and resistant tobramycin results, perhaps accounts for the inability to show any highly statistically significant differences among methods when *P. aeruginosa* is tested against tobramycin.

Method-related trends, similar to those observed for gentamicin, were noted when testing amikacin. Autobac and agar dilution results tended to impart a more susceptible character to the *P. aeruginosa* population tested, although compared with gentamicin data in general, less resistance to amikacin was noted by any method. The Avantage data with

amikacin were not statistically different from disk diffusion or broth microdilution data. If the differences between Avantage and disk diffusion data for gentamicin are attributable to cation deficiency, the agreement between Avantage and disk diffusion data for amikacin is surprising and is perhaps due to other factors. Sensititre AutoBP and Vitek data for amikacin were not shown to differ statistically from the results of disk diffusion or the results of testing with CSMHB microdilution.

Data collected for the beta-lactams (carbenicillin and piperacillin) showed a much greater homogeneity of results among methods than did data for the aminoglycosides. Frequency distribution curves of MIC categories generated by agar dilution and broth microdilution could be superimposed, suggesting that cation content has little influence on the activity of these two agents.

The study has provoked several interesting and complex issues. Clearly, AST of *P. aeruginosa* against aminoglycosides, particularly gentamicin, is technique or method dependent. Present standards set criteria for variables such as inoculum size, medium composition, antimicrobial agent concentration, and incubation time. It is also suggested that the cation content of the medium be monitored by either biological performance for agar-based methods or absolute levels for broth dilution. However, during testing in an experienced clinical laboratory, following national consensus procedures and guidelines, one NCCLS reference method (agar dilution) did not produce gentamicin results comparable to those of two other methods listed as or based upon reference procedures (disk diffusion and broth microdilution). The inability to control the level of biologically active cations in agar should perhaps prompt consideration of testing *Pseudomonas* spp. and aminoglycosides only in broth, in which the impact of cation content on test results appears to be at least consistent.

Importantly, such variation in AST testing directly influences the susceptibility profiles of *P. aeruginosa* to aminoglycosides in a given hospital and makes difficult the comparison of these data among hospitals whose laboratories use different AST methodologies. In addition, any laboratory that switches from a rapid turbidometric method, which tends to underestimate resistance, to an overnight incubation method or to the Vitek instrument is likely to observe an abrupt shift in the apparent resistance of the *P. aeruginosa* population in that hospital, particularly with respect to gentamicin. In one of our laboratories (J.L.S.), a change from Autobac to a CSMHB microdilution method resulted in a change in the gentamicin statistics among *P. aeruginosa* from 89% susceptible one year to 55% susceptible the next. No doubt, adoption of a change in cation content tentatively proposed by the NCCLS M7-T2 document (19) would push the susceptibility percentages upward again. Given the dilemmas involved in predicting from a test tube the success or failure of antibiotic therapy, laboratory workers must caution physicians that the clinical implications of such method-dependent shifts are uncertain. However, such data, rightly or wrongly, will affect prescribing patterns and possibly pharmacy inventory policies. Few physicians would be comfortable treating patients with antibiotics to which the organism is characterized as resistant or even intermediate by the laboratory when other similar antibiotics, to which the organism is reported as susceptible, are available. Therefore, laboratories are prompted to proceed with the greatest of caution in the interpretation of *P. aeruginosa* AST data and to be cognizant of the influence of the method by which such data are generated.

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LITERATURE CITED

- Barry, A. L., G. H. Miller, C. Thornsberry, R. S. Hare, R. N. Jones, R. R. Lorber, R. Ferraresi, and C. Cramer. 1987. Influence of cation supplements on activity of netilmicin against *Pseudomonas aeruginosa* in vitro and in vivo. *Antimicrob. Agents Chemother.* 31:1514-1518.
- Barry, A. L., F. D. Schoenknecht, R. Norton, T. F. O'Brien, J. M. Matsen, C. Thornsberry, L. D. Thrupp, E. M. Markley, and T. L. Gavan. 1976. Inter- and intralaboratory variability in antibiotic susceptibility tests with *Pseudomonas aeruginosa* and Enterobacteriaceae. *J. Infect. Dis.* 134:328-335.
- Black, H. R., and R. S. Griffith. 1971. Preliminary studies with nebramycin factor 6, p. 314-321. *Antimicrob. Agents Chemother.* 1970.
- Casillas, E., M. A. Kenny, B. H. Minshew, and F. D. Schoenknecht. 1981. Effect of ionized calcium and soluble magnesium on the predictability of the performance of Mueller-Hinton agar susceptibility testing of *Pseudomonas aeruginosa* with gentamicin. *Antimicrob. Agents Chemother.* 19:987-992.
- Cherne, J. E., and L. R. Petersen. 1980. Microdilution aminoglycoside susceptibility testing of *Pseudomonas aeruginosa* and *Escherichia coli* with a cation-supplemented inoculum. *Antimicrob. Agents Chemother.* 18:210-211.
- D'Amato, R. F., C. Thornsberry, C. N. Baker, and L. A. Kirven. 1975. Effects of calcium and magnesium ions on the susceptibility of *Pseudomonas* species to tetracycline, gentamicin, polymyxin B, and carbenicillin. *Antimicrob. Agents Chemother.* 7:596-600.
- Davis, S. D., and A. Iannetta. 1972. Antagonistic effect of calcium in serum on the activity of tobramycin against *Pseudomonas*. *Antimicrob. Agents Chemother.* 1:466-469.
- Davis, S. D., and A. Iannetta. 1973. Relative antagonism in vitro of calcium in serum to the bactericidal activities of gentamicin and tobramycin on *Pseudomonas aeruginosa*. *Chemotherapy* 19:243-253.
- Dorn, G. V., J. L. Stanek, C. Needham, and T. Tubert. 1987. Sensititre Autoreader for same-day breakpoint broth microdilution susceptibility testing of members of the family Enterobacteriaceae. *J. Clin. Microbiol.* 25:1481-1485.
- Garrod, L. P., and P. M. Waterworth. 1969. Effect of medium composition on the apparent sensitivity of *Pseudomonas aeruginosa* to gentamicin. *J. Clin. Pathol.* 22:534-538.
- Gilbert, D. N., E. Kutcher, P. Ireland, J. A. Barnett, and J. P. Sanford. 1971. Effects of the concentration of magnesium and calcium on the in vitro susceptibility of *Pseudomonas aeruginosa* to gentamicin. *J. Infect. Dis.* 124:S37-S45.
- Kenny, M. A., H. M. Pollock, B. H. Minshew, E. Casillas, and F. D. Schoenknecht. 1980. Cation components of Mueller-Hinton agar affecting testing of *Pseudomonas aeruginosa* susceptibility to gentamicin. *Antimicrob. Agents Chemother.* 17:55-62.
- Mayo, J. B., T. E. Kiehn, B. Wong, E. M. Bernard, and D. Armstrong. 1982. Examination of *Pseudomonas aeruginosa*-gentamicin discrepancies encountered in an Autobac I-disk diffusion comparison. *Antimicrob. Agents Chemother.* 21:412-415.
- Medeiros, A. A., T. F. O'Brien, W. E. C. Wacker, and N. F. Yulug. 1971. Effect of salt concentration of the apparent in vitro susceptibility of *Pseudomonas* and other Gram-negative bacilli to gentamicin. *J. Infect. Dis.* 124:S59-S64.
- National Committee for Clinical Laboratory Standards. 1979. Performance standards for antimicrobial disk susceptibility tests, 2nd ed. Approved standard ASM-2. National Committee for Clinical Laboratory Standards, Villanova, Pa.

16. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **National Committee for Clinical Laboratory Standards.** 1985. Performance standards for antimicrobial disk susceptibility tests, 3rd ed. Approved standard M2-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **National Committee for Clinical Laboratory Standards.** 1986. Evaluating production lots of dehydrated Mueller-Hinton agar. Proposed standard M6-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **National Committee for Clinical Laboratory Standards.** 1988. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Tentative standard M7-T2, vol. 8, no. 8. National Committee for Clinical Laboratory Standards, Villanova, Pa.
20. **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.
21. **Preston, D. A., and W. E. Wick.** 1971. Preclinical assessment of the antibacterial activity of nebramycin factor 6, p. 322-327. *Antimicrob. Agents Chemother.* 1970.
22. **Reller, L. B., R. D. Schoenknecht, M. A. Kenny, and J. C. Sherris.** 1984. 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.
23. **Sanders, C. C., and W. E. Sanders, Jr.** 1975. Effects of procedural variations on the activity of aminoglycosides in vitro. *Am. J. Clin. Pathol.* **63**:438-445.
24. **Thornsberry, C., A. L. Barry, R. N. Jones, C. N. Baker, R. E. Badal, and R. R. Packer.** 1981. Antibacterial activity of fortimicin A compared with those of five other aminoglycosides, and factors affecting susceptibility tests. *Antimicrob. Agents Chemother.* **19**:122-129.
25. **Tilton, R. C., L. Lieberman, and E. H. Gerlach.** 1973. Microdilution antibiotic susceptibility testing: examination of certain variables. *Appl. Microbiol.* **26**:658-665.
26. **Walonick, D. S.** 1984. Statpac—statistical analysis package. Walonick and Associates, Minneapolis.
27. **Washington, J. A., R. J. Snyder, P. C. Kohner, C. G. Wiltse, D. M. Ilstrup, and J. T. McCall.** 1978. Effect of cation content of agar on the activity of gentamicin, tobramycin, and amikacin against *Pseudomonas aeruginosa*. *J. Infect. Dis.* **137**:103-111.
28. **Woolfrey, B. F., J. M. Fox, and C. O. Quall.** 1981. A comparison of minimum inhibitory concentration values determined by three antimicrobial dilution methods for *Pseudomonas aeruginosa*. *Am. J. Clin. Pathol.* **75**:39-44.
29. **Woolfrey, B. F., R. T. Lally, M. N. Ederer, and C. O. Quall.** 1984. Evaluation of the AutoMicrobic system for susceptibility testing of *Pseudomonas aeruginosa* to gentamicin, tobramycin, and amikacin. *J. Clin. Microbiol.* **19**:502-505.
30. **Woolfrey, B. F., W. A. Ramadei, and C. O. Quall.** 1978. Inability of the standardized disk agar-diffusion test to measure susceptibility to the fluorescent group of pseudomonads to gentamicin. *Am. J. Clin. Pathol.* **70**:337-342.
31. **Zimelis, V. M., and G. G. Jackson.** 1973. Activity of aminoglycoside antibiotics against *Pseudomonas aeruginosa*: specificity and site of calcium and magnesium antagonism. *J. Infect. Dis.* **127**:663-669.