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In Vitro Studies of Tobramycin

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The in vitro activity of tobramycin was studied against 457 clinical isolates of gram-negative bacilli and 151 clinical isolates of gram-positive cocci. The vast majority of the gram-negative bacilli was inhibited by tobramycin at a concentration of 1.56 μg or less per ml. Only a few isolates of *Staphylococcus aureus* and no isolates of *Streptococcus pyogenes* or *Diplococcus pneumoniae* were susceptible to this drug. Tobramycin was generally more active than gentamicin sulfate against gram-negative bacilli, although organisms resistant to gentamicin sulfate were also resistant to tobramycin. The major difference between the two drugs was the greater activity of tobramycin against *Pseudomonas aeruginosa*.

Gram-negative bacilli have become a frequent cause of infection in hospitalized patients. The aminoglycoside group of antibiotics have the broadest spectrum of activity against these organisms. Among the aminoglycoside antibiotics used for the treatment of systemic infections, gentamicin sulfate has the broadest spectrum of activity, including most species of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Recently, a new aminoglycoside antibiotic, tobramycin, was isolated from a complex produced by *Streptomyces tenebrarius* (2, 6). In vitro studies have demonstrated that the spectrum of activity of this drug is similar to that of gentamicin (8; N. Moreland, M. B. Pearson, and T. W. Williams, Jr., Abstr. 11th Intersci. Conf. Antimicrob. Ag. Chemother., p. 42, 1971). However, a major advantage of tobramycin is its greater activity against *P. aeruginosa* (5). Infections caused by gram-negative bacilli, and especially *P. aeruginosa*, are common in cancer patients. Consequently, an in vitro study of tobramycin was conducted against isolates of these organisms cultured from cancer patients.

MATERIALS AND METHODS

Susceptibility tests were conducted on 457 clinical isolates of gram-negative bacilli and 151 clinical isolates of gram-positive cocci, by use of the broth dilution technique (4). Gram-negative bacilli were inoculated into Mueller-Hinton broth (Difco) and incubated at 37 C for 18 hr. A 0.1-ml sample of a 10^{-4} dilution of this broth (approximately 10^{3} colony-forming units) was used as the inoculum for susceptibility testing of gram-negative bacilli. Isolates of coagulase-positive *Staphylococcus aureus* were inoculated into Tryptose phosphate broth (Difco), and isolates of group A *Streptococcus pyogenes* and *Diplococcus pneumoniae* were inoculated into Tryptose phosphate broth containing 1.5% human blood; incubation was at 37 C for 18 hr (7). A 0.1-ml sample of a 10^{-1} dilution of these preparations (approximately 10^{6} colony-forming units) was used as the inoculum for susceptibility testing of gram-positive cocci.

Tobramycin used in this study was supplied as a solution containing 1,000 μg/ml by Eli Lilly & Co., Indianapolis, Ind. Twofold serial dilutions of the antibiotic were made in Mueller-Hinton broth (for gram-negative bacilli), Tryptose phosphate broth (for *S. aureus*), or Tryptose phosphate broth and 1.5% human blood (for *S. pyogenes* and *D. pneumoniae*). The final volume of broth in each tube was 1.0 ml. The minimal inhibitory concentration (MIC) was determined after incubation at 37 C for 18 hr. All tubes containing no discernible growth were subcultured on sheep blood-agar. A wire loop calibrated to deliver 0.01 ml was used to transfer the inoculum. Tobramycin was considered to be bactericidal against those isolates which yielded no growth on subculture of broth from the tube containing the MIC.

All gram-negative bacilli used in this study were cultured from blood specimens obtained from patients between 1966 and 1971. The majority of the patients were hospitalized at this institution and had underlying malignant disease. A total of 100 isolates each of *P. aeruginosa*, *Escherichia coli*, and *Klebsiella* spp., 64 isolates of *Proteus* spp., 52 isolates of *Enterobacter* spp., and 41 isolates of *Serratia marcescens* were studied. All gram-positive cocci used in this study were cultured from hospitalized patients, most of whom did not have cancer. A total of 49 isolates of *S. pyogenes*, 19 isolates of *D. pneumoniae*, and 83 isolates of *S. aureus* were studied. The susceptibility of isolates of *S. aureus* to penicillin G was determined, by means of the broth-dilution technique. Those isolates inhibited by less than 0.10 μg/ml were considered to be penicillin G-susceptible and those isolates re-

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sistant to more than 25 μg/ml were considered to be penicillin G-resistant.

The MIC against 290 isolates of gram-negative bacilli was compared with the zone of inhibition around discs containing 10 μg of tobramycin. The disc studies were performed according to the methods of Bauer et al. (1). The discs were supplied by Eli Lilly & Co.

RESULTS

The in vitro activity of tobramycin against gram-negative bacilli and gram-positive cocci is shown in Fig. 1. The MIC of tobramycin was 1.56 μg or less per ml against all of the isolates of *Klebsiella* spp. and indole-positive *Proteus* spp., 99% of *P. aeruginosa*, 94% of *Enterobacter* spp., 93% of *E. coli*, 92% of *P. mirabilis*, and 86% of *S. marcescens*. Only 14% of penicillin G-resistant *S. aureus* strains and 9% of penicillin G-susceptible *S. aureus* strains were inhibited by 3.12 μg or less per ml. All of the isolates of *S. pyogenes* and *D. pneumoniae* were resistant to 3.12 μg of tobramycin per ml. The antibiotic was bactericidal against 45% of isolates of *E. coli*, 26% of *Klebsiella* spp., 7% of *Enterobacter* spp., 16% of *Proteus* spp., 11% of *S. marcescens*, 2% of *P. aeruginosa*, and 4% of *S. aureus*.

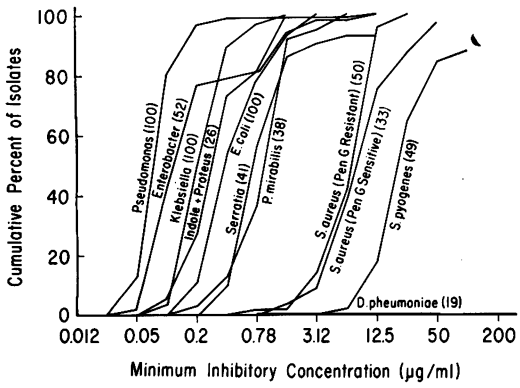

FIG. 1. *In vitro activity of tobramycin against clinical isolates of bacteria.*

The effect of inoculum size on the MIC of tobramycin was determined against 10 isolates each of *P. aeruginosa*, *Klebsiella* spp., and *E. coli* (Fig. 2). The inocula used were 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions of an 18-hr broth culture of the test organisms (containing approximately 10^8 colony-forming units/ml). In general, tobramycin was slightly less active against the larger inocula. The greatest differences were observed with *P. aeruginosa*.

The in vitro activity of tobramycin was compared with that of gentamicin sulfate (Fig. 3). Tobramycin was more active than gentamicin sulfate against most gram-negative bacilli. At a concentration of 1.56 μg/ml, tobramycin inhibited 93% of isolates of *E. coli* and 94% of *Enterobacter* spp., whereas gentamicin sulfate inhibited 80 and 81%, respectively. At the same

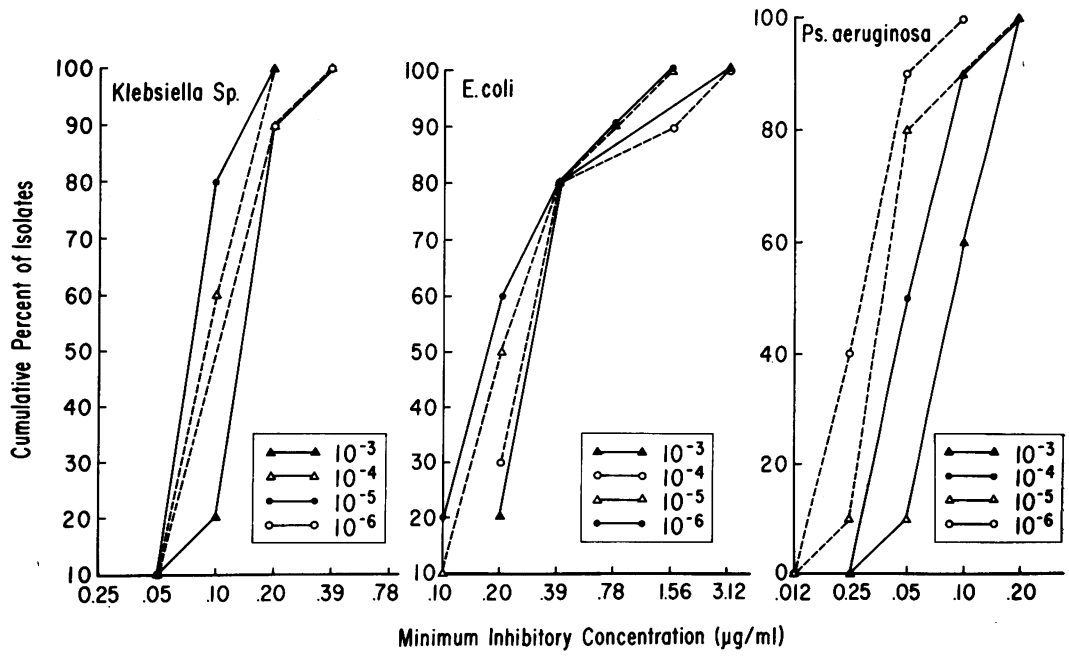

FIG. 2. *Effect of inoculum size on the activity of tobramycin.*

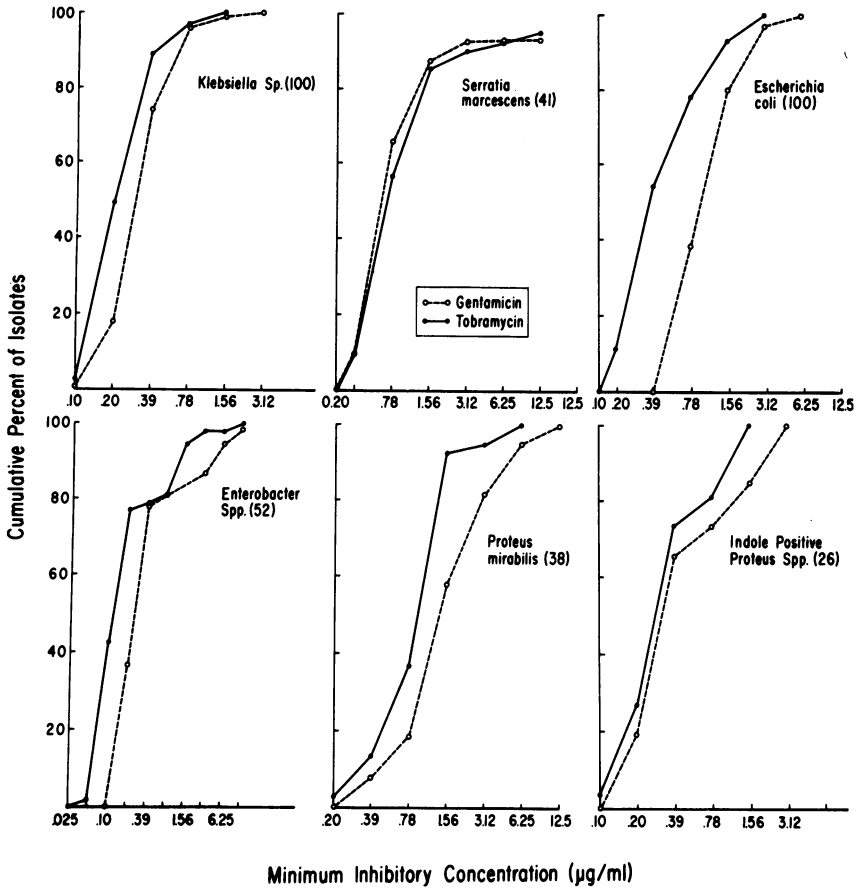

FIG. 3. *Comparison of activity of tobramycin and gentamicin sulfate against gram-negative bacilli.*

concentration, tobramycin inhibited 92% of *P. mirabilis* strains and 100% of indole-positive strains of *Proteus* spp., whereas gentamicin sulfate inhibited 58 and 85%, respectively. The greatest differences between the two drugs were observed with *P. aeruginosa* isolates (Fig. 4), among which 80% were inhibited by 0.10 μg of tobramycin per ml but only 4% were inhibited by the same concentration of gentamicin sulfate. At a concentration of 6.25 μg/ml, tobramycin inhibited 29% of penicillin G-susceptible isolates of *S. aureus* and 42% of penicillin G-resistant isolates of *S. aureus*, whereas gentamicin sulfate inhibited 79 and 36%, respectively (Fig. 5).

Nine isolates of gram-negative bacilli resistant to gentamicin sulfate concentrations of 6.25 μg/ml or greater were tested for their susceptibility to tobramycin (Table 1). Only one isolate of *Klebsiella* sp. and one of *P. aeruginosa* were found to be substantially more susceptible to tobramycin.

The MIC against 290 isolates of gram-negative bacilli was compared with the zones of inhibition around discs containing 10 μg of tobramycin

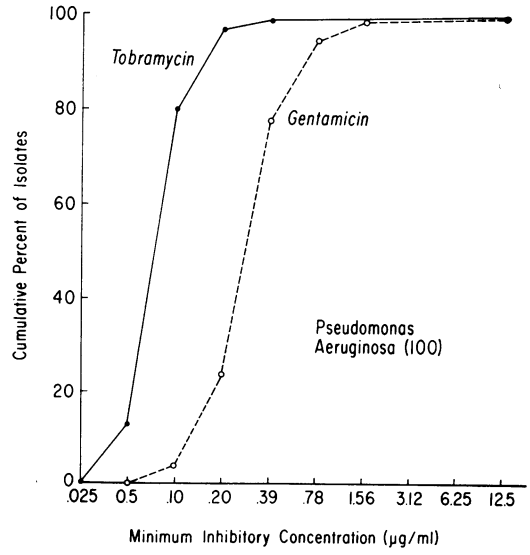

FIG. 4. *Comparison of activity of tobramycin and gentamicin sulfate against Pseudomonas aeruginosa.*

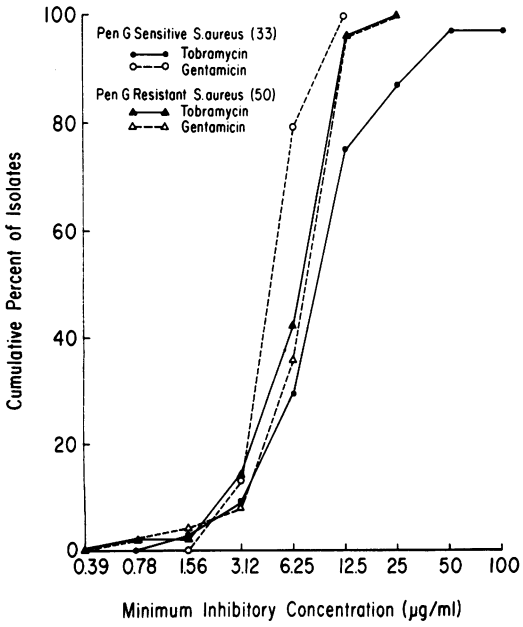

FIG. 5. *Comparison of activity of tobramycin and gentamicin sulfate against Staphylococcus aureus.*

TABLE 1. *Activity of tobramycin against gram-negative bacilli resistant to gentamicin sulfate*

Isolate	Source	Minimal inhibitory concn (μg/ml)	
		Gentamicin	Tobramy
Klebsiella	Stool	>50	50
Klebsiella	Urine	50	50
Klebsiella	Blood	12.5	3.12
Klebsiella	Blood	12.5	6.25
E. coli	Stool	25	>50
E. coli	Blood	12.5	6.25
Pseudomonas	Blood	50	12.5
Pseudomonas	Skin	50	50
Serratia	Leg	50	50

(Table 2). Four of the five isolates resistant to 3.12 μg of tobramycin per ml had zones less than 14 mm in diameter. Of the 227 isolates susceptible to 0.78 μg or less/ml, 196 (86%) had zones 17 mm or more in diameter.

DISCUSSION

In general, the results of our in vitro tests of tobramycin against gram-negative bacilli were similar to those reported by other investigators (3, 5; Moreland et al., Abstr. 11th Intersci. Conf. Antimicrob. Ag. Chemother., p. 42, 1971). However, our isolates of *S. marcescens* and *P. mirabilis* were more susceptible to tobramycin than those reported by Dienstag and Neu (3). Both studies were done with isolates from institutions where gentamicin sulfate and other aminoglycoside antibiotics have been used widely.

TABLE 2. *Activity of tobramycin against gram-negative bacilli as determined by broth dilution and agar diffusion tests*

MIC (μg/ml)	Diam of zone of inhibition (mm)[a]			
	<14	15-16	17-18	>18
>3.12	4[b]	0	0	1
1.56-3.12	9	5	18	25
0.39-0.78	1	7	51	45
0.10-0.20	0	24	44	52
<0.10	0	0	0	4

[a] Discs containing 10 μg of tobramycin were used.
[b] Number of isolates.

Tobramycin was somewhat more active than gentamicin sulfate against our clinical isolates of gram-negative bacilli. The greater activity of tobramycin was most pronounced against isolates of *P. aeruginosa*. Other investigators have also reported the superiority of tobramycin against *P. aeruginosa* but have found gentamicin sulfate to be slightly more active against other gram-negative bacilli. This latter difference in results is probably due to technical factors such as the relative potency of antibiotic powders, and it is unlikely that it will be of any clinical significance.

Tobramycin is not very active against *D. pneumoniae* and *S. pyogenes*, but other investigators have found it to be active against *S. aureus* (2, 3). In these other studies, all isolates of *S. aureus* were inhibited by 6.25 μg or less of tobramycin per ml. Our isolates were somewhat more resistant, but this was probably a result of our use of a larger inoculum and a richer broth.

The in vitro studies of tobramycin suggest that it will be a useful antibiotic for the treatment of infections due to gram-negative bacilli, and especially those caused by *P. aeruginosa*.

ACKNOWLEDGMENTS

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