Interrelationships Between Disk and Tube Dilution Sensitivity Tests for the Aminoglycoside Antibiotics Gentamicin, Kanamycin, Sisomicin, and Tobramycin

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Received for publication 12 July 1973

Poor statistical correlation was obtained between tube dilution test results and disk test results by using standard procedures. Significant regressions were obtained although they were not linear. Different regressions were obtained with different bacterial species. It is suggested that for the aminoglycosides studied (gentamicin, kanamycin, tobramycin, and sisomicin) both disk and dilution tests are useful in separating resistant from sensitive organisms. However, poor relationships were obtained between the two test types among sensitive organisms.

The increasing acceptance and use of the standard single disk susceptibility test method known as the Bauer-Kirby procedure (1) and its acceptance by federal regulatory agencies (3) has contributed meaningfully to standardization of susceptibility test procedures as performed in clinical laboratories. Paralleling the increasing use of the disk test have been efforts to relate disk test results to tube dilution results. The recent report of an international collaborative study on sensitivity testing (2) attempts to standardize both disk and dilution methods for sensitivity testing. This report recommends, as an acceptable method of reporting out results of disk tests, the reporting of minimal inhibitory concentrations (MIC) values estimated by extrapolation from appropriate regression line data. This view has moved some steps beyond the initial recommendations of Bauer et al. (1) in which organisms are classified as sensitive, resistant, or intermediate. The Ericsson Commission recommendations (2) suggest that there exists for many antibiotics a distinctive relationship between disk test results and agar or broth dilution test results expressed as MICs. Numerous authors have presented results of studies of such relationships. Previously (6), our laboratory showed that such relationships between tube dilution tests and disk test results for gentamicin and *Pseudomonas* are difficult to demonstrate.

There were separations between resistant and sensitive organisms with both techniques; however, among the sensitive organisms poor correlation existed between MIC values and disk test results. Similar difficulties have been observed recently for another aminoglycoside, tobramycin (4). The graph, in the latter paper correlating MIC values with disk results, shows considerable clustering of points, and differences were seen between different bacterial species. In the course of evaluating the comparative activity of four aminoglycoside antibiotics, kanamycin, gentamicin, sisomicin, and tobramycin (5), we have had the opportunity to relate disk and tube dilution results for a variety of organisms; such studies are the subject of this report.

MATERIALS AND METHODS

Gentamicin, kanamycin, and sisomicin were used in the form of the sulfate, whereas tobramycin was used as the base; all values are corrected in terms of the base. Kanamycin was obtained from Bristol Laboratories and tobramycin was obtained from Eli Lilly and Co. The strains of bacteria used in the study were from a variety of sources but in almost all cases represent recent clinical isolates. Identification of all strains was confirmed by usual biochemical test procedures. Disk sensitivity tests were done by using commercially available 10- μ g gentamicin disks and 30- μ g kanamycin disks. The 10- μ g sisomicin disk was prepared for use by BBL (Cockeysville, Md.); the

10- μ g tobramycin disks were prepared in our laboratories. The disk test procedure used was identical to that described by Bauer et al. (1) as modified by the Food and Drug Administration (3) and is conventionally known as the Bauer-Kirby procedure. Mueller-Hinton agar (BBL) was used in plastic disposable petri dishes (Falcon). Tube dilution tests were done in Mueller-Hinton broth (BBL) in a volume of 3 ml per tube with an inoculum of approximately 5×10^4 to 1×10^6 organisms. This inoculum was obtained by appropriate dilution of an 18-h broth culture (0.05 ml of a 1:1,000 dilution). For tube dilution tests, stock concentrations were freshly prepared in sterile distilled water and added to 100-ml quantities of broth in order to obtain the desired antibiotic concentrations. Based on 1 μ g/ml, these concentrations ranged from 0.03 to 256 μ g/ml in terms of the base. Tubes were read visually after incubation for 18 to 24 h at 37 C. For convenience in statistical evaluation, MIC values were converted to $\log_2$ as recommended by Ericsson and Sherris (2). For our purposes, 0.015 μ g/ml was represented as $\log_2 = 0$, 0.03 as $\log_2 = 1$, and so forth. MIC was considered the independent variable, whereas zone diameter was considered the dependent variable. Regression lines, slopes, intercepts, and correlation coefficients were calculated by standard statistical procedures. A total of 246 bacterial strains was studied with kanamycin, 330 with tobramycin and gentamicin, and 427 with sisomicin. For almost all strains the four antibiotics were evaluated in parallel with both techniques. For kanamycin, *Pseudomonas* strains were not included.

RESULTS

For each antibiotic, the overall regression relationship was determined for all strains, as well as for each of the individual bacterial types

alone. The results of such studies in terms of the y intercept (α), slope (β), and correlation coefficient (R), with 95% confidence limits for slope and intercept estimates, are shown in Tables 1 through 4. The α values given are the intercepts of $\log_2 = 0$ or at an MIC of 0.015 μ g/ml. For the four antibiotics the overall correlation coefficients ranged from a low of 0.63 with sisomicin to 0.77 with gentamicin, reflecting the difficulty in demonstrating relationships between disk tests and MIC values. In none of the cases were the calculated regression lines significantly linear although in each case a significant regression was indicated. The data for individual species of bacteria varied from aminoglycoside to aminoglycoside; however, in general, correlation coefficients for the four aminoglycosides were low for gram-positive and higher for gram-negative bacteria.

The data were further examined individually for each bacterial species. In each of these subsequent studies, the regression lines calculated for all strains and for a particular species are shown graphically on a plot with the individual points for that particular species. In Fig. 1 such data are shown for *Staphylococcus* strains and indicate the similarity in response for the four aminoglycoside antibiotics. In each case the slope for *Staphylococcus* was not as steep as the slope for all organisms together. Marked clustering of points existed for each antibiotic but was most dramatic for gentamicin. The results with enterococci and *Streptococcus* are shown in Fig. 2 and illustrate

TABLE 1. Intercept (α), slope (β), and correlation coefficient (R) of calculated regression lines for kanamycin^a

Organism	No. of strains	Kanamycin		
		α^b	β	R
All strains	246	36.3 (35.7-36.9)	-2.27 (-1.98 to -2.56)	0.70
<i>Staphylococcus</i>	43	35.6 (34.2-37.0)	-1.79 (-1.07 to -2.52)	0.61
<i>Streptococcus</i>	24	15.0 (14.0-16.0)	-0.67 (0.28 to -1.61)	0.29
<i>Enterococcus</i>	15	23.9 (21.9-25.8)	-1.32 (0.35 to -3.00)	0.43
<i>Escherichia coli</i>	52	37.7 (37.1-38.4)	-2.45 (-1.95 to -2.94)	0.81
<i>Klebsiella</i>	71	34.2 (33.6-34.9)	-2.13 (-1.86 to -2.40)	0.88
<i>Proteus</i> sp.	26	34.9 (33.5-36.2)	-1.49 (-0.61 to -2.37)	0.58
<i>Salmonella</i>	15	30.3 (28.9-31.7)	-0.61 (1.32 to -2.53)	0.18

^a $\pm$ 95% confidence limits.

^b Intercept at a MIC of 0.015 μ g/ml ($\log_2 = 0$).

TABLE 2. Intercept (α), slope (β), and correlation coefficient (R) of calculated regression lines for tobramycin^a

Organism	No. of strains	Tobramycin		
		α^b	β	R
All strains	330	33.5 (33.1-33.9)	-2.18 (-1.97 to -2.38)	0.76
<i>Staphylococcus</i>	43	28.5 (27.7-29.2)	-0.52 (-0.05 to -0.98)	0.33
<i>Streptococcus</i>	24	17.7 (17.1-18.6)	-0.77 (-0.03 to -1.52)	0.42
<i>Enterococcus</i>	15	33.0 (30.4-35.6)	-2.49 (0.77 to -5.77)	0.42
<i>Escherichia coli</i>	52	38.5 (37.9-39.3)	-2.67 (-2.05 to -3.29)	0.78
<i>Klebsiella</i>	71	28.9 (28.4-29.7)	-1.69 (-1.39 to -2.01)	0.79
<i>Proteus</i> sp.	26	26.5 (25.8-27.1)	-1.09 (-0.60 to -1.58)	0.68
<i>Pseudomonas aeruginosa</i>	84	34.1 (33.4-34.8)	-2.26 (-1.92 to -2.61)	0.82
<i>Salmonella</i>	15	24.7 (23.8-25.5)	-0.65 (0.32 to -1.61)	0.37

^a $\pm$ 95% confidence limits.^b Intercept at an MIC of 0.015 μ g/ml ($\log_2 = 0$).TABLE 3. Intercept (α), slope (β), and correlation coefficient (R) of calculated regression lines for gentamicin^a

Organism	No. of strains	Gentamicin		
		α^b	β	R
All strains	330	35.9 (35.4-36.2)	-2.34 (-2.13 to -2.54)	0.77
<i>Staphylococcus</i>	43	29.6 (28.9-30.3)	-0.84 (-0.18 to -1.87)	0.25
<i>Streptococcus</i>	24	19.2 (18.7-19.8)	-0.49 (0.01 to -0.98)	0.40
<i>Enterococcus</i>	15	23.3 (22.4-24.0)	-1.09 (0.41 to -2.59)	0.40
<i>Escherichia coli</i>	52	33.6 (32.9-34.1)	-1.74 (-1.06 to -2.42)	0.59
<i>Klebsiella</i>	71	30.1 (29.4-30.8)	-1.57 (-1.21 to -1.93)	0.73
<i>Proteus</i> sp.	26	26.4 (25.6-27.1)	-0.69 (-0.20 to -1.18)	0.52
<i>Pseudomonas aeruginosa</i>	84	44.6 (43.8-45.3)	-3.39 (-3.04 to -3.75)	0.90
<i>Salmonella</i>	15	27.4 (26.4-28.5)	-0.55 (0.82 to -1.92)	0.23

^a $\pm$ 95% confidence limits.^b Intercept at an MIC of 0.015 μ g/ml ($\log_2 = 0$).

further the poor relationship between the regression line calculated for all species and the regression lines calculated for enterococci and streptococci individually. In all cases the points lie below the overall regression line and markedly different slopes were obtained. There are striking similarities in the placement of points

for each of the four antibiotics as opposed to the overall regression lines.

The results with strains of *Escherichia coli* are shown in Fig. 3. With this species, the points for sisomicin deviated more markedly from the overall regression line than did those for tobramycin, gentamicin, and kanamycin. The

TABLE 4. Intercept (α), slope (β), and correlation coefficient (R) of calculated regression lines for sisomicin^a

Organism	No. of strains	Sisomicin		
		α^b	β	R
All strains	427	31.3 30.9-31.7)	-1.68 (-1.48 to -1.87)	0.63
<i>Staphylococcus</i>	53	30.1 (29.4-30.8)	-1.03 (-0.56 to -1.49)	0.53
<i>Streptococcus</i>	36	30.1 (29.5-30.8)	-2.06 (-1.29 to -2.83)	0.68
<i>Enterococcus</i>	25	14.9 (13.9-15.9)	-0.05 (1.40 to -1.50)	0.14
<i>Escherichia coli</i>	64	25.1 (24.6-25.7)	-0.45 (-0.11 to -0.78)	0.32
<i>Klebsiella</i>	82	29.9 (29.3-30.7)	-1.54 (-1.18 to -1.89)	0.70
<i>Proteus</i> sp.	48	29.1 (28.6-29.6)	-1.23 (-0.86 to -1.60)	0.70
<i>Pseudomonas aeruginosa</i>	104	33.1 (32.1-34.1)	-1.99 (-1.55 to -2.43)	0.66
<i>Salmonella</i>	15	25.9 (24.8-26.9)	-0.18 (1.52 to -1.90)	0.16

^a $\pm$ 95% confidence limits.^b Intercept at an MIC of 0.015 μ g/ml ($\log_2 = 0$).

kanamycin data suggest clustering about the overall regression line with a slope for *E. coli* similar to the slope for all organisms. Gentamicin and tobramycin exhibited a tighter clustering than did kanamycin or sisomicin. The data for *Klebsiella* are shown in Fig. 4 and demonstrate a better correlation between disk test and tube dilution results than for some other bacterial species. All four antibiotics showed points clustering about the overall regression line with only gentamicin showing a significant deviation from the overall regression line. Indeed, the lines calculated for the *Klebsiella* strains for kanamycin and sisomicin are remarkably similar to those for all organisms together. The results of tests with *Salmonella* and *Proteus* are shown in Fig. 5, indicating for all of the aminoglycoside antibiotics a poor relationship between the individual species regression lines and the overall regression and as well a poor relationship between the zone size and minimal inhibitory concentration for these two bacterial groups. Possibly the results with *Salmonella* and *Proteus* are influenced by the rather small number of strains used.

The results of tests with a large number of *Pseudomonas* strains are shown in Fig. 6 (kanamycin was not included in the *Pseudomonas* evaluation). For the three aminoglycoside antibiotics studied, gentamicin, sisomicin, and tobramycin, the regression lines calculated for

Pseudomonas were fairly close to that calculated for all organisms. Substantial clustering of points existed for both gentamicin and sisomicin, whereas the tobramycin points were more evenly distributed along the regression line.

DISCUSSION

The results of this study suggest that distinct generalized regression lines relating zone diameter and tube dilution results are difficult to demonstrate for the four aminoglycosides studied. This may be a unique situation with aminoglycosides since many workers have found good relationships between disk and dilution tests for other antibiotic types.

The marked differences seen with different bacterial species suggest that the calculation of a relationship for all bacterial species is highly dependent on the unique collection of bacterial strains used and, in particular, the relative numbers of each species included. It is obvious that any such mixture is artificial with reference to the relative frequency of species isolated in a particular laboratory and to the "true" relationships between test results.

Resorting to separate regression lines for individual bacterial species does not markedly improve these relationships. It seems clear, for aminoglycosides at least, that both disk and dilution tests are useful in separating resistant from sensitive organisms. However, determin-

FIG. 1. Relationship between Bauer-Kirby disk test and tube dilution results for *Staphylococcus aureus* strains. Number in parentheses is the number of strains tested. Solid lines are calculated regression lines for all bacterial species; broken lines are calculated lines for *Staphylococcus*.

FIG. 2. Relationship between Bauer-Kirby disk test and tube dilution results for *Streptococcus* and *Enterococcus* strains. Solid lines are calculated regression lines for all bacterial species; broken lines are calculated lines for *Enterococcus* and *Streptococcus*. The number of strains studied for *Enterococcus* and *Streptococcus*, respectively, was 15 and 24 for gentamicin, kanamycin, and tobramycin, and 25 and 36 for sisomicin.

FIG. 3. Relationship between Bauer-Kirby disk test and tube dilution results for *Escherichia coli* strains. Number in parentheses is the number of strains tested. Solid lines are calculated regression lines for all bacterial species; broken lines are calculated for *Escherichia coli*.

FIG. 4. Relationship between Bauer-Kirby disk test and tube dilution results for *Klebsiella* strains. Number in parentheses is the number of strains tested. Solid lines are calculated regression lines for all bacterial species; broken lines are calculated lines for *Klebsiella*.

FIG. 5. Relationship between Bauer-Kirby disk test and tube dilution results for *Proteus* and *Salmonella* strains. Solid lines are calculated regression lines for all bacterial species; broken lines are calculated lines for *Proteus* and *Salmonella*. The number of strains studied for *Proteus* and *Salmonella*, respectively, was 15 and 24 for gentamicin, kanamycin and tobramycin and 25 and 36 for sisomicin.

FIG. 6. Relationship between Bauer-Kirby disk test and tube dilution results for *Pseudomonas aeruginosa* strains. Number in parentheses is the number of strains tested. Solid lines are calculated regression lines for all bacterial species; broken lines are calculated lines for *Pseudomonas*.

ing unique relationships between results of dilution and disk tests among sensitive bacteria may be more of use as a statistical exercise than as a technique for generating information of a useful practical nature.

ACKNOWLEDGMENTS

Eugene L. Moss, Jr., Frank Sabatelli, and Frederick Menzel provided the laboratory data; Sandra Forman provided statistical assistance.

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