

In Vitro Activity of Netilmicin Compared with Gentamicin, Tobramycin, Amikacin, and Kanamycin

THEODORE C. EICKHOFF* AND JOSEPHINE M. EHRET

Division of Infectious Disease, Department of Medicine, University of Colorado Medical Center, Denver, Colorado 80262

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The in vitro activity of netilmicin was compared with that of gentamicin, tobramycin, amikacin, and kanamycin against 636 strains of bacteria recently isolated from clinical sources. Gentamicin was the most active antibiotic, but netilmicin and tobramycin closely paralleled it. Netilmicin was generally four- to eightfold less active than gentamicin against *Serratia* and group A streptococci, and was twofold less active against *Pseudomonas aeruginosa*. When effects of inoculum size and concentration of divalent cations in the media were evaluated, netilmicin was shown to be similar to gentamicin in vitro. Minimum inhibitory concentrations for *P. aeruginosa* were increased as much as 18-fold when the Mg^{2+} and Ca^{2+} concentrations were increased to physiological levels in Mueller-Hinton broth.

The increasing frequency of nosocomial infections caused by gram-negative bacilli has become one of the major infectious disease problems of the 1970s (7). *Escherichia*, *Klebsiella*, *Enterobacter*, *Pseudomonas*, and *Serratia* now account for well over half of all hospital-acquired infections (5). The aminoglycosides are among the most useful drugs currently available for treating serious infections due to these genera, but the problems associated with their use, that is, development of resistant strains (10, 14, 15, 20) and potential toxicity (2, 3, 6, 9, 11, 12, 13, 19, 21), are well known.

Because of its broad spectrum, gentamicin has been particularly useful in the treatment of such infections. One of the most worrisome factors in the use of gentamicin is the narrow margin between an effective dose and a toxic dose. Netilmicin, *O*-2,6-diamino-2,3,4,6,-tetra-deoxy- α -D-glycerol-hex-4-enopyranosyl-(1 \rightarrow 4)-*O*-[3-deoxy-4-C-methyl-3-(methylamino)- β -L-arabinopyranosyl-(1 \rightarrow 6)]-2-deoxy-1-*N*-ethyl-D-streptamine, is a new semisynthetic aminoglycoside antibiotic that, based on subacute toxicity studies done in rats and dogs, may have less nephrotoxic and ototoxic potential than gentamicin (17). The purpose of this study is to compare the in vitro activity of netilmicin with that of gentamicin, tobramycin, amikacin, and kanamycin.

MATERIALS AND METHODS

Bacteria. A total of 636 strains of bacteria were tested. These were distributed as follows: 70 *Escherichia coli*, 35 *Enterobacter*, 52 *Klebsiella*, 35 *Proteus*

mirabilis, 46 *Proteus rettgeri*, 17 *Serratia*, 35 *Salmonella*, 10 *Shigella*, 75 *Pseudomonas aeruginosa*, 75 *Staphylococcus aureus*, 52 *Staphylococcus epidermidis*, 26 group D streptococci, 18 alpha-hemolytic streptococci (non-group D), 23 group A beta-hemolytic streptococci, 14 *Streptococcus pneumoniae*, 19 *Neisseria gonorrhoeae*, 18 *Neisseria meningitidis*, and 16 *Haemophilus influenzae*. Most of these organisms were recently isolated from clinical sources and identified in the Clinical Microbiology Laboratory of Colorado General Hospital under the direction of L. Barth Reller. The strains of *N. gonorrhoeae* were supplied by the Colorado General Hospital Venereal Disease Laboratory under the direction of Peter E. Dans. Most of the strains of *Salmonella* and *Shigella* were obtained from the Colorado State Public Health Laboratory. The *P. rettgeri* isolates were provided by F. Marc LaForce of the Denver Veterans Administration Hospital.

Aminoglycosides. Standard laboratory reference powders of netilmicin and gentamicin sulfate were provided by the Schering Corp., amikacin and kanamycin sulfate were donated by Bristol Laboratories, and tobramycin standard solution was provided by Eli Lilly & Co.

Susceptibility testing methods. (i) **Broth minimal inhibitory concentration.** The minimal inhibitory concentrations (MICs) of *E. coli*, *Enterobacter*, *Proteus*, *Salmonella*, *Shigella*, *Klebsiella*, *Serratia*, *P. aeruginosa*, *S. aureus*, *S. epidermidis*, group D streptococci, and *S. pneumoniae* were determined by a microtiter broth dilution technique. All tests were performed in standard Mueller-Hinton broth (pH 7.4, 7.2 mg of magnesium and 12 mg of calcium per liter), with the exception of group D streptococci and *S. pneumoniae*, which were tested in Trypticase soy broth (pH 7.35). The group D streptococci were also tested in Mueller-Hinton broth supplemented with 2% defibrinated sheep erythrocytes. All strains of

Pseudomonas were additionally tested in Mueller-Hinton broth (pH 7.38) adjusted to contain 31.2 mg of magnesium and 76.0 mg of calcium per liter. Magnesium and calcium levels in both the adjusted and unadjusted broth were determined in the Pediatric Microchemistry Laboratory of the University of Colorado Medical Center by means of atomic absorption spectrophotometry.

Serial twofold dilutions of freshly prepared antibiotics were made in the appropriate broth, and 0.05 ml of each dilution was dropped into the wells of a microtiter plate (Cooke Engineering Co.). With the exception of *S. pneumoniae*, which was diluted 10^{-3} , overnight broth cultures of the organisms were diluted 10^{-5} in the appropriate broth, and 0.05 ml was then added to the diluted antibiotic. The plates were placed on a shaker for 5 min, and then incubated overnight at 35°C in ambient air. The MIC was defined as the lowest concentration of antibiotic in which there was no visible growth. The minimal bactericidal concentration (MBC) was determined by using an adaptation of the Steers replicator (18), which delivered approximately 0.003 ml from each well of the microtiter plates to Mueller-Hinton agar. The strains of group D streptococci and *S. pneumoniae* were subcultured on Mueller-Hinton agar containing 4% defibrinated sheep erythrocytes. Subcultures of *S. pneumoniae* were incubated overnight at 35°C in a 5% CO₂ atmosphere. All other subcultures were incubated overnight at 35°C in ambient air.

(ii) Agar dilution method. The strains of *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, group A beta-hemolytic streptococci and alpha-hemolytic streptococci (non-group D) were all tested by the twofold agar dilution method with the Steers inocula replicator (18). *Neisseria* and *Haemophilus* were inoculated onto plates containing GC medium base (Difco) supplemented with 1% hemoglobin (BBL) and 1% IsoVitaleX (BBL). Strains of the non-group D alpha-hemolytic streptococci and the group A beta-hemolytic streptococci were tested on Trypticase soy agar containing 4% defibrinated sheep erythrocytes.

As inoculum, a volume of approximately 0.003 ml of an overnight undiluted broth culture was applied to the surface of the antibiotic-containing plates with the Steers replicator. Strains of *Neisseria* and *Haemophilus* were grown overnight in Mueller-Hinton broth supplemented with 1% hemoglobin and 1% IsoVitaleX at 35°C in a 5% CO₂ atmosphere. For *N. gonorrhoeae* this provided an inoculum of approximately 1.5×10^8 colony-forming units (CFU) per ml; for *N. meningitidis*, 1.3×10^6 CFU/ml; and for *H. influenzae*, 1.5×10^8 CFU/ml.

Group A beta-hemolytic streptococci and alpha-hemolytic streptococci were grown overnight in Trypticase soy broth supplemented with 2% defibrinated sheep erythrocytes. For the group A streptococci, this resulted in an inoculum of approximately 5×10^8 CFU/ml and, for the alpha-hemolytic streptococci, an inoculum of 2×10^7 CFU/ml. All of the plates were incubated at 35°C in a 5% CO₂ atmosphere for 24 to 36 h.

RESULTS

There were only rare instances of significant difference between MICs and MBCs; therefore, only MICs are shown. The activity of the five aminoglycosides against members of *Enterobacteriaceae* is shown in the cumulative distribution curves of Fig. 1 and 2. In general, there appears to be little significant in vitro difference among netilmicin, gentamicin, and tobramycin, except in the case of *Serratia*, for which gentamicin demonstrates clearly greater activity. These aminoglycosides were consistently the most active antibiotics against the *Enterobacteriaceae*, with amikacin the next most active and kanamycin the least active. The one striking exception to this was found when 46 strains of *P. rettgeri* were tested. Amikacin was fourfold more active than any of the other aminoglycosides tested, with 100% of strains in-

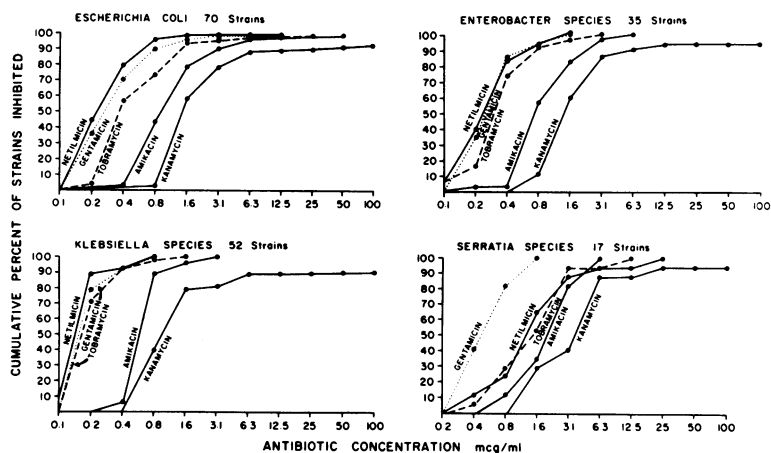


FIG. 1. Susceptibility of *E. coli*, *Enterobacter*, *Klebsiella*, and *Serratia* to netilmicin, gentamicin, tobramycin, amikacin, and kanamycin.

hibited at 6.3 $\mu\text{g/ml}$. This is particularly worthy of note, since disk susceptibility testing by the Kirby-Bauer method (1) had previously demonstrated that 55% of these strains were resistant to cephalothin, 73% to kanamycin, 100% to nitrofurantoin, 54% to ampicillin, 47% to gentamicin, and 43% to carbenicillin.

Figure 3 demonstrates the activity of the aminoglycosides when tested against 75 strains of *P. aeruginosa* in standard Mueller-Hinton broth and in a calcium- and magnesium-adjusted Mueller-Hinton broth. These strains were divided among two groups; the first consisted of 31 strains reported as susceptible to

gentamicin when tested by the Kirby-Bauer method; the second consisted of 44 strains reported as intermediate or resistant by the Kirby-Bauer method. Tobramycin was the most active aminoglycoside against both groups of *P. aeruginosa*. Gentamicin, netilmicin, and amikacin were very similar in their activity. The Ca^{2+} and Mg^{2+} content of the broth was most significant when netilmicin and gentamicin were tested. In Mueller-Hinton broth unadjusted for divalent cation concentration, netilmicin had an activity against *P. aeruginosa* of approximately one dilution less than gentamicin, and was slightly more active than amika-

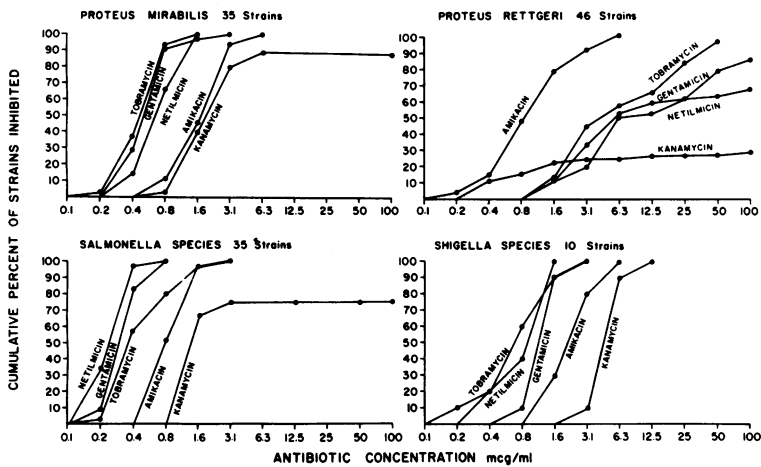


FIG. 2. Susceptibility of *P. mirabilis*, *P. rettgeri*, *Salmonella*, and *Shigella* to netilmicin, gentamicin, tobramycin, amikacin, and kanamycin.

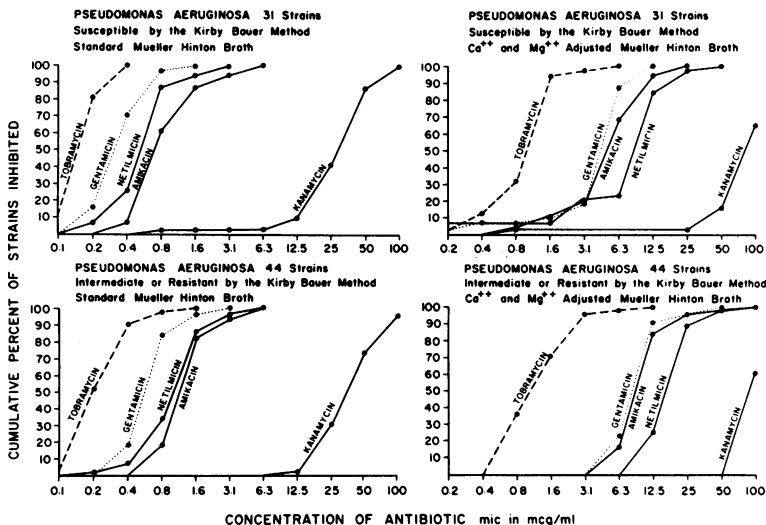


FIG. 3. Susceptibility to gentamicin-susceptible and intermediate or resistant strains of *P. aeruginosa* to netilmicin, gentamicin, tobramycin, amikacin, and kanamycin in standard and calcium-magnesium-adjusted Mueller-Hinton broth.

cin. In broth adjusted to physiological levels of Ca^{2+} and Mg^{2+} , netilmicin remained one dilution less active than gentamicin but showed less activity than amikacin. The geometric mean MIC of netilmicin against *P. aeruginosa* in unadjusted broth was 0.73 $\mu\text{g}/\text{ml}$ for gentamicin-susceptible strains and 1.35 $\mu\text{g}/\text{ml}$ for gentamicin-resistant strains. In Ca^{2+} - and Mg^{2+} -adjusted broth, the netilmicin MIC for gentamicin-susceptible *P. aeruginosa* was 9.6 $\mu\text{g}/\text{ml}$, and for gentamicin-resistant strains it was 23.1 $\mu\text{g}/\text{ml}$, an 18-fold increase.

Despite the Kirby-Bauer disk susceptibility reports, only a one-dilution difference in the gentamicin MIC was found between those strains of *P. aeruginosa* reported as susceptible and those reported as resistant. The "resistant" group had a geometric mean gentamicin MIC of 0.81 $\mu\text{g}/\text{ml}$, as compared to 0.45 $\mu\text{g}/\text{ml}$ for the susceptible group when tested in standard Mueller-Hinton broth. In broth adjusted to physiological levels of Ca^{2+} and Mg^{2+} , geometric mean MICs of gentamicin were 5 $\mu\text{g}/\text{ml}$ for the susceptible group and 11.8 $\mu\text{g}/\text{ml}$ for the resistant group. In view of the lack of strong correlation between the reported disk susceptibility test results and the broth dilution MICs, the effect of inoculum size was also examined. Table 1 demonstrates that inoculum size has a more significant effect when the divalent cation content of the broth is low. This is more marked with susceptible strains than with resistant ones. The significance of increased cation con-

tent of the broth was not as great when tobramycin was tested. In standard Mueller-Hinton broth, tobramycin was approximately twofold more active against *P. aeruginosa* than gentamicin. In Ca^{2+} - and Mg^{2+} -adjusted broth, tobramycin was four- to sixfold more active. Kanamycin was consistently the least active aminoglycoside.

Gentamicin, netilmicin, and tobramycin continued to be the most active aminoglycosides when tested against staphylococci and group D streptococci, as illustrated in Fig. 4. When tested in Trypticase soy broth, group D streptococci continued to demonstrate their characteristic resistance to aminoglycosides. A striking medium effect was found, however, when MICs in Trypticase soy broth were compared with those determined in Mueller-Hinton broth supplemented with 2% defibrinated sheep erythrocytes. The MICs were 7- to 22-fold higher when measured in Trypticase soy broth.

Figure 5 illustrates the results of testing *S. pneumoniae*, alpha-hemolytic streptococci (non-group D), group A beta-hemolytic streptococci, *N. gonorrhoeae*, *N. meningitidis*, and *H. influenzae*. Once again, gentamicin, netilmicin, and tobramycin were consistently more active than amikacin and kanamycin.

DISCUSSION

The results of this study show that, with few exceptions, the in vitro activity of netilmicin closely parallels that of gentamicin. The major

TABLE 1. Effect of inoculum size on the MIC of four aminoglycoside antibiotics

Antibiotic ($\mu\text{g}/\text{ml}$)	Gentamicin MIC for <i>P. aeruginosa</i> (with given dilution) classified by Kirby-Bauer method as:											
	Susceptible (5 strains)						Intermediate or resistant (5 strains)					
	10^{-2} ^a		10^{-5} ^a		$10^{-2}/10^{-5}$		10^{-2} ^a		10^{-5} ^a		$10^{-2}/10^{-5}$	
	Range	Mean ^b	Range	Mean ^b	Range	Mean ^b	Range	Mean ^b	Range	Mean ^b	Range	Mean ^b
Gentamicin												
MHB-S ^c	6.3-25	12.5	0.4-0.8	0.53	16-62	24	1.6-12.5	5	0.4-1	0.9	2-16	5.7
MHB-A ^d	6.3-25	16.5	3.1-6.3	4.7	3.1-6.3	3.5	12.5-25	22	6.3-25	12.5	1-4	1.8
Netilmicin												
MHB-S ^c	50	50	0.4-1.6	0.6	32-125	72.5	3.1-25	9.9	0.8-3.1	1.8	2-8	5.7
MHB-A ^d	25-50	37.9	6.3-12.5	10.9	2-8	3.5	25-50	39.7	25-50	31.5	0.5-2	1.3
Tobramycin												
MHB-S ^c	25-50	43.5	0.2-0.4	0.26	62.5-250	104	1.6-3.1	2.5	0.2-0.8	0.28	4-16	9
MHB-A ^d	1.6-12	5.5	0.8-1.6	1.1	2-8	4.6	0.4-3.1	1.1	0.8-3.1	1.3	0.3-2	0.9
Amikacin												
MHB-S ^c	25	25	0.8-1.6	1.1	16-32	24.3	6.3-25	8.9	0.8-6.3	1.8	2-8	5
MHB-A ^d	12.5-25	21.8	3.1-12.5	9.2	2-8	3.5	25-50	28	6.3-25	14.1	1-4	2

^a Dilution of an overnight Mueller-Hinton broth culture containing 10^9 organisms per ml.

^b Geometric mean.

^c Unadjusted Mueller-Hinton broth (Difco), pH 7.4, 7.2 mg and 12 mg of calcium per liter.

^d Adjusted Mueller-Hinton broth (Difco), pH 7.38, adjusted to contain 31.2 mg of magnesium and 76 mg of calcium per liter.

exceptions to this were found when strains of *Serratia* and group A streptococci were tested. In both instances, gentamicin showed approximately a two- to threefold greater activity than netilmicin. Netilmicin was approximately one dilution less active against *P. aeruginosa* than

gentamicin. Overall, gentamicin, netilmicin, and tobramycin were the most active antibiotics tested. Amikacin, however, was significantly more active against multi-drug-resistant strains of *P. rettgeri*. Kanamycin was consistently the least active antibiotic against all or-

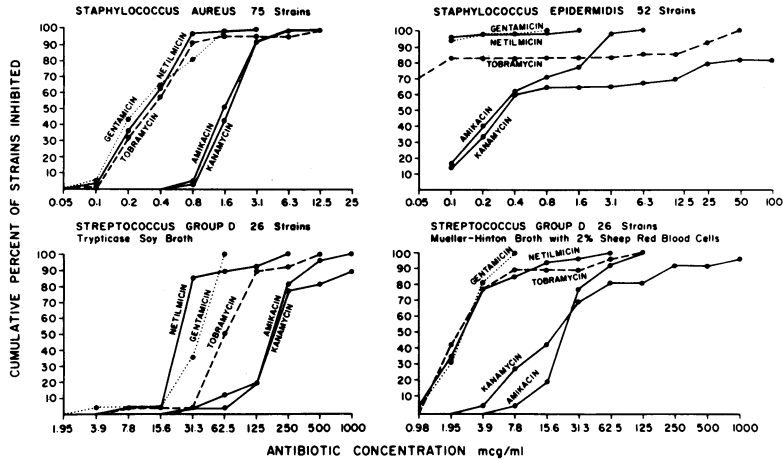


FIG. 4. Susceptibility of *S. aureus*, *S. epidermidis*, and group D streptococci to netilmicin, gentamicin, tobramycin, amikacin, and kanamycin.

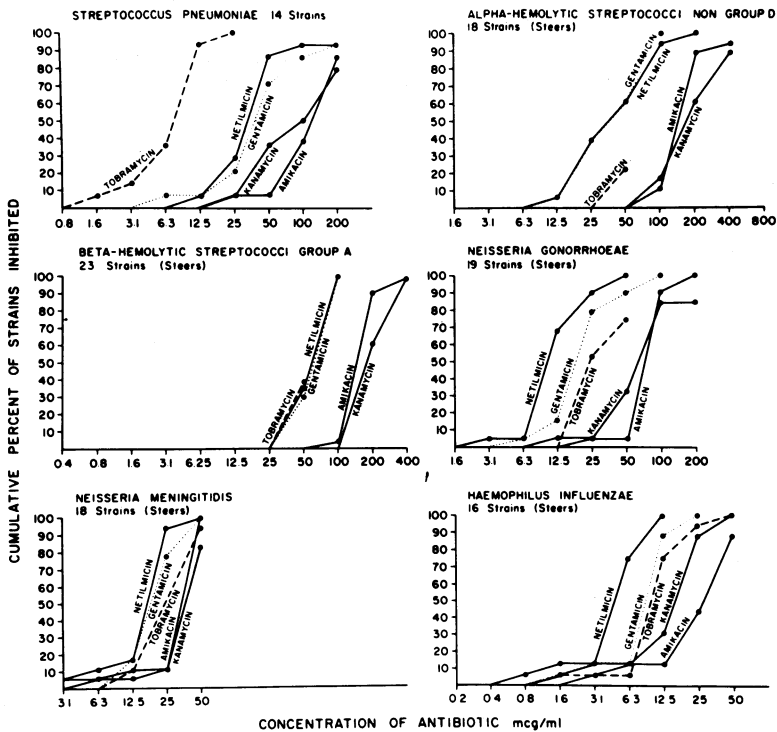


FIG. 5. Susceptibility of *S. pneumoniae*, alpha-hemolytic streptococci (non-group D), group A streptococci, *N. gonorrhoeae*, *N. meningitidis*, and *H. influenzae* to netilmicin, gentamicin, tobramycin, amikacin, and kanamycin.

ganisms tested. As has been reported with other aminoglycoside antibiotics (4, 8), the Ca^{2+} and the Mg^{2+} concentration of the medium was shown to have an important effect on the MIC of netilmicin when *P. aeruginosa* was tested. Our data also demonstrate an increased importance of inoculum size when broth low in Mg^{2+} and Ca^{2+} is used. This is particularly true of susceptible strains.

Another significant effect is found when media supplemented with sheep blood are used to test aminoglycoside activity against enterococci. This has previously been reported to enhance the activity of gentamicin against enterococci (16). Our data confirm this and extend it to other aminoglycosides.

LITERATURE CITED

1. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized disk method. *Am. J. Clin. Pathol.* 45:493-496.
2. Bulger, R. J., S. Sidell, and W. M. M. Kirby. 1963. Laboratory and clinical studies of gentamicin. *Ann. Intern. Med.* 59:593-604.
3. Chang, J. C., and B. Reyes. 1975. Agranulocytosis associated with gentamicin. *J. Am. Med. Assoc.* 232:1154-1155.
4. Davis, S. D., and A. Iannetta. 1973. Relative antagonism *in vitro* of calcium in serum to the bactericidal activities of gentamicin and tobramycin on *Pseudomonas aeruginosa*. *Chemotherapy* 19:243-253.
5. Eickhoff, T. C. 1975. New antibacterial treatment of nosocomial infections. *Bull. N.Y. Acad. Med.* 51:1056-1064.
6. Falco, F. G., H. M. Smith, and G. M. Arcieri. 1969. Nephrotoxicity of aminoglycosides and gentamicin. *J. Infect. Dis.* 119:406-409.
7. Finland, M. 1970. Changing ecology of bacterial infections as related to antibacterial therapy. *J. Infect. Dis.* 122:419-431.
8. Gilbert, D. N., E. Kutscher, P. Ireland, J. A. Barnett, and J. P. Sanford. 1971. Effect of the concentrations of magnesium and calcium on the *in vitro* susceptibility of *Pseudomonas aeruginosa* to gentamicin. *J. Infect. Dis.* 124(Suppl.):S37-S45.
9. Holtzman, J. L. 1976. Gentamicin and neuromuscular blockade. *Ann. Intern. Med.* 84:55.
10. Houang, E. T., and E. McKay-Ferguson. 1976. Activities of tobramycin and amikacin against gentamicin resistant gram-negative bacilli. *Lancet* 1:423-424.
11. Jackson, G. G. 1967. CCXXXIV. - Gentamicin. *Curr. Ther.* 198:855-866.
12. Jackson, G. G., and G. Arcieri. 1971. Ototoxicity of gentamicin in man: a survey and controlled analysis of clinical experience in the United States. *J. Infect. Dis.* 124(Suppl.):S130-S137.
13. Klein, J. O., T. C. Eickhoff, and M. Finland. 1964. Gentamicin: activity *in vitro* and observations in 26 patients. *Am. J. Med. Sci.* 248:528-543.
14. Martin, C. M., N. S. Ikuri, J. Zimmerman, and J. A. Waitz. 1971. A virulent nosocomial *Klebsiella* with a transferable R factor for gentamicin: emergence and suppression. *J. Infect. Dis.* 124(Suppl.):S24-S29.
15. Meyer, R. D., R. P. Lewis, J. Halter, and M. White. 1976. Gentamicin resistant *Pseudomonas aeruginosa* and *Serratia marcescens* in a general hospital. *Lancet* 1:580-583.
16. Raymond, E. A., and W. H. Traub. 1971. Medium-dependent activity of gentamicin sulfate against enterococci. *Appl. Microbiol.* 21:192-194.
17. Schering Corporation. 1975. Investigational brochure - informational material for the investigational drug Sch 20569. Schering Corp., Bloomfield, N.J.
18. Steers, E., E. L. Foltz, and E. S. Graves. 1959. Inocula replicating apparatus for routine testing of bacterial sensitivity to antibiotics. *Antibiot. Chemother.* 9:307-311.
19. Tjernstrom, O., G. Banck, S. Belfrage, I. Juhlin, L. Nordstrom, and N. G. Taremalm. 1973. The ototoxicity of gentamicin. *Acta. Pathol. Microbiol. Scand.* 81(Suppl.):73-78.
20. Warren, R. E., and S. O. B. Roberts. 1976. Gentamicin-resistant staphylococci. *Lancet* 1:543-544.
21. Wilfert, J. N., J. P. Burke, H. A. Bloomer, and C. B. Smith. 1971. Renal insufficiency associated with gentamicin therapy. *J. Infect. Dis.* 124(Suppl.):S148-S153.