Correlation of Netilmicin Agar Dilution and Disk Diffusion Susceptibilities

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A study of 283 isolates of gram-negative bacilli revealed a good correlation ($r = -0.74$) between disk diffusion zones of inhibition and agar dilution minimal inhibitory concentrations. Regression analysis suggested that strains with zone sizes ≤ 11 mm should be considered resistant, but 34 of 45 strains resistant by minimal inhibitory concentration (including 27 strains of *Pseudomonas aeruginosa*) would have been called susceptible using this break point.

Netilmicin (Sch 20569), the 1-*N*-ethyl derivative of sisomycin, is a new aminoglycoside with a pharmacokinetic profile similar to that of gentamicin (8). Netilmicin has been reported to have considerable activity against gram-negative clinical isolates (2, 3, 5, 6, 8-10) and to be effective against some gentamicin-resistant strains (3, 7-9). This in vitro study evaluates the correlation between netilmicin agar dilution minimal inhibitory concentrations (MICs) and disk diffusion zone diameters.

Two hundred eighty-three clinical isolates of gram-negative bacilli were studied: 69 strains of *Pseudomonas aeruginosa*, 6 strains of *Pseudomonas* sp., 20 strains of indole-positive *Proteus*, 28 strains of *Enterobacter*, 64 strains of *Klebsiella*, 10 strains of *Serratia*, 10 strains of *Citrobacter*, and 76 strains of *Escherichia coli*. MICs were performed by the agar dilution method as recommended by the International Collaborative Study of the World Health Organization (4). Bacteria were grown overnight in Mueller-Hinton broth (BBL). Serial twofold dilutions of the antibiotics to be tested were prepared in Mueller-Hinton agar (BBL). The Mueller-Hinton agar plates were inoculated with approximately 10^4 organisms using a Steers replicator. Plates were read after 24 h of incubation at 37°C. Gentamicin resistance was defined as an MIC ≥ 8 $\mu\text{g/ml}$. Disk diffusion susceptibility testing was performed according to the method of Bauer et al. (1) using a 10- μg netilmicin disk. Correlation between netilmicin zone diameters and MICs was examined by linear regression analysis. Netilmicin sulfate and netilmicin sulfate disks were supplied by the Schering Corp.

There was good correlation between $\log_2$ MICs for netilmicin obtained by the agar dilu-

tion technique and zone diameters obtained by the Bauer-Kirby method. The linear regression curve for all 283 strains is shown in Fig. 1 ($\log_2$ MIC = $6.39 - 0.31$ zone diameter; correlation coefficient = -0.74 ; $P = <0.01$). Extrapolating from this curve, MICs ≥ 8 $\mu\text{g/ml}$ (resistant to netilmicin) correspond to zone sizes ≤ 11 mm. However, using this 11-mm break point, 34 of our 45 strains with MICs ≥ 8 $\mu\text{g/ml}$ would have been called susceptible to netilmicin. Of these 34 strains, 27 were *P. aeruginosa*.

A separate linear regression curve for *P. aeruginosa* ($\log_2$ MIC = $5.44 - 0.19$ zone diameter; correlation coefficient = -0.71 ; $P = <0.01$) suggested a break point ≤ 13 mm for resistance to netilmicin. However, 18 strains of *P. aeruginosa* with MICs ≥ 8 $\mu\text{g/ml}$ would still have been called susceptible using this break point, and 2 strains with MICs < 8 $\mu\text{g/ml}$ would have been called resistant. The difficulty in establishing a reliable break point for *P. aeruginosa* is due to the wide variation in zone size for strains with MICs of 4 $\mu\text{g/ml}$ (zones, 11 to 25 mm) and 8 $\mu\text{g/ml}$ (zones, 10 to 23 mm). The problem is compounded by the fact that the MICs for our strains of *P. aeruginosa* have a unimodal rather than bimodal distribution, with most strains having MICs of 4 to 8 $\mu\text{g/ml}$ (Fig. 2). Thus, given the usual one-dilution error in the reproducibility of agar dilution testing, it may be difficult to categorize most strains of *P. aeruginosa* as clearly susceptible or resistant to netilmicin by either the agar dilution or disk diffusion method.

Few of our strains demonstrated high-level resistance to netilmicin, and it is possible that the break point for disk diffusion testing of gram-negative bacilli can be refined by including a greater number of resistant strains in the

FIG. 1. Regression curve for gram-negative bacilli ($\log_2 \text{MIC} = 6.39 - 0.31 \text{ zone diameter}$; $r = -0.74$).

FIG. 2. Distribution of *P. aeruginosa* MICs.

linear regression analysis. However, our data suggest that it will be difficult to establish a reliable break point for *P. aeruginosa* using a 10- μg netilmicin disk.

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