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Effect of Different Lots of Mueller-Hinton Agar on the Interpretation of the Gentamicin Susceptibility of *Pseudomonas aeruginosa*

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Population distributions and quality control data for strains of *Pseudomonas aeruginosa* tested for gentamicin susceptibility on six lots of Mueller-Hinton agar were analyzed. The lots of agar were used in three University of Washington hospitals from April 1975 through October 1977. The analyses indicated that the performance of members of the *P. aeruginosa* populations in each hospital closely followed the performance of the quality control strain, *P. aeruginosa* ATCC 27853, when tested on each lot of Mueller-Hinton medium. The variability of zone diameters with the *P. aeruginosa* populations and the quality control strain indicated that a fixed indeterminate range (13 to 16 mm) of gentamicin susceptibility was not applicable to these organisms as it was with the *Enterobacteriaceae*. Variability in gentamicin susceptibility results was demonstrated in both minimal inhibitory concentration and disk diffusion tests when eight selected *P. aeruginosa* strains and the quality control strain were tested on each lot of medium. This variation in susceptibility to gentamicin was not related to the total Ca^{2+}, Mg^{2+}, or Zn^{2+} content of each lot of medium. The data demonstrated that a moving indeterminate range of gentamicin susceptibility, 3 to 6 mm below the mean zone diameter of the quality control strain, was a suitable criterion for strains tested on a single medium lot. These results illustrate the importance of defining stringent performance standards for media used in the susceptibility testing of *P. aeruginosa* with gentamicin and other aminoglycoside antibiotics.

Reproducible criteria for determining the susceptibility of *Pseudomonas aeruginosa* to gentamicin and other aminoglycoside antibiotics are critical for the appropriate interpretation of antibiotic susceptibility tests. Unfortunately, the media used and their cation content contribute significantly to the technical variables encountered with these bacterial and antibiotic combinations. Standards of *P. aeruginosa* ATCC 27853 on Mueller-Hinton agar were adopted to help control these variables, and limits of 16 to 21 mm for individual determinations with gentamicin disks were recommended (10). A break point of ≥13 mm had previously been recommended to define susceptibility to gentamicin on media performing within the recommended control limits for *Escherichia coli* and *Staphylococcus aureus* (10). Occasionally, strains of *P. aeruginosa* and other organisms were encountered that were apparently not correctly classified as susceptible or resistant to gentamicin by this criterion (9, 13). Several investigators, therefore, proposed alternate criteria for interpreting the results (7, 9, 13).

In a previous study, Minshew et al. (9) suggested that the *Enterobacteriaceae* giving zone diameters of 13 to 16 mm with a standard gentamicin (10 μg) disk be considered intermediate or indeterminant in their response to this antimicrobial agent. This recommendation was limited to the *Enterobacteriaceae,* however, since examination of more recent data indicated that this criterion was apparently not always suitable for *P. aeruginosa*. Variable results with this species were observed with different lots of Mueller-Hinton agar (9), and we have suggested that an intermediate category relating interpretation of zone size to the zone diameter obtained with the quality control organism might provide a solution.

In this report we have analyzed the variation in gentamicin disk diffusion susceptibility results

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with *P. aeruginosa* obtained in three separate laboratories using different lots of Mueller-Hinton agar. The application of the intermediate criterion previously suggested is evaluated. Both the susceptibility testing results with routine isolates and an experimental evaluation of selected strains on different lots of media confirmed that the problem of criteria for intermediate susceptibility of *P. aeruginosa* isolates to gentamicin may be resolved by relating intermediate criteria to the mean zone diameter observed with the quality control strain, *P. aeruginosa* ATCC 27853, on each lot of Mueller-Hinton agar used.

MATERIALS AND METHODS

Bacteria and media. Strains of *E. coli* and *P. aeruginosa* were isolated from specimens submitted to the diagnostic microbiology laboratories at University Hospital (UH), Harborview Medical Center (HMC), and the Veterans Administration Hospital (VAH) in Seattle. Burn Center isolates from HMC were excluded from the population analysis because most of these isolates were resistant to gentamicin (9). From April 1976 through August 1977, population data for these two organisms and quality control data for *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were analyzed for variations in disk susceptibility test results with gentamicin on each lot of Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). The following lots of Mueller-Hinton agar were included: Difco control numbers 620275 (275) previously described (9), 625309 (309), 633389 (389), 633724 (724), 634947 (947), and 640657 (657). Population data for lots 564372 (372), 603207 (207), and 594436 (436), used in 1974 to 1975, were also evaluated for purposes of comparison. The media used at all three hospitals were prepared in the UH media laboratory.

Eight strains of *P. aeruginosa* were selected to compare minimal inhibitory concentrations (MICs) of gentamicin with zone diameters on four lots of Mueller-Hinton agar (275, 309, 947, and 657) used during different time periods in the clinical laboratories. The isolates included two very susceptible UH strains, three strains with intermediate susceptibility as measured by MIC and disk diffusion tests, obtained from the Burn Center at HMC, and three strains from UH general hospital patients that were interpreted as having intermediate susceptibility to gentamicin during a later time period when tested on a different lot of Mueller-Hinton than the HMC strains.

Disk diffusion susceptibilities. The standard disk diffusion susceptibility test (1, 7) was used for isolates in all three hospitals. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were included daily for quality control. Zone diameters were determined with a Vernier caliper by reading from the back of the plate with indirect light and recorded to the nearest millimeter. With the eight selected strains, zone diameters surrounding three separate gentamicin disks were measured to the nearest 0.1 mm by two readers. The mean of the six determinations was used to compare the results obtained with the four different lots of Mueller-Hinton agar tested.

MICs. MICs were determined for eight selected *P. aeruginosa* strains by a standard agar dilution method (3, 6), with the same four lots of agar used for the disk diffusion test. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were also included as controls. The inocula (10^4 organisms) were applied to the plates using a Steers' replicator (14), and each strain was tested twice at separate times on each lot of agar.

Chemical analysis of media. Samples of prepared media for calcium and magnesium analysis were dehydrated and "dry" ashed in a muffel oven as described previously (12), except that 7-ml media samples were used. Mineral ash was redissolved in 2 N HCl. The calcium and magnesium contents of the acid-soluble residue were simultaneously determined by atomic absorption spectrophotometry (IL 353; Instrumentation Laboratory Inc., Lexington, Mass.). Recovery of calcium and magnesium added to prepared media samples was 100% (6, 11, 16).

Samples (20 ml) of prepared media for copper and zinc analysis were completely dehydrated at 80°C. After 3 to 4 days, the residue was dissolved in 4.5 ml of 8 N HNO_3. The "wet digestion" was considered complete in 6 h at 26°C with frequent shaking. After addition of 2 drops of bromocresol green (tetrabromo-*m*-cresol-sulfonphthalein, 0.04 g/100 ml of water containing 0.3 ml of saturated NaOH) and 1.8 ml of water, the acid hydrolysate was titrated to the indicator's blue color at pH > 5.4 by adding solid tris(hydroxymethyl)aminomethane base (ultra pure grade, Schwartz/Mann, Orangeburg, N.Y.). Each digest was then diluted to 10 ml, and the volume was measured exactly.

Samples of the digest were then assayed for copper and zinc content by atomic absorption spectrophotometry (IL 353). The method of Meret and Henkin (8) with 6% L-butanol as solvent was used, except that 0.1% Triton X-100 in 6% L-butanol was used to rinse the probe and atomizer. This method was accurate between 5 and 400 μg/dl in our hands. Between-day precision (n = 12 days) was 4.8% coefficient of variation at 25 μg/dl. Recovery of copper and zinc added to prepared media samples was 95 to 101% over the range being tested.

All glassware used in these experiments was acid washed. Batches of plastic test tubes were specifically tested and found free of metal contamination before use. Water used for media preparation and analysis contained no detectable calcium, magnesium, copper, or zinc.

RESULTS

Evaluation of quality control data. The mean and standard deviation was calculated for disk diffusion susceptibility results with gentamicin for *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 on each of six lots of Mueller-Hinton agar tested at UH, HMC, and VAH (Table 1). The standard deviation with these quality control strains was 1.2 mm or less in each laboratory. The variation between laboratories

was 1.4 mm or less for the mean of the zone diameters on each lot of medium tested. Although the gentamicin test results were reasonably stable with *E. coli* ATCC 25922 on all lots of media, results with *P. aeruginosa* varied remarkably (Table 1). The mean of all the quality control data from all hospitals was used to evaluate the significance of the variation between the lots of media. The pooled results indicated that the quality control values obtained with *P. aeruginosa* ATCC 27853 on lots 309, 389, 724, 947, and 657 were significantly different from 275 ($P < 0.05$) when comparing the mean and standard deviation for each lot. Similar analysis of the quality control data with *E. coli* ATCC 25922 demonstrated no significant variation between lots except for lot 309, which consistently yielded larger zone diameters. Medium lot 275 was used as a basis for comparison, since we had previously derived recommendations for intermediate zone criteria for gentamicin using this lot of agar.

A comparison of population zone diameters with quality control data. The zone diameters with gentamicin for strains of *P. aeruginosa* and *E. coli* isolated from clinical specimens were grouped by the lot of Mueller-Hinton agar on which the results were obtained. The mean and standard deviation for the zone diameters from each of the hospitals was analyzed for each lot and compared with a similar analysis of the quality control strain (Table 2). These analyses revealed that the means of the zone diameters for routine isolates of *P. aeruginosa* changed approximately the same number of millimeters and in the same direction, either smaller or larger, as the mean zone diameter for the quality control strain with each lot of Mueller-Hinton agar used (Table 2). The principal exception to this general trend was with lot 309 at HMC. A greater number of resistant strains during this time period lowered the mean zone diameter for the population in this hospital. Consequently, the mean differences in results between lot 309 and lots 275 and 389 were not as great as those observed at UH and VAH. Only lot 309 showed significant differences when zone diameters for *E. coli* were analyzed in a similar fashion. This reflected the results with the quality control strain (Table 1).

We applied the 13- to 16-mm intermediate zone diameter criterion previously described for gentamicin (9) to populations of *E. coli* and *P. aeruginosa* isolated at the three hospitals. Susceptible strains of *E. coli* were adequately distinguished with all lots of Mueller-Hinton agar, as illustrated by the data from UH (Fig. 1); the results from HMC and VAH were similar. The population of *E. coli* isolates was described by an essentially normal distribution corresponding to the susceptible population described in a previous report and was identified as susceptible

TABLE 1. *Comparison of quality control data for gentamicin on different lots of Mueller-Hinton agar, obtained in three hospitals*[a]

Lot no.	*P. aeruginosa* ATCC 27853			*E. coli* ATCC 25922		
	UH	VAH	HMC	UH	VAH	HMC
275	20.1 ± 0.9 (42)	19.7 ± 1 (34)	20.2 ± 1 (30)	21.8 ± 1 (61)	21.3 ± 0.9 (37)	22.3 ± 1.2 (31)
309	22.3 ± 1.2 (41)	21.3 ± 1 (50)	21.7 ± 1 (46)	24.0 ± 0.9 (81)	22.6 ± 1 (59)	23.7 ± 1.2 (51)
389*[b]	19.0 ± 1.1 (25)	18.3 ± 0.9 (36)	18.7 ± 1 (32)	22.5 ± 0.8 (29)	21.9 ± 0.9 (36)	22.7 ± 1 (32)
724*	18.2 ± 1.1 (46)	16.8 ± 0.8 (55)	16.8 ± 1.1 (41)	22.6 ± 1 (55)	22.6 ± 1 (50)	23.1 ± 1.1 (43)
947*	17.5 ± 0.7 (20)	16.6 ± 0.8 (20)	17.3 ± 0.5 (19)	23.6 ± 0.6 (17)	22.4 ± 1 (15)	23.1 ± 1.2 (16)
657*	18.2 ± 1.1 (16)	17.2 ± 1.0 (16)	17.2 ± 0.6 (16)	21.9 ± 1.0 (16)	20.9 ± 0.8 (16)	22.5 ± 1.0 (16)

[a] The means of the zone diameters determined at three hospitals on the different agar lots were averaged and compared to the average mean for lot 275. The differences were significant ($P < 0.05$) by a t test for *P. aeruginosa* tested on all lots and *E. coli* tested on lot 309. The number of observations are indicated in parentheses.

[b] *, The differences in the comparison of these lots with lot 275 were not statistically significant for *E. coli*.

TABLE 2. *Comparison of the means of the zone diameters with gentamicin disks for P. aeruginosa ATCC 27853 with the means of the general hospital populations of P. aeruginosa tested on different lots of Mueller-Hinton agar*[a]

Lot no.	UH		VAH		HMC	
	QC	P	QC	P	QC	P
275	20.1 ± 1.3 (42)	18.2 ± 3.0 (96)	19.7 ± 1.0 (34)	20.4 ± 2.7 (60)	20.2 ± 1.0 (30)	18.4 ± 2.8 (364)
309	22.3 ± 1.2 (41)	20.6 ± 3.2 (240)	21.3 ± 1.1 (59)	21.4 ± 3.0 (143)	21.7 ± 1.1 (46)	18.4 ± 2.8 (175)
389	19.0 ± 1.1 (25)	17.4 ± 4.4 (86)	18.3 ± 0.9 (36)	17.6 ± 4.5 (44)	18.7 ± 1.0 (32)	18.6 ± 3.0 (56)
724	18.2 ± 1.1 (46)	16.4 ± 3.4 (156)	16.8 ± 0.8 (55)	16.2 ± 4.4 (97)	16.8 ± 1.1 (41)	15.8 ± 2.8 (69)
947	17.5 ± 0.7 (20)	15.9 ± 2.6 (34)	16.6 ± 0.8 (20)	14.7 ± 4.8 (28)	17.3 ± 0.5 (19)	15.7 ± 1.9 (43)
657	18.2 ± 1.1 (16)	16.1 ± 4.2 (79)	17.2 ± 1.0 (16)	15.3 ± 4.5 (52)	17.2 ± 0.6 (16)	14.4 ± 3.4 (58)

[a] QC, Means of disk diffusion test results with quality control strain *P. aeruginosa* ATCC 27853 ± one standard deviation. P, Means of disk diffusion test results for general hospital isolates ± one deviation. Parentheses indicate number of observations.

with all media batches. When these same zone criteria were applied to *P. aeruginosa,* an entirely different picture emerged. Whereas a 13- to 16-mm intermediate criterion appeared appropriate for strains tested on lots 275, as previously described (Fig. 1) (9), and 389 (Fig. 2), nearly one-half of the susceptible general hospital isolates were classified as intermediate by this criterion when they were tested on lots 724 (Fig. 1) and 436 and 657 (Fig. 2). It also appeared that many strains may have been classified as falsely "susceptible" to gentamicin when they were tested on lot 309. It is readily apparent from Fig. 1 that the entire population of *P. aeruginosa* moved to larger (lot 309) or smaller (lots 389 and 724) zone diameters to approximately the same degree as the quality control strain *P. aeruginosa* ATCC 27853 (arrows).

The population distributions of *P. aeruginosa* strains isolated in 1974 and 1975 were also grouped according to the lot of Mueller-Hinton agar on which they were tested. When these data were compared with the populations tested on media giving similar quality control performances in 1977 (Fig. 2), the majority of general hospital isolates gave a similar range of zone diameters for both time periods. Nevertheless, a real increase in resistant strains was apparent when 1977 populations were compared with those from 1974 to 1976 (Fig. 2).

These plots also demonstrated that the previously suggested fixed criterion for an intermediate zone of 13 to 16 mm, as defined by MICs on lot 275 (9), was not suitable for *P. aeruginosa* tested on other media lots (lot 436 for 1974 and lot 657 for 1977, Fig. 2). A large proportion of the general hospital isolates demonstrated zones of inhibition that would have been in the intermediate category (13 to 16 mm) around a gentamicin (10 μg) disk. This occurred with populations from 1974 as well as those from 1977. This phenomenon was not due to increased resistance of the isolates, but occurred in addition to a slight increase in resistance (Fig. 2).

The zone diameter distributions for susceptible and low-level resistant strains were previously defined on lot 275 (9). Isolates of *E. coli, Klebsiella,* and *P. aeruginosa* giving zones of inhibition (13 to 16 mm) smaller than the range described by the normal general hospital isolates were clearly resistant by MIC test (9). It appears that application of an intermediate zone for gentamicin derived from regression analysis on lot 275 (Fig. 1) (9) cannot be uniformly applied to all lots of Mueller-Hinton agar (Fig. 1 and 2) to define *Pseudomonas* strains with low-level resistance. Lots of agar such as 309, which demonstrate large gentamicin zone diameters with the quality control strain (21 to 22 mm), will make organisms having low-level resistance and zone diameters only slightly smaller than those of the normal susceptible population appear susceptible. Lots of agar on which organisms give smaller zone diameters would cause a dispropor-

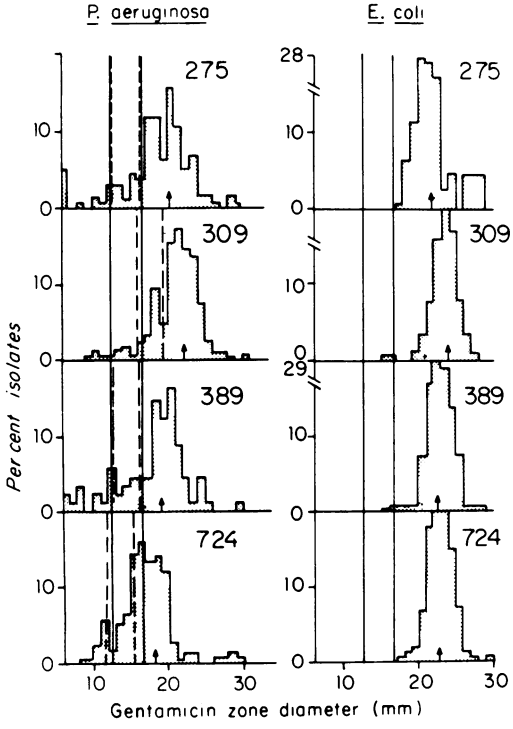

FIG. 1. *Comparison of the population distributions of general hospital isolates of E. coli and P. aeruginosa tested for gentamicin susceptibility on different lots of Mueller-Hinton agar. Shown are the effects of a 13- to 16-mm indeterminate range (solid lines) and a variable indeterminate range (broken lines) 3 to 6 mm below the mean value (arrow) for P. aeruginosa ATCC 27853 on the interpretation of the standard disk test.*

tionate number to be classified as intermediate by the 13- to 16-mm criterion (Fig. 1).

Comparison of zone diameters and MICs on different lots of Mueller-Hinton agar. Eight strains of *P. aeruginosa* and *P. aeruginosa* ATCC 27853, selected to give a range of values, were tested for gentamicin susceptibility on four representative lots of Mueller-Hinton agar (275, 309, 947, and 657) to compare the relationships of the zone diameters and MICs (Fig. 3). The organisms were tested twice on separate days with good reproducibility (Table 3). Regression lines were fitted to the nine paired determinations for each lot with the $\log_2$ of the MIC from one comparison as the independent variable. A comparison of the positions of the points along the separate regression lines for the four lots of media indicates, as expected, that there is a correlative variation in the zone diameters and MICs. All of the strains performed in the same way on the duplicate analysis, giving significantly smaller zone diameters with lots 947 and 657 than with lots 275 and 309 ($P < 0.05$). The means of the zone diameters were 17.35 ± 1.2 for lot 275, 18.2 ± 1.2 for lot 309, 14.3 ± 1.4 for lot 947, and 15.0 ± 1.4 for lot 657. Higher MICs were obtained with lots 947 and 657 than with lots 275 and 309 (Table 3). The geometric mean of the MICs with lot 947 (8) was significantly greater than those of lots 275 (4) and 309 (2). There were no significant differences in the zone diameters or MICs for *E. coli* ATCC 25922 on the four lots of agar. A statistical analysis of the four separate regression lines (Fig. 3) revealed that a single line described all data points; thus, all the MIC and zone diameter paired observations may be described by a single regression line with a slope of −2.44 and have a coefficient of correlation −0.941. Clearly, differences in zone diameters were reflected in differences in MICs on the four lots of media. Several strains were susceptible by MIC test on lot 309 but resistant on lots 947 and 657. Lot 275 was used to define susceptibillty in a previous report; again, several isolates were susceptible on lot 309 but resistant on lot 275 (Table 3). Like the zone diameter distributions of the populations (Fig. 1), the MICs demonstrate that lot 309 provides false susceptibility with various strains as compared to lot 275.

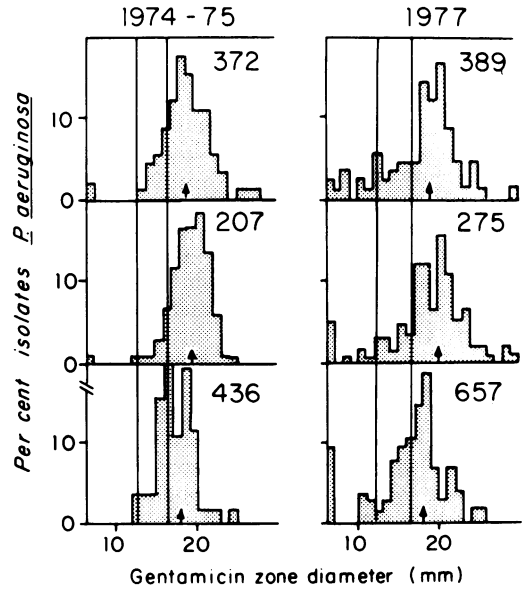

FIG. 2. *Comparison of the population distributions of P. aeruginosa, tested for gentamicin susceptibility on media lots that exhibited similar quality control performances with P. aeruginosa ATCC 27853 (arrow) in 1974 to 1975 and 1977, with the effect of a 13- to 16-mm indeterminate range of susceptibility.*

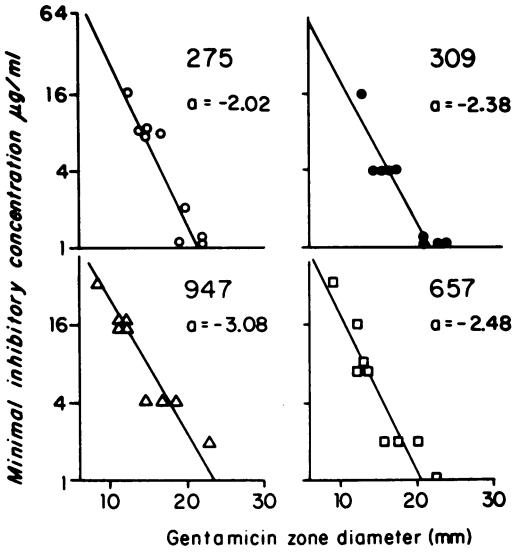

FIG. 3. *Comparison of MICs and zone diameters for gentamicin with four different lots of Mueller-Hinton agar and nine strains of P. aeruginosa.*

Cation content of four lots of Mueller-Hinton agar. Four lots of Mueller-Hinton agar were analyzed for their respective cation contents (Table 4). Total Mg^{2+} and Ca^{2+} contents were within the ranges suggested by Reller et al. (12) for the testing of aminoglycoside antibiotics, except for lot 657, in which values for both cations were below the suggested range. The differences for Ca^{2+} and Zn^{2+} for lots 947 and 657 compared with lots 275 and 309 were greater than would be expected on the basis of the chance variability of the methods. Lots which contained lower total cation content produced the smaller zone diameters around gentamicin disks. This was the reverse of expectations from the results of previous reports (2, 4, 5, 12).

DISCUSSION

In a previous study (9), we suggested an intermediate or indeterminate range of 13- to 16-mm zones of inhibition with the standard gentamicin (10 µg) disks and the *Enterobacteriaceae*. Although "intermediate" is the term generally used, this range of zone diameters was also observed with a group of organisms that were borderline in their susceptibility. Thus organisms within this group may be of indeterminate susceptibility. These limits also were appropriate for strains of *P. aeruginosa* tested in the previous study in which the mean of the gentamicin zone diameters for *P. aeruginosa* ATCC 27853 was toward the upper end of the acceptable limits (20.1 ± 1.1) on the lot of Mueller-Hinton agar (275) used, but other observations indicated enough lot-to-lot variability with Mueller-Hinton agar that the criteria could not be applied to *P. aeruginosa* without further modification (Fig. 1 and 2). We have shown in the present study that an unacceptable number of susceptible strains would be classified as intermediate on some lots of Mueller-Hinton agar if the 13- to 16-mm intermediate range were used. This would occur even if the method was controlled (Fig. 1 and 2), as was the case here.

An approach similar to that suggested by Garrod and Waterworth (4) was applied to the data from three hospitals. An intermediate range 3 to 6 mm below the mean was defined by the performance of the quality control strain *P. aeruginosa* ATCC 27853. We found that susceptible isolates could be clearly separated from strains possessing low-level resistance by this method. Thus, with an intermediate zone 3 to 6 mm below the quality control strain, a lot of Mueller-Hinton agar giving a quality control mean zone diameter of 19 mm would have an intermediate zone range of 13 to 16 mm; lots of agar with quality control values of 17 mm would have an intermediate zone range of 11 to 14 mm. The dashed lines in Fig. 1 represent the application of an intermediate range 3 to 6 mm less than the mean zone diameter of the quality control strain

TABLE 3. *Comparison of MICs of gentamicin determined on four different lots of Mueller-Hinton agar with selected strains of P. aeruginosa for duplicate tests*

Strain	Test no.	Mueller-Hinton agar lot no.			
		275	309	947	657
UW 27	1	2	1	2	2
	2	1	1	4	2
UW 421	1	8	4	16	8
	2	8	4	16	8
UW 427	1	2	1	4	4
	2	2	1	4	2
UW 429	1	4	4	8	4
	2	8	4	16	16
HMC 55	1	16	16	32	32
	2	16	16	32	32
HMC 57	1	8	4	16	8
	2	8	4	16	8
HMC 262	1	16	8	16	8
	2	8	4	16	8
P. aeruginosa ATCC 27853	1	1	1	4	2
	2	2	1	4	2

TABLE 4. *Relationship between total cationic content of four lots of Mueller-Hinton agar and gentamicin zones of inhibition with P. aeruginosa ATCC 27853*

Lot no.	Gentamicin QC zone[a] (mm)	Magnesium (mg/liter)	Calcium (mg/liter)	Zinc (μg/liter)	Copper (μg/liter)
275	20.1	31	89	738	47
309	22.3	30	77	576	40
947	17.5	35	49	371	92
657	18.2	19	37	373	72

[a] Mean of the gentamicin zone diameters given by *P. aeruginosa* ATCC 27853 at UH.

(arrows) on each lot of media for strains of *P. aeruginosa* tested at UH. It is apparent from this figure that a standard defined by the quality control strain (dotted lines) is more appropriate than a fixed range (solid lines) for the classification of gentamicin susceptibility with *P. aeruginosa* populations. For example, if we were to define a fixed indeterminate range based on susceptibility tests with a lot of medium such as 724, on which *P. aeruginosa* strains typically give smaller zone diameters with gentamicin disks, we would expect to find an unacceptable number of strains falsely classified as susceptible when tested on a lot of medium such as 309 or 275 where larger zone diameters are the rule (Fig. 1). The limits on 275 have been defined by MIC determinations.

MICs do not solve the problem of determining the susceptibility of *Pseudomonas* to the aminoglycosides on different lots of Mueller-Hinton agar. The same variability was seen with gentamicin agar dilution tests as occurred with disk diffusion tests (Fig. 3). The MICs correlated inversely with the zone diameters, and on a given lot of Mueller-Hinton agar the MIC was always as would be expected for the accompanying zone diameter, but did not uniformly define susceptibility (Table 3).

Several investigators have related increased resistance of *P. aeruginosa* to aminoglycoside antibiotics with increased concentrations of Mg^{2+} and Ca^{2+} in the media (1, 2, 4, 5, 12). Conversely, the antibacterial activity of these antibiotics was shown to be enhanced in media with low cationic content. Analysis of four lots of Mueller-Hinton agar for total metal ions did not provide a satisfactory explanation for our results based on current knowledge. Total calcium and zinc contents were lower in the lots of agar demonstrating less gentamicin activity; i.e., higher MICs by agar dilution and smaller zones of inhibition by disk diffusion. The magnesium contents of the four lots were essentially the same (Table 4). These data do not demonstrate any relationship between total metal ion content and gentamicin susceptibility (Tables 3 and 4). Washington et al. (18) also described a lack of correlation between the total cation contents of various lots of Mueller-Hinton agar and susceptibility as demonstrated by MIC. This may be a phenomenon unique to agar-based media. When investigators have added Mg^{2+} and Ca^{2+} to a test medium, the inverse relationship between Mg^{2+} and Ca^{2+} concentrations and susceptibility has been clear. It may be that measuring total cationic content in agar is not relevant to the explanation of the phenomenon of decreased susceptibility with increased concentrations of cations, but that the ion which is active is only the readily available ion in the medium. Methods for measuring available cation in agar media are currently under investigation. Preliminary evidence indicates that the proportion of cation available varies from lot to lot.

Data from our institutions are only complete for gentamicin; however, initial investigations indicate that tobramycin and amikacin behave in the same way as gentamicin with respect to the direction and degree of variation between lots of media and selected isolates of *P. aeruginosa*. In practice, most isolates do not have zone diameters with tobramycin disks close to the recommended break points, and problems of misinterpretation with this antibiotic may not be very common. Nevertheless, isolates which do have a zone diameter around 14 mm may also be misinterpreted as a fixed intermediate zone in a manner similar to that observed for gentamicin. We are currently investigating the validity of applying an interpretation similar to that suggested for gentamicin to disk diffusion susceptibility tests with these other aminoglycosides. Evidence suggests that the same criteria may be applied.

It is apparent that lot-to-lot variation in Mueller-Hinton agar and the emergence of isolates of *P. aeruginosa* possessing low-level resistance to aminoglycosides (9) have made the application of a fixed interpretative range inappropriate for this combination at the present time. The application of a moving intermediate range that is dependent on the quality control organism is a reasonably effective temporary guideline for the interpretation of disk susceptibility tests, but a

much more satisfactory solution would be the use of media that give reproducible results with aminoglycosides and *Pseudomonas* as well as with other antibiotics and organisms. We have previously indicated the need for manufacturing performance standards for susceptibility testing media in the case of aminoglycosides and *Pseudomonas* (9). These performance standards should define a narrower range for zone diameters than the presently recommended limits for the standard *Pseudomonas* strain.

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