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Studies on the Susceptibility of 150 Consecutive Clinical Isolates of *Pseudomonas aeruginosa* to Tobramycin, Gentamicin, Colistin, Carbenicillin, and Five Other Antimicrobials

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The in vitro susceptibilities of 150 clinical isolates of *Pseudomonas aeruginosa* to the aminoglycoside tobramycin and eight other antimicrobials were evaluated. The Food and Drug Administration standardized disk diffusion method showed that tobramycin inhibited 100% of the 150 isolates with gentamicin inhibiting only 90.7%. The difference between colistin (97.3%) and tobramycin was less marked. Carbenicillin (87.3%) was found to be slightly less active than gentamicin. Only a small percentage of the isolates were inhibited by the other drugs used. By using a minimal inhibitory concentration of 4 μg/ml in brain heart infusion broth as the level of susceptibility, it was found that only three of the isolates showed resistance to tobramycin, whereas 20 of the isolates were resistant to gentamicin. Approximately half of the isolates were inhibited by 0.5 μg/ml or less of tobramycin, whereas 1.5 μg of gentamicin per ml was required to inhibit 50% of susceptible strains.

Tobramycin (17) has been evaluated by both the disk diffusion method and the broth dilution method with regard to its in vitro activity against a variety of gram-negative bacilli. Included in many studies are isolates of *Pseudomonas aeruginosa* (3-5, 7, 8, 12, 13, 15, 16, 19). This study was undertaken to evaluate a sample of consecutive clinical isolates of *P. aeruginosa* for their susceptibility to tobramycin, gentamicin, colistin, carbenicillin, ampicillin, kanamycin, cephalothin, chloramphenicol, and tetracycline hydrochloride. Due to conflicting reports concerning the comparable efficacy of tobramycin and gentamicin (3, 5, 7, 15, 16), both the disk method and broth dilution method were used for these two drugs with only the disk diffusion method being used for the other seven drugs.

MATERIALS AND METHODS

Isolates. Through the cooperation of the Director of Clinical Laboratories of the University Hospital, University of Mississippi Medical Center, consecutive isolates of *Pseudomonas* from clinical specimens were obtained. Each isolate was plated on eosin methylene blue agar (Difco) to assure purity, and a mixed colonial population was transferred to triple sugar iron (TSI) agar (Difco) slants for initial confirmation of identification. The isolates were then inoculated to pigment production medium A and B of King et al. (11). A total of 150 isolates producing pyocyanin on the A medium (10) with colonial morphology and reactions on TSI typical of *P. aeruginosa* were used in subsequent antibiotic testing. After confirmation of identification, the isolates were transferred to brain heart infusion agar (Difco) slants, incubated overnight at 37 C, and stored at 4 C until tested. The storage time of an isolate usually was less than 2 weeks and never exceeded 6 weeks.

Disk diffusion. The standardized disk diffusion method using 6-mm disks described by Bauer et al. (2) as modified by the Food and Drug Administration (FDA) (9) was used. Commercially prepared disks (BBL) were used with the exception of tobramycin disks (10 μg), which were supplied by R. S. Griffith of Eli Lilly & Co. Each isolate was tested against tobramycin, gentamicin, colistin, carbenicillin, ampicillin, cephalothin, chloramphenicol, kanamycin, and tetracycline hydrochloride at the disk strength recommended by the FDA (9). Susceptibility was based on the zone diameters recommended by the FDA (9). In the case of tobramycin, the zone size (14 mm) recommended by Eli Lilly & Co. was used.

Broth dilution. Only gentamicin and tobramycin were evaluated by the broth dilution method. Gen-

tamicin was obtained as a standardized powder and a stock solution containing 1,000 μg/ml was prepared in phosphate buffer 0.1 M, pH 8.0, as recommended by the manufacturer (Schering Corp.). Tobramycin was provided by Eli Lilly & Co. as a solution containing 1,000 μg/ml. Serial twofold dilutions ranging from 4 to 0.03 μg/ml were made in 2 ml of brain heart infusion broth (Difco) in tubes (13 by 100 mm). The dilutions and culture control were inoculated with 0.01 ml of a 10^{-2} dilution of an overnight culture and incubated for 18 h at 37 C, and the minimal inhibitory concentration (MIC) was read as the lowest concentration showing no visible turbidity. A MIC of 4 μg/ml or less was interpreted as an indication of susceptibility (6).

RESULTS

Disk diffusion. By the disk diffusion method most of the isolates were susceptible to tobramycin and colistin with a lesser percentage susceptible to gentamicin and carbenicillin (Table 1 and Fig. 1). The order of susceptibility was tobramycin (100%) as most active followed closely by colistin (97.3%), then gentamicin (90.7%) and carbenicillin (87.3%). Only an occasional isolate was susceptible to ampicillin, chloramphenicol, kanamycin, tetracycline hydrochloride, and cephalothin (Table 1), and these data were not included in Fig. 1.

Broth dilution. The cumulative percentages of tobramycin- and gentamicin-susceptible *P. aeruginosa* at each concentration (μg/ml) are shown in Fig. 2. A 4 μg/ml susceptibility level was selected based on a concentration easily obtained in the serum of normal adults by nontoxic doses (6). It can be noted that more than half (53%) of the 150 isolates had an MIC of 0.5 μg or less of tobramycin per ml, whereas 1.5 μg of gentamicin per ml was required to inhibit 50% of the susceptible isolates. Only three isolates had an MIC of 4 μg of tobramycin per ml, with all others being 2 μg or less per ml. It has been suggested that a broth dilution MIC for tobramycin of 3.13 μg/ml (16) is indicative of susceptibility. The data reported herein cannot be compared exactly with the earlier study because the starting concentrations were not the same.

TABLE 1. *Percentage of susceptibility for 150 isolates of Pseudomonas aeruginosa*

Antimicrobial	Disk diffusion[a]	Broth dilution[b]
Tobramycin	100.0	98
Gentamicin	90.7	86.7
Colistin	97.3	
Carbenicillin	87.3	
Chloramphenicol	2.7	
Tetracycline hydrochloride	3.3	
Kanamycin	0.0	
Cephalothin	0.0	
Ampicillin	0.67	

[a] Susceptibility was based on zone sizes as recommended by the FDA with the tobramycin zone at 14 mm.

[b] MIC of 4 μg/ml or less.

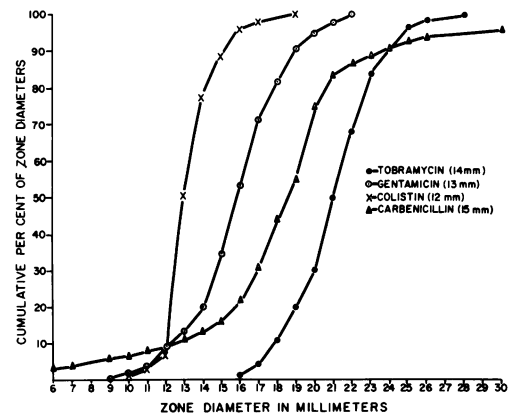

FIG. 1. *Rectilinear plots of cumulative totals of 150 Pseudomonas aeruginosa isolates at each zone size for the drugs tobramycin, gentamicin, colistin, and carbenicillin. The zone diameter indicating susceptibility is indicated by the name of each drug.*

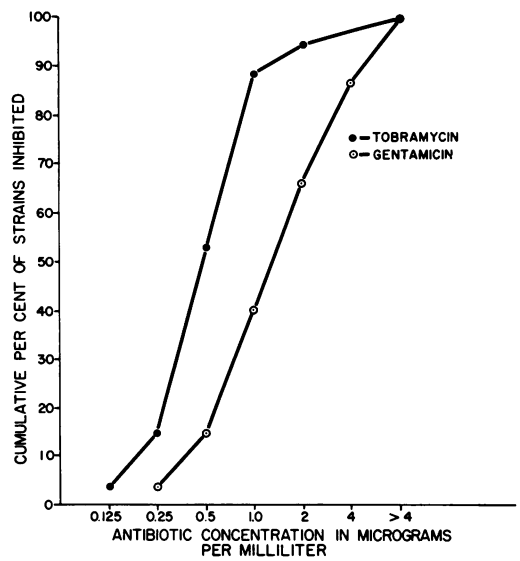

FIG. 2. *Rectilinear plots of cumulative percentage totals of 150 Pseudomonas aeruginosa isolates at each concentration (μg/ml) for the drugs tobramycin and gentamicin. A concentration of 4 μg/ml was selected as the level for susceptibility.*

Comparison of disk diffusion and broth dilution methods. With the stated criteria for susceptibility, it appeared that good agreement

was obtained for the two methods with only a 2 to 3% difference (Table 1). When individual isolates were examined, however, the correlation of the disk and broth methods was found to be much better for tobramycin than for gentamicin.

There were 31 isolates which showed resistance to either tobramycin or gentamicin. Only three of the 31 resistant isolates showed resistance to tobramycin by the broth method with none resistant by disk diffusion. One of the three showed resistance by broth dilution to both tobramycin and gentamicin as well as to gentamicin by disk diffusion. This isolate had a zone size of 19 mm to tobramycin, which was well above the recently recommended minimal zone size for susceptibility (15, 16, 18). The other two were resistant to gentamicin by broth dilution but had zone sizes of 16 mm to gentamicin by the disk method.

Of the 20 isolates resistant to gentamicin by the broth method, only two were resistant by the disk method. Conversely, the disk method indicated that 11 isolates were resistant to gentamicin with zone sizes ranging from 12 to 9 mm; however, the broth dilution test indicated all 11 to be susceptible to the drug. Three of these 11 had an MIC of 2 μg/ml and two had an MIC of 1 μg/ml, with the other six requiring 4 μg/ml for inhibition. Traub (18) has suggested that *P. aeruginosa* isolates giving a gentamicin zone of less than 12 mm be subjected to broth dilution study. The above results seem to support his observation.

DISCUSSION

Reports by others (8, 12, 13, 15, 16) have shown tobramycin to be slightly more active in vitro against *Pseudomonas* than gentamicin. The results of this study corroborate their findings. The surprising finding of 100% susceptibility to tobramycin by the disk diffusion method may have been due to the criteria used to select the isolates studied. Since only isolates producing pigment on medium A (11) were tested, and consequently no nonpigmented strains were included, it may have been that resistant isolates as reported by others (5) were systematically excluded. Even if this is so, tobramycin emerged as inhibiting a slightly higher percentage than gentamicin. It has been suggested (16) that 16 mm be the minimal zone size for susceptibility to tobramycin. In this study all tobramycin zones were 16 mm or greater so that the susceptibility remained 100% even if the more stringent criterion were used. The results obtained with gentamicin, carbenicillin, and colistin are in close agreement with the results of others (1).

The finding of only minimal differences between the disk and broth dilution studies for the same drug may be related to the use of brain heart infusion broth. Others have shown that the results of such studies are greatly influenced by the composition of the medium (16, 19). In an earlier unpublished study of 25 *Pseudomonas* isolates in this laboratory using the broth dilution method with Mueller-Hinton broth (Difco), gentamicin broth susceptibilities correlated much better with the disk diffusion method. In this laboratory the disk diffusion technique seemed to lend itself more readily to duplication, with the broth dilution method subject to greater variability even in experienced hands. When individual isolates are to be examined, the broth dilution method may prove useful for determining gentamicin susceptibility for those showing a zone size under 12 mm (18).

A recent editorial (6) called attention to the need for definitive data on the efficacy of antibiotic regimens used in treatment of *Pseudomonas* infections. Even though the data reported herein indicated that tobramycin was more active against *P. aeruginosa* than the other antimicrobials in vitro, it should not be assumed that these in vitro sensitivity patterns necessarily correspond to results to be obtained in vivo.

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