

Activity of Netilmicin Compared with Those of Gentamicin and Tobramycin Against Enterobacteria and *Pseudomonas aeruginosa*

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The inhibitory activity of netilmicin against 500 isolates of gram-negative bacteria was compared with those of gentamicin and tobramycin. Netilmicin was considerably less active than tobramycin and slightly less inhibitory than gentamicin for *Pseudomonas aeruginosa* but was at least as active against *Escherichia coli* and *Klebsiella pneumoniae* as were the other two antibiotics. A few *Klebsiella* and *Serratia* isolates resistant to gentamicin and tobramycin were inhibited by netilmicin. All three antibiotics were strongly bactericidal for *E. coli*, *K. pneumoniae* and *P. aeruginosa* but had less lethal activity against the otherwise susceptible *Serratia* isolates tested. Some necessary precautions in reading minimal inhibitory concentrations on agar media are stressed, and some possible advantages of a 4-h bactericidal test, using a constant antibiotic concentration, are defined.

Although gentamicin is an extremely useful agent for the treatment of infections due to enterobacteria and *Pseudomonas aeruginosa*, its use is limited by toxicity for the kidney and the eighth cranial nerve. Peak drug concentrations in serum after recommended dosage may be less than four times the minimal inhibitory concentration (MIC) for the infecting organism, and increased dosage carries serious risk of undesirable side effects.

The development of netilmicin (*N*-ethyl-sisomicin), an aminoglycoside that in animal experiments appears to be much less toxic than gentamicin for animal cells (14), is therefore of considerable interest. Several publications (1, 6, 8, 9, 11) now record the activity of netilmicin against enterobacteria and *P. aeruginosa*. Although some organisms may be less susceptible to netilmicin than to the established aminoglycosides, the difference in activity appears small.

In this laboratory, the activity of netilmicin was tested against 400 isolates of enterobacteria and 100 of *P. aeruginosa*. Gentamicin and tobramycin were tested in parallel. The bactericidal activity of the three aminoglycosides against representative isolates was also investigated.

MATERIALS AND METHODS

Organisms. The 500 cultures were isolates from separate patients collected over a 4-month period. Enterobacteria were identified primarily by the replica plate method described by Chadwick et al. (2)

and by additional reactions in fluid media where necessary. *P. aeruginosa* isolates were identified by methods recommended by Cowan and Steel (3).

Antibiotics. Netilmicin and gentamicin were obtained in powder form, with respective potencies of 592 and 565 $\mu\text{g}/\text{mg}$, from the Schering Corp., Pointe Claire, Quebec. Tobramycin was obtained as a solution in 2-mg vials from Eli Lilly & Co., Indianapolis, Ind. Stock solutions of each antibiotic, of 1-mg/ml strength, were prepared in sterile distilled water and maintained frozen until required for incorporation in the test media.

Determination of MIC. MICs of the antibiotics were measured in Mueller-Hinton agar (BBL). Test organisms were initially grown for 6 h in Mueller-Hinton broth (BBL) at 37°C. The magnesium concentrations of agar and broth were, respectively, 1.1 and 0.75 mg/100 ml, and the calcium concentrations were 5.1 and 1.5 mg/100 ml. After the 6-h incubation period, samples of undiluted broth culture and of 10⁻² dilutions were inoculated onto agar plates containing antibiotic by means of a replicator. For each antibiotic the concentrations in the agar were 0.5, 1, 2, and 4 $\mu\text{g}/\text{ml}$. Subsequently, resistant strains were tested at concentrations up to and including 32 $\mu\text{g}/\text{ml}$. A control plate containing no antibiotic was included in each series, and two control organisms, *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, were inoculated in parallel with each set of 16 fresh isolates. The plates were incubated at 37°C for 18 h. For both undiluted and 10⁻²-diluted inocula (approximately 10⁶ and 10⁴ bacteria), the MIC was recorded in two ways: (i) the smallest tested concentration causing complete inhibition of growth and (ii) the smallest tested concentration causing reduction in growth to 10 or fewer discrete colonies.

Determination of bactericidal activity. The bactericidal activity of the antibiotics was estimated by exposing various-sized bacterial populations to gentamicin, tobramycin, or netilmicin at the single concentration of 4 $\mu\text{g/ml}$ in Mueller-Hinton broth. Overnight Mueller-Hinton broth cultures of enterobacteria were diluted 1:400 in broth and then incubated in flasks for 4 h at 37°C. The 4-h cultures were diluted 10^{-1} and 10^{-2} in broth, also in flasks, and viable counts were performed on these cultures by the Miles and Misra method (10). Immediately, one antibiotic was added to each of a series of three flask cultures to give a final concentration of 4 $\mu\text{g/ml}$. For *P. aeruginosa* the procedure was similar except that the overnight broth culture was diluted only 1:10. After addition of the antibiotics, all cultures were reincubated at 37°C for 4 h. Further viable counts, using Mueller-Hinton agar without antibiotic as plating medium, were performed on these cultures after 1, 2, 3, and 4 h in preliminary experiments, and only at 4 h in subsequent work. Count plates were incubated at 37°C overnight. The bactericidal activity of an antibiotic was expressed as the difference in logarithm of viable counts performed immediately before and at specified times after addition of the antibiotic to the culture. The maximum lethal activity recorded was a 3-log reduction in population; when initial bacterial inocula were around $10^6/\text{ml}$, detection of lethal activity greater than 3 logs would necessitate using such low dilutions of the culture that significant amounts of antibiotic would be carried over onto the agar.

RESULTS

MICs of the three antibiotics for all the organisms were recorded for both heavy and light inocula in terms of complete inhibition and reduction in growth to 10 or fewer colonies. For reasons presented in the Discussion, the lighter inoculum and the reduced-growth end point were selected as providing the most meaningful readings of MIC. The responses of the principal bacterial species to the selected concentrations of the antibiotics are summarized in Table 1. Virtually all the *E. coli* strains were inhibited by 2 μg or less of any antibiotic per ml; genta-

micin and netilmicin were somewhat more active than tobramycin at the lower concentrations. All but three of the *Klebsiella* isolates were inhibited by 2 μg or less of each antibiotic per ml, although tobramycin was again somewhat less active than the others. The remaining three isolates were inhibited by netilmicin at 4 $\mu\text{g/ml}$, but not by gentamicin or tobramycin. The *Enterobacter* isolates included representatives of *Enterobacter aerogenes*, *Enterobacter hafniae*, and *Enterobacter cloacae*, which did not differ from each other in overall response. This was a very susceptible group of organisms; gentamicin was, by a slight margin, the most active antibiotic. The *Serratia* isolates were relatively few but showed interesting differences from the other organisms; whereas only 9 of the 15 isolates were inhibited by 4 μg of gentamicin or tobramycin per ml, netilmicin inhibited 13 of them at 1 $\mu\text{g/ml}$ and 14 at 2 $\mu\text{g/ml}$. *Proteus mirabilis* was inhibited to a very similar degree by all three antibiotics, all strains being suppressed by concentrations of 4 $\mu\text{g/ml}$. The 11 *Proteus rettgeri* isolates were less responsive to the drugs, as only 7 or 8 of them, depending on the antibiotic, were inhibited by concentrations of 4 $\mu\text{g/ml}$. *P. aeruginosa* showed a pattern different from those of the other bacterial species, as tobramycin was clearly the most active against it. However, all isolates of *P. aeruginosa* were susceptible to 4 μg of gentamicin or tobramycin per ml, and only two isolates were resistant to the same concentration of netilmicin. The isolates whose MICs were not incorporated in this table included seven *Citrobacter* isolates, five isolates of *Proteus vulgaris*, eight *Proteus morganii*, and 1 *Providencia stuartii*. With the *Providencia* strain the MIC of all three drugs was 4 $\mu\text{g/ml}$; the other 20 isolates were all inhibited by 2 μg or less of each antibiotic per ml; gentamicin and netilmicin were both more active than tobramycin against *Proteus vulgaris* and *Proteus morganii*.

TABLE 1. Response of gram-negative bacteria to gentamicin, tobramycin, and netilmicin in terms of reduced-growth MIC for inoculum of 10^4 organisms

Bacterial species	No. of isolates	Cumulative % of isolates susceptible to stated concentrations ($\mu\text{g/ml}$)											
		Gentamicin				Tobramycin				Netilmicin			
		0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
<i>E. coli</i>	131	87	98	100	100	56	88	99	100	95	98	100	100
<i>K. pneumoniae</i>	116	89	96	97	97	72	94	97	97	87	97	98	100
<i>Enterobacter</i> species	49	96	100	100	100	78	98	100	100	92	98	100	100
<i>S. marcescens</i>	15	40	60	60	67	7	20	40	67	53	87	93	93
<i>Proteus mirabilis</i>	57	53	89	96	100	49	82	93	100	44	86	95	100
<i>Proteus rettgeri</i>	11	9	45	63	63	9	24	54	72	18	27	54	63
<i>P. aeruginosa</i>	100	16	47	82	100	72	92	97	100	11	25	58	98

TABLE 2. Organisms resistant to 4 μg of one or more aminoglycosides per ml

Bacterial species	Isolate no.	Reduced-growth MIC ($\mu\text{g}/\text{ml}$) for 10^4 inoculum of:			Resistance to all three drugs at 4 $\mu\text{g}/\text{ml}$
		Gentamicin	Tobramycin	Netilmicin	
<i>K. pneumoniae</i>	1	>32	>32	1	-
	2	>32	>32	1	-
	3	>32	>32	4	-
<i>S. marcescens</i>	1	>32	>32	0.5	-
	2	>32	>32	1	-
	3	>32	>32	0.5	-
	4	>32	>32	0.5	-
	5	8	16	>32	+
<i>Proteus rettgeri</i>	1	16	4	32	-
	2	8	32	32	+
	3	8	32	32	+
	4	16	32	32	+
<i>P. aeruginosa</i>	1	4	1	8	-
	2	4	2	8	-
Total isolates resistant to 4 $\mu\text{g}/\text{ml}$		12	11	7	4

The control *E. coli* ATCC 25922 showed modal MICs of 0.5 μg of gentamicin and netilmicin per ml and 1 μg of tobramycin per ml. Modal MICs of gentamicin, tobramycin, and netilmicin for *P. aeruginosa* ATCC 27853 were, respectively, 1, 0.5, and 2 $\mu\text{g}/\text{ml}$.

Table 2 lists the bacterial isolates that were resistant to 4 μg of one or more of the aminoglycosides per ml. These isolates were tested further against concentrations of 4, 8, 16, and 32 $\mu\text{g}/\text{ml}$, and the MICs required for growth inhibition of them are shown in the table. Resistance of this order was confined to four bacterial species. The numbers of isolates resistant to gentamicin, tobramycin, and netilmicin were, respectively, 12, 11, and 7. Four strains were resistant to all three drugs.

The MICs required to reduce growth to 10 or fewer colonies were compared for the two bacterial inocula, differing by a factor of 100. For most of the 500 isolates, the MICs were identical or showed a difference of one tested drug concentration. There was a slightly greater inoculum effect with tobramycin, mainly accounted for by the responses of *E. coli* and *Klebsiella* isolates. These observations are summarized in Table 3.

Table 4 shows how the apparent number of resistant isolates in this series varied when inoculum size and method of reading were altered. The most obvious effects of changing these two parameters were to increase the number of isolates of *Proteus mirabilis* and *P. aeruginosa* resistant to 4- $\mu\text{g}/\text{ml}$ concentrations of the antibiotics. In most cases the apparent resistance of the heavy inoculum was due to persistence of small numbers of discrete colo-

nies. However, it was interesting to note that the proportion of *P. aeruginosa* isolates "resistant" to netilmicin increased considerably when a heavy inoculum was used, whether the MIC was read in terms of "reduced growth" or complete inhibition. Reading for complete inhibition of the heavy inoculum also increased the number of *E. coli* isolates resistant to tobramycin.

In preliminary experiments on bactericidal activity, when viable counts were performed at hourly intervals during the 4 h after addition of the antibiotic, isolates for which antibiotics were bactericidal showed progressive decreases in viable counts over this period. The results suggested that *P. aeruginosa* was most susceptible and *Serratia marcescens* least susceptible to the lethal activity of the drugs. However, as the initial *P. aeruginosa* populations were considerably smaller than those of the other organisms, further experiments were designed to include an assessment of the influence of population size on bactericidal activity. In these experiments two viable counts were performed on each culture, one immediately before and the other 4 h after addition of the antibiotics. Tables 5, 6, 7, and 8 illustrate, respectively, the bactericidal activity of the three aminoglycosides on varying populations of representative isolates of *E. coli*, *K. pneumoniae*, *S. marcescens*, and *P. aeruginosa*. All the isolates used in these experiments had MICs in the agar medium of 4 $\mu\text{g}/\text{ml}$ or less for all three antibiotics. Table 9 shows the expected colony counts on agar media inoculated with either 10^6 or 10^4 organisms when the population is suppressed to different degrees by the antibiotic.

TABLE 3. Differences in reduced-growth MIC when bacterial inoculum on agar surface was increased from 10^4 to 10^6 organisms

Antibiotic	No. of isolates tested	No. of isolates showing difference in MIC of:			
		0 TC ^a	1 TC	2 TC	3 TC
Gentamicin	500	239	224	33	4
Tobramycin	500	169	269	58	4
Netilmicin	500	249	216	33	2

^a TC, Tested concentration.

TABLE 4. Variation in numbers of isolates of each species resistant to 4 μ g of the aminoglycosides per ml, with alteration in bacterial inoculum and method of defining MIC

Bacterial inoculum and MIC definition	Antibiotic	No. of resistant isolates of: ^a										
		EC	Kpn	Ent	SM	Citro	PM	PV	Pmg	PR	Prst	Psa
10^6 , complete inhibition	Gentamicin	2	5	1	7	0	20	1	0	8	1	29
	Tobramycin	22	8	1	13	0	30	3	1	9	1	13
	Netilmicin	1	4	1	5	0	15	1	0	8	1	56
10^6 , reduced growth	Gentamicin	0	3	0	6	0	2	0	0	5	1	13
	Tobramycin	2	4	0	7	0	4	0	0	7	1	3
	Netilmicin	0	3	0	2	0	2	0	0	7	1	32
10^4 , complete inhibition	Gentamicin	0	2	0	6	0	2	0	0	5	0	4
	Tobramycin	2	4	0	6	0	2	0	0	5	0	1
	Netilmicin	0	2	0	2	0	2	0	0	6	1	19
10^4 , reduced growth	Gentamicin	0	3	0	5	0	0	0	0	4	0	0
	Tobramycin	0	3	0	5	0	0	0	0	3	0	0
	Netilmicin	0	0	0	1	0	0	0	0	4	0	2
Total isolates		131	116	49	15	7	57	5	8	11	1	100

^a EC, *E. coli*; Kpn, *K. pneumoniae*; Ent, *Enterobacter* species; SM, *S. marcescens*; Citro, *Citrobacter* species; PM, *Proteus mirabilis*; PV, *Proteus vulgaris*; Pmg, *Proteus morganii*; PR, *Proteus rettgeri*; Prst, *Providencia stuartii*; Psa, *P. aeruginosa*.

The salient points emerging from these tables are as follows. (i) *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were much more susceptible than *S. marcescens* to the lethal action of all three drugs. (ii) When the initial bacterial population was increased to the order of 10^6 /ml, bactericidal activity of all three drugs was much reduced. (iii) The bactericidal activities of the three antibiotics were similar. Insofar as differences were detectable, tobramycin was least active against *E. coli*, netilmicin was least active against *K. pneumoniae*, and gentamicin was most lethal for the relatively resistant *Serratia* isolates. (iv) The 4-h incubation period was adequate for demonstration of significant bactericidal activity.

DISCUSSION

The measurement of antibiotic activity in terms of the MIC is conventional but open to criticism (13). Several factors influence the level of the MIC, among them being the bacterial population size, composition of the basal

medium and its physical state (solid or fluid), incubation time, and method of reading. Whereas the effect of inoculum size is less important for the aminoglycosides than for the β -lactam antibiotics, the cationic content of the basal medium has been shown to have considerable influence on the MIC of gentamicin for *P. aeruginosa* (6) and also for other gram-negative organisms (5). Duncan (4) showed that the MIC of gentamicin for *P. aeruginosa* differed as the basal agar was altered and recommended Mueller-Hinton agar (BBL) or brain heart infusion agar as the basal medium giving the most realistic results. The MIC may also vary according to its definition in terms of complete inhibition or significant reduction in growth. Of workers who have already published results on the activity of netilmicin, Briedis and Robson (1), Kabins et al. (7), and Marks et al. (8) read their MICs in terms of complete inhibition, whereas Fu and Neu (5) accepted the growth of five colonies or less on their agar media as indicating susceptibility. Meyer et al. (9) considered

the formation of a fine haze of growth to be within the limits of susceptibility. Kabins et al. (7), however, did report that MICs recorded in terms of 90% reduction of growth were the same as those for complete inhibition in 97% of their tested strains.

In this investigation, MICs for all the organisms were recorded with two inocula differing by a factor of 100, in terms of complete inhibition or reduction in growth to 10 or fewer colonies. The smaller inoculum in our experiments corresponded closely to the larger of two inocula used by Briedis and Robson (1), Fu and Neu (5), Kabins et al. (7), and Marks et al. (8). Although Table 3 indicates that the effect of inoculum on MIC was small in terms of inhibitory level, the figures in Table 4 show that the number of isolates termed resistant to a critical concentration of antibiotic varied considerably with inoc-

TABLE 5. Bactericidal activity of aminoglycosides against various-sized populations of three *E. coli* isolates

Iso- late	Initial population per ml		Logarithmic reduction in viable population after 4-h exposure to drug at 4 µg/ml		
	Count	Log count	Genta- micin	Tobra- mycin	Netil- micin
1	8×10^8	8.9	0.9	0.3	1.7
	8×10^7	7.9	2.9	0.4	2.9
	8×10^6	6.9	>3.0	>3.0	>3.0
2	5×10^8	8.7	>3.0	2.1	>3.0
	5×10^7	7.7	>3.0	>3.0	>3.0
	5×10^6	6.7	>3.0	>3.0	>3.0
3	4×10^8	8.6	0.1	0.3	0.3
	4×10^7	7.6	1.3	>3.0	>3.0
	4×10^6	6.6	>3.0	2.3	>3.0

TABLE 6. Bactericidal activity of aminoglycosides against various-sized populations of three *K. pneumoniae* isolates

Iso- late	Initial population per ml		Logarithmic reduction in viable population after 4-h exposure to drug at 4 µg/ml		
	Count	Log count	Genta- micin	Tobra- mycin	Netil- micin
1	2×10^9	9.3	2.5	1.7	1.4
	2×10^8	8.3	>3.0	>3.0	>3.0
	2×10^7	7.3	>3.0	>3.0	>3.0
2	3×10^8	8.5	1.2	1.3	0.0
	3×10^7	7.5	>3.0	>3.0	1.8
	3×10^6	6.5	>3.0	>3.0	2.2
3	3×10^8	8.5	2.7	1.9	1.0
	3×10^7	7.5	>3.0	>3.0	>3.0
	3×10^6	6.5	>3.0	>3.0	>3.0

TABLE 7. Bactericidal activity of aminoglycosides against various-sized populations of three *S. marcescens* isolates

Iso- late	Initial population per ml		Logarithmic reduction in viable population after 4-h exposure to drug at 4 µg/ml		
	Count	Log count	Genta- micin	Tobra- mycin	Netil- micin
1	5×10^8	8.7	0.7	0.0	0.0
	5×10^7	7.7	1.2	0.0	0.0
	5×10^6	6.7	>3.0	1.9	1.1
2	5×10^8	8.7	0.0	0.0	0.0
	5×10^7	7.7	0.0	0.0	0.0
	5×10^6	6.7	>3.0	2.7	1.4
3	1.5×10^8	8.2	0.6	0.0	0.0
	1.5×10^7	7.2	0.9	0.0	0.0
	1.5×10^6	6.2	>3.0	1.0	0.3

TABLE 8. Bactericidal activity of aminoglycosides against various-sized populations of three *P. aeruginosa* isolates

Iso- late	Initial populations per ml		Logarithmic reduction in viable population after 4-h exposure to drug at 4 µg/ml		
	Count	Log count	Genta- micin	Tobra- mycin	Netil- micin
1	2×10^8	8.3	2.0	2.0	2.3
	2×10^7	7.3	>3.0	>3.0	>3.0
	2×10^6	6.3	>3.0	>3.0	>3.0
2	2×10^8	8.3	1.0	1.2	0.9
	2×10^7	7.3	>3.0	>3.0	>3.0
	2×10^6	6.3	>3.0	>3.0	>3.0
3	3×10^8	8.5	1.0	1.2	0.9
	3×10^7	7.5	>3.0	>3.0	>3.0
	3×10^6	6.5	>3.0	>3.0	>3.0

ulum and method of reading. A reduced-growth MIC seems more logical than one based on complete inhibition because it represents the response of the majority of the bacterial population and is not raised artificially by the presence of a few resistant organisms in a predominantly susceptible culture. Use of the reduced-growth MIC, however, necessitates care in the choice of bacterial inoculum. Table 9 shows the expected colony counts on agar media inoculated with either 10^6 or 10^4 organisms when the population is suppressed to different degrees by the antibiotic. Because the amount of visible growth on a small area of agar will be the same whether the initial population is as small as 10^2 or as large as 10^5 organisms, the uninhibited segment of the population must be nearer 10^1 than 10^2 bacteria before unequivocal sensitivity can be detected. With a starting population of

TABLE 9. Observed responses to antibiotics in agar inoculated with 10^6 or 10^4 organisms depending on size of residual uninhibited population^a

% Residual population	Colonies from 10^6 inoculum	Observed response	Colonies from 10^4 inoculum	Observed response
10	10^5	R	10^3	R
1	10^4	R	10^2	R
0.1	10^3	R	10^1	DC
0.01	10^2	R	1	DC/S
0.001	10^1	DC	0	S
0.0001	1	DC/S	0	S
0.00001	0	S	0	S

^a R, Resistant; DC, discrete colonies; S, susceptible.

10^6 , this requires a 5-log inhibition, whereas with an initial inoculum of 10^4 susceptibility would become obvious when the number of viable organisms on the antibiotic medium is reduced by a factor of 3 logs. What constitutes a significant reduction in bacterial growth may be open to argument, but clearly the use of too large an inoculum may mask an important degree of susceptibility.

The results of the inhibition tests reported in this paper indicate that netilmicin is comparable in antibacterial activity to gentamicin and tobramycin. It was considerably less active than tobramycin and slightly less active than gentamicin for *P. aeruginosa* but at least as inhibitory as the other two drugs against *E. coli* and *K. pneumoniae*. Netilmicin was active against some *Klebsiella* and *Serratia* isolates, which were resistant to both gentamicin and tobramycin.

The approach to measurement of bactericidal activity also warrants comment. Methods for estimating such activity are not well standardized. A common procedure consists in exposing a standard bacterial inoculum to graded concentrations of an antibiotic in broth and, after overnight incubation, subculturing known volumes from tubes that show no turbidity to demonstrate either absence of growth or a preselected reduction of viability, usually a 2- to 3-log drop in viable population. In this work the antibiotic concentration was constant at 4 $\mu\text{g}/\text{ml}$, the period of exposure to the drug (4 h) was comparatively short, and differences between isolates were recorded in terms of the degree of killing, expressed logarithmically. The advantages of this approach are: (i) the concentration of antibiotic is one that is easily achievable in the bloodstream after recommended dosage, but is lower than the peak value, so that significant bactericidal activity at this level would suggest the probability of effective killing in

vivo; (ii) in practical therapeutics the lethal effect of an antibiotic is presumably exerted within the first few hours after its administration; (iii) the result of the test for bactericidal activity is available only 1 day after setting up the test.

The optimal inoculum for bactericidal tests of this type may be of the order of 10^7 bacteria per ml, as this would allow detection of as much as a 4-log kill without danger of carryover of antibiotic onto count plates through the use of low dilutions of the antibiotic-treated cultures. With the possibility of lethal effect varying from 0 to 4 logs, grading of the bactericidal effect of the different antibiotics against a given isolate would become practicable.

In this work no attempt was made to relate bactericidal activity to MIC, as the two measurements were carried out in different media whose cation concentrations were not comparable. The measurement of bactericidal activity is normally more complicated than an MIC estimation, so it is not usually undertaken as a routine procedure. Possibly however, the use of standardized inocula and a constant drug concentration, in a test designed to reveal a defined degree of population reduction in a 4-h period, might provide a practicable approach to routine measurement of bactericidal activity. Whether such tests are undertaken in the medical microbiology laboratory will depend upon the clinical significance of bactericidal activity as compared with growth inhibition.

The relative insusceptibility of the *Serratia* isolates to bactericidal activity of the antibiotics was surprising. Further investigations are in progress to assess whether this is a commonly occurring feature of *Serratia*; at this stage it would be unwise to generalize from a small series of observations.

If reports on the comparative lack of toxicity of netilmicin are substantiated and if in the clinical field it brings about rapid resolution of infection without producing undesirable side effects, then it promises to be a useful agent for treatment of infections due to enterobacteria and *P. aeruginosa*. Its somewhat lesser antibacterial activity against some species may be compensated by increase in dosage above that normally used for established aminoglycosides.

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LITERATURE CITED

- Briedis, D. J., and H. G. Robson. 1976. Comparative activity of netilmicin, gentamicin, amikacin, and tobramycin against *Pseudomonas aeruginosa* and *En-*

- terobacteriaceae*. Antimicrob. Agents Chemother. 10:592-597.
2. Chadwick, P., G. J. Delisle, and M. Byer. 1974. Biochemical identification of hospital enterobacteria by replica agar plating. Can. J. Microbiol. 20:1653-1664.
 3. Cowan, S. T., and K. J. Steel. 1974. Cowan and Steel's manual for the identification of medical bacteria, p 90-93, 2nd ed. Cambridge University Press, Cambridge.
 4. Duncan, I. B. R. 1974. Susceptibility of 1,500 isolates of *Pseudomonas aeruginosa* to gentamicin, carbenicillin, colistin, and polymyxin B. Antimicrob. Agents Chemother. 5:9-15.
 5. Fu, K. P., and H. C. Neu. 1976. In vitro study of netilmicin compared with other aminoglycosides. Antimicrob. Agents Chemother. 10:526-533.
 6. Garrod, L. P., and P. M. Waterworth. 1969. Effect of medium composition on the apparent sensitivity of *Pseudomonas aeruginosa* to gentamicin. J. Clin. Pathol. 22:534-538.
 7. Kabins, S. A., C. Nathan, and S. Cohen. 1976. In vitro comparison of netilmicin, a semisynthetic derivative of sisomicin, and four other aminoglycoside antibiotics. Antimicrob. Agents Chemother. 10:139-145.
 8. Marks, M. I., S. Hammerberg, G. Greenstone, and B. Silver. 1976. Activity of newer aminoglycosides and carbenicillin, alone and in combination, against gentamicin-resistant *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 10:399-401.
 9. Meyer, R. D., L. L. Kraus, and K. A. Pasierzuik. 1976. In vitro susceptibility of gentamicin-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* to netilmicin and selected aminoglycoside antibiotics. Antimicrob. Agents Chemother. 10:677-681.
 10. Miles, A. A., and S. S. Misra. 1938. The estimation of the bactericidal power of the blood. J. Hyg. 38:732-740.
 11. Phillips, I., A. King, C. Warren, and B. Watts. 1976. The activity of penicillin and eight cephalosporins on *Neisseria gonorrhoeae*. J. Antimicrob. Chemother. 2:31-39.
 12. Schering Corporation. 1975. Informational material for the investigational drug Sch. 20569. Schering Corp., Bloomfield, N.J.