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Gentamicin and Amikacin Disk Susceptibility Tests with *Pseudomonas aeruginosa*: Definition of Minimal Inhibitory Concentration Correlates for Susceptible and Resistant Categories

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With currently recommended disk susceptibility tests, minimal inhibitory concentration correlates for amikacin were >16 μg/ml for resistance and ≤12 μg/ml (not ≤8 μg/ml) for susceptibility. For gentamicin, they were >8 μg/ml for resistance and ≤6 μg/ml (not ≤4 μg/ml) for susceptibility. Minor discrepancies between disk tests and minimal inhibitory concentration determinations will occur if only doubling dilutions of drug are used for measuring minimal inhibitory concentration values.

When testing the susceptibility of *Pseudomonas aeruginosa* to gentamicin or amikacin, disk tests do not always correlate well with minimal inhibitory concentrations (MICs) (1-5, 7, 10). Much attention has been placed upon the need for standardized broth and agar media (2, 4, 7-9). Performance standards for media have been developed by defining the responses expected with a control strain of *P. aeruginosa* (ATCC 27853) (6-9). Even with standardized media, a poor correlation between zone diameters and MIC values is often observed, and thus regression analysis of such data is frequently inappropriate (1). In the present report, we describe our efforts to improve the correlation by determining MICs with more closely spaced dilution intervals and by plotting mean values of duplicated tests performed in two separate laboratories.

Gentamicin blood levels should not exceed 8 to 10 μg/ml, because of the potential toxicity of the drug. Thus, strains with MICs >8 μg/ml are clearly resistant. Strains with MICs ≤4 μg/ml are often considered susceptible, and those inhibited by 8 μg/ml but not by 4 μg/ml are, at best, intermediate in susceptibility. Strains with MICs of 8 μg/ml could be susceptible to levels substantially less than 8 μg/ml but greater than 4 μg/ml. An MIC breakpoint of ≤6 μg/ml for the susceptible category might be just as logical to use as the commonly used MIC breakpoint of ≤4 μg/ml which is an artifactually low value derived from the normal practice of testing only doubling dilutions, i.e. 2, 4, 8, 16 μg/ml, etc.

Amikacin blood levels rarely exceed 25 to 30 μg/ml and, consequently, resistance is often defined as an MIC >32 μg/ml. Strains with MICs of 16 μg/ml may be considered intermediate in susceptibility. The MIC breakpoint for the susceptible category could be placed at ≤12 μg/ml rather than at ≤8 μg/ml if an intermediate concentration between 8 and 16 μg/ml is tested when determining MIC values.

We first defined the distribution of MICs when an unselected series of *P. aeruginosa* isolates was tested in microdilution panels which incorporated concentrations intermediate to the usual doubling dilutions (Table 1). A series of 520 clinical isolates was collected from five geographically separate institutions. These strains were contributed by P. C. Fuchs and R. N. Jones (Portland, Oreg.), A. L. Barry (Sacramento, Calif.), E. H. Gerlach (Wichita, Kans.), and H. R. Sommers (Chicago, Ill.). The lot of microdilution panels used in this phase of the study was found to perform satisfactorily when pretested with the control strain of *P. aeruginosa* (ATCC 27853). Nearly half (47%) of our isolates were inhibited by 8 μg of gentamicin per ml but not by 4 μg/ml (intermediate in susceptibility). However, most of those strains were actually inhibited by 5 or 6 μg/ml, and relatively few required 7 or 8 μg/ml for inhibition. With amikacin, 23% of the isolates would have had intermediate MICs (16 μg/ml) if only doubling dilutions had been tested. The majority of those

TABLE 1. *Inhibition of 520 P. aeruginosa clinical isolates by gentamicin and amikacin: effect of testing concentrations intermediate to the usual doubling dilution scheme*

Concentration tested	Inhibition by:	
	Doubling dilution[a]	Intermediate
Gentamicin		
≥32	5	
16	7	2
12		5
8	43	6
7		9
6		10
5		18
4	36	
≤2	8	
Amikacin		
≥64	2	
32	3	1
24		2
16	23	5
12		18
8	45	
4	20	
2	3	
≤1	3	

[a] Distribution of MICs that would have been recorded if only doubling dilutions of antimicrobic had been tested.

isolates were actually inhibited by 12 μg/ml (about half the normal peak blood level).

To establish the relative precision of MIC values obtained with closely spaced intermediate concentrations, 77 *P. aeruginosa* strains were distributed to all three authors, along with microdilution test panels containing duplicated series of gentamicin and amikacin dilutions (as listed in Table 1). Modal gentamicin MICs of 4, 5, 6, 7, or 8 μg/ml were recorded with 48 of the 77 strains; these isolates were free to vary over the most closely spaced dilution intervals. Precision of the dilution tests with these 48 strains (Table 2) was quite satisfactory, considering the fact that the dilution intervals were so closely spaced. Precision is generally considered acceptable if 95% of all repeated tests vary no more than ±1 doubling dilution. Most of our tests varied over a range of two intermediate dilution intervals (±1 dilution). Similar precision was observed with amikacin dilution tests and with the other gentamicin tests which involved $\log_2$ or half-$\log_2$ dilution intervals.

TABLE 2. *Precision of gentamicin MIC determinations, using microdilution panels with closely spaced dilution intervals*[a]

Strain no.	MIC (μg/ml)		Strain no.	MIC (μg/ml)	
	Mode	Min-max (*R*)[b]		Mode	Min-max (*R*)[b]
1-19	4	4-4 (0)	34	5	4-6 (2)
20	4	2-4 (1)	35	5	4-6 (2)
21	4	2-4 (1)	36	5	4-7 (3)
22	4	2-4 (1)	37	5	4-7 (3)
23	4	4-5 (1)	38	5	4-7 (3)
24	4	4-5 (1)	39	6	6-6 (0)
25	4	4-5 (1)	40	6	5-6 (1)
26	4	4-5 (1)	41	6	6-7 (1)
27	4	4-5 (1)	42	6	6-8 (2)
28	4	4-5 (1)	43	6	5-8 (3)
29	4	1-4 (2)	44	6	5-12 (4)
30	5	5-5 (0)	45	7	6-7 (1)
31	5	4-5 (1)	46	7	7-8 (1)
32	5	4-5 (1)	47	8	8-12 (1)
33	5	5-6 (1)	48	8	7-12 (2)

[a] Test panels contained 0.5, 1, 2, 4, 5, 6, 7, 8, 12, 16, and 32 μg/ml. Data include only those from the 48 *P. aeruginosa* strains with modal MIC values of 4, 5, 6, 7, or 8 μg/ml.

[b] *R* = range (dilution intervals) of MIC values recorded when duplicate tests were performed in three separate laboratories, six values per strain. Min, Minimum; max, maximum.

The 77 isolates were also tested in two laboratories by the agar diffusion technique (6) with 10-μg gentamicin disks (lot #909041; BBL Microbiology Systems) and 30-μg amikacin disks (lot #808101; BBL). Performance of the agar and broth media used in this phase of the study was satisfactory. Fifteen separate tests with *P. aeruginosa* (ATCC 27853) produced gentamicin zones which averaged 18.1 mm (17 to 19 mm) and amikacin zones which averaged 21.1 mm (20 to 23 mm). Thirty gentamicin MICs were either 4 or 5 μg/ml (mean, 4.1 μg/ml), and amikacin MICs were either 4 or 8 μg/ml (mean, 5.7 μg/ml).

Table 3 summarizes the results of these disk tests; zone-size breakpoints currently recommended for 30-μg amikacin disks (3, 6, 10) and the gentamicin zone standards which have recently been proposed (A. L. Barry, C. Thornsberry, R. N. Jones, and E. H. Gerlach, Amer. J. Clin. Pathol., in press) were used. The majority of strains with zones ≥16 mm were inhibited by 6 μg/ml, whereas most of the strains that were resistant to 6 μg/ml gave zones ≤15 mm in diameter. Very few strains were resistant to 12 μg of amikacin per ml, but most of the isolates that were inhibited by 12 μg/ml produced zones of 17 mm or greater. If only doubling dilutions of each antimicrobial agent were tested to define

MICs, a significant number of strains would have appeared to be intermediate in susceptibility but yet produced zones in the susceptible range. Such apparent errors with the disk test occurred more frequently with gentamicin than with amikacin because of the larger proportion of isolates with intermediate susceptibility to gentamicin.

The method of least squares was used to mathematically express the correlation between the average zone diameters and the geometric mean ($\log_2 + 9$) MIC values. Regression formulae for the data of two laboratories were nearly identical (Table 4). MIC correlates were calculated for those zone diameters which represent midpoints between zone-size interpretive breakpoints. With a 10-μg gentamicin disk, a zone of 15.5 mm (halfway between 15 and 16 mm) correlated with an MIC of 6.4 μg/ml (between 6 and 7 μg/ml). A gentamicin zone of 12.5 mm (between 12 and 13 mm) correlated with an MIC of 10.7 μg/ml (between 8 and 12 μg/ml). With 30-μg amikacin disks, a zone of 16.5 mm correlated

TABLE 3. *Correlation between disk test categories and MIC values (intermediate concentrations), 77 P. aeruginosa tested in two separate laboratories*

MIC (μg/ml)[a]	Gentamicin 10-μg disk test						MIC (μg/ml)	Amikacin 30-μg disk test					
	Laboratory 1			Laboratory 2				Laboratory 1			Laboratory 2		
	≤12[b]	13–15	≥16	≤12	13–15	≥16		≤14[b]	15–16	≥17	≤14	15–16	≥17
≥16	5[c]	1		6			≥64	1[c]			1		
12		3			4		32						
							24					1	
8		4			2	1							
7		2			5	1	16		1	1		4	1
6		3	3		4	3	12		5	8		1	11
5			9		3	4	8			26			24
4			31		1	27	4			24			22
≤2			16			16	≤2			11			12

[a] Horizontal lines represent MIC break points between interpretive categories.
[b] Zone size (mm).
[c] Number of isolates.

TABLE 4. *Regression formulae correlating gentamicin and amikacin MICs with zone diameters and calculated MIC correlates for selected zone diameters*[a]

Data source for given antimicrobial agent	Regression formula[b]	Correlation coefficient	MIC correlates (μg/ml) for zones of the following diameter:				
			6 mm[c]	12.5 mm[d]	14.5 mm[e]	15.5 mm[d]	16.5 mm[e]
Gentamicin							
Laboratory 1	$Y = 15.3 - 0.24X$	0.87	29.2	9.9		6.0	
Laboratory 2	$Y = 15.6 - 0.26X$	0.83	33.6	10.4		6.1	
Pooled data	$Y = 15.5 - 0.25X$	0.88	33.1	10.7		6.4	
Amikacin							
Laboratory 1	$Y = 16.5 - 0.24X$	0.81	66.2		16.1		11.6
Laboratory 2	$Y = 16.6 - 0.24X$	0.83	71.5		17.4		12.5
Pooled data	$Y = 16.6 - 0.24X$	0.83	69.2		16.7		12.0

[a] *P. aeruginosa* isolates (77 in number) were tested in two separate laboratories, one at the University of California, Davis Medical Center, Sacramento, Calif., and the other at the Centers for Disease Control, Atlanta, Ga.
[b] $Y = \log_2 + 9$ of the MIC (μg/ml), X = zone diameter (mm).
[c] MIC correlates for a 6-mm zone represent the theoretical maximal MIC level that could be detected with the disk test.
[d] Strains are considered resistant to gentamicin if the zone is ≤12 mm and susceptible if the zone is ≥16 mm, thus MIC correlates are calculated for zones between 12 and 13 mm (12.5 mm) and between 15 and 16 mm (15.5 mm).
[e] Strains are considered resistant to amikacin if the zone is ≤14 mm and susceptible if the zone is ≥17 mm, thus MIC correlates are calculated for zones between 14 and 15 mm (14.5 mm) and between 16 and 17 mm (16.5 mm).

with an MIC of 12 μg/ml, and a zone of 14.5 mm corresponded to an MIC of 16.7 μg/ml.

Clinical experience in treating *P. aeruginosa* infections generally seems to support the value of disk tests for predicting susceptibility to the aminoglycosides. MIC values which correspond to the disk test susceptible and resistant categories are as follows. Gentamicin was resistant at >8 μg/ml and susceptible of ≤6 μg/ml, and amikacin was resistant at >16 μg/ml and susceptible at ≤12 μg/ml. These MIC breakpoints represent intermediate concentrations halfway between the usual $\log_2$ dilution intervals. Laboratorians routinely performing dilution susceptibility tests should consider deviating from the normal practice of testing only doubling dilutions of the aminoglycosides. This is particularly important with *P. aeruginosa* because of the distribution of MICs among clinical isolates.

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