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In Vitro Bactericidal Effectiveness of Four Aminoglycoside Antibiotics

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The antibacterial activity of four aminoglycoside antibiotics (gentamicin, Sch 13706, tobramycin, and sisomicin) was tested against eight gram-negative and three gram-positive species. A total of 323 strains were studied by the broth dilution technique. Tobramycin and sisomicin had greater bacteriostatic and bactericidal activity against *Pseudomonas* strains than did gentamicin and Sch 13706. Of the four antibiotics, sisomicin was most active against *Klebsiella*, *Enterobacter*, *Escherichia coli*, indole-negative and -positive *Proteus*, and *Streptococcus pyogenes*. Gentamicin was most effective against *Serratia*. A fourfold or greater difference existed frequently between the minimal inhibitory and bactericidal concentrations of all antibiotics against *Enterobacter* and *Serratia*. This difference was greatest with tobramycin. *Staphylococcus aureus* was highly susceptible, *Providencia* relatively resistant, and enterococcus uniformly resistant to the antibiotics studied. Agar diffusion susceptibility testing with gentamicin and tobramycin showed that organisms susceptible to less than 6.2 μg/ml usually yielded zones 17 to 26 mm in diameters. Zones of 15 to 16 mm represented intermediate susceptibility which varied with the organism and antibiotic. Several *Serratia* strains required 6.2 to 12.5 μg of gentamicin/ml or 25 to 50 μg of tobramycin/ml for bactericidal activity despite minimal inhibitory concentrations of 0.09 to 3.1 μg/ml and zone sizes greater than 13 and 17 mm, respectively. Studies with *Enterobacter* and tobramycin yielded similar results.

The continuing clinical problem of gram-negative infection has stimulated an intense search for more effective broad-spectrum antibiotics. Among currently available agents, gentamicin provides the widest bactericidal spectrum against gram-negative bacilli. Recently, three related aminoglycosides with antimicrobial spectra similar to gentamicin have been developed. These are sisomicin, isolated from *Micromonospora inyuensis* (8, 9), Sch 13706, produced from *Micromonospora purpurea* (10) and tobramycin (nebramycin factor 6), a product of *Streptomyces tenebrarius* (5, 11). The purpose of this study was to compare, under identical conditions, the in vitro effectiveness of sisomicin, Sch 13706, tobramycin, and gentamicin sulfate against 11 bacterial species.

MATERIALS AND METHODS

Organisms. Thirty clinical isolates of each species were tested. Those studied were *Pseudomonas*, *Klebsiella*, *Enterobacter*, indole-negative *Proteus*, indole-positive *Proteus*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and enterococcus. Twenty-three strains of *Providencia* were also evaluated. Identification of the organisms was determined in duplicate by the clinical bacteriology laboratory, on the basis of the criteria of Edwards and Ewing.

Antibiotics and discs. Tobramycin laboratory standard solution (1,000 μg/ml) and 10-μg antibiotic-impregnated discs were furnished by Eli Lilly & Co., Indianapolis, Ind. The powdered standards of gentamicin sulfate (579 μg/mg), Sch 13706 (615 μg/mg), and sisomicin (623 μg/mg), as well as gentamicin and sisomicin discs (10 μg), were supplied by Schering Laboratories, Bloomfield, N.J.

Antibiotic susceptibility assays. Antibiotic susceptibility of the bacterial isolates was determined by the broth dilution method. An overnight culture grown in Trypticase soy broth (BBL) was diluted to 10^{-4} (10^{-2} for *S. pyogenes*) in Mueller-Hinton broth (Difco), and 0.5-ml amounts were inoculated into tubes containing serial twofold dilutions of antibiotic in the same medium to give a final volume of 1.0 ml. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic preventing visible turbidity. After incubation for 16 to 18 hr at 37 C, all clear tubes were subcultured with a

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calibrated loop (0.01 ml) onto Trypticase soy agar (BBL) and reincubated for 18 to 24 hr at 37 C. The minimal bactericidal concentration (MBC) was defined as that yielding growth of fewer than 10 colonies. For subculture of *S. pyogenes*, 2% citrated horse blood was added to the agar. When differences between the MIC and MBC were predominantly two broth dilutions or less, only the latter is reported.

The inoculum size of each species was randomly monitored by making 10-fold serial dilutions of the overnight culture and seeding 0.1 ml into 10 ml of heart infusion agar. Colonies were enumerated after 18 hr of incubation at 37 C. The inoculum for each organism was 5×10^4 organisms with the exception pf *S. pyogenes* (5×10^2). Disc susceptibility testing was performed in duplicate according to the procedure of Bauer et al. (1).

RESULTS

Broth dilution susceptibility tests. *Klebsiella* and *E. coli* were the most susceptible gram-negative species tested. MBC values for the four antibiotics against these species are compared in Fig. 1. Twenty-eight isolates (93%) of *Klebsiella* and *E. coli* were killed by 0.39 and 1.56 μg of sisomicin/ml, by 0.78 and 3.1 μg of gentamicin/ml, and by 3.1 and 6.2 μg of Sch 13706 and tobramycin/ml, respectively.

Sisomicin was the most effective agent against *Enterobacter* (Fig. 2). Tobramycin concentrations greater than 6.2 μg/ml were required to kill 30% of strains. Among the *Serratia* strains, 60% were killed by 3.1 μg of sisomicin/ml, and 85% were killed by that concentration of Sch 13706 or gentamicin. However, for 60% of these strains, more than 12.5 μg of tobramycin/ml was required for a bactericidal effect (Fig. 3). A fourfold or greater difference between the MIC and MBC of the four antibiotics was frequently noted when *Enterobacter* and *Serratia* strains were tested. In particular, the MBC of tobramycin against these groups was often 8- to 32-fold higher than the respective MIC (Fig. 2 and 3).

Indole-negative and -positive *Proteus* strains were less susceptible to all four antibiotics, although sisomicin was again most active (Fig. 4). Among the indole-negative group, 90% were killed by 3.1 μg of sisomicin/ml, by 6.2 μg of Sch 13706/ml, and by 12.5 μg of gentamicin and tobramycin/ml; 30 to 33% of indole-positive *Proteus* strains were resistant to 6.2 μg of gentamicin, tobramycin, and Sch 13706/ml.

Tobramycin and sisomicin were equally effective against *Pseudomonas* (Fig. 5), with 93% of strains killed by 3.1 μg or less of these antibiotics/ml; 86% were killed by 3.1 μg of gentamicin/ml and only 37% by the same concentration of Sch 13706.

Providencia was the most resistant gram-negative species evaluated (Fig. 6). The MBC of gentamicin of Sch 13706, and tobramycin was 12.5 μg/ml or greater for 78% of all strains. However, two-thirds were killed by 6.2 μg of sisomicin/ml.

S. aureus was quite susceptible to all four antibiotics. Concentrations of 0.78 μg or less/ml were bactericidal for more than 90% of strains. *S. pyogenes* was most susceptible to sisomicin, with which the highest MBC was

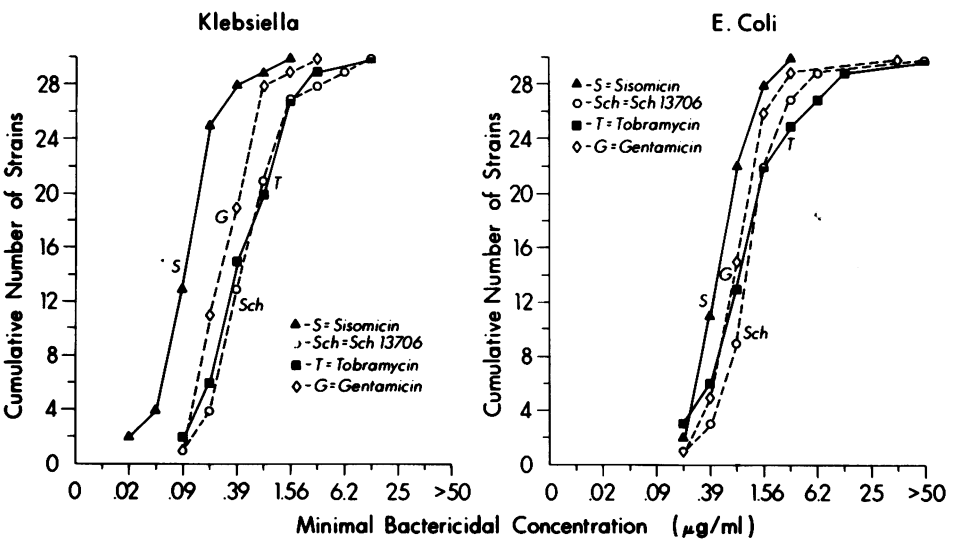

FIG. 1. *Minimal bactericidal concentrations of sisomicin, SCH 13706, tobramycin, and gentamicin for 30 strains of Klebsiella and E. coli.*

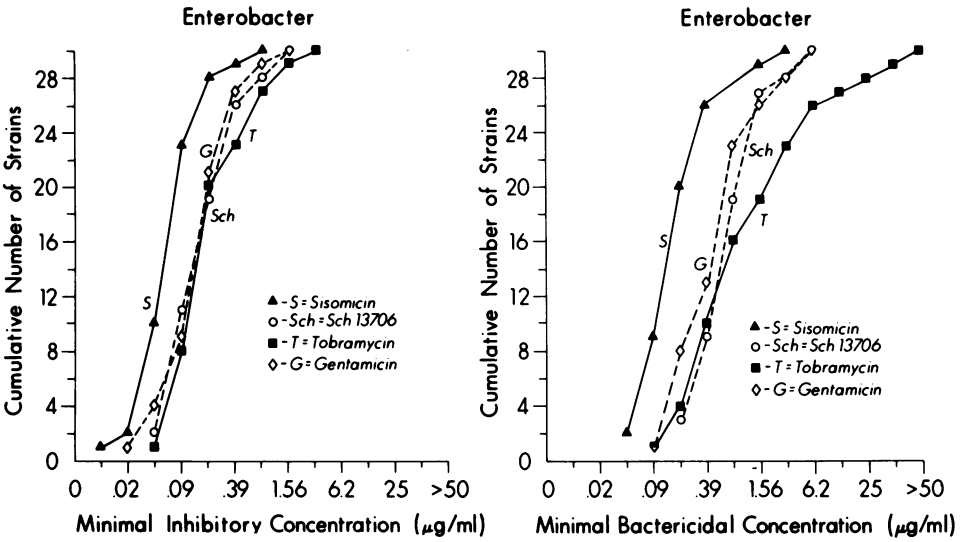

FIG. 2. *Minimal inhibitory and bactericidal concentrations of sisomicin, SCH 13706, tobramycin, and gentamicin for 30 strains of Enterobacter.*

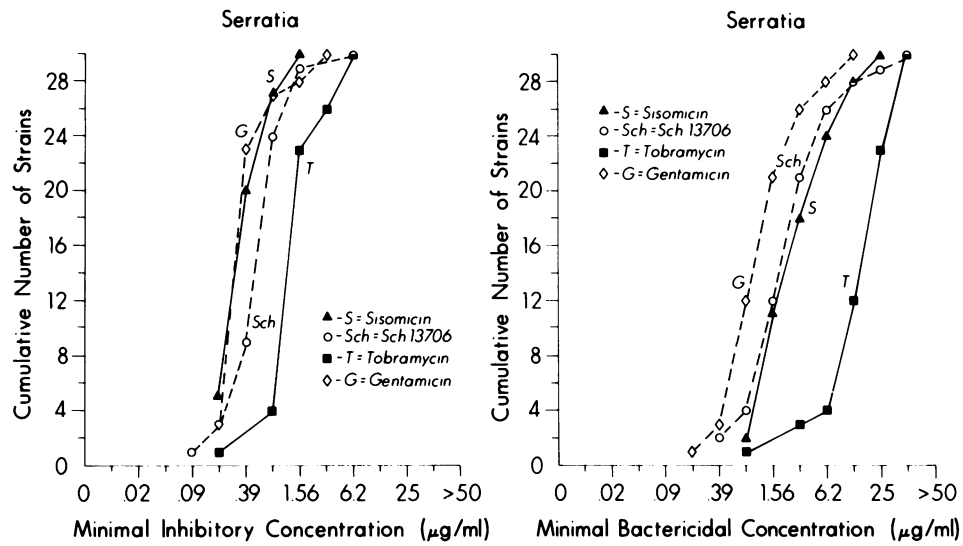

FIG. 3. *Minimal inhibitory and bactericidal concentrations of sisomicin, SCH 13706, tobramycin, and gentamicin for 30 strains of nonpigmented Serratia.*

1.56 μg/ml; the highest MBC of gentamicin and Sch 13706 was 12.5 μg/ml, and that of tobramycin was 50 μg/ml. Enterococci were resistant to all antibiotics tested (Fig. 7).

Disc susceptibility tests. The relationship between zone sizes produced by disc susceptibility tests and MBC values for gentamicin and tobramycin are represented in Fig. 8–11. Almost all *E. coli* and *Klebsiella* strains were highly susceptible to gentamicin; MIC and MBC values of 0.09 to 6.2 μg/ml were scattered randomly throughout zones 16 to 22 mm in diameter (Fig. 8). A small number of *Klebsiella* strains were slightly less susceptible to tobramycin and yielded smaller zones. Indole-positive *Proteus* strains demonstrated a wider range of susceptibility to both drugs. Again, both MIC and MBC values were similar, and zone sizes were generally inversely proportional to the broth dilution results (Fig. 8).

Although *Pseudomonas* was generally susceptible to both tobramycin and gentamicin (tobramycin being more effective), a greater discrepancy between MIC and MBC values

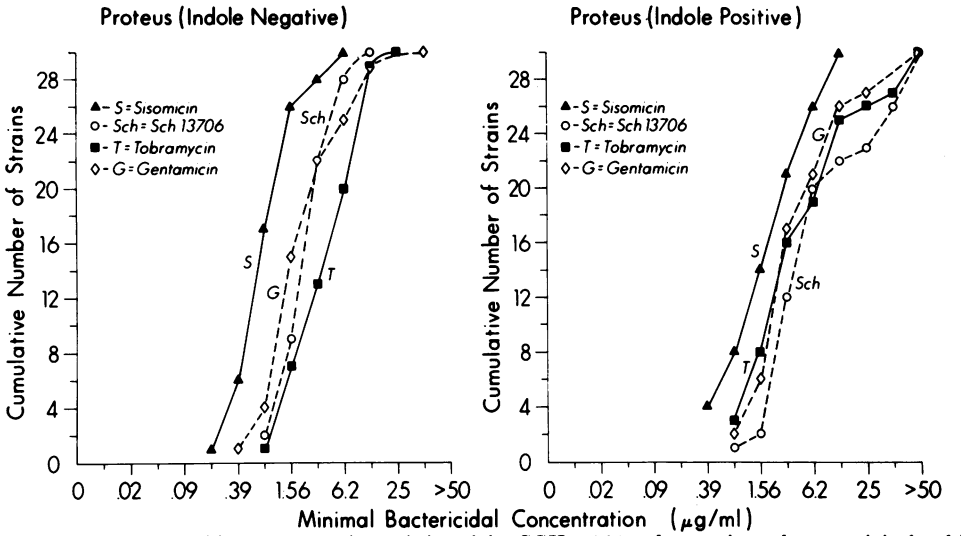

FIG. 4. *Minimal bactericidal concentrations of sisomicin, SCH 13706, tobramycin and gentamicin for thirty strains of indole-negative Proteus and indole-positive Proteus.*

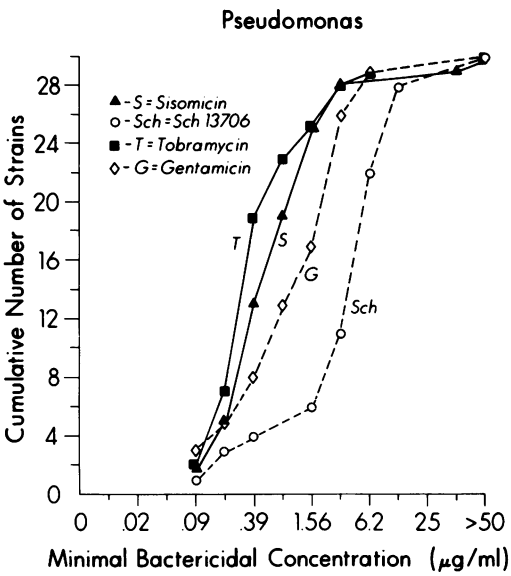

FIG. 5. *Minimal bactericidal concentrations of sisomicin, SCH 13706, tobramycin, and gentamicin for 30 strains of Pseudomonas aeruginosa.*

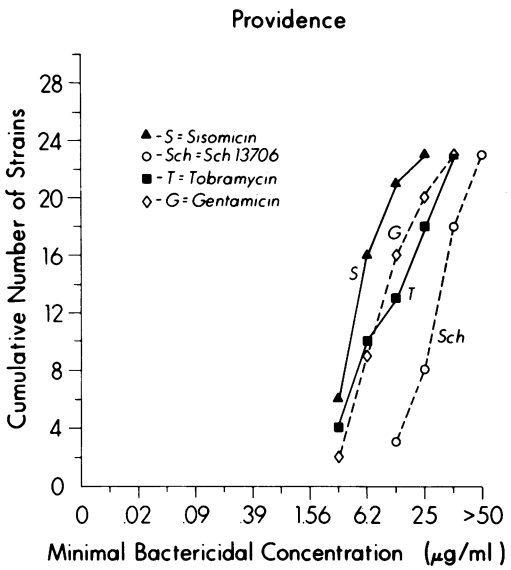

FIG. 6. *Minimal bactericidal concentrations of sisomicin, SCH 13706, tobramycin, and gentamicin for 23 strains of Providencia.*

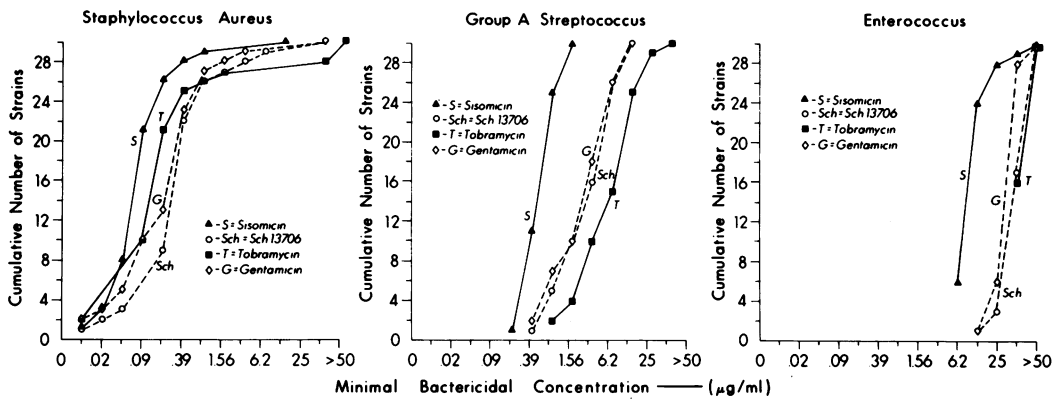

FIG. 7. *Minimal bactericidal concentrations of sisomicin, SCH 13706, tobramycin, and gentamicin for 30 strains of Staphylococcus aureus, group A streptococcus, and enterococcus.*

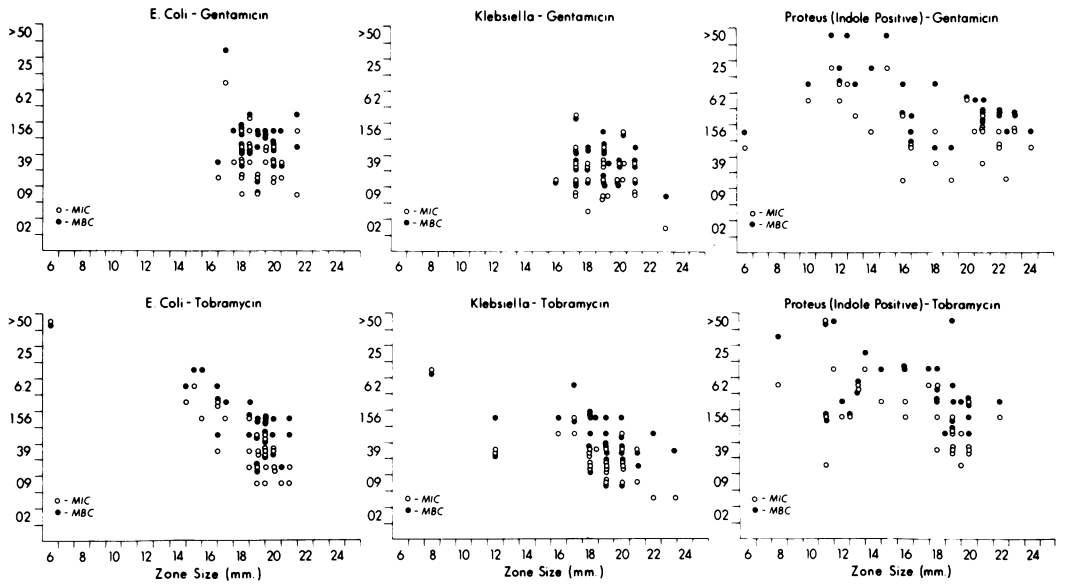

FIG. 8. *Comparison of MIC and MBC values for gentamicin and tobramycin with disc susceptibility zone sizes against E. coli, Klebsiella, and indole-positive Proteus.*

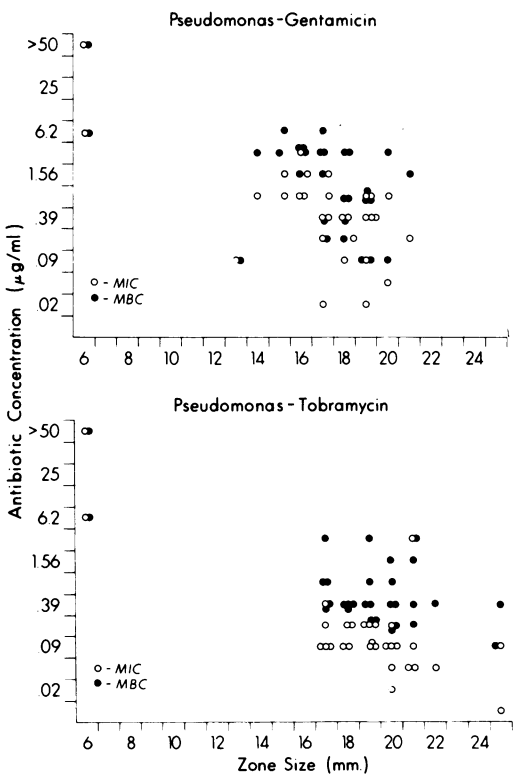

FIG. 9. *Comparison of MIC and MBC values for gentamicin and tobramycin with disc susceptibility zone sizes against Pseudomonas aeruginosa.*

for both drugs is evident in Fig. 9. Thus, a four-fold difference existed between the bacteriostatic and bactericidal concentration required for several strains. There was no correlation between the higher MBC values (3.1 to 6.2 μg/ml) and zone sizes, which were frequently greater than 13 mm with gentamicin and 16 mm with tobramycin. This discrepancy was also observed when *Serratia* and *Enterobacter* were tested against tobramycin and gentamicin. The MBC of tobramycin for several *Serratia* and a few *Enterobacter* strains was 12.5 to 50 μg/ml, although the MIC was usually 3.1 μg or less/ml. Zone sizes did not distinguish between the wide range of MBC values (Fig. 10). Indole-negative *Proteus* strains were usually killed by 12.5 μg or less of either gentamicin or tobramycin/ml; MIC values were one to two broth dilutions lower, and most zones of inhibition were 14 mm or greater in diameter (Fig. 11). *Providencia* strains were relatively resistant to these two antibiotics, and zone diameters were less than 14 mm.

Gentamicin discs produced larger zone diameters (22 to 26 mm) when tested against staphylococci than did tobramycin (18 to 22 mm), despite similar broth dilution susceptibility (Fig. 7). For group A streptococci, gentamicin and tobramycin zones were similar (12 to 22 mm and 11 to 20 mm, respectively). Neither antibiotic yielded inhibitory zones when enterococci were tested.

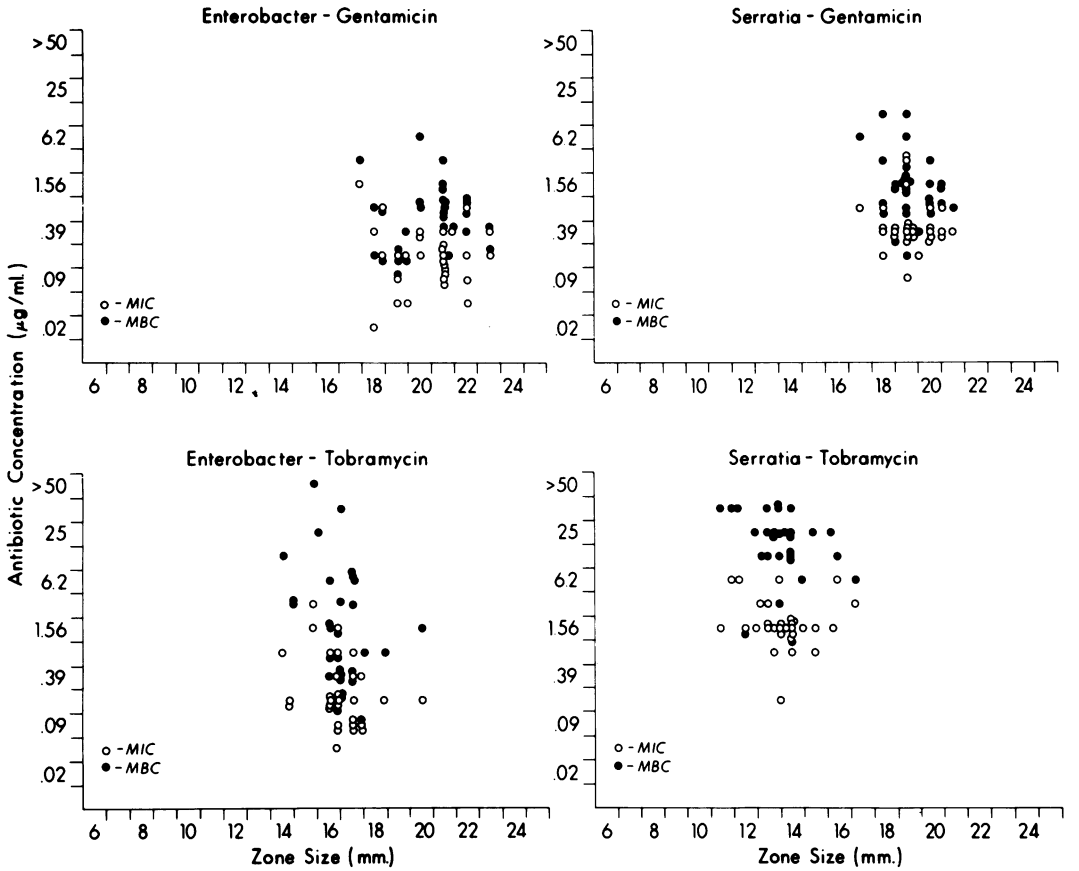

FIG. 10. *Comparison of MIC and MBC values for gentamicin and tobramycin with disc susceptibility zone sizes against Enterobacter and Serratia.*

DISCUSSION

The in vitro efficacy of four aminoglycoside antibiotics—gentamicin, Sch 13706, sisomicin, and tobramycin—was tested simultaneously against 323 clinical isolates. Although tobramycin and sisomicin were equally active against *P. aeruginosa*, sisomicin was the most effective of the four against *Klebsiella*, *Enterobacter*, *E. coli*, indole-negative and -positive *Proteus* strains, and *S. pyogenes*. *S. aureus* was highly susceptible, *Providencia* strains were relatively resistant, and enterococci were uniformly resistant to the antibiotics tested.

The results of our comparative susceptibility studies with gentamicin and tobramycin are similar to those of earlier studies, in which tobramycin was found to be more active than gentamicin against *P. aeruginosa* and usually ineffective at clinically attainable concentrations against *S. marcescens* and *Providencia* (2, 3, 7). Our data and those of Dienstag and Neu (3) indicate that *Enterobacter* and indole-positive *Proteus* species are less frequently susceptible to tobramycin than to gentamicin. The present study also indicates that sisomicin is as active as tobramycin against *Pseudomonas*, slightly less effective than gentamicin against *Serratia*, and two to four times as effective as either antibiotic against other *Enterobacteriaceae*, *Proteus*, and *Providencia*. Sch 13706 is similar to gentamicin in its activity against all species with the exception of a two- to fourfold lesser bactericidal effect on *Pseudomonas*. The potential clinical value of the latter two drugs will depend upon their toxicity relative to gentamicin and tobramycin. Studies by Weinstein, Waitz, and colleagues (8) indicated that the acute lethal toxicity of sisomicin in mice is twice that of gentamicin. Sisomicin is also approximately 1.3 times as toxic as gentamicin in the production of ataxia in cats. These findings, if applicable to man, may negate the in vitro superiority of sisomicin over the other

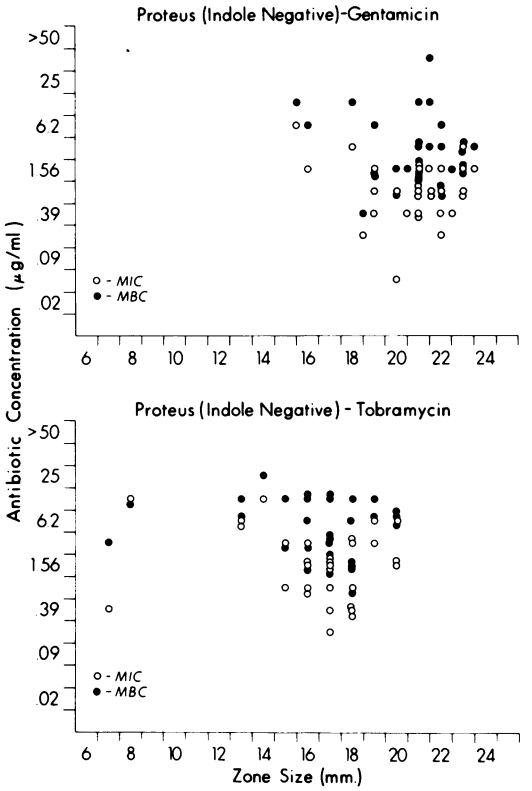

FIG. 11. *Comparison of MIC and MBC values for gentamicin and tobramycin with disc susceptibility zone sizes against indole-negative Proteus.*

three aminoglycosides tested in this study. Conversely, Sch 13706 may prove clinically useful should toxicological studies allow significantly greater serum levels than those safely attainable with gentamicin and tobramycin.

Most in vitro susceptibility studies have demonstrated an inverse correlation between MIC values of gentamicin and tobramycin and zone sizes produced by disc diffusion susceptibility tests in agar. Exceptions have been reported by Traub, using gentamicin against *Pseudomonas* (6), and by Dienstag and Neu, using tobramycin against *Proteus* (3). Most of our *Pseudomonas* strains were susceptible to both gentamicin and tobramycin and yielded zone sizes of 14 mm and 16 mm or more with the two drugs, respectively. Indole-positive *Proteus* strains showed a broader susceptibility range, which included some highly resistant strains of *P. rettgeri*. The more resistant strains generally yielded zones less than 14 mm in diameter. Of further interest, however, was the narrow range of zone sizes produced by gentamicin against *Serratia* (17 to 21 mm) and by tobramycin against *Enterobacter* (14 to 18 mm) and *Serratia* (11 to 17 mm), reflecting the usually low MIC (3.1 μg/ml or less for most strains). However, the MBC values were frequently high and often exceeded the MIC by 8- to 32-fold. This was most apparent with tobramycin and less so with gentamicin. Organisms for which the MBC of tobramycin was 6.2 to 50 μg/ml yielded the same zone sizes as those for which the MIC was 1.56 μg or less/ml. Although the MIC and MBC values for aminoglycosides have usually been considered identical for practical purposes (within one or two broth dilutions), other studies have indicated differences as high as eightfold among many strains (4, 6). Further, several strains for which the MIC varies 8- to 16-fold may yield the same zone size (2, 6, 7). These differences may be of little clinical significance when the range of MIC values is 0.39 to 3.12 μg/ml. However, when strains for which the MBC ranges between 3.12 and 25 μg/ml yield the same zone size, disc diffusion tests may be misleading. Our data indicate that *E. coli* and *Klebsiella* produce zone sizes reflecting a narrow range of MIC and MBC values and that *Proteus* and *Pseudomonas* may demonstrate slightly wider differences between MIC and MBC values (four- to eightfold). However, *Serratia* strains frequently require 6.2 to 12.5 μg of gentamicin/ml or 25 to 50 μg of tobramycin/ml for a bactericidal effect, despite MIC values of 0.09 to 3.1 μg/ml and zone sizes greater than 13 mm. The susceptibility of *Enterobacter* to tobramycin is similar to that of *Serratia*. Clinical isolates belonging to these two groups may be relatively resistant to therapy despite apparent in vitro susceptibility by disc testing.

ACKNOWLEDGMENTS

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