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In Vitro Susceptibility of Gentamicin-Resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* to Netilmicin and Selected Aminoglycoside Antibiotics

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Netilmicin (Sch 20569), a semisynthetic aminoglycoside related to gentamicin C_{1a}, was evaluated in vitro in agar dilution testing against 224 different clinical isolates of gentamicin-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* in parallel with amikacin, gentamicin, sisomicin, and tobramycin. Netilmicin showed a very high degree of activity against gentamicin-resistant organisms, but amikacin was more active in vitro, particularly against *Providencia stuartii* and *P. aeruginosa*. Sisomicin and tobramycin were consistently less active than either netilmicin or amikacin. Netilmicin was bactericidal in broth testing against *P. aeruginosa*. Netilmicin showed a greater difference between results with agar and broth dilution testing than did amikacin.

Resistance to aminoglycoside antibiotics has commonly followed clinical use of these agents (4). Resistance to gentamicin among the *Enterobacteriaceae* and *Pseudomonas aeruginosa* appears to be a problem of increasing importance (5, 12). At the Wadsworth VA Hospital amikacin was shown to be the most effective aminoglycoside in vitro against gentamicin-resistant *Enterobacteriaceae* and *P. aeruginosa* (8, 12). Likewise, it has been highly effective in treating infections caused by gentamicin-resistant, gram-negative bacilli (11).

Netilmicin (Sch 20569) is a new semisynthetic aminoglycoside related to sisomicin, a dehydrogenated analogue of gentamicin C_{1a}, but it contains an additional ethyl group on the central ring (15). Rahal et al. have compared netilmicin in vitro with amikacin and gentamicin against gentamicin-susceptible and gentamicin-resistant gram-negative bacilli and have shown considerable activity (15). Early clinical investigation with netilmicin indicates that the blood levels achieved are equal to or greater than those achieved with gentamicin, which it closely resembles pharmacologically (L. S. Young, personal communication, 1976). This study was designed to compare the in vitro effectiveness of netilmicin with other aminoglycosides currently licensed or under clinical investigation, i.e., amikacin (8, 9, 11, 12, 15, 19, 20), sisomicin (8, 9, 20), and tobramycin (8, 9, 13, 20). Two-hundred and twenty-four different recent clinical isolates of gentamicin-resistant *Enterobacteriaceae* and *P. aeruginosa* were available.

MATERIALS AND METHODS

Different clinical isolates of *Enterobacteriaceae* and *P. aeruginosa* resistant to gentamicin by standardized disk testing (1) in the Microbiology Laboratory of Wadsworth VA Hospital were collected from August 1974 to March 1976 and identified by standard criteria. All isolates within a genus were from different patients. *Serratia marcescens* was identified to species by the fermentation of arabinose. Organisms showing a zone size of ≤12 mm to a 10-μg gentamicin disk on subsequent standardized disk testing (1) as recommended for *P. aeruginosa* (18) were tested by the agar plate dilution method (3) recommended by the International Collaborative Study of the World Health Organization. Approximately 10^4 organisms grown overnight at 37°C in Mueller-Hinton broth culture were inoculated with a replicating device (17) onto media prepared from Mueller-Hinton broth solidified with 1.5% agar (Difco) and 5% defibrinated sheep blood prepared to contain amikacin, gentamicin, netilmicin, sisomicin, or tobramycin in twofold dilutions from 128 to 1 μg/ml. Plates identical except for lack of antibiotic were used as controls. Amikacin sulfate was supplied by E. Yevak of Bristol Laboratories, and tobramycin base was supplied by R. S. Griffith of Eli Lilly & Co. Gentamicin sulfate, netilmicin, and sisomicin sulfate were gifts of George Hough of the Schering Corporation. A single lot of Mueller-Hinton broth was utilized because of the effect of varied cation activity on aminoglycoside testing (6, 10). Freeze-thaw extraction of the agar media was performed. The magnesium content of the liquid extracted from

the media, which was repeatedly frozen and thawed, was from 0.71 to 1.54 mg/100 ml (mean, 1.23 mg/100 ml on three determinations) as measured by atomic absorption spectrophotometry; these results are similar to previous measurements (12). The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic showing only a haze, one colony, or no growth after overnight incubation (3). Reference strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included in parallel tests. All determinations were made in duplicate or triplicate, and the MICs were expressed as averages. Only organisms with a gentamicin MIC $\geq$16 μg/ml in agar dilution testing or $\geq$8 μg/ml in broth dilution testing were included. Two hundred and twenty-four isolates fulfilled these criteria.

Because of the known effect of cation concentration and especially magnesium (6, 10; K. P. Fu and H. C. Neu, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A30, p. 6) on susceptibility testing of aminoglycosides with *P. aeruginosa*, as well as our initial results of higher MICs to netilmicin with *P. aeruginosa* than with *Enterobacteriaceae*, broth dilution testing was performed in parallel with netilmicin, amikacin, and gentamicin. MICs in broth were performed as outlined (3) with an inoculum of approximately 5×10^5 organisms/ml. Minimal bactericidal concentrations were performed by inoculating approximately 0.01 ml from tubes showing no turbidity onto antibiotic-free agar plates and recording the concentration of antibiotic at which five or fewer colonies grew after overnight incubation. This corresponds to 99.9% or greater killing. The magnesium content of the broth was 0.35 to 0.53 mg/100 ml (mean, 0.42 mg/100 ml on 11 determinations).

RESULTS

P. aeruginosa. Amikacin showed the greatest degree of in vitro activity in agar testing (Fig. 1). Gentamicin and the two most active agents in agar testing, amikacin and netilmicin, were also utilized in broth dilution testing (Fig. 2). A greater difference between agar and broth MICs was seen with netilmicin than with either amikacin or gentamicin (Fig. 2). Netilmicin broth MICs ranged from 2 to 16 times lower than agar MICs, but a smaller range was seen with amikacin. Various *P. aeruginosa* isolates that required 32 to 128 μg of netilmicin per ml for inhibition in broth were inhibited by relatively lower concentrations of amikacin ($\leq$16 μg/ml), but cross-resistance was noted in some strains. Conversely, no isolate of *P. aeruginosa* resistant to amikacin in broth (MIC $\geq$32 μg/ml) was inhibited by netilmicin at a concentration of $\leq$16 μg/ml, and almost all of these organisms were inhibited by netilmicin only at a concentration of 64 to 128 μg/ml.

S. marcescens. Both netilmicin and amikacin were highly effective in vitro. Sisomicin and tobramycin were considerably less active (Fig. 3).

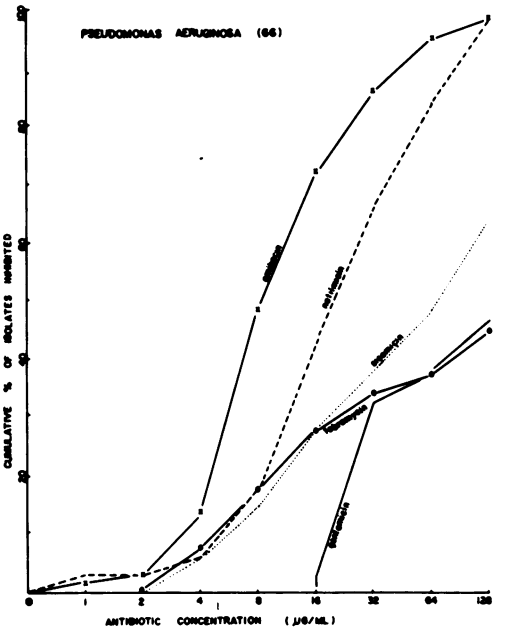

FIG. 1. *Antibiotic susceptibility patterns of 66 different clinical isolates of gentamicin-resistant P. aeruginosa in agar dilution testing.*

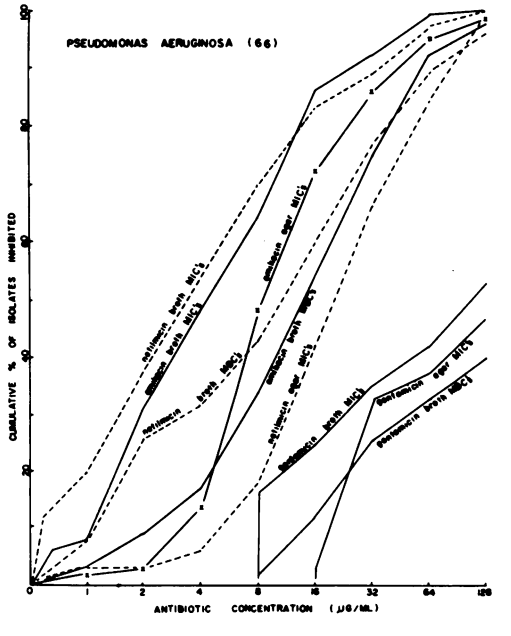

FIG. 2. *Comparison of agar MICs, broth MICs, and broth minimal bactericidal concentrations (MBCs) of 66 different clinical isolates of gentamicin-resistant P. aeruginosa with amikacin, gentamicin, and netilmicin.*

Klebsiella pneumoniae. As with *S. marcescens*, netilmicin and amikacin were the most active agents in vitro (Fig. 4).

Providencia stuartii. Amikacin was the most active agent. Netilmicin and sisomicin showed less activity than amikacin but were similar to each other (Fig. 5).

***Enterobacter* sp.** Netilmicin and amikacin were both highly active in vitro. Sisomicin was less active; tobramycin results were generally parallel to gentamicin (Fig. 6).

E. coli. Netilmicin was the most active agent in vitro. Amikacin was also highly effective, but sisomicin and tobramycin again were less active (Fig. 7).

Indole-positive *Proteus* sp. Amikacin was the most effective in vitro agent (Fig. 8). All five strains of *P. morganii* were inhibited by 2 μg or less of netilmicin per ml, but three strains of *P. rettgeri* required high levels of netilmicin for inhibition while showing inhibition at low levels of amikacin.

DISCUSSION

Our results show that netilmicin is highly active in vitro against almost all strains of gentamicin-resistant *Enterobacteriaceae*, with the exception of *P. stuartii*. It is bactericidal against *P. aeruginosa* in broth testing, but the medium affects results markedly. Agar dilution testing with netilmicin certainly appears suitable for gram-negative bacilli other than *P. aeruginosa*. The marked differences, however, between agar and broth MICs in netilmicin testing with *P. aeruginosa* indicate that more information is required before either agar or broth dilution testing with netilmicin can be

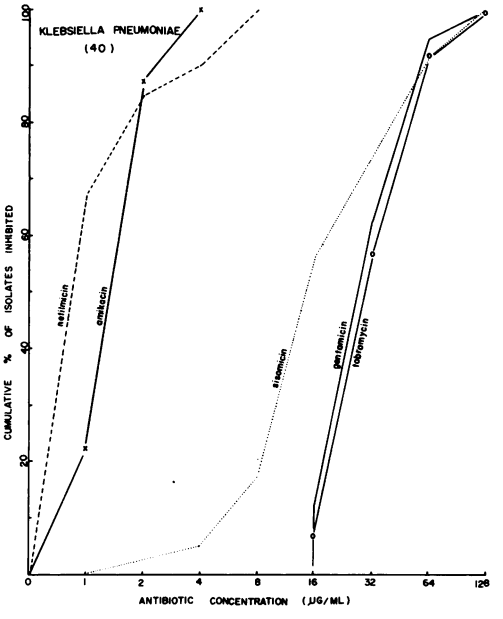

FIG. 4. *Antibiotic susceptibility patterns of 40 different clinical isolates of gentamicin-resistant Klebsiella pneumoniae in agar dilution testing.*

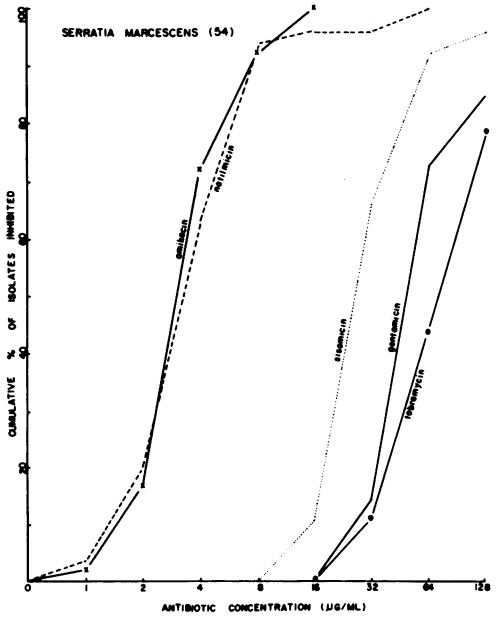

FIG. 3. *Antibiotic susceptibility patterns of 54 different clinical isolates of gentamicin-resistant S. marcescens in agar dilution testing.*

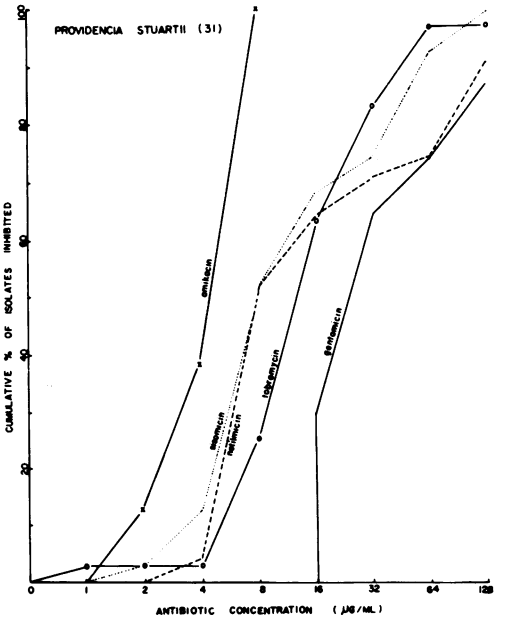

FIG. 5. *Antibiotic susceptibility patterns of 31 different clinical isolates of gentamicin-resistant P. stuartii in agar dilution testing.*

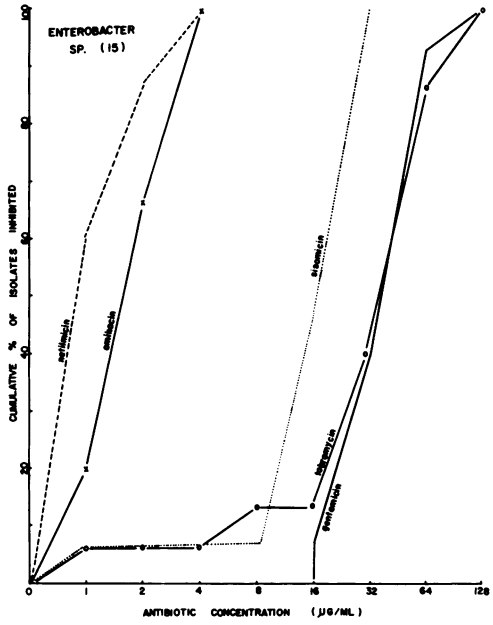

FIG. 6. *Antibiotic susceptibility patterns of 15 different clinical isolates of gentamicin-resistant Enterobacter sp. in agar dilution testing.*

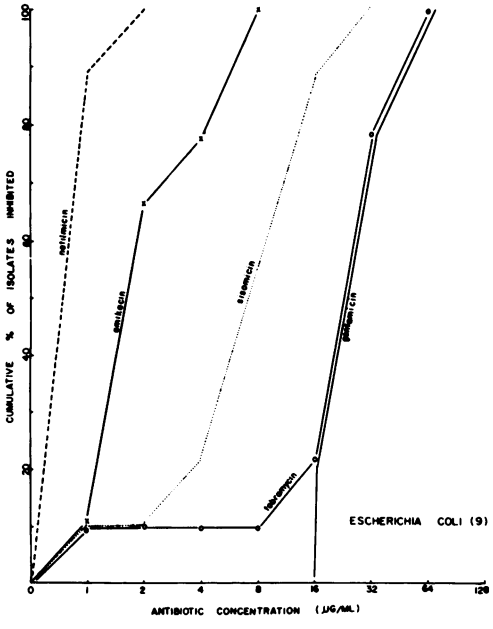

FIG. 7. *Antibiotic susceptibility patterns of nine different clinical isolates of gentamicin-resistant E. coli in agar dilution testing.*

recommended. Standardization of media, more in vitro data, and clinical correlation of in vitro test results with serum levels and therapeutic responses will be of value. Certainly the differences between agar and broth results were

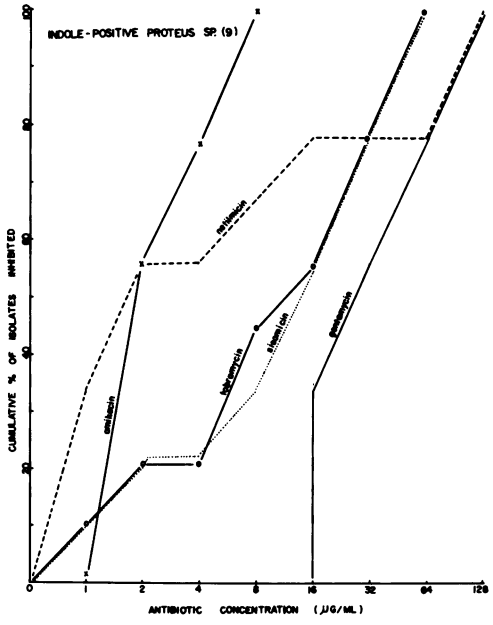

FIG. 8. *Antibiotic susceptibility patterns of nine different clinical isolates of gentamicin-resistant, indole-positive Proteus sp. in agar dilution testing.*

more noteworthy with netilmicin than those with amikacin or gentamicin. The disparity between results with agar and broth testing with *P. aeruginosa* with netilmicin has been noted by others (L. S. Young, personal communication, 1976). Although calcium content of the media, which also has been shown to affect in vitro results (16), was not measured, magnesium concentrations of our media from a single lot were similar. Magnesium content has been noted to affect in vitro testing with netilmicin more than other ions (Fu and Neu, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, A30, p. 6). Our results agree with those of Rahal et al., who found amikacin to be the most active agent against *Providencia* and found a high degree of activity of both netilmicin and amikacin against gentamicin-resistant *E. coli*, *Klebsiella*, and *Enterobacter* sp. (15). The findings of Rahal et al. (15) *Serratia* and indole-positive *Proteus* sp. with cross-resistance between netilmicin and amikacin differ from ours in that all of our gentamicin-resistant isolates of these genera were susceptible to amikacin, but not all were susceptible to netilmicin.

Our results also differ from those of Rahal et al. (15), who demonstrated similar activity with netilmicin and amikacin against *P. aeruginosa* in broth testing. Our results show amikacin to be the most active against gentamicin-resistant *P. aeruginosa* when the anticipated blood levels are considered. Our previous studies showed

a mean 1-h peak blood level of 27.6 μg/ml with amikacin (11). On comparison on a weight basis, however, our results with *P. aeruginosa* are similar to those of Rahal et al. (15) in showing that netilmicin is the most active agent until the higher concentrations, when amikacin appears so.

The high degree of in vitro activity of amikacin against gentamicin-resistant *Enterobacteriaceae* and *P. aeruginosa* agrees with our previous findings reported in a smaller number of different clinical isolates (8, 12). Although gentamicin and tobramycin are active against some strains of *Providencia* (7), our experience agrees with that of Overturf et al., who found gentamicin-resistant strains that were susceptible to amikacin (14).

The wider range of amikacin than netilmicin against gentamicin-resistant organisms may be due to either permeability factors or to resistance to inactivation by enzymes produced by R-factors. Amikacin is inactivated only by kanamycin acetyltransferase (2), which is also known as aminoglycoside acetyltransferase (6'). Netilmicin is inactivated not only by aminoglycoside acetyltransferase (6') but also by aminoglycoside acetyltransferase (2') and by aminoglycoside acetyltransferase (3')-II (Kenneth E. Price, personal communication, 1976). These observations may explain the patterns of resistance noted in our isolates, although assays for specific enzymes have not been performed.

Netilmicin has a wide range of activity in vitro against gentamicin-resistant *Enterobacteriaceae* and *P. aeruginosa*. Amikacin, however, remains the currently available agent that has the widest range of activity against these gentamicin-resistant organisms. Clinical studies with netilmicin are merited; studies of efficacy and toxicity comparing it to amikacin and other aminoglycosides are needed to determine its exact role.

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