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Comparative Activity of Netilmicin, Gentamicin, Amikacin, and Tobramycin Against *Pseudomonas aeruginosa* and *Enterobacteriaceae*

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Netilmicin (Sch 20569), a semisynthetic aminoglycoside antibiotic, was compared with gentamicin, tobramycin, and amikacin against 242 clinical isolates of *Pseudomonas* and *Enterobacteriaceae*. The minimum inhibitory concentration (MIC) was determined in both solid and liquid media. Netilmicin exhibited typical aminoglycoside properties, such as little effect of inoculum size on MIC, relatively small gap between MIC and minimum bactericidal concentration, and potentiation of anti-*Pseudomonas* activity in the presence of carbenicillin. Netilmicin provided no advantage in antimicrobial activity over gentamicin for either *Pseudomonas* or *Enterobacteriaceae*. Nearly complete cross-resistance to netilmicin was encountered with isolates resistant to gentamicin in either solid or liquid media. Netilmicin was less active than gentamicin against isolates of *Pseudomonas* and *Providencia*. Major discrepancies between MIC values determined in agar as opposed to those determined in broth were encountered for most isolates of *Pseudomonas* but also, depending upon antibiotic tested, for between 15 and 40% of isolates of *Enterobacteriaceae*. This new aminoglycoside agent will be useful clinically only if it is shown to be significantly less toxic than presently available analogues.

Gentamicin, with or without the concomitant administration of carbenicillin, is widely used in the treatment of hospital-acquired, gram-negative bacillary infection, including those caused by *Pseudomonas aeruginosa*. Gentamicin is far from an ideal antimicrobial agent. Peak serum levels after administration of recommended doses may barely exceed the inhibitory concentrations for frequently isolated gram-negative bacilli, whereas the administration of larger doses may lead to significant toxicity. Further, the emergence of isolates with high-level resistance to gentamicin has been well documented (3).

Accordingly, the search continues for new aminoglycoside antibiotics that might exhibit greater antibacterial activity and less toxicity. Netilmicin (Sch 20569), an agent differing from sisomicin by the presence of an ethyl group at the 1-N position of the deoxystreptamine moiety, has recently been provided for in vitro work. Initial animal toxicity studies indicated that this agent may be less oto- and nephrotoxic than gentamicin (5). Our study compares the in vitro activity of netilmicin with those of gentamicin, tobramycin, and amikacin against recent isolates of *P. aeruginosa* and various *Enterobacteriaceae*. In addition, the potentiation of the activity of the four aminoglycosides against *P. aeruginosa* by carbenicillin was evaluated.

MATERIALS AND METHODS

Bacterial isolates. Two hundred and forty-two clinical isolates were obtained from the diagnostic microbiology laboratory of the Royal Victoria Hospital. These isolates included *Escherichia coli* (35), *Klebsiella pneumoniae* (36), *Enterobacter* (36), *Proteus mirabilis* (37), indole-positive *Proteus* (25), *Providencia stuartii* (10), and *P. aeruginosa* (63). The identity of all isolates was confirmed by standard methods. Not more than one isolate of a given species per patient was included.

Antibiotics. Gentamicin sulfate and netilmicin sulfate were supplied by the Schering Corp. Amikacin was supplied by Bristol Laboratories, tobramycin by Eli Lilly and Co., and carbenicillin disodium by Ayerst Laboratories. Antibiotic stock solutions were stored at −70°C.

Agar dilution susceptibility. Minimal inhibitory concentrations (MIC) were determined for all isolates by an agar dilution method in Mueller-Hinton agar. Parallel series of antibiotic-containing plates were inoculated for each antibiotic by a multiple inoculator apparatus (8) using both 10^{-4} and 10^{-2} dilutions of an overnight Mueller-Hinton broth

(MHB) culture. The Mueller-Hinton agar batch used had concentrations of calcium and magnesium of 9.1 and 4.25 mg/dl, respectively, as determined by atomic absorption (Perkin-Elmer atomic absorption spectrophotometer, model 303).

Broth dilution susceptibility. Twenty resistant or relatively resistant isolates each of *P. aeruginosa* and *Enterobacteriaceae*, as determined by agar dilution testing, were selected for broth dilution susceptibility testing. The aminoglycoside antibiotics were serially diluted in twofold steps in MHB (final volume, 2.0 ml). Each tube was then inoculated with 0.05 ml of a 10^{-2} dilution of an overnight MHB culture. The lowest concentration that inhibited visible growth after overnight incubation at 37°C was taken as the MIC. A sample (0.01 ml) of each clear tube was subcultured to antibiotic-free Mueller-Hinton agar, and the minimal bactericidal concentration (MBC) was taken to be the lowest antibiotic concentration to yield fewer than five colonies after overnight incubation at 37°C. The MHB contained 1.18 mg of calcium and 0.208 mg of magnesium per dl, again measured by atomic absorption.

Activity of aminoglycoside-carbenicillin combinations. The 20 isolates of *P. aeruginosa* selected for aminoglycoside susceptibility tests in MHB were also evaluated for susceptibility to aminoglycoside-carbenicillin combinations. To each tube in a twofold aminoglycoside dilution series in MHB a fixed amount of carbenicillin was added to give a final carbenicillin concentration of 50 μg/ml in a volume of 2.0 ml. Each tube was inoculated with 0.05 ml of a 10^{-2} dilution of an overnight MHB culture and incubated aerobically overnight at 37°C. The resulting MIC of each aminoglycoside for each isolate was compared in the presence and absence of carbenicillin.

RESULTS

Agar dilution MIC. Varying the inoculum size had relatively little effect on the results. In almost all instances increasing the inoculum 100-fold resulted in either no change in MIC or a twofold increase. Accordingly, the results presented are those obtained with the larger inoculum, as a similar inoculum was used in the subsequent broth dilution tests.

MICs of the four aminoglycosides tested against the large inocula of *E. coli*, *K. pneumoniae*, and *Enterobacter* are shown in Table 1. The in vitro activities of netilmicin and gentamicin were almost identical, inhibiting 90% or more of isolates at a concentration of 3.1 μg/ml. A small percentage of *K. pneumoniae* and *Enterobacter* (3 to 5%) had netilmicin or gentamicin MICs ≥ 12.5 μg/ml. Tobramycin inhibited all *E. coli* and *K. pneumoniae* at a concentration of 1.6 μg/ml, but about 3% of *Enterobacter* isolates had MICs ≥ 12.5 μg/ml. Amikacin inhibited all isolates at a concentration of 12.5 μg/ml or less.

The MIC values of the four agents for isolates of *P. mirabilis*, indole-positive *Proteus*, and *P. stuartii* are presented in Table 2. In each instance netilmicin and gentamicin demonstrated similar activity. Netilmicin inhibited 70.3% of *P. mirabilis* and 80% of indole-positive *Proteus* at a concentration of 3.1 μg/ml, compared to 78.4 and 76%, respectively, inhibited by gentamicin. Tobramycin showed greater activity, inhibiting 86.5% of *P. mirabilis* and 92.0% of indole-positive *Proteus* at a concentration of 3.1 μg/ml. None of these three agents showed significant activity against *P. stuartii*. Amikacin was highly active against these isolates, inhibiting 83.7% of *P. mirabilis*, 96% of indole-positive *Proteus*, and all *P. stuartii* at a concentration of 12.5 μg/ml.

Almost complete cross-resistance was exhibited to netilmicin by isolates of *Enterobacteriaceae* highly resistant to gentamicin (MIC ≥ 25 μg/ml). One of the 14 such isolates had a netilmicin MIC of 6.3 μg/ml; all other MIC values were ≥12.5 μg/ml (Table 3). Tobramycin also showed little activity against most gentamicin-resistant isolates but amikacin was

TABLE 1. *Agar dilution MIC of aminoglycoside antibiotics for E. coli, Klebsiella, and Enterobacter*

Organism (no. tested)	Antibiotic	Cumulative % isolates inhibited at (μg/ml):								
		0.2	0.4	0.8	1.6	3.1	6.3	12.5	25.0	50.0
E. coli (35)	Netilmicin	0	8.6	62.8	100.0					
	Gentamicin	0	2.9	74.3	97.2	100.0				
	Tobramycin	0	28.6	91.4	100.0					
	Amikacin	0	0	2.9	28.6	82.9	91.4	100.0		
K. pneumoniae (36)	Netilmicin	2.8	30.6	86.0	97.2	97.2	97.2	100.0		
	Gentamicin	5.6	38.9	83.2	97.2	97.2	97.2	97.2	100.0	
	Tobramycin	11.1	77.8	91.6	100.0					
	Amikacin	0	0	5.6	63.8	91.6	100.0			
Enterobacter (36)	Netilmicin	0	4.2	55.6	86.0	91.6	94.5	97.2	97.2	97.2
	Gentamicin	0	11.1	63.8	94.5	94.5	94.5	94.5	97.2	97.2
	Tobramycin	0	27.8	86.0	97.2	97.2	97.2	97.2	100.0	
	Amikacin	0	0	0	44.4	94.5	100.0			

TABLE 2. *Agar dilution MIC of aminoglycoside antibiotics for P. mirabilis, indole-positive Proteus, and P. stuartii*

Organism (no. tested)	Antibiotic	Cumulative % isolates inhibited at (μg/ml):								
		0.2	0.4	0.8	1.6	3.1	6.3	12.5	25.0	50.0
P. mirabilis (37)	Netilmicin	0	0	5.4	45.9	70.3	86.5	89.2	100.0	
	Gentamicin	0	0	29.7	54.1	78.4	86.5	89.2	97.3	100.0
	Tobramycin	0	21.6	67.6	81.1	86.5	89.2	89.2	89.2	100.0
	Amikacin	0	0	0	8.1	37.8	59.4	83.7	89.1	89.1
Indole-positive	Netilmicin	0	4.0	36.0	60.0	80.0	92.0	92.0	96.0	100.0
Proteus (25)	Gentamicin	0	8.0	28.0	60.0	76.0	88.0	88.0	100.0	
	Tobramycin	8.0	28.0	60.0	80.0	92.0	100.0			
	Amikacin	0	0	4.0	32.0	64.0	84.0	96.0	100.0	
P. stuartii (10)	Netilmicin	0	0	0	0	0	20.0	30.0	70.0	90.0
	Gentamicin	0	0	0	0	0	20.0	60.0	90.0	100.0
	Tobramycin	0	0	0	10.0	20.0	40.0	60.0	90.0	100.0
	Amikacin	0	0	10.0	60.0	90.0	100.0			

TABLE 3. *MIC by agar dilution of netilmicin, tobramycin, and amikacin for highly gentamicin-resistant Enterobacteriaceae (MIC ≥ 25 μg/ml)*

Organism (no. highly resistant/no. tested)	MIC (μg/ml)			
	Gentamicin	Netilmicin	Tobramycin	Amikacin
K. pneumoniae (1/36)	25	12.5	1.6	1.6
Enterobacter spp. (2/36)	>50	>50.0	25.0	3.1
	25	12.5	1.6	1.6
Indole-positive *Proteus* (3/25)	25	25.0	6.3	1.6
	25	50.0	3.1	1.6
	25	6.3	6.3	12.5
P. mirabilis (4/37)	50	25.0	50.0	>50.0
	25	25.0	50.0	>50.0
	25	25.0	50.0	>50.0
	25	25.0	50.0	>50.0
P. stuartii (4/10)	25	50.0	25.0	1.6
	25	50.0	25.0	1.6
	25	>50.0	50.0	6.3
	50	25.0	12.5	3.1

highly active, inhibiting 10 isolates at a concentration of 12.5 μg/ml. The four amikacin-resistant *P. mirabilis* isolates were also resistant to the other agents tested by this method.

Netilmicin exhibited little activity against *P. aeruginosa* when tested by the agar dilution method, inhibiting only 7.9% of isolates at a concentration of 3.1 μg/ml compared to 22.2% inhibited by gentamicin (Fig. 1). Tobramycin was the most active anti-*Pseudomonas* agent; 87.3% of isolates had MICs of 3.1 μg/ml or less. Amikacin inhibited 68.3% of *P. aeruginosa* at a concentration of 12.5 μg/ml. Of 18 gentamicin-resistant *P. aeruginosa* (MIC ≥ 25 μg/ml), none had a netilmicin MIC lower than 25 μg/ml. In contrast, tobramycin inhibited 11 such isolates at 3.1 μg/ml. Only one gentamicin-resistant *P. aeruginosa* was inhibited by amikacin at a concentration of 12.5 μg/ml.

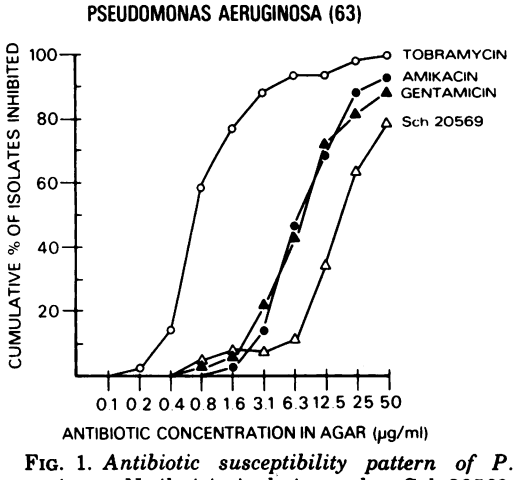

FIG. 1. *Antibiotic susceptibility pattern of P. aeruginosa. Netilmicin is designated as Sch 20569.*

Broth dilution susceptibility tests. The MIC of the four aminoglycosides was determined in MHB for 20 isolates each of *Enterobacteriaceae* and *P. aeruginosa*. Eighteen of the 20 *Enterobacteriaceae* and all 20 *P. aeruginosa* selected had gentamicin MICs ≥ 12.5 μg/ml by the agar dilution method.

The correlations between MICs determined in agar or broth for *Enterobacteriaceae* are shown in Fig. 2. Broth dilution MIC values were one-quarter or less of those determined in agar for 8 of 20 isolates tested with netilmicin and gentamicin, 5 of 20 tested with tobramycin, and 3 of 20 tested with amikacin. Agar dilution MIC values of netilmicin for all 20 *Enterobacteriaceae* were ≥6.3 μg/ml; when retested in broth, only 8 showed MICs of 3.1 μg/ml or less. Three of the four *P. mirabilis* isolates that exhibited high-level resistance to the four anti-

biotics by the agar dilution method (Table 3) were tested by broth dilution. All had netilmicin, gentamicin, and tobramycin MICs ≤ 3.1 μg/ml and an amikacin MIC ≤ 12.5 μg/ml.

Twelve isolates of *Enterobacteriaceae* had gentamicin MICs ≥ 6.3 μg/ml by tube dilution (Table 4). Only one of these isolates had a netilmicin MIC < 6.3 μg/ml, and only two had a tobramycin MIC < 6.3 μg/ml. In contrast, the amikacin MIC of 10/12 gentamicin-resistant isolates was <12.5 μg/ml.

The relationships between the MIC of the agents determined by agar dilution and those determined by broth dilution for *P. aeruginosa* are shown in Fig. 3. In the great majority of

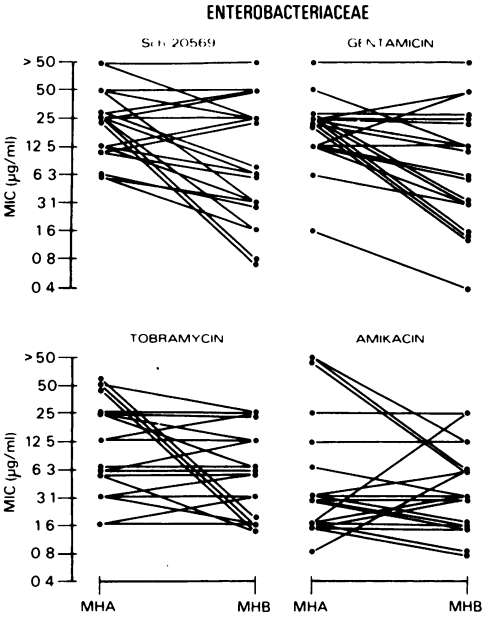

FIG. 2. *Comparison of MICs of antibiotics for Enterobacteriaceae as determined in Mueller-Hinton agar (MHA) and MHB. Each line represents a single isolate. Netilmicin is designated as Sch 20569.*

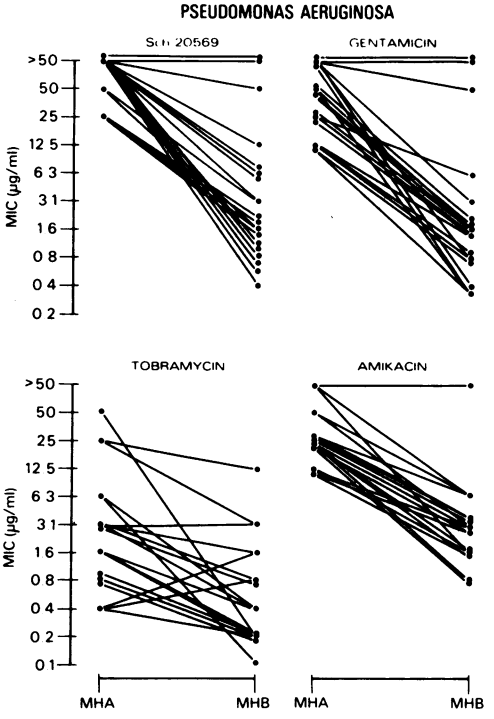

FIG. 3. *Comparison of MICs of antibiotics for P. aeruginosa as determined in Mueller-Hinton agar (MHA) and MHB. Each line represents a single isolate. Netilmicin is designated as Sch 20569.*

TABLE 4. *Activity of netilmicin, tobramycin, and amikacin in MHB against gentamicin-resistant[a] Enterobacteriaceae*

Isolate	Gentamicin		Netilmicin		Tobramycin		Amikacin	
	MIC[b]	MBC[b]	MIC	MBC	MIC	MBC	MIC	MBC
1. *Providencia*	12.5	50	25	100	25	50	0.8	1.6
2. *Providencia*	25.0	100	25	100	25	50	3.1	12.5
3. *Providencia*	25.0	100	50	50	25	50	3.1	12.5
4. *Providencia*	6.3	12.5	6.3	12.5	25	25	6.3	12.5
5. *Providencia*	50.0	100	50	>100	6.3	50	25.0	25.0
6. *Providencia*	12.5	100	25	100	12.5	50	1.6	12.5
7. *Providencia*	12.5	25	12.5	50	6.3	12.5	1.6	12.5
8. *Klebsiella*	50.0	50	25	50	3.1	6.3	1.6	1.6
9. *Enterobacter*	12.5	25	6.3	12.5	1.6	3.1	0.8	1.6
10. *Enterobacter*	>100.0	>100	100	>100	12.5	25	1.6	6.3
11. *P. mirabilis*	6.3	12.5	3.1	12.5	6.3	12.5	25.0	50.0
12. Indole-positive *Proteus*	25.0	100	50	100	12.5	25	1.6	6.3

[a] Gentamicin MIC >6.3 μg/ml in tube dilution test.
[b] MICs and MBCs are given in micrograms per milliliter.

cases, a fourfold or greater decrease in the MIC as determined in broth was noted: this occurred for netilmicin and gentamicin with 17 of 20 isolates, for tobramycin with 14 of 20 isolates, and for amikacin with 18 of 20 isolates. The numbers of isolates out of 20 tested with broth dilution MICs > 3.1 μg/ml for netilmicin, gentamicin, and tobramycin were 7, 4, and 1, respectively. Only 1 of the 20 isolates tested had an amikacin MIC > 6.3 μg/ml under the same conditions.

Comparison of MBC with MIC for the 40 isolates evaluated showed that in only three instances was there a greater than fourfold increase in MBC_4 over MIC for netilmicin and gentamicin, whereas 7 of 40 isolates had tobramycin or amikacin MBCs fourfold or more greater than the corresponding MIC value.

Potentiation of aminoglycoside activity by carbenicillin. Eighteen of the 20 *P. aeruginosa* isolates whose MIC to the aminoglycosides had been determined by the tube dilution method were resistant to 50 μg of carbenicillin per ml alone in MHB. The effect of added carbenicillin (50 μg/ml) on the MICs of the four aminoglycosides for these 18 isolates is shown in Fig. 4.

Netilmicin and gentamicin MICs determined in the presence of carbenicillin were lowered to one-quarter or less of the value obtained in its absence for 15 of 18 isolates. The netilmicin MICs measured in the presence of carbenicillin were 3.1 μg/ml or less for 16 of 18 isolates; two remained resistant, with MICs of 50 and >100 μg/ml, respectively. Similar results were obtained with gentamicin; MICs of 15 of 18 isolates were <3.1 μg/ml. In no instance was there evidence of drug antagonism.

A similar fourfold or greater reduction of MIC was observed in 9 of 18 isolates for tobramycin and 16 of 18 isolates for amikacin.

DISCUSSION

In contrast to previously published data (5), we found netilmicin provided no advantage in antimicrobial activity over gentamicin for either *P. aeruginosa* or *Enterobacteriaceae*. Nearly complete cross-resistance to netilmicin was encountered with isolates resistant to gentamicin when tested in solid or liquid media. Netilmicin was less active than gentamicin against isolates of *P. aeruginosa* and *P. stuartii*. Netilmicin exhibits typical aminoglycoside characteristics, such as little effect of inoculum size on MIC, relatively small gap between MIC and MBC, and the potentiation of its anti-*Pseudomonas* activity when combined with carbenicillin. Major discrepancies between MIC values determined in agar as opposed to those determined in broth for *P. aeruginosa*, well described for other aminoglycosides, are also found with netilmicin. These discrepancies have been attributed in the case of *P. aeruginosa* to a cell wall-stabilizing effect of high concentrations of divalent cations, especially

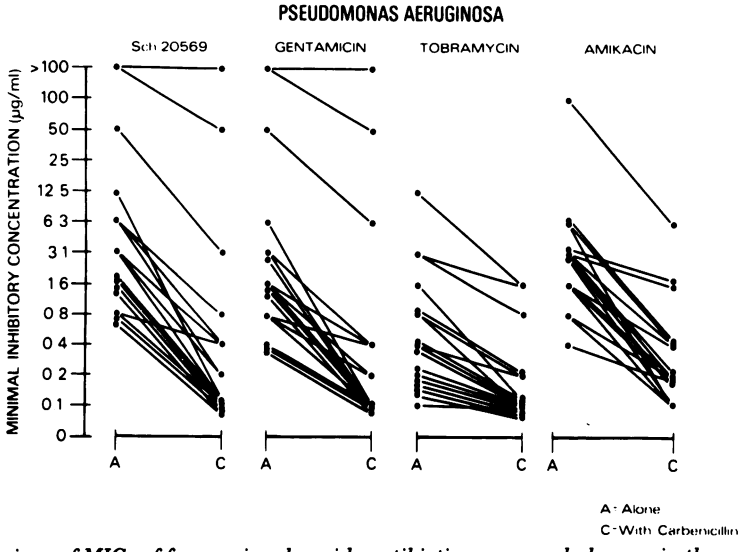

FIG. 4. *Comparison of MICs of four aminoglycoside antibiotics measured alone or in the presence of 50 μg of carbenicillin per ml for P. aeruginosa. Each line represents a single isolate. Netilmicin is designated as Sch 20569.*

Ca^{2+} and Mg^{2+}, which are usually present in the solid but not the liquid media (1, 2, 7). Supporting this is the fact that markedly increased binding and presumably cell wall penetration of ^3H-labeled gentamicin to *Pseudomonas* occurs in media with low divalent ion concentrations (6). In addition, the "protective" effect of high divalent cation concentrations is lost when the organism being tested is a carbenicillin-induced spheroplast in hypertonic medium (9). Previous investigators have concluded that the divalent cation concentration in the medium has little or no effect in susceptibility tests of *Enterobacteriaceae* (2, 4, 9). These conclusions, however, rest upon the testing of relatively small numbers of isolates. We found that, depending upon which aminoglycoside was used, between 15 and 40% of the isolates of *Enterobacteriaceae* exhibited fourfold or greater diminutions of MIC when tested in broth as compared to agar. For some *Enterobacteriaceae* differences in media may create as significant a problem in interpretation of in vitro susceptibility test results, as already exists for *P. aeruginosa*.

If further studies in experimental animals and humans demonstrate that netilmicin indeed possesses less toxic potential than other currently available aminoglycoside antibiotics, this agent may become a useful therapeutic tool.

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