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Interpretation of the Disk Diffusion Susceptibility Test for Amikacin: Report of a Collaborative Study

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Because excessively high rates of false resistance have been encountered with the 10-μg amikacin disk in diffusion susceptibility tests, a study was performed to examine existing zone diameter interpretative criteria and to compare the accuracy of 10- and 30-μg amikacin disks by the error rate-bounded classification scheme. Although current zone diameter interpretative criteria eliminate false susceptibles, there is an unacceptably high rate of false resistants. This problem can be resolved in most instances by revising the zone diameter interpretative criteria for the 10-μg disk (resistant, ≤9 mm; indeterminate, 10 to 11 mm; susceptible, ≥12 mm) or, preferably, by replacing the 10-μg disk with a 30-μg disk and adopting new interpretative criteria (resistant, ≤14 mm; indeterminate, 15 to 16 mm; susceptible, ≥17 mm). Because of significant differences in performance among media, it is necessary to include *Pseudomonas aeruginosa* ATCC 27853 among controls routinely tested and to exclude from use lots of Mueller-Hinton agar yielding results outside the 75% tolerance (90% confidence) limits for amikacin.

Concerns about existing zone diameter interpretative criteria for determining resistance to amikacin have been expressed by Moellering et al. (11) and, increasingly, by reference laboratories that are unable to confirm by dilution techniques resistance encountered in the disk diffusion test. Moellering et al. (11) found that only 67.5% of organisms found to be resistant by disk testing were resistant by dilution testing.

The purposes of this study were to examine the existing zone diameter interpretative criteria for the 10-μg amikacin disk and to determine whether more reliable results could be obtained with a 30-μg disk.

MATERIALS AND METHODS

Four laboratories participated concurrently in two separate phases of study. Each study center collected approximately 200 isolates from clinical specimens, subcultured these onto sheep blood agar to ensure purity, and then suspended each isolate into each of three vials containing defibrinated sheep blood to be stored at −60°C. Before testing, vials were thawed, and their contents were subcultured onto sheep blood agar plates. Quotas for various species were established for each study center to ensure that a variety of organisms would be available for testing. In addition, each study center was supplied with 56 stock cultures previously determined to have been resistant by Kenneth E. Price (Bristol Laboratories, Syracuse, N.Y.). These organisms were stored in a manner similar to that used for the clinical isolates, as were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, which were used as controls.

In the first phase of the study, one lot (lot B9DEMM; BBL Microbiology Systems, Cockeysville, Md.) of Mueller-Hinton agar (MHA) was distributed to all study centers for agar dilution and disk diffusion tests. This lot was chosen on the basis of a determination that the minimal inhibitory concentration (MIC) of gentamicin was 0.5 μg/ml when tested against the Mayo Clinic control strain of *P. aeruginosa* (17). In addition, all study centers received the same lot numbers of Mueller-Hinton broth (MHB) and Trypticase soy broth for microdilution testing and inoculum preparation, respectively.

For the second phase of the study, 12 different lots of MHA were purchased from four different manufacturers (BBL Microbiology Systems; Difco Laboratories, Detroit, Mich.; Grand Island Biological Co., Grand Island, N.Y.; and Scott Laboratories, Fiskeville, R.I.). Three lots were distributed to each study center for disk diffusion tests.

Amikacin powder for dilution tests and 10- and 30-μg amikacin disks were prepared and distributed to all study centers by Bristol Laboratories. The assayed potencies of the 10- and 30-μg disks were 12 and 36 μg, respectively.

Disk diffusion tests were performed according to procedures described in the published standard of the National Committee for Clinical Laboratory Standards (12). Microdilution tests were performed in an

automated device (MIC-2000, Cooke Laboratory Products, Alexandria, Va.), with the use of unsupplemented MHB, by the method described by Thornsberry et al. (16).

In the first phase of the study, each study center determined the MIC by both agar dilution and microdilution methods and the zone diameter of inhibition with both the 10- and 30-μg disks for each clinical isolate and stock culture. In the second phase of the study, the same organisms were retested with the 10- and 30-μg disks on three different lots of MHA.

All data were submitted to Bristol Laboratories for statistical analysis by the error rate-bounded method of classification described by Metzler and DeHaan (9). Separate analyses were made for agar dilution and broth dilution. Susceptibility to amikacin was defined by inhibition at ≤16 μg/ml.

In a final phase of study at Bristol Laboratories, 93 strains were selected from stock cultures on the basis of MICs previously determined to be distributed relatively evenly over the range of 1 to 64 μg/ml. In addition to the original (reference) lot of MHA, three lots of MHA were selected for MIC determinations on the basis of wide variations in zone diameters in previous studies. The MIC of each strain was also determined in three lots of MHB. Finally, each strain was tested against both the 10- and the 30-μg amikacin disks on the reference lot of MHA (lot A) and on two of the other selected lots of MHA.

RESULTS

The clinical isolates and stock cultures tested by all of the study centers are shown in Table 1. Of the 56 strains provided to each laboratory by Bristol Laboratories, 21 were resistant to 16 μg of amikacin per ml. There were, in addition, 77 of 773 clinical isolates collected in the four study centers which were resistant to this concentration of amikacin. A partial listing of the data obtained in the first study from the classification scheme for all zone diameters and analyzed by the error rate-bounded method is shown in Table 2. With the 10-μg disks, the observed rates of error for false susceptibles and for false resistants and the percentage of strains classified as indeterminate were the least when compared with either agar dilution or microdilution with breakpoints of ≤9 and ≥12 mm for resistance and susceptibility, respectively. Similarly, breakpoints of ≤14 and ≥17 mm yielded the smallest rates of false susceptibles and false resistants and the smallest percentage of intermediates with the 30-μg disks.

Results of the second study are shown in Table 3. The breakpoints that provided the best results in the first study also provided the best results in the second study.

During the course of the second study, there were 88 zone diameter determinations in the four study centers with the 10- and 30-μg disks in 12 lots of MHA against *P. aeruginosa* ATCC 27853. For all determinations in all lots of MHA, the 75% tolerance limits with 90% confidence (2) for the 10-μg disks were 16 to 21 mm; those for the 30-μg disks were 20 to 25 mm.

Results of the final phase of study involving the determination of MIC and zone diameters of 93 strains on four lots of MHA and three lots of MHB are shown in Tables 4 and 5.

TABLE 1. *Clinical isolates and stock cultures tested*

Organism	No. of isolates tested
E. coli	112 (10)[a]
K. pneumoniae	79
Enterobacter spp.	74 (1)
Serratia marcescens	22 (9)
Proteus mirabilis	61
Proteus vulgaris	15
Proteus morganii	17
Proteus rettgeri	14 (6)
Providencia stuartii	13
Other enterics[b]	69
Pseudomonas aeruginosa	116 (30)
Other pseudomonads[c]	35
S. aureus	51
Staphylococcus epidermidis	37
Streptococcus group D	40
Miscellaneous[d]	18

[a] Numbers in parentheses represent the number of Bristol Laboratories stock cultures that were tested by all four study centers.

[b] This group included 13 *Salmonella*, 12 *Shigella*, 1 *Arizona*, 22 *Citrobacter diversus*, 17 *Citrobacter freundii*, 1 *Enterobacter agglomerans*, and 3 *Yersinia enterocolitica* isolates.

[c] This group included 17 *Pseudomonas maltophilia*, 4 *Pseudomonas stutzeri*, 2 *Pseudomonas putida*, 2 *Pseudomonas cepacia*, 4 *Pseudomonas fluorescens*, 1 *Pseudomonas testosteroni*, 1 *Pseudomonas diminuta*, 1 *Pseudomonas alcaligenes*, 1 *Pseudomonas mendocina*, and 2 *Pseudomonas acidovorans* isolates.

[d] This group included 1 *Alcaligenes*, 12 *Acinetobacter calcoaceticus*, 1 *Acinetobacter lwoffi*, 1 *Listeria monocytogenes* and 3 *Aeromonas hydrophila* isolates.

DISCUSSION

The stimulus for this study was the frequency of occurrence of strains of *P. aeruginosa* found to be falsely resistant to amikacin in hospitals using the 10-μg disk in diffusion testing. Many such strains have not been confirmed as demonstrating resistance when tested by dilution procedures.

False resistance to amikacin may be attributed to several factors: (i) disk potency below its specified 10-μg content, (ii) excessively large zone diameter criteria for defining susceptibility, (iii) variations in the composition of lots of MHA, (iv) slight shifts in the susceptibility of

TABLE 2. *Abbreviated classification scheme by error rate-bounded method for zone diameters obtained from a single lot of MHA*[a]

Disk content (μg)	Interpretative criteria[b]			% of isolates in the following groups:					
				Agar dilution			Microdilution		
	R	I	S	E_R	I	E_S	E_R	I	E_S
10	≤8	9–12	>13	0.7	11.7	0.1	2.5	11.7	0.1
	≤9	10–11	≥12	1.8	5.1	0.2	4.8	5.1	0.4
	≤10	11–12	≥13	3.8	5.2	0.1	6.8	5.2	0.1
	≤11	12–13	≥14	5.4	5.1	0.1	8.4	5.1	0.1
	≤12	13	≥14	8.7	1.8	0.1	11.4	1.8	0.1
30	≤12	13–15	≥16	0.3	9.3	1.0	1.3	9.3	1.4
	≤13	14–16	≥17	0.6	9.5	0.5	2.5	9.5	0.6
	≤14	15–16	≥17	2.2	6.4	0.5	4.8	6.4	0.6
	≤14	15–17	≥18	2.2	9.1	0.3	4.8	9.1	0.2
	≤14	15–18	≥19	2.2	11.3	0.1	4.8	11.3	0.1

[a] Abbreviations: R, resistant; I, indeterminate; S, susceptible; E_R, observed rate of error for false resistants; E_S, observed rate of error for false susceptibles.
[b] Breakpoints of zone diameters are given in millimeters.

TABLE 3. *Abbreviated classification scheme by error rate-bounded method for zone diameters obtained in 12 different lots of MHA*[a]

Disk content (μg)	Interpretative criteria[b]			% of isolates in the following groups:					
				Agar dilution			Microdilution		
	R	I	S	E_R	I	E_S	E_R	I	E_S
10	≤8	9–10	≥11	1.0	4.8	0.9	3.6	4.8	1.4
	≤9	10–11	≥12	1.8	5.2	0.3	5.0	5.2	0.7
	≤10	11–12	≥13	3.3	5.6	0.1	6.4	5.6	0.4
	≤11	12–13	≥14	5.5	6.0	0.03	8.6	6.0	0.2
	≤12	13	≥14	8.0	3.2	0.03	11.0	3.2	0.2
30	≤13	14–16	≥17	1.2	8.7	0.4	3.7	8.7	0.7
	≤13	14–17	≥18	1.2	11.9	0.1	3.7	11.9	0.2
	≤14	15–16	≥17	2.5	5.9	0.4	5.4	5.9	0.7
	≤14	15–17	≥18	2.5	9.1	0.1	5.4	9.1	0.2
	≤15	16–17	≥18	4.2	6.4	0.1	7.5	6.4	0.2

[a] Abbreviations: R, resistant; I, indeterminate; S, susceptible; E_R, observed rate of error for false resistants; E_S, observed rate of error for false susceptibles.
[b] Breakpoints of zone diameters are given in millimeters.

bacterial populations within hospitals, and (v) technical errors, including an improperly standardized inoculum and the lack of use of a *P. aeruginosa* control strain by many laboratories. The first two factors are unique to amikacin, whereas the last three are common to gentamicin, tobramycin, and amikacin.

It would, therefore, seem desirable to replace the 10-μg disk with a 30-μg disk because low-potency disks are more difficult to manufacture reliably and their potency is more difficult to maintain during storage. Moreover, zone diameters are less reliably determined if most of the strains are in the range of 12 mm or less.

Susceptibility to amikacin is now defined by a zone diameter of ≥14 mm. That this zone diameter is excessively large is abundantly clear. In the first study, 5.1% of all strains tested in agar were erroneously classified as resistant when the existing interpretative criteria were used; however, in the second study, as many as 12.4% of strains tested in one lot of MHA were found to be falsely resistant when existing interpretative criteria were used. When the data from all four study centers and all lots of media were analyzed together, 8.6% of all strains were falsely resistant. Practically no strains were falsely susceptible with the existing ≤11-mm breakpoint.

Central to this issue is the question of whether 16 μg/ml is acceptable as the upper limit for defining susceptibility to amikacin. There have been no data presented in two symposia on amikacin suggesting that this concentration is excessive (3, 15). Moreover, Klastersky et al. (8)

TABLE 4. *Classification scheme by error rate-bounded analysis of zone diameters and MICs obtained with 93 strains in different lots of MHA*[a]

Medium	Disk diffusion: Disk content (μg)	Zone diam (mm) R	I	S	Analysis by agar medium used for MIC[b]: Lot A E_R	Lot A I	Lot A E_S	Lot B E_R	Lot B I	Lot B E_S	Lot C E_R	Lot C I	Lot C E_S	Lot D E_R	Lot D I	Lot D E_S	Totals E_R	Totals I	Totals E_S
A	10	≤9	10–11	≥12	1	7	1	1	7	0	0	7	3	1	7	1	3 (0.8)[c]	28 (7.5)	5 (1.3)
	10	≤11	12–13	≥14[d]	6	7	0	4	7	0	2	7	0	3	7	0	5 (4.0)	28 (7.5)	0
	30	≤14	15–16	≥17	4	8	0	2	8	0	1	8	2	2	8	1	9 (2.4)	32 (8.6)	3 (0.8)
C	10	≤9	10–11	≥12	7	11	0	6	11	0	3	11	0	5	11	0	21 (5.6)	44 (11.8)	0
	10	≤11	12–13	≥14[d]	17	20	0	16	20	0	12	20	0	14	20	0	59 (15.9)	80 (21.5)	0
	30	≤14	15–16	≥17	6	12	0	5	13	0	1	13	0	3	13	0	15 (4.0)	51 (13.7)	0
D	10	≤9	10–11	≥12	4	4	3	2	4	2	1	4	5	2	4	3	9 (2.4)	16 (4.3)	13 (3.5)
	10	≤11	12–13	≥14[d]	8	20	0	6	20	0	3	21	0	5	20	0	22 (5.9)	81 (21.8)	0
	30	≤14	15–16	≥17	4	5	2	2	5	1	1	5	4	2	5	2	9 (2.4)	20 (5.4)	9 (2.4)
Totals	10	≤9	10–11	≥12	12 (4.3)[e]	22 (7.9)	4 (1.4)	9 (3.2)	22 (7.9)	2 (0.7)	4 (1.4)	22 (7.9)	8 (2.9)	8 (2.9)	22 (7.9)	4 (1.4)			
	10	≤11	12–13	≥14[d]	31 (11.1)	47 (16.8)	0	26 (9.3)	47 (16.8)	0	17 (6.1)	48 (17.2)	0	22 (7.9)	47 (16.8)	0			
	30	≤14	15–16	≥17	14 (5.0)	25 (9.0)	2 (0.7)	9 (3.2)	26 (9.3)	1 (0.4)	3 (1.1)	26 (9.3)	6 (2.2)	7 (2.5)	26 (9.3)	3 (1.1)			

[a] Abbreviations: R, resistant; I, indeterminate; S, susceptible; E_R, observed rate of error for false resistants; E_S, observed rate of error for false susceptibles.
[b] Results are expressed as number of strains by category; numbers in parentheses are percentages of totals.
[c] Based on 372 tests.
[d] Current zone diameter interpretative criteria.
[e] Based on 279 tests.

TABLE 5. *Classification scheme by error rate-bounded analysis of zone diameters and MICs obtained with 93 strains in different lots of MHA and MHB*[a]

Disk diffusion					Analysis by broth medium used for MIC[b]									Totals		
Medium	Disk content (μg)	Zone diam (mm)			MHB lot E			MHB lot F			MHB lot G					
		R	I	S	E_R	I	E_S	E_R	I	E_S	E_R	I	E_S	E_R	I	E_S
MHA lot A	10	≤9	10–11	≥12	4	7	3	4	8	1	3	6	3	11 (3.9)[c]	21 (7.5)	7 (2.5)
	10	≤11	12–13	≥14[d]	7	7	0	8	8	1	8	7	1	23 (8.2)	22 (7.9)	2 (0.7)
	30	≤14	15–16	≥17	6	8	0	6	9	1	6	8	1	18 (6.5)	25 (9.0)	2 (0.7)
MHA lot C	10	≤9	10–11	≥12	9	11	2	11	11	2	9	11	1	29 (10.4)	33 (11.8)	5 (1.8)
	10	≤11	12–13	≥14[d]	17	20	0	21	20	0	18	20	0	56 (20.1)	60 (21.5)	0
	30	≤14	15–16	≥17	8	13	0	10	13	0	7	13	1	25 (9.0)	39 (14.0)	1 (0.4)
MHA lot D	10	≤9	10–11	≥12	7	4	7	7	4	4	6	4	5	20 (7.2)	12 (4.3)	16 (5.7)
	10	≤11	12–13	≥14[d]	11	20	1	11	20	1	10	19	1	32 (11.5)	59 (21.1)	3 (1.1)
	30	≤14	15–16	≥17	7	5	6	8	4	3	6	5	5	21 (7.5)	14 (5.0)	14 (5.0)
Totals	10	≤9	10–11	≥12	20 (7.2)[e]	22 (7.9)	12 (4.3)	22 (7.9)	23 (8.2)	7 (2.5)	18 (6.5)	21 (7.5)	9 (3.2)			
	10	≤11	12–13	≥14[d]	35 (12.5)	47 (16.8)	1 (0.4)	40 (14.3)	48 (17.2)	2 (0.7)	36 (12.9)	46 (16.5)	2 (0.7)			
	30	≤14	15–16	≥17	21 (7.5)	26 (9.3)	6 (2.2)	24 (8.6)	26 (9.3)	4 (1.4)	19 (6.8)	26 (9.3)	7 (2.5)			

[a] Abbreviations: R, resistant; I, indeterminate; S, susceptible; E_R, observed rate of error for false resistants; E_S, observed rate of error for false susceptibles.

[b] Results expressed as number of strains by category; numbers in parentheses are percentages of totals. MHB in lots E and F was unsupplemented; lot G was supplemented with Ca^{2+} and Mg^{2+}.

[c] Based on 279 tests.

[d] Current zone diameter interpretative criteria.

[e] Based on 279 tests.

were unable to demonstrate that the in vitro susceptibility of a gram-negative bacillus to amikacin alone or in combination with penicillin or carbenicillin influenced the clinical results in gram-negative sepsis. Although there does not, therefore, appear to be any compelling reason at this time to alter the existing criterion for susceptibility, this issue is complicated by the facts that the MIC of *P. aeruginosa* against amikacin may vary considerably with the lot of MHA used for susceptibility testing (19) and that aminoglycoside resistance can be unstable and lost during storage (7). Which of these two variables might have accounted for our finding that only 21 of the 56 previously resistant stock strains used in this study remained resistant on retesting is unknown.

Linear regression and correlation have been used to relate MICs and zone diameters and to define zone diameters that are interpretable as signifying susceptibility, intermediate (or indeterminate) susceptibility, and resistance. An alternative method has been proposed by Metzler and DeHaan (9), in which maximal tolerable rates of error of 1 and 4% can be specified for false-resistant and for false-susceptible classifications, respectively, whereas the rate of intermediate susceptibility is kept small.

Analysis of the amikacin data from the first study by this scheme showed that, by defining susceptibility with a zone diameter of ≥12 mm, the rate of false resistance would be reduced to 1.8%, whereas the rate of false susceptibility remained at an acceptably low level of 0.2% (resistance equals a zone diameter of ≤9 mm). In addition, the percentage of indeterminate strains was approximately 5%. A similar analysis of results obtained with the 30-μg disk demonstrated comparable rates of error when susceptibility and resistance were defined by zone diameters of ≥17 and ≤14 mm, respectively (Table 2).

Minshew et al. (10) have suggested that there may be a need to change zone diameter interpretative standards for amikacin, and especially for gentamicin and tobramycin, because of the emergence in a burn center of more resistant strains of *E. coli, Klebsiella pneumoniae,* and *P. aeruginosa.* They proposed, as did Quinn (14), that a broader indeterminate range might represent a reasonable solution to this problem. In a graph correlating the MICs of amikacin with zone diameters of 168 organisms collected by Minshew et al. (10), one strain (0.6%) was falsely susceptible and eight (4.8%) were falsely resistant with the existing zone diameter interpretative criteria. Three isolates of *P. aeruginosa* and one of *K. pneumoniae* with MICs of 32 μg/ml would have been erroneously classified as susceptible (false-susceptible rate, 4 of 168 [2.4%]) if 12 mm had been used as the breakpoint for susceptibility. Likewise, 3 of 168 (1.8%) of their strains with MICs of 16 μg/ml would have been erroneously classified as resistant. This percentage of false susceptibles only slightly exceeds the maximum recommended by Metzler and DeHaan (9) and the rate encountered with the isolates tested in our study in all 12 lots of MHA.

The effects of the composition of the medium on aminoglycoside activity in vitro are complex. Numerous reports have demonstrated an inverse relationship between activity and the Ca^{2+} and Mg^{2+} content of liquid media. A similar relationship between the total cation content of solid media and aminoglycoside activity has not, however, been demonstrated (19). Waterworth (20) has recently reviewed the influence of the phosphate content of the medium, the formation of stable pyrophosphate complexes of Mg^{2+} and Ca^{2+} during autoclaving, and the differences in the dissociation of various calcium and magnesium salts in solution on aminoglycoside zone diameters.

Pollock et al. (13) have questioned whether defined zone diameter interpretative criteria represent a valid means of determining the susceptibility or resistance of *P. aeruginosa* to gentamicin and possibly to tobramycin and amikacin. They have proposed a flexible indeterminate range for gentamicin that is dependent on mean zone diameters obtained with *P. aeruginosa* ATCC 27853 in each lot of MHA. Although specific guidelines for determining this intermediate range were not given, a similar recommendation was made by Garrod and Waterworth in 1969 (6).

Even though a flexible indeterminate range is not at all unreasonable, it does constitute a rather profound departure from the approach that has been advocated in the United States since 1966 (1) and has been published as a standard by both the Food and Drug Administration (4, 5) and the National Committee for Clinical Laboratory Standards (12). In this study, therefore, an alternative approach was sought by attempting to determine whether revised zone diameter interpretative criteria with a higher content disk would reduce the rate of false-susceptible and false-resistant results to acceptable levels, as defined by the error rate-bounded technique (9). By using one lot of MHA to define the MIC of all strains tested, it was possible to bring the false-susceptible and false-resistant rates within acceptable ranges when zone diameters were determined with the same medium and with 12 additional lots of MHA. One possible objection to this approach is that all MICs originated from a single lot of MHA that had been

specifically selected on the basis of the acceptability of an MIC of gentamicin in this medium when tested against a control strain of *P. aeruginosa*. Because the MIC is related in an approximately linear fashion to the zone diameter, it seems likely that the false-susceptible and false-resistant rates would change significantly if the MIC were determined in other lots of MHA. The final phase of this study was designed to explore this problem in detail (Tables 4 and 5). The population of strains used for this phase included 28 strains with MICs in the range of 8 to 32 μg/ml.

For any given medium used for obtaining the MIC in agar (Table 4), the rate of false susceptibility was zero with the existing interpretative criteria; however, the rates of false resistance and intermediate susceptibility were quite large with these criteria. With the zone diameter interpretative criteria selected on the basis of the first two studies (Tables 2 and 3), the rates of intermediate susceptibility and particularly false resistance were reduced substantially; however, false susceptibility increased to 1.1 and 1.4% in one of the lots (lot D) and to 2.2 and 2.9% in another (lot C), with consistently lower figures when the criteria proposed for the 30-μg disk were used.

A similar analysis relating zone diameters in three lots of MHA to MIC determinations in three lots of MHB by the broth dilution technique is shown in Table 5. In general, the rates of false susceptibility, indeterminate susceptibility, and false resistance were higher than those encountered with agar dilution MIC determinations, and there were no substantial differences among the results obtained with the three lots of MHB (Table 5), although one (lot G) was supplemented with cations and the other two were not. Obviously, the results presented in Tables 4 and 5 are skewed by the selection of strains and media that were tested, and it is unlikely that this combination of circumstances would occur very often in most clinical laboratories. Nonetheless, such strains were similar to those described by Minshew et al. (10), and the lots of MHA selected for this part of the study were purchased from commercial sources.

In conclusion, there are several possible solutions to the problems that have been encountered with use of amikacin disk susceptibility tests. First, whereas the current zone diameter interpretative criteria virtually eliminate false susceptibles, there is clearly an unacceptably high rate of false resistants. Most often, this problem can be resolved either by revising the zone diameter interpretative criteria for the 10-μg disk (resistant, ≤9 mm; intermediate, 10 to 11 mm; susceptible, ≥12 mm) or by using a 30-μg disk (resistant, ≤14 mm; intermediate, 15 to 16 mm; susceptible, ≥17 mm). The latter disk seems preferable because 9 mm, used to define resistance with the 10-μg disk, approaches the least measurable zone diameter (disk diameters are approximately 6 mm). That this solution will not apply to all circumstances, however, is evident from the data in Tables 4 and 5, although these data do represent a "worst case" type of situation. Second, clinical laboratories should use *P. aeruginosa* ATCC 27853 for control purposes. Currently, many do not. If the zone diameters of the control are less than or exceed the 75% tolerance (90% confidence) limits, evidence is provided that something is wrong with the medium, the disk, or the technique. Third, the manufacturers should institute control measures so that lots of MHA that are approved for distribution and sale comply with the tolerance limits proposed for the control organism. Fourth, manufacturers should refrain from adjusting the total cation content of MHA. Two of the manufacturers whose media were used in this study admitted to making such adjustments. Such adjustments obviously compound the problems posed by variations in the components of agar, influence of autoclaving, dissociation of salts, and so forth. Finally, it may be feasible to adjust zone diameter interpretative criteria in a flexible manner, as has been suggested for gentamicin, based on the zone diameters found with the control strain in a given lot of MHA.

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