Modification of Interpretive Breakpoints for Netilmicin Disk Susceptibility Tests with \textit{Pseudomonas aeruginosa}

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Regression analysis of data correlating 30-µg netilmicin disk zone diameters with microdilution MICs, obtained by testing close-interval dilution steps, was performed with 77 selected strains of \textit{Pseudomonas aeruginosa}, each tested in three independent laboratories. A zone of \( \geq 15 \text{ mm} \) correlated with an MIC of \( \leq 12 \text{ µg/ml (susceptible)} \), and a zone of \( \leq 12 \text{ mm} \) correlated with an MIC of \( > 16 \text{ µg/ml (resistant)} \). Additional disk tests were performed with 256 strains having known resistance mechanisms and 280 susceptible strains: the majority were appropriately categorized by these interpretive zone standards. The previously recommended standards of \( \geq 17 \text{ mm (MIC, } \leq 8.0 \text{ µg/ml)} \) for the susceptible category inappropriately placed a significant number of truly susceptible \textit{P. aeruginosa} strains in the intermediate category.

Netilmicin is a newer aminoglycoside with pharmacological properties similar to those of gentamicin, tobramycin, and sisomicin (8, 11, 12). It reportedly has a decreased potential for oto- and nephrotoxicity in humans (6) and thus may be used in somewhat elevated doses, achieving levels in blood higher than those obtained with gentamicin, tobramycin, or sisomicin. Consequently, for in vitro susceptibility testing, tentative MIC breakpoints separating susceptible and resistant categories should be between those used for kanamycin-like aminoglycosides and those used for gentamicin or tobramycin. In the testing of aminoglycosides, the tradition of using only twofold dilutions causes some difficulty; closer dilution intervals seem more appropriate for this group of drugs. We have recently recommended that for gentamicin, tobramycin, and sisomicin, the MIC breakpoint for the susceptible category should be \( \leq 6.0 \text{ rather than } \leq 4.0 \text{ µg/ml (3, 4).} \)

In 1980, netilmicin susceptibility tests were evaluated by Jones et al. (5), and 30-µg disks were recommended with zone standards of \( \geq 17 \text{ mm} \) for the susceptible category (MIC, \( \leq 8.0 \text{ µg/ml} \)) and \( \geq 13 \text{ mm} \) for the resistant category (MIC, \( > 16 \text{ µg/ml} \)). In that study, a fairly large proportion of \textit{Pseudomonas aeruginosa} strains provided intermediate MICs of \( 16 \text{ µg/ml} \) and intermediate zones of 14 to 16 mm in diameter. In the current study, we reexamined netilmicin disk tests with \textit{P. aeruginosa}, applying a midrange MIC breakpoint of \( \leq 12 \text{ µg/ml} \) for the susceptible category, as has been done with other aminoglycosides (3, 4).

MATERIALS AND METHODS

Laboratories directed by three of us (A. L. Barry, R. N. Jones, and C. Thornsberry) each tested 77 \textit{P. aeruginosa} strains, selected to provide a uniform distribution of MICs over the range of concentrations tested. All three laboratories performed tests with a single lot of microdilution trays containing cation-supplemented Mueller-Hinton broth (10) and duplicated close-interval dilutions of netilmicin (1, 2, 4, 5, 6, 7, 8, 9, 10, 12, 16, and 32 µg/ml). Two laboratories also performed standard disk diffusion tests (9) with 30-µg netilmicin disks. Microdilution tests were performed according to the National Committee for Clinical Laboratory Standards (10). Each laboratory generated two MICs for each strain tested. Thus, six MICs were accumulated for each strain, and geometric mean MICs (log₂) were calculated and plotted against the arithmetic mean of two zone diameters (millimeters). Precision of data generated in this manner was very acceptable, as documented in reports of other aminoglycosides, cephalosporins, and semisynthetic penicillins (1-3).

To further evaluate the test results obtained from the study outlined above, data from the resistance mechanism surveillance program of Schering Corp. were provided by two of us (G. H. Miller and R. S. Hare). Between January 1980 and August 1982, 538 isolates of \textit{P. aeruginosa} were submitted from 75 hospitals worldwide. Each strain was studied to identify its aminoglycoside resistance mechanism (7; K. Shimizu, T. Kimada, W. Hsieh, H. Y. Chung, Y. Chong, R. Hare, G. H. Miller, F. Sabatelli, and J. Howard, 13th Int. Congr. Chemother., 1983, abstr. no. SE 2.6, p. 52). This was done by correlating the pattern of susceptibility of each strain to six or more different aminoglycosides with the patterns obtained with strains containing known aminoglycoside-modifying enzymes. Broth dilution tests were performed with gentamicin, tobramycin, amikacin, netilmicin, 2'-N-ethyl-netilmicin, and 6'-N-ethyl-netilmicin. Supplemental tests were also performed against sisomicin, 5-episisomicin, SCH 21420, SCH 27598, fortimicin A, and dibekacin when needed to confirm mechanisms identified on the basis of tests with the initial set of six drugs. Strains resistant to the first six aminoglycosides were considered to be permeability mutants. Netilmicin, gentamicin, tobramycin, and amikacin disk tests were performed, and their zone diameters were recorded. Included in the collection were 209 normal strains with no identifiable resistance pattern: they were susceptible to all tested aminoglycosides. Strains with patterns indicating aminoglycoside-modifying enzymes were considered susceptible to each drug when the enzyme was known to be inactive against that aminoglycoside and resistant to each drug when the enzyme was capable of inactivat-
ing the aminoglycoside. The distribution of the zone diameters recorded by two of us (G. H. Miller and R. S. Hare) was calculated, comparing the resistant and susceptible populations of strains.

**RESULTS**

Data were obtained from the three collaborating laboratories, each using duplicated close-interval dilutions for determining MICs. Geometric means of six MICs were plotted against arithmetic means of two zone diameters (Fig. 1A). To depict the results that would have been obtained if close-interval dilution schedules had not been tested and if geometric mean MICs had not been calculated, the data are also displayed by rounding off the geometric mean MICs to the next even log₂ dilution interval (Fig. 1B). Six strains gave MICs of ≤1 μg/ml (not included in Fig. 1); they produced zones 24 to 28 mm in diameter. Six other strains provided MICs of >32 μg/ml; their zone sizes are included in Fig. 1 but were not used for regression analysis. If even log₂ dilution intervals had been tested (Fig. 1B), a zone size breakpoint of ≥17 mm accurately predicted susceptibility to 8.0 μg/ml or less and a zone of ≤13 mm indicated an MIC of >16 μg/ml. This confirms the data previously presented by Jones and colleagues (5). Intermediate MICs (16 μg/ml) were recorded with 22 of the 77 strains; 19 of the 22 strains gave intermediate zones of >13 but <17 mm. Intermediate-sized zones were also observed with 11 of the 24 susceptible strains (MIC, 8 μg/ml) and with 7 of the 17 resistant strains (MIC, >16 μg/ml). Using the criteria of Jones et al. (5), we obtained truly equivocal or indeterminant disk test results with 37 of the 77 strains. Regression analysis of these data provided a relatively poor correlation coefficient (0.74), but appropriate MIC correlates for 13- and 17-mm zones were calculated (Fig. 1B). A 15-mm zone correlated with an MIC of just below 16 μg/ml (15.6 μg/ml).

By plotting intermediate MICs obtained by close-interval dilution tests, the correlation coefficient was improved (0.82 versus 0.74). Examination of these data (Fig. 1A) revealed that strains with zones in the 15- to 16-mm range were actually inhibited by 14 μg/ml or less and that most required MICs of ≤12 μg/ml, whereas the majority of strains with zones of <15 mm required MICs of >12 μg/ml. These facts suggest that strains with zones of ≥15 mm might be considered susceptible to 12 μg or less of netilmicin per ml.

Strains with known resistance mechanisms were next examined to clearly define the susceptibility breakpoint. Table 1 shows the P. aeruginosa isolates that have been studied to date. They can be separated into resistant and susceptible populations according to the type of aminoglycoside-modifying enzyme that was identified. The distribution of zone sizes that were recorded is shown in Fig. 2. With

**TABLE 1.** *P. aeruginosa* analyzed for aminoglycoside resistance mechanisms

<table>
<thead>
<tr>
<th>Resistance mechanism*</th>
<th>No. of strains (S, susceptible to the drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Netilmicin</td>
</tr>
<tr>
<td>None</td>
<td>209 (S)</td>
</tr>
<tr>
<td>AAC(3)-I</td>
<td>8 (S)</td>
</tr>
<tr>
<td>AAC(3)-Ia</td>
<td>9</td>
</tr>
<tr>
<td>AAC(3)-II</td>
<td>17</td>
</tr>
<tr>
<td>AAC(6')-I</td>
<td>22</td>
</tr>
<tr>
<td>AAC(6')-II</td>
<td>182</td>
</tr>
<tr>
<td>ANT(2)</td>
<td>63 (S)</td>
</tr>
<tr>
<td>Permeability</td>
<td>28</td>
</tr>
<tr>
<td>Total susceptible</td>
<td>280</td>
</tr>
<tr>
<td>Total resistant</td>
<td>258</td>
</tr>
</tbody>
</table>

* Aminoglycoside-modifying enzymes or permeability mutants identified by susceptibility patterns (7; Shimizu et al., 13th Int. Congr. Chemother., 1983). AAC, Acetylating enzymes; ANT, adenylating enzymes; permeability, resistant to all tested aminoglycosides but no identifiable enzymatic inactivating mechanism.

* The strains were assumed to be susceptible to the drug if the enzyme is known to be inactive against that aminoglycoside.
gentamicin and tobramycin, few strains gave intermediate zone sizes and very few resistant strains would have been reported to be susceptible. With amikacin, the separation of resistant and susceptible populations was less clearly defined, but the total number of resistant strains was extremely small. Only 2 of 50 amikacin-resistant strains would have been reported to be susceptible by the disk test. With 30-µg netilmicin disks, 54 strains gave zones of 15 to 16 mm in diameter and >98% of those were susceptible (no detectable resistance patterns). Zones of 13 to 14 mm in diameter were observed with 31 of the 536 strains: 7 were resistant and 24 were susceptible to netilmicin. Eight susceptible strains gave zones of ≤12 mm, and six resistant strains gave zones of ≥15 mm (five had zones of ≥17 mm).

**DISCUSSION**

To be consistent with the criteria currently being applied for categorizing other aminoglycosides, MIC breakpoints for netilmicin should involve intermediate, short-interval dilutions, i.e., MIC of ≤12 rather than ≥8 µg/ml for the susceptible category. Such breakpoints are not inconsistent with pharmacological data that are currently available (8, 11, 12). MIC breakpoints for gentamicin, tobramycin, and sisomicin have been defined as ≤6.0 µg/ml for the susceptible category and >8.0 µg/ml for the resistant category (3, 4). For all of the above aminoglycosides, the breakpoint for the susceptible category is very close to the maximal level in serum that can be achieved with a reasonable degree of safety.

Zone size breakpoints for gentamicin and tobramycin (10-µg disks) have been set at ≤12 mm for resistant, 13 to 14 mm for intermediate, and ≥15 mm for susceptible categories (9). The same interpretive standards can be applied to tests with 30-µg netilmicin disks, although the disk potency and the MIC correlates differ. The current study considers tests only with *P. aeruginosa*, and applicability to other gram-negative bacilli remains to be reported.

Revision of interpretive zone standards for tests with *P. aeruginosa* from ≥17 to ≥15 mm for the susceptible category is supported by the data accumulated during the resistance pattern survey. With the zone standards previously proposed by Jones et al. (5), an undesirably large proportion of susceptible *P. aeruginosa* isolates produce zones in the intermediate (equivocal) range. In a recent survey of *P. aeruginosa* disk susceptibility tests performed in 35 hospitals throughout the United States, 18% of 2,365 tests with *P. aeruginosa* gave intermediate zones of 14 to 16 mm in diameter (BAC DATA survey, G. Miller, personal communication). Data presented here suggest that the vast majority of those isolates with intermediate zones are truly susceptible. Clinical response data that are currently available (Miller, personal communication) support the contention that strains with 15- to 16-mm zones should be considered clinically susceptible to netilmicin. These observations lead us to conclude that the following interpretive zone standards should be applied when testing *P. aeruginosa* with 30-µg netilmicin disks: susceptible, ≥15 mm (MIC, ≤12 µg/ml); intermediate, 13 to 14 mm (MIC, 16 µg/ml); resistant, <12 mm (MIC, >16 µg/ml).

These recommendations are based on our studies with *P. aeruginosa*. Since very few members of the family Enterobacteriaceae produce zones in the 15- to 16-mm range, the above modified zone size breakpoints might be applied also to tests with the enteric bacilli and other species. However, limited data with aminoglycoside-resistant enteric bacilli suggest that a susceptible breakpoint of ≥17 mm might be more appropriate for 30-µg netilmicin disks when testing species other than *P. aeruginosa* (Miller, personal communication). Until appropriate studies have been reported indicating that the same set of zone size breakpoints may be applied to all species, we recommend breakpoints of ≤12 and ≥15 mm for tests with *P. aeruginosa* and ≥13 and ≥17 mm for all other gram-negative bacilli.

**LITERATURE CITED**