Correlation of Netilmicin Disk Diffusion Susceptibility and Agar Dilution Susceptibility

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The minimal inhibitory concentration of netilmicin determined by the agar dilution method was correlated with the disk diffusion zone of inhibition against 322 clinical isolates. Regression line analysis revealed good correlation, $r = -0.90$, and suggested that isolates with zone sizes ≤ 14 mm should be considered resistant.

We have reported that netilmicin, an ethyl derivative of sisomicin, inhibits many aerobic gram-negative bacteria and is active against many gentamicin-resistant organisms (2). Studies in our laboratory have shown that netilmicin has pharmacokinetic properties similar to those of gentamicin (6). Furthermore, we have demonstrated that netilmicin is an effective and safe therapy for serious infections in hospitalized patients (7).

In view of the potential clinical usefulness of netilmicin, we evaluated the correlation of agar dilution minimal inhibitory concentrations (MICs) of netilmicin with disk sensitivity against 84 *Pseudomonas aeruginosa*, 34 *Proteus*, 31 *Klebsiella pneumoniae*, 31 *Escherichia coli*, 23 *Serratia marcescens*, 9 *Acinetobacter*, 17 *Providencia*, 18 *Enterobacter*, 16 *Citrobacter*, 20 *Salmonella*, 18 *Shigella*, and 21 *Staphylococcus aureus* isolates. MICs were determined by the agar dilution method with a 10^{-2} dilution of an overnight culture in Mueller-Hinton broth (BBL), using a final inoculum to agar of approximately 10^5 colony-forming units.

Disk diffusion studies were performed according to the method of Bauer et al. (1). Overnight cultures were diluted with sterile Mueller-Hinton broth and were adjusted to an 0.5 MacFarland opacity standard. A control stain of *S. aureus* ATCC 25923 was included for each experiment. Disks containing 10 μ g of netilmicin were supplied by the Schering Corp., Bloomfield, N.J. Correlation between agar dilution MIC and zone diameter was examined by linear regression analysis.

The linear regression curve plotting MICs and zone diameters is shown in Fig. 1. There was a good correlation with regression line: zone size = $22.73 - 2.22 \log_2 \text{MIC}$. The calculated correlation coefficient was -0.90 , and the P value was <0.01 . Serum levels of 6 to 10 μ g/ml are achieved after doses of 2 mg/kg (6). Utilizing regression

line analysis, isolates resistant to netilmicin ($\text{MIC}, \geq 12.5 \mu\text{g/ml}$) correspond to zone sizes of ≤ 14 mm. With this breakpoint, however, 11 *Pseudomonas* isolates susceptible to netilmicin would have been considered resistant and 8 *Pseudomonas* isolates resistant to netilmicin would have been considered susceptible. The use of these criteria also revealed two "false" resistant and six "false" susceptible organisms among the *Enterobacteriaceae*.

This is in contrast to the results of Goldmann et al. (3), who stated that isolates with zone sizes ≤ 11 mm should be considered resistant to netilmicin. This difference undoubtedly is because they had few resistant isolates. We found 10% of isolates with netilmicin MICs $\geq 25 \mu\text{g/ml}$, whereas they found $<1\%$ resistant to 25 μ g of netilmicin per ml. This is one of the reasons that the regression line found by Goldmann et al. (3) does not intersect the ordinate well above the critical MIC for resistance. We also experienced difficulty in establishing a breakpoint for *P. aeruginosa*, as did Goldmann et al. (3). A separate regression curve for *P. aeruginosa* (zone size = $2.172 - 2.2 \log_2 \text{MIC}$; correlation coefficient = -0.89 ; P value, <0.01) also suggested a break-point of ≤ 14 mm for resistance, with 19 of 83 isolates yielding incorrect results.

The breakpoint reported by Greenstone et al. (4) was ≤ 18 mm for resistance. However, this group selected highly resistant *Pseudomonas* isolates (98% of the isolates with netilmicin MICs $\geq 12.5 \mu\text{g/ml}$) and otherwise used all *Enterobacteriaceae* with MICs $\leq 6.3 \mu\text{g/ml}$. Moreover, no regression line was analyzed to give the 18-mm breakpoint.

Our results agree with those of Habwe and Shadomy (5), who also suggested a 14-mm zone size as the breakpoint. This regression line had a low correlation coefficient ($r = 0.6584$) and only one false resistant organism. The false resistant and susceptible readings in our study

FIG. 1. Regression line between zone of inhibition of 10- μ g netilmicin disk and netilmicin MIC. Symbols: ($\square$) *Pseudomonas*; ($\bullet$) *P. mirabilis*; ($\circ$) *Proteus*, indole-positive; ($\blacktriangle$) *Klebsiella*; ($\triangle$) *E. coli*; ($\times$) *Enterobacter*; ($\blacksquare$) Other (*S. aureus*, *Serratia*, *Acinetobacter*, *Providencia*, *Citrobacter*, *Salmonella*, and *Shigella*).

TABLE 1. Regression line analysis of susceptibility data by netilmicin agar dilution studies

Organism	No. of strains tested	Disk size (μ g)	Correlation coefficient	Slope	Y intercept ^a
<i>P. aeruginosa</i>	84	10	-0.89	-2.27	+21.72
<i>P. aeruginosa</i>	84	20	-0.88	-2.41	+24.39
All organisms	322	10	-0.90	-2.22	+22.73
All organisms	322	20	-0.88	-2.04	+23.90

^a Expressed in $\log_2$ MIC.

occurred since we selected many intermediate organisms for which the netilmicin MIC ranged from 6.3 to 25 μ g/ml. Nonetheless, the correlation coefficient for the large group (322 isolates) was -0.90.

Use of a 20- μ g disk does not obviate the aforementioned problems (see Table 1). Thus, the use of the 10- μ g disk with inhibition zones of ≤ 14 mm being resistant, 15 mm being intermediate, and ≥ 16 mm being susceptible seems optimal. Use of these criteria would eliminate most false resistance.

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