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

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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-μg gentamicin disks and 30-μg kanamycin disks. The 10-μg sisomicin disk was prepared for use by BBL (Cockeysville, Md.); the
10-μg 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 × 10^5 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 μg/ml, these concentrations ranged from 0.03 to 256 μg/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 log₂, as recommended by Ericsson and Sherris (2). For our purposes, 0.015 μg/ml was represented as log₂ = 0, 0.03 as log₂ = 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 log₂ = 0 or at an MIC of 0.015 μg/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 aminoglycosides 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>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>Kanamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>α</td>
</tr>
<tr>
<td>All strains</td>
<td>246</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(35.7-36.9)</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>43</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(34.2-37.0)</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>24</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14.0-16.0)</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>15</td>
<td>23.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(21.9-25.8)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>52</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(37.1-38.4)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>71</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33.6-34.9)</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>26</td>
<td>34.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33.5-36.2)</td>
</tr>
<tr>
<td>Salmonella</td>
<td>15</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(28.9-31.7)</td>
</tr>
</tbody>
</table>

*α ± 95% confidence limits.

*Intercept at a MIC of 0.015 μg/ml (log₂ = 0).*
DISK AND TUBE DILUTION SENSITIVITY TESTS

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>Tobramycin</th>
<th>α</th>
<th>β</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>All strains</td>
<td>330</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

α ± 95% confidence limits.
β Intercept at an MIC of 0.015 μg/ml (log₂ = 0).

Table 3. Intercept (α), slope (β), and correlation coefficient (R) of calculated regression lines for gentamicin

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>Gentamicin</th>
<th>α</th>
<th>β</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>All strains</td>
<td>330</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

α ± 95% confidence limits.
β Intercept at an MIC of 0.015 μg/ml (log₂ = 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
Enterococcus
Streptococcus
Staphylococcus
strains
All
Escherichia coli
Klebsiella
Proteus sp
Pseudomonas
aeruginosa

predicted
regression
overall
448
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the
Pseudomonas data
strains for
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and
tube dilution results
similar
The data
clustering
than did
and
tamicin
nisms
gerotics
species regression
evaluation).
For the three
and Proteus

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demonstrate
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and
relationship
for these
in
Fig.
6
Resorting
to
the marked differences
were fairly close to that calculated
for all organisms. Substantial clustering of
points existed for both gentamicin and sisomi-
cin, 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 diam-
eter and tube dilution results are difficult to
demonstrate for the four aminoglycosides stud-
died. 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" relations-
ships between test results.

Resorting to separate regression lines for indi-
vidual 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-

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of</th>
<th>Sisomicin</th>
<th>Intercept (a)</th>
<th>Slope (b)</th>
<th>Correlation coefficient (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All strains</td>
<td>427</td>
<td></td>
<td>31.3</td>
<td>-1.68</td>
<td>0.63</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>53</td>
<td></td>
<td>30.1</td>
<td>-1.03</td>
<td>0.53</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>36</td>
<td></td>
<td>29.4-30.8</td>
<td>-0.56 to -1.49</td>
<td>0.68</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>25</td>
<td></td>
<td>13.9-15.9</td>
<td>-0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>64</td>
<td></td>
<td>24.6-25.7</td>
<td>-0.11 to -0.78</td>
<td>0.32</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>82</td>
<td></td>
<td>29.3-30.7</td>
<td>-1.18 to -1.89</td>
<td>0.70</td>
</tr>
<tr>
<td>Proteus sp.</td>
<td>48</td>
<td></td>
<td>28.6-29.6</td>
<td>-0.86 to -1.60</td>
<td>0.70</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>104</td>
<td></td>
<td>32.1-34.1</td>
<td>-1.55 to -2.43</td>
<td>0.66</td>
</tr>
<tr>
<td>Salmonella</td>
<td>15</td>
<td></td>
<td>24.8-26.9</td>
<td>-0.18</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* ± 95% confidence limits.
* Intercept at an MIC of 0.015 μg/ml (log₂ = 0).

kanamycin data suggest clustering about the
overall regression line with a slope for E. coli
'similar to the slope for all organisms. Gent-
amycin 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 organ-
isms together. The results of tests with
Salmonella and Proteus are shown in Fig. 5,
indicating for all of the aminoglycoside antibi-
otics a poor relationship between the individual
species regression lines and the overall regres-
sion 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 influ-
enced by the rather small number of strains
used.

The results of tests with a large number of
Pseudomonas strains are shown in Fig. 6 (kana-
mycin was not included in the Pseudomonas
evaluation). For the three aminoglycoside anti-
biotics 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 sisomi-
cin, whereas the tobramycin points were more
evenly distributed along the regression line.

Table 4. Intercept (a), slope (b), and correlation coefficient (R) of calculated regression lines for sisomicin*
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.

ACKNOWLEDGMENTS
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LITERATURE CITED