Evaluation of the In Vitro Activity of Tobramycin as Compared with That of Gentamicin Sulfate

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The in vitro activity of tobramycin was quantitatively compared with that of gentamicin sulfate against 195 bacterial isolates from clinical material. Tobramycin was found to be twice as active as gentamicin against isolates of Pseudomonas aeruginosa. Conversely, gentamicin proved fourfold more active than tobramycin against isolates of Serratia marcescens. Both drugs were of comparable activity against isolates of Staphylococcus aureus and the majority of the enterobacterial isolates other than S. marcescens. On the basis of the obtained data, the following criteria are proposed for the interpretation of diffusion susceptibility tests with 10-μg discs of gentamicin and tobramycin. Enterobacteriaceae and isolates of S. aureus are designated as susceptible to gentamicin and tobramycin if the zones of inhibition measure 15 mm or more in diameter; zones of 14 mm or less are indicative of resistance. Pseudomonadaceae are interpreted as sensitive to tobramycin and gentamicin if the inhibition zones measure at least 15 and 12 mm in diameter, respectively.

Tobramycin, factor 6 of the nebramyin complex of aminoglycoside antibiotics elaborated by the actinomycete Streptomyces tenebrarius, is of interest since preliminary investigations have shown this broad-spectrum antibiotic to be effective against isolates of multiple-drug-resistant S. aureus, as well as against isolates of Enterobacteriaceae, including Proteus sp., and Pseudomonadaceae (5, 12). This study served to evaluate quantitatively the in vitro activity of tobramycin as compared with that of gentamicin sulfate.

MATERIALS AND METHODS

Bacteria. A total of 195 bacterial isolates from clinical material were examined; the majority of these had been isolated during the past 2 years and stored at −65 C. Enterobacteriaceae were represented by 122 of the isolates: Escherichia coli (25 isolates), Klebsiella pneumoniae (25 isolates), Enterobacter (E. cloacae and E. aerogenes, 12 isolates), S. marcescens (17 isolates), Proteus mirabilis (14 isolates), P. vulgaris (1 isolate), P. morganii (15 isolates), P. rettgeri (3 isolates), Providencia stuartii (2 isolates), and Citrobacter freundii (8 isolates). The enterobacterial isolates were identified as described previously (11). Gram-negative, nonfermenting organisms involved 28 isolates of P. aeruginosa, identified as before (10), and 5 isolates of P. maltophilia, which were characterized through their hydrolysis of deoxyribonucleic acid (DNA), liquefaction of gelatin, motility, lack of oxidase, prompt oxidative utilization of maltose (Hugh-Leifson O-F test), delayed utilization of glucose and inertia in xylose, reduction of nitrate, and lack of pyocyanin and pyoverdin production. The two isolates of Acinetobacter anitratum tested were identified through their lack of oxidase, motility, and nitrate reduction. The isolates oxidatively utilized glucose and xylose but not maltose. The 25 isolates of S. aureus were identified by previously published criteria (6), as were the 15 isolates of enterococci (7). The control organisms were S. aureus ATCC 25923, E. coli ATCC 25922, and a laboratory control strain of P. aeruginosa.

Media. Mueller Hinton broth (MHB; pH 7.4) and agar (MHA; pH 7.4) were purchased from Difco, as were Brain Heart Infusion broth (BHB; pH 7.4), nutrient broth (NB; pH 6.8), and tryptic soy broth (TSB; pH 7.3). The organisms were maintained on slants of Brain Heart Infusion agar (Difco) at 4 C.

Antibiotics. The Lilly Research Laboratories, Indianapolis, Ind., furnished tobramycin in the form of a stock solution with 1,000 μg/ml activity and 10-μg tobramycin discs (lots P69790 and YV1390AMV). Nonsterile gentamicin sulfate powder (lot GMC-8-M-65-1) was a gift from the Schering Corp., Union, N.J.; 10-μg gentamicin discs (lot 506308) were obtained from Difco. Gentamicin was dissolved in sterile distilled water to yield 2,000 μg/ml, passed through 0.2-μm membrane filters (Nalgé Sybron
stored pregrown in TSB at 35°C for 5 hr. Disc diffusion tests were performed by the method of Bauer et al. (1), except that the large plates (100 by 15 mm) contained 60 ml of MHA, which resulted in an agar depth of roughly 4 mm (2). Duplicate discs of tobramycin and gentamicin each were used per isolate. For the enterococcal isolates, MHA with 5% added sheep blood was used. Simultaneous broth dilution tests were performed, employing a previously described Microtiter method (10). Briefly, use was made of sterile, disposable, U-shaped Microtiter plates, MHB (BHIB in the case of enterococci), and bacterial inocula adjusted to yield approximately 1.5 \times 10^4 colony-forming units (CFU)/ml (i.e., 7.5 \times 10^4 CFU/0.05 ml) at zero time. The isolates were exposed to serial twofold dilutions of the drugs; 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, and 0.05 \mu g/ml (final concentrations) of tobramycin and gentamicin, respectively. The S. aureus isolates were exposed to serial twofold dilutions of both drugs over the range of 6.3 to 0.006 \mu g/ml, respectively. The plates were incubated for 16 to 18 hr at 35°C. The minimal inhibitory concentration (MIC) of each of the drugs was defined as the lowest concentration of antibiotic that completely inhibited growth as judged by visual inspection (10). The Microtiter broth dilution and tube broth dilution (8) techniques were employed against the three control organisms to assess the accuracy of the former method. Finally, the three control organisms were examined for their susceptibility to the two drugs in four different broths, namely BHIB, MHB, NB, and TSB, for the purpose of detecting any major media-dependent variation in activity of any of the two drugs.

RESULTS

Tobramycin was twice as effective as gentamicin against the laboratory control strain of P. aeruginosa in MHB; the strain was inhibited by 0.4 \mu g of tobramycin per ml as compared with 0.8 \mu g of gentamicin per ml. Both drugs were of equal activity against the control strains of S. aureus and E. coli: the control S. aureus was inhibited by 0.1 \mu g of tobramycin and gentamicin per ml, respectively, whereas both drugs inhibited the control strain of E. coli at 0.8 \mu g/ml. The MIC values obtained with the Microtiter method were identical to those of the tube broth dilution technique. The MIC values of the two drugs against the three control organisms in BHIB, MHB, NB, and TSB are shown in Table 1. The two drugs were most active in NB and least so in TSB. Again, tobramycin was twofold more active than gentamicin against the control strain of P. aeruginosa, regardless of the particular broth employed. The control S. aureus was characterized by MIC levels in BHIB that varied twofold upon repeated testing, a finding attributed to experimental error in day-to-day runs.

The MIC values of tobramycin and gentamicin against all bacterial isolates examined, with the exception of the enterococcal isolates, are depicted in Fig. 1 and 2, respectively, together with the diameters (in mm) of the resultant zones of inhibition. Tobramycin was found to be twice as active as gentamicin against isolates of P. aeruginosa, confirming the observations of the investigators at the Lilly Research Laboratories (5, 12). On the other hand, gentamicin was approximately fourfold more active than tobramycin against isolates of S. marcescens. Gentamicin appeared to be slightly more active than tobramycin against some of the isolates of E. coli, P. pneumoniae, Enterobacter, P. mirabilis, and C. freundii. The five isolates of P. maltophilia proved resistant to both drugs.

The two drugs were of identical activity against isolates of S. aureus. The 13 enterococcal isolates examined proved uniformly resistant to both tobramycin and gentamicin in BHIB, in that they tolerated 50 or more \mu g/ml of each of the two drugs; yet all but two isolates yielded zones of inhibition of 16 mm or more around tobramycin discs and zones measuring 14 mm or more in diameter around gentamicin discs. In view of our earlier observation, namely that the addition of sheep blood to solid media markedly enhanced the activity of gentamicin against enterococci (7), the results obtained for these isolates with tobramycin and gentamicin were not plotted in the two figures.

DISCUSSION

The results obtained for gentamicin sulfate and isolates of P. aeruginosa are in agreement with our earlier findings (8). We recently described a unique, multiple-drug-resistant strain of P. rettgeri (9). With one exception, the isolates comprising this particular strain required 25 to 50 \mu g of gentamicin per ml for inhibition; however, these isolates yielded zones of inhibition around 10-\mu g discs of gentamicin that measured 10 to 13 mm in diameter. In fact, one of the 21 isolates of indole-positive Proteus, Providencia (Fig. 1 and 2) was such a P. rettgeri which required 50 \mu g of tobramycin and 25 \mu g of gentamicin per ml for inhibition. The corresponding zones of inhibition obtained with both drugs measured 13 mm in diameter. This is why we recommend
TABLE 1. Comparative minimum inhibitory concentrations (MIC) of tobramycin and gentamicin against three control organisms in four different broths

| Organism                      | MIC of tobramycin (µg/ml)* in | MIC of gentamicin (µg/ml)* in |
|-------------------------------|-------------------------------|-------------------------------|
|                               | BHIB  MHB  NB  TSB        | BHIB  MHB  NB  TSB        |
| Staphylococcus aureus ATCC 25923 | 0.4/0.8  0.1  0.05  0.8   | 0.4/0.8  0.1  0.05  0.8   |
| Escherichia coli ATCC 25922    | 6.3    0.8  0.2  12.5  | 6.3    0.8  0.2  12.5  |
| Pseudomonas aeruginosa         | 1.6    0.4  0.2  1.6   | 3.2    0.8  0.4  3.2   |

* Abbreviations: BHIB, Brain Heart Infusion broth; MHB, Mueller Hinton broth; NB, nutrient broth; TSB, tryptic soy broth.
* Determined with the Microtiter procedure.

![Figure 1](image-url)  
**Fig. 1.** Activity of tobramycin against 182 clinical isolates of Enterobacteriaceae, Pseudomonadaceae, A. anitratum, and S. aureus, as determined with the broth dilution and disc diffusion methods of susceptibility testing.

that those enterobacterial isolates be reported as susceptible to gentamicin that yield zones of inhibition measuring at least 15 mm in diameter (Table 2). Independently, Matsen and co-workers (4) likewise had proposed that inhibition zones of 15 mm or more be considered the breakpoint, in contrast to the tentative criterion of 13 mm or more, as originally proposed by the Seattle group of workers (3). However, our previous (8) and current data for gentamicin and isolates of *P. aeruginosa* indicate that one has to apply a different zone criterion for this particular organism. This is why we recommend a zone diameter of 12 mm or more as indicative of susceptibility of this organism to gentamicin. On the other hand, it is proposed that *Enterobacteriaceae* as well as *Pseudomonadaceae* and isolates of *S. aureus* be designated as susceptible to tobramycin if the zones of inhibition around 10-µg discs of tobramycin amount to 15 mm or more in diameter, because tobramycin was found to be twice as effective as gentamicin against isolates of *P. aeruginosa* (5, 12), an observation confirmed by our data. Although tobramycin was fourfold less active than gentamicin against isolates of *S. marcescens*, all isolates yielded inhibition zones of 16 mm or more in diameter around 10-µg tobramycin discs.

The proposed zone criteria for tobramycin and gentamicin appear to be generally valid with the exception of occasional isolates. For example, one isolate of *P. stuartii* was found to require 25 µg of tobramycin per ml and 12.5 µg
of gentamicin per ml for inhibition, yet yielded inhibition zones that measured 16 mm in diameter.

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![Fig. 2. Activity of gentamicin against 182 clinical isolates of Enterobacteriaceae, Pseudomonadaceae, A. anitratum, and S. aureus, as determined with the broth dilution and disc diffusion methods of susceptibility testing.](image)

**Table 2. Proposed zone criteria for 10-μg tobramycin and 10-μg gentamicin discs**

| Organisms                          | Tobramycin (mm) of inhibition zones with |
|------------------------------------|-----------------------------------------|
|                                    | Susceptible | Resistant |
| Enterobacteriaceae, Staphylococcus aureus | – 14 | 15 or more | – 14 | 15 or more |
| Pseudomonadaceae                   | – 14 | 15 or more | – 11 | 12 or more |