

Tobramycin: In Vitro Activity and Comparison with Kanamycin and Gentamicin

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The in vitro activity of the aminoglycoside antibiotic tobramycin was demonstrated by broth dilution and single-disc methods on 50 isolates each of *Staphylococcus aureus*, *Klebsiella* or *Enterobacter*, indole-positive and -negative *Proteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. All organisms were inhibited by 6.25 µg or less of the drug/ml. *Pseudomonas* strains resistant to kanamycin or gentamicin or both were susceptible to tobramycin. Those strains which were inhibited by 6.25 µg of tobramycin/ml by the broth dilution method had zone diameters of 16 mm or more by the single-disc method. Of 313 organisms tested by the disc method, 3 strains were found to be resistant to tobramycin, 73 were resistant to kanamycin, and 18 were resistant to gentamicin. Tobramycin was found to have satisfactory in vitro activity against many clinically important organisms, including strains resistant to gentamicin and kanamycin.

Nebramycin is an antibiotic compound derived from the soil saprophyte *Streptomyces tenebrarius*. This compound is an aminoglycoside similar in structure to kanamycin, gentamicin, and neomycin and can be separated into eight active factors. After preliminary testing of each, factor 6, tobramycin, was found to have the highest specific activity and broadest spectrum.

This study was undertaken to determine the susceptibility of clinically important species of gram-negative bacilli and *Staphylococcus aureus* to tobramycin in vitro, using both broth dilution and single-disc techniques.

MATERIALS AND METHODS

Organisms. The strains tested (from the Clinical Bacteriology Laboratory, Grady Memorial Hospital, Atlanta, Ga.) were obtained from specimens of pus, urine, and blood. Included were 50 strains each of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* or *Enterobacter*, indole-positive *Proteus*, and indole-negative *Proteus*, a total of 300.

Thirteen gentamicin-resistant strains of *P. aeruginosa* (provided by J. A. Shulman) were also studied. These were isolated from burned patients.

Standard strains *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 (provided by R. S. Griffith, Lilly Laboratories) were used as controls.

Tube dilutions. Overnight cultures in Trypticase soy broth (BBL) were diluted 1:10,000, and 0.5 ml

was added to serial twofold dilutions of the drug being tested in Mueller-Hinton broth (BBL). For each organism, a growth control was included. The total volume in each tube was 1 ml, and the final concentrations of drug ranged from 50 µg/ml to 0.1 µg/ml. The results were read after 18 hr of incubation at 37 C. The minimal inhibitory concentration (MIC) was the lowest concentration of drug in which there was no visible turbidity.

Disc susceptibility tests. Disc susceptibility tests were done as described by Bauer et al. (1) with the modifications proposed in the *Federal Register* (3).

RESULTS

Susceptibility to tobramycin. All fifty of the coagulase-positive strains of staphylococci tested were susceptible to 0.39 µg or less of the drug/ml, the mode of the MIC values being ≤0.1 µg/ml (Table 1). The MIC values of the 50 indole-positive *Proteus* strains tested ranged from 0.39 to 3.12 µg/ml with a mode of 0.78 µg/ml, whereas the indole-negative *Proteus* strains were susceptible to 0.78 to 3.12 µg/ml, the mode being 1.56 µg/ml. For the strains of *E. coli* tested, the range of MIC values of tobramycin was 0.39 to 6.25 µg/ml; the mode was 0.78. Eight *Enterobacter* and 42 *Klebsiella* strains were studied; these will be described as a group since all were equally susceptible to the antibiotics tested. The modal MIC of tobramycin for these organisms was 0.2 µg/ml; the range was ≤ 0.1 to 1.56 µg/ml.

Fifty freshly isolated strains of *P. aeruginosa* and 13 strains known to be resistant to 6.25 µg

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TABLE 1. Minimal inhibitory concentration of tobramycin (313 isolates)

Organism	No. tested	Tobramycin ($\mu\text{g/ml}$)						
		≤ 0.10	0.20	0.39	0.78	1.56	3.12	6.25
<i>Proteus</i>								
Indole +	50			6	21	13	10	
Indole -	50				15	28	7	
<i>Escherichia coli</i>	50			8	17	19	4	2
<i>Enterobacter</i>	50	2	27	16	4	1		
<i>Pseudomonas</i>	50	13	30	5			2	
<i>Pseudomonas</i> Genta. resistant	13	2	7	4				
<i>Staphylococcus aureus</i>	50	30	16	4				

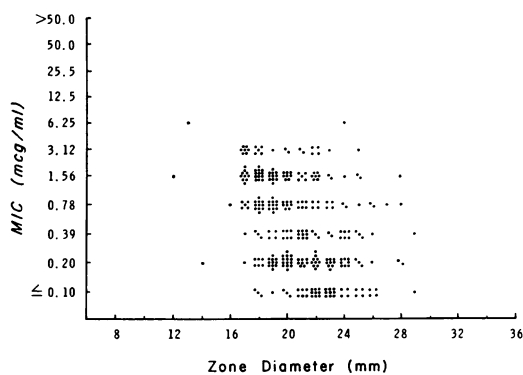


FIG. 1. Correlation between minimal inhibitory concentration of tobramycin by the broth dilution method and zone size by the disc method with 300 clinical strains.

or greater of gentamicin were tested with the broth dilution method. The MIC of tobramycin for all strains was below $0.78 \mu\text{g/ml}$ (range $\leq 0.1 - 0.39$; mode, 0.2). The gentamicin-resistant strains showed the same susceptibility to tobramycin as the gentamicin-susceptible strains of *Pseudomonas*.

Correlation of disc and broth dilution methods with tobramycin. Figure 1 shows the correlation between MIC of tobramycin and zone size for 300 clinical laboratory isolates. Except for four strains, all showed MIC values of $3.12 \mu\text{g}$ or less/ml and zone sizes of 16-mm diameter or greater. Two *E. coli* strains, a strain of *P. aeruginosa* and an indole-negative *Proteus* strain were the exceptions.

Susceptibility to kanamycin, gentamicin, and tobramycin by the single-disc method. Three hundred and thirteen strains were tested for their susceptibility to each drug (Table 2). Three (1%) were resistant to tobramycin (*P. aeruginosa*, indole-negative *Proteus*, and *E. coli*). Seventy-three strains were judged resistant to kanamycin since

TABLE 2. Comparison of susceptibility to tobramycin, kanamycin, and gentamicin by the disc method^a

Organism	No. tested	No. resistant to:		
		Tob.	Kan.	Gen.
<i>Pseudomonas aeruginosa</i>	63	1	61	16
<i>Proteus</i> : indole +	50	0	3	1
indole -	50	1	5	0
<i>Escherichia coli</i>	50	1	1	1
<i>Enterobacter</i> , <i>Klebsiella</i>	50	0	3	0
<i>Staphylococcus aureus</i>	50	0	0	0

^a Susceptible zone: Tobramycin (Tob.; $10 \mu\text{g}$)—16 mm; kanamycin (Kan.; $30 \mu\text{g}$)—18 mm (1); gentamicin (Gen.; $10 \mu\text{g}$)—13 mm (3).

the zone sizes were less than 18 mm. Ten of these were in the intermediate range and showed zones between 14 and 18 mm (two indole-positive and five indole-negative strains of *Proteus*, two strains of *Klebsiella*, and one strain of *Pseudomonas*). The remaining kanamycin-resistant strains showed zone diameters of less than 14 mm (usually no zone) and included 60 strains of *P. aeruginosa*, and one each of indole-positive *Proteus*, *E. coli*, and *Enterobacter*. Eighteen of the strains showed zone sizes of less than 13 mm when tested with gentamicin ($10 \mu\text{g}$) discs. Of these, five were part of the unselected group of *P. aeruginosa* obtained from the hospital laboratory, and the remaining 13 were strains of *P. aeruginosa* isolated from burn patients and selected for study because of their known resistance to gentamicin.

DISCUSSION

In this study, tobramycin was found to be an effective inhibitor of growth of a wide variety of organisms. Of 300 freshly isolated strains of *S.*

aureus, *Proteus*, *E. coli*, *Klebsiella* or *Enterobacter*, and *P. aeruginosa*, all but four had an MIC of tobramycin of 3.12 $\mu\text{g}/\text{ml}$ and a zone diameter of 16 mm or more (Fig. 1). All strains of *S. aureus* were inhibited by 0.39 μg of tobramycin/ml; *Proteus* strains were inhibited by 3.12 $\mu\text{g}/\text{ml}$; all but two *E. coli* strains were inhibited by less than 6.25 $\mu\text{g}/\text{ml}$; all *Klebsiella* and *Enterobacter* strains were inhibited by less than 3.12 $\mu\text{g}/\text{ml}$; and all *Pseudomonas* strains were inhibited by less than 6.25 $\mu\text{g}/\text{ml}$ (Table 1). These results are only slightly different than those of Black and Griffith (2) who found all of 99 gram-negative strains to be inhibited by less than 3 μg of tobramycin/ml.

The correlation between the broth dilution MIC of 6.25 $\mu\text{g}/\text{ml}$ and a zone diameter of 16 mm or greater around a 10 μg tobramycin disc has previously been shown by Preston and Wick (5). These authors suggest an MIC of 8 $\mu\text{g}/\text{ml}$ and inhibitory zone diameter of 14 mm or less as the levels at which resistant bacteria are not likely to respond to an ordinary dosage of tobramycin. Thus, the single-disc method of Bauer et al. (1) is clinically applicable for tobramycin susceptibility testing.

The data presented here show that many strains which are resistant to kanamycin, gentamicin, or both, are susceptible to tobramycin. None of the organisms found resistant to tobramycin were susceptible to kanamycin, but two of the three were susceptible to gentamicin. Of the 13 gentamicin-resistant *Pseudomonas* chosen for study, 9 were obtained from burn patients previously exposed to topical gentamicin in large amounts, and 5 of these were pyocine type 5, which was endemic on the burn unit at that time (6). These organisms were resistant to 6.25 to 50 μg of gentamicin (mode 50) and showed no zone of growth inhibition about a 10 μg gentamicin disc. When tested to tobramycin by disc and broth dilution methods, no difference could be seen between these 13 strains and the 50 *Pseudomonas* strains isolated in the clinical laboratory. This indicates a lack of cross-resistance in these 13 gentamicin-resistant *Pseudomonas* strains between

gentamicin and tobramycin, a potentially useful asset of tobramycin.

In one strain of *P. aeruginosa*, the two methods of testing did not show correlation. In this case, a zone diameter of 14 mm and an MIC of 0.2 μg tobramycin/ml (clearly indicating susceptibility) were found. A similar finding has been previously reported by Traub in 18.6% of *Pseudomonas* tested with gentamicin (7).

Preliminary multiple-dose studies in human volunteers with tobramycin showed that blood levels above those required to inhibit the organisms we tested in vitro are obtained with reasonable intramuscular doses without evidence of otic or renal toxicity (*Clinical Investigation Manual*; personal communication, R. S. Griffith, Eli Lilly and Company).

Thus, judging from preliminary clinical data and in light of the in vitro data presented here, it appears that tobramycin is a promising aminoglycoside antibiotic for use against clinically important bacteria, including gentamicin-resistant *Pseudomonas*.

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