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Activity of Newer Aminoglycosides and Carbenicillin, Alone and in Combination, Against Gentamicin-Resistant *Pseudomonas aeruginosa*

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The in vitro activity of the aminoglycoside antibiotics tobramycin, sisomicin, amikacin, gentamicin, and netilmicin (SCH 20569) were compared against 26 gentamicin-resistant isolates of *Pseudomonas aeruginosa* cultured from hospitalized children. Tobramycin had the greatest activity on a weight basis, followed by sisomicin, gentamicin, amikacin, and netilmicin. All isolates were resistant to achievable concentrations of netilmicin and gentamicin, but 23% were inhibited by achievable concentrations of tobramycin, 8% by amikacin, and 4% by sisomicin. The combinations carbenicillin/tobramycin, carbenicillin/sisomicin, and carbenicillin/amikacin were synergistic for 92% of strains; antagonism was not encountered. These in vitro results suggest that tobramycin, sisomicin, or amikacin in combination with carbenicillin would be the safest initial regimen in the therapy of gentamicin-resistant *Pseudomonas* infections pending susceptibility studies.

Pseudomonas aeruginosa resistant to currently available antibiotics, including gentamicin, are becoming more prevalent. A study was undertaken to investigate the activity of several new aminoglycosides against a population of gentamicin-resistant *P. aeruginosa* to determine the extent of cross-resistance between tobramycin, sisomicin, amikacin, netilmicin (SCH 20569), and gentamicin. The possibility of carbenicillin/aminoglycoside synergism against this resistant population of *P. aeruginosa* was also examined.

MATERIALS AND METHODS

Twenty-six strains of *P. aeruginosa* were isolated from 23 patients at the Montreal Children's Hospital and identified by using standard microbiological techniques. At least 14 different types of *Pseudomonas* were represented among the isolates studied, as determined by serological and phage-typing methods (kindly performed by N. Hinton, Toronto). All strains were screened for resistance to gentamicin by a standardized disk diffusion test (16) and were considered resistant if the zone diameter was <13 mm and the agar dilution minimum inhibitory concentration (MIC) was ≥6.25 μg/ml. Bacteria were stored in sheep blood at −20°C until tested.

Tobramycin sulfate was supplied as a solution of 1,000 μg/ml and was stored at 4°C. Gentamicin, sisomicin, amikacin, netilmicin, and carbenicillin were supplied as dry powders and were reconstituted immediately before use. Agar dilution MICs were performed according to the method of Steers et al. (19) using Mueller-Hinton agar (Ca^{2+}, 7 mg/100 ml, Mg^{2+}, 3.6 mg/100 ml) and an inoculum of 10^6 to 10^7 bacteria/ml. The MIC was defined as the lowest concentration of drug allowing no colonial growth on the surface of the agar.

Plates for single antibiotic agar dilution studies were prepared 48 h in advance and stored at 4°C. Plates for combination studies were prepared 2 h before use. Synergism was tested by adding either 25 or 50 μg of carbenicillin per ml to each dilution of four of the aminoglycosides listed above. The combination was considered synergistic if there was a decrease of four double dilutions or greater in the aminoglycoside MIC and antagonistic if an increase of four double dilutions or greater in MIC was noted.

RESULTS

Susceptibilities of the 26 isolates of gentamicin-resistant *P. aeruginosa* are plotted in Fig. 1. At a concentration of ≤3.12 μg/ml, tobramycin inhibited 23% of all isolates, versus 4% for sisomicin and 0 for gentamicin, amikacin, and netilmicin. At ≤12.5 μg/ml (a readily achievable serum concentration), amikacin inhibited 8% of isolates. Ninety-two percent of isolates were inhibited by ≥50 μg of carbenicillin per ml.

The results of adding 25 or 50 μg of carbenicillin per ml to each concentration of four of the aminoglycosides are summarized in Table 1. Eighty-one percent of the organisms were susceptible to the combination of tobramycin and

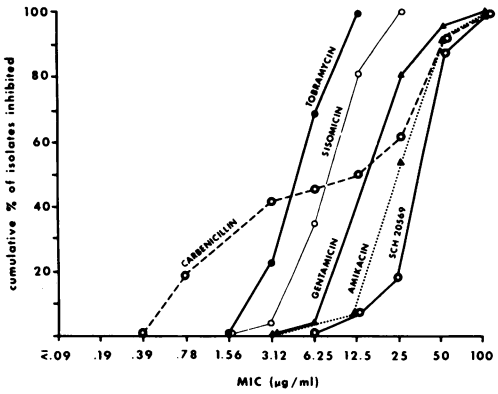

FIG. 1. *Activity of five aminoglycosides and carbenicillin against 26 recent clinical isolates of gentamicin-resistant P. aeruginosa (agar dilution method, 10^6 to 10^7-colony-forming units/ml inoculum). Sch 20569 is netilmicin.*

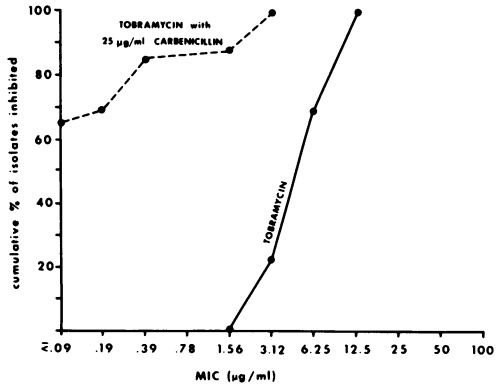

FIG. 2. *Effect of adding 25 μg of carbenicillin per ml on the activity of tobramycin against 26 recent clinical isolates of gentamicin-resistant P. aeruginosa (method as in Fig. 1).*

TABLE 1. *Effect of carbenicillin on the anti-pseudomonas activity of four aminoglycosides*

Antibiotic	% of strains inhibited	
	25 μg of carbenicillin per ml	50 μg of carbenicillin per ml
Tobramycin	81	92
Sisomicin	77	92
Gentamicin	73	88
Amikacin	85	92

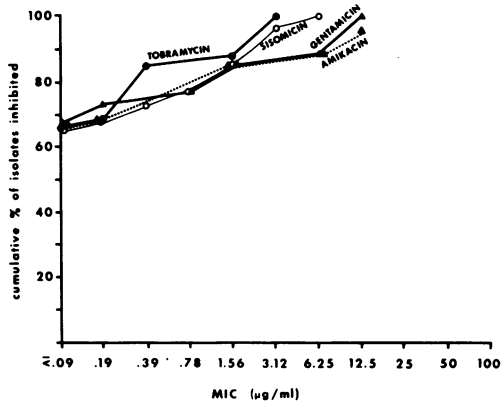

FIG. 3. *Effect of adding 25 μg of carbenicillin per ml on activity of four aminoglycosides against 26 recent clinical isolates of gentamicin-resistant P. aeruginosa (method as in Fig. 1).*

25 μg of carbenicillin per ml (Fig. 2). Seventy-seven percent of the organisms were susceptible to the sisomicin/carbenicillin combination, 85% to amikacin/carbenicillin, and 73% to gentamicin/carbenicillin combinations (Fig. 3). Synergy was more marked (88 to 92%) when 50 μg of carbenicillin per ml was added (Table 1). When the 15 highly gentamicin-resistant isolates (MIC >25 μg/ml) were considered, synergy was demonstrated against 87% with each of tobramycin, amikacin, gentamicin, and sisomicin combined with carbenicillin. Antagonism was not demonstrable between any of the four aminoglycosides and carbenicillin.

DISCUSSION

This study evaluated the susceptibility of a population of gentamicin-resistant strains of *P. aeruginosa* to tobramycin, sisomicin, gentamicin, amikacin, netilmicin, and carbenicillin. Tobramycin exhibited the greatest activity against *Pseudomonas*, as previously reported (8, 9, 12, 13, 15, 21). Sisomicin was active against gentamicin-resistant *Pseudomonas* but to a lesser degree than tobramycin (20). There was complete cross-resistance between amikacin, netilmicin, and gentamicin on a weight comparison basis but higher concentrations of amikacin were achievable in serum. Nevertheless, only 8% of the isolates were inhibited by ≥12.5 μg of amikacin per ml. This agrees with previous reports of tobramycin, gentamicin, and amikacin in vitro anti-*Pseudomonas* activity (14, 18, 20, 21). Two studies, using broth dilution methods, have reported higher amikacin activity against these strains (4, 11). The lack of complete cross-resistance between tobramycin and gentamicin is in accord with previous reports (3, 5–7, 12).

Although many strains of *Pseudomonas* are susceptible to achievable concentrations of carbenicillin, emergence of resistance is a limiting factor to the use of carbenicillin alone in the treatment of *Pseudomonas* infections. Synergism against *Pseudomonas* has been reported

previously with combinations of tobramycin/carbenicillin (1, 9–11), gentamicin/carbenicillin (1, 9–11, 15, 17), amikacin/carbenicillin (10, 11), and sisomicin/carbenicillin (10). In this study, 73 to 92% of strains exhibited some potential for synergism when tobramycin was combined with 25 or 50 μg of carbenicillin per ml; however, no antagonism was noted. Kluge et al. (11) reported that highly gentamicin-resistant *Pseudomonas* were not inhibited by combinations of tobramycin or gentamicin with carbenicillin. As recently reported by Anderson et al. (1), however, we found that synergism could not be predicted by the degree of gentamicin resistance. Differences in methods (Kluge et al. [11] used Trypticase soy agar broth in microtiter; Anderson and our studies used Mueller-Hinton agar dilution) may be responsible.

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