In Vitro Susceptibility Studies with Netilmicin: Comparison of a 10-μg Netilmicin Disk with a Standardized 10-μg Gentamicin Disk

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Netilmicin is a new, semisynthetic aminoglycoside antibiotic active against some gentamicin-resistant gram-negative bacteria. In this study we compared a 10-μg netilmicin disk with the standardized 10-μg gentamicin disk in terms of their abilities to predict probable clinical susceptibility to netilmicin. The agar dilution procedure of the International Collaborative Study of the World Health Organization and the U.S. Food and Drug Administration standardized disk test procedure were used. The gentamicin disk failed to predict the clinical susceptibility to netilmicin of 26 of 118 isolates previously shown by the agar dilution technique to be netilmicin susceptible. The netilmicin disk correctly predicted probable susceptibility of all 26 isolates, including 20 shown by the agar dilution procedure to be resistant to gentamicin. These studies demonstrate the need for a separate netilmicin disk for use in agar diffusion disk susceptibility tests.

Netilmicin (Sch 20569) is a new, semisynthetic aminoglycoside derived by ethylation of the 1-N position of the deoxystreptamine ring of sisomicin (9). It has been shown to have in vitro activity similar to that of sisomicin and gentamicin against gram-negative pathogens (4, 5, 7, 8) and also to be active against some gentamicin-resistant strains of gram-negative bacteria (6–8). Netilmicin also has been shown to have a pharmacokinetic profile similar to that of gentamicin (8). This in vitro study compared a 10-μg netilmicin disk with the standardized 10-μg gentamicin disk in terms of their abilities to predict probable clinical susceptibility or resistance to netilmicin.

One hundred eighteen clinical isolates of gram-negative bacilli were studied. These included 25 strains of Pseudomonas aeruginosa, of which 7 were clinically resistant to gentamicin (minimum inhibitory concentration [MIC], ≥8 μg/ml); 28 strains of Serratia marcescens, including 5 resistant to gentamicin; 26 strains of Klebsiella species, of which 8 were resistant to gentamicin; 14 strains of Enterobacter species; and 25 strains of Escherichia coli. All were recent clinical isolates recovered from laboratory specimens including urines, bloods, and sputa.

MICs were determined with the agar dilution method recommended by the International Collaborative Study of the World Health Organization (2). Bacteria were grown overnight in Mueller-Hinton broth (BBL). Serial twofold dilutions of netilmicin sulfate (Schering, batch 7733-15, potency of 603 μg/mg) and gentamicin sulfate (Schering, batch GMC-5-7048, potency of 558 μg/mg) were prepared in Mueller-Hinton agar (BBL), with final concentrations ranging from 128 to 0.063 μg of active drug per ml. The agar plates, with and without antibiotic, were inoculated with approximately 10⁶ organisms by using a mechanical replicator device. Plates were examined after 18 to 24 h of incubation at 35°C, and the MIC was defined as the lowest concentration of antibiotic inhibiting visible growth. Disk diffusion susceptibility tests were performed according to the method of Bauer et al. as modified by the U.S. Food and Drug Administration (3) using an experimental 10-μg netilmicin disk (Schering, lot X-2017) and a standard, certified 10-μg gentamicin disk (Schering, lot 6AMW101). Correlations between resulting zone diameters and MICs were examined by linear regression analysis.

Figures 1 and 2 show the linear correlations between results obtained with the 10-μg netilmicin and the 10-μg gentamicin disks, respectively, with paired MICs of netilmicin as determined by agar dilution tests. With the netilmicin disk there was good correlation between netilmicin log₂ MICs and paired zone diameters ($r = -0.6584, P < 0.001$). In contrast, results with the gentamicin disk revealed poor correlation ($r$
FIG. 1. Relationship between zonal responses to a 10-μg netilmicin disk (U.S. Food and Drug Administration disk test) and paired netilmicin MICs (World Health Organization International Collaborative Study agar dilution test).

FIG. 2. Relationship between zonal responses to the standardized 10-μg gentamicin disk (U.S. Food and Drug Administration disk test) and paired netilmicin MICs (World Health Organization International Collaborative Study agar dilution test).
= −0.3532) between paired netilmicin log₂ MICs and gentamicin zone diameters. The coefficient of correlation for paired gentamicin MICs and zone diameters was −0.883. The lesser degree of correlation between paired netilmicin MICs and zone diameters, as compared with the correlation between paired gentamicin MICs and zone diameters, reflects the presence of organisms totally resistant to gentamicin but susceptible to netilmicin.

Comparison of the graphic data plots for the two regression lines (Fig. 1 and 2) indicated that the 10-μg gentamicin disk could not be used for prediction of probable clinical susceptibility of gram-negative pathogens to netilmicin over a wide range of MICs. Using an MIC of 8 μg/ml as the upper limit of probable clinical susceptibility to netilmicin (G. Arcieri, Schering Corp., personal communication) and zone diameters of ≥14 mm for the netilmicin disk and ≥13 mm for the gentamicin disk as the “break points” for probable clinical susceptibility, it was observed (Fig. 2) that 26 gram-negative organisms susceptible to netilmicin as determined by agar dilution MICs were “resistant” according to results obtained with the gentamicin disk. All were “susceptible” according to the netilmicin disk test. Therefore, the ability of the standardized 10-μg gentamicin disk to predict probable susceptibility or resistance of bacterial pathogens to the newer, enzymatically stable aminoglycosides such as netilmicin and amikacin appears to be limited. These findings demonstrate the need for standardization of a separate netilmicin disk for use in agar diffusion susceptibility tests.

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