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In Vitro Activity of Gentamicin, Amikacin, and Netilmicin Alone and in Combination with Carbenicillin Against *Serratia marcescens*

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The inhibitory and bactericidal effects of gentamicin, amikacin, netilmicin (Sch 20569), and carbenicillin were tested against 55 clinical isolates of *Serratia marcescens* that had been subtyped into 26 strains by biotyping and serotyping. Three major patterns of resistance to gentamicin, netilmicin, and carbenicillin were recognized among these isolates. (i) Most of the 27 isolates that were susceptible to gentamicin (minimal bactericidal concentration [MBC] ≤6.25 μg/ml) were susceptible to carbenicillin (MBC ≤125 μg/ml) and resistant to netilmicin (MBC ≥12.5 μg/ml). (ii) Most of the 11 isolates with moderate resistance to gentamicin (MBC of 12.5 to 25 μg/ml) were also susceptible to carbenicillin and resistant to netilmicin. (iii) The 17 isolates with high-level resistance to gentamicin (MBC ≥ 50 μg/ml) were all highly resistant to carbenicillin (MBC ≥8,000 μg/ml) but susceptible to netilmicin (MBC ≤6.25 μg/ml). The susceptibility to amikacin was unpredictable among these groups of isolates but, overall, 80% of the isolates were killed by 25 μg of amikacin/ml, which is within the range of peak serum concentrations used therapeutically. Clinically attainable subinhibitory concentrations of carbenicillin enhanced the activity of the three aminoglycosides against all isolates with MBCs of carbenicillin ≤2,000 μg/ml. The 17 isolates with high-level resistance to carbenicillin and gentamicin, as well as the four isolates with high-level resistance to carbenicillin but not to gentamicin, were not susceptible to such enhancement of aminoglycoside activity by carbenicillin.

Serratia marcescens has been isolated with increasing frequency from patients who are hospitalized or subject to intravenous drug abuse (18, 21, 22, 37, 43, 44). At the University of Chicago Hospitals and Clinics, approximately 600 isolates of *S. marcescens* have been recovered from clinical specimens annually. Although most of these isolates were from sites of colonization, some were implicated in a variety of serious infections.

Antibiotic therapy of serious *Serratia* infections has been difficult. The advent of gentamicin improved inhibition of many *Serratia* strains in vitro over results achieved with other agents such as kanamycin, chloramphenicol, and nalidixic acid (8, 30, 39, 41, 43, 44). This made possible more effective treatment of some clinical infections (44). However, treatment of infections in relatively inaccessible sites, such as cardiac valvular vegetations, may be limited by a requirement for higher serum levels of gentamicin, which may potentiate the risk of toxicity. Furthermore, gentamicin resistance has lately become an increasingly serious problem for the treatment of *Serratia* infections. At this institution, in particular, disk susceptibility testing of *S. marcescens* isolates showed a dramatic increase in the incidence of resistance to gentamicin during successive 6-month periods from January 1973 through June 1975 (0, 5, 17, 30, and 50%, respectively).

The addition of sublethal concentrations of carbenicillin has been shown to produce a synergistic bactericidal effect with gentamicin and other aminoglycoside antibiotics against some strains of *Pseudomonas* and other gram-negative bacilli, both in vitro (1, 2, 12, 14-16, 24, 33, 35, 40) and in animal-model infections (2). By reducing the effective bactericidal concentration of gentamicin, synergistic enhancement of gentamicin activity may increase its clinical effectiveness in the treatment of infections caused by marginally susceptible strains as well as those caused by resistant strains. Also, the risks of toxicity may be reduced by the use of lower concentrations of gentamicin in serum. For these reasons, carbenicillin has been used with gentamicin in the treatment of infections

due to *Serratia* (6, 7, 9, 21, 27) as well as other gram-negative organisms (2, 13, 23, 32, 34), although synergism against *Serratia* has only rarely been examined in vitro (7, 42).

Newer aminoglycoside antibiotics, such as tobramycin and amikacin, have exhibited greater in vitro activity than gentamicin against some strains of *Pseudomonas* and other gram-negative bacilli (4, 5, 11, 15, 17, 25, 45). Amikacin, in particular, has been shown to be effective against *S. marcescens* both in vitro (4, 25, 35, 44) and in clinical infections (10, 19, 36). Although most strains of *Serratia* that have been tested with amikacin were very susceptible to gentamicin, there have been occasional reports of its effectiveness against gentamicin-resistant strains (25, 35). In a recent report, netilmicin (Sch 20569), a new semisynthetic aminoglycoside, was shown to have activity against many gentamicin-resistant gram-negative bacilli, although it was less active than gentamicin against some of the 20 *Serratia* strains tested (26). Synergism with carbenicillin may enhance the bactericidal activity of these two aminoglycosides.

The present in vitro study was undertaken to examine the effects of two therapeutic alternatives to the use of gentamicin alone against each of a series of 55 recent clinical isolates of *S. marcescens*. (i) The activities of amikacin and netilmicin were compared with that of gentamicin. (ii) The activity of each of the three aminoglycosides in combination with sublethal concentrations of carbenicillin was compared with the activity of each aminoglycoside alone.

MATERIALS AND METHODS

Bacterial strains. Fifty-five clinical isolates of *S. marcescens* were obtained for this study from the Clinical Microbiology Laboratory of the University of Chicago Hospitals and Clinics. Isolates were chosen to include approximately 50% resistant to gentamicin by disk susceptibility testing. These isolates were grouped into 26 strains by biotype and serotype testing (28) carried out by F. Kocka and E. Roemisch of the Clinical Microbiology Laboratory. One strain included seven isolates, two had six, and the other thirty-six isolates were distributed among the remaining twenty-three strains, with fifteen strains (58%) each having only one isolate. The subtyping of this collection of *Serratia* isolates assured the diversity of this population in which the response to various antibiotics was studied. Subtyping also indicated that colonization and infection with this organism were not limited to a predominant hospital strain.

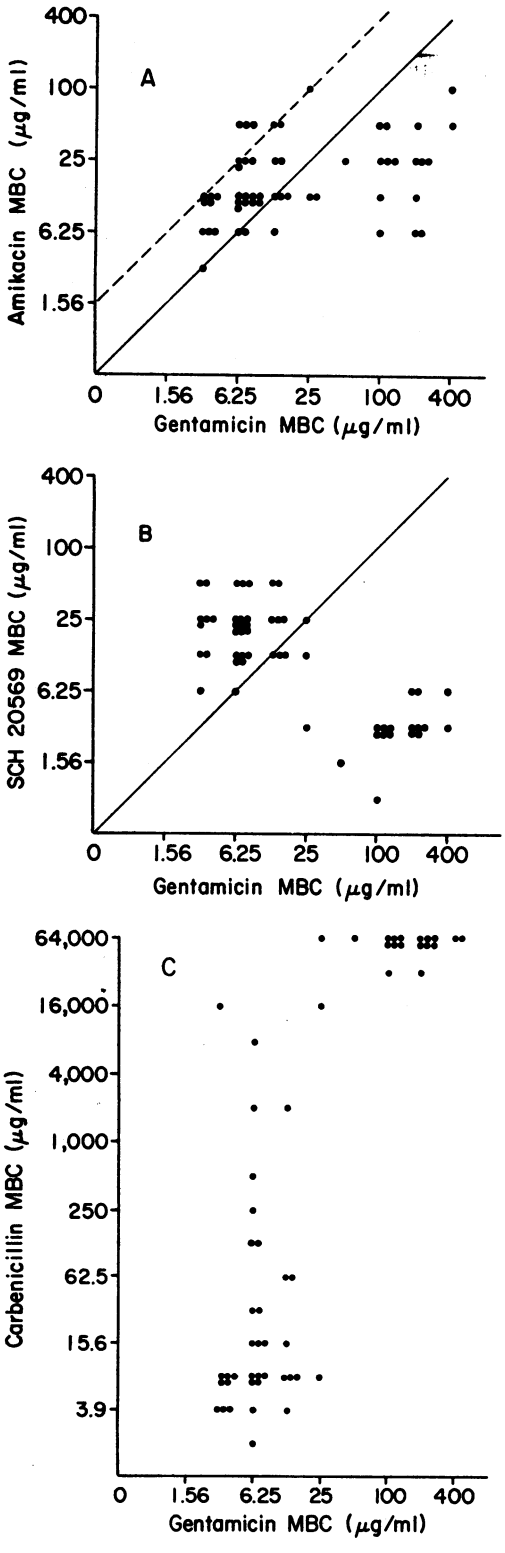
Antibiotics. Gentamicin and netilmicin were gifts of Schering Corp.; amikacin was a gift of Bristol Laboratories; and carbenicillin was a gift of Beecham, Inc. Stock solutions of the aminoglycoside antibiotics were made up in water according to concentrations of their bases and were kept at −20°C. These solutions were diluted in Trypticase soy broth (TSB) (BBL) for use in susceptibility testing. Carbenicillin solutions were prepared fresh before each experiment by dissolving disodium carbenicillin in water and diluting further in TSB.

Antibiotic susceptibility testing. The susceptibility of the *Serratia* isolates to the aminoglycosides and to carbenicillin was determined by a broth dilution method in a single experiment. A culture of each isolate grown overnight in TSB at 37°C was diluted to 10^{-4} with TSB for inoculation. Portions of 0.5 ml were added to sets of tubes, each of which contained 0.5 ml of a twofold dilution of one of the antibiotics in TSB. The minimal inhibitory concentration (MIC) was the lowest concentration that prevented the development of visible turbidity during incubation at 37°C for 18 h. Cultures without visible growth were subcultured by streaking with a 0.01-ml calibrated loop onto Columbia sheep blood agar plates (BBL), which were then incubated at 37°C for 18 h. The minimal bactericidal concentration (MBC) of an antibiotic was the lowest concentration in a culture from which fewer than 10 colonies grew. In the majority of cases, MICs were within two dilutions of the MBCs.

Testing of synergism. The effects of sublethal concentrations of carbenicillin on the bactericidal activities of the aminoglycosides for each *S. marcescens* isolate was determined by a variation of the checkerboard technique (29). Twofold serial dilutions of carbenicillin in TSB (0.25 ml) were mixed with 0.25 ml of twofold serial dilutions of each aminoglycoside in TSB. Each tube was inoculated with 0.5 ml of a 10^4-fold TSB dilution of a culture that had grown in TSB for 18 h at 37°C. All tubes that remained clear after incubation at 37°C for 18 h after inoculation were subcultured with a 0.01-ml calibrated loop onto blood agar plates. For all isolates with MBC of carbenicillin ≤500 μg/ml, the MBC of an aminoglycoside alone was compared with its MBC in the presence of one-fourth of the MBC of carbenicillin. For isolates with MBC of carbenicillin >500 μg/ml, the reduction of the aminoglycoside MBCs was scored in the presence of carbenicillin at the clinically attainable concentration of 125 μg/ml (and also at 500 μg/ml). Synergism was defined as a fourfold or greater reduction of the aminoglycoside MBC in the presence of these subinhibitory concentrations of carbenicillin. For each isolate, the MBCs of each drug in the synergism tests were within one tube dilution of the MBCs obtained previously.

RESULTS

Susceptibility to gentamicin, amikacin, netilmicin, and carbenicillin. No isolates were killed at concentrations of gentamicin <3.12 μg/ml (Fig. 1). Only 27 isolates (49%) were killed, and 32 (58%) were inhibited by 6.25 μg of gentamicin per ml, which is within the range of peak serum concentrations used to treat patients with this drug. Eleven isolates (20%) were moderately resistant to the antibiotic, re-



quiring 12.5 to 25 μg/ml for bactericidal effect. Seventeen (31%) were highly resistant to gentamicin, with MBCs of 50 to 400 μg/ml.

In comparison, 44 *Serratia* isolates (80%) were killed and 52 (94%) were inhibited by 25 μg of amikacin per ml (Fig. 1A), a peak concentration in serum that has been used in the treatment of patients (3, 10, 19, 36). The remaining 11 isolates all had MBCs of 50 to 100 μg/ml.

Netilmicin showed a greater difference between MICs and MBCs. At a concentration of 6.25 μg/ml, 40 isolates (73%) were inhibited, but only 20 (36%) were killed (Fig. 1B). All isolates were killed at 50 μg/ml.

There was little difference between MICs and MBCs of carbenicillin for this series of isolates. Carbenicillin was able to inhibit and kill 30 (54%) at a concentration of 125 μg/ml (Fig. 1C), which is clinically attainable in serum. Even at only 15.6 μg/ml, carbenicillin was bactericidal to 44% of the isolates. Although only four isolates had MBCs in the range of 250 to 2,000 μg/ml, 21 (38%) were found to be highly resistant, with MBCs greater than 2,000 μg/ml (8,000 to 64,000 μg/ml).

Figures 1A and 1B compare the MBCs of amikacin and of netilmicin with the MBC of gentamicin for each *Serratia* isolate. Amikacin is compared at four times the concentration of gentamicin, since the clinically achievable peak serum levels of amikacin are approximately four times higher than those of gentamicin (3, 36, 41). For all but three isolates, the MBCs of amikacin were at or less than four times the comparable MBCs of gentamicin. Furthermore, 8 of 11 isolates with MBCs of gentamicin at 12.5 to 25 μg/ml, as well as 12 of 17 with higher MBCs of gentamicin, were shown to be susceptible to amikacin (MBC ≤25 μg/ml). There was, however, no correlation between the levels of susceptibility to these two drugs.

In contrast, there was an inverse correlation between the susceptibility to gentamicin and to netilmicin for most isolates. All isolates that were highly resistant to gentamicin (MBC ≥ 50 μg/ml) were susceptible to netilmicin (MBC ≤6.25 μg/ml). On the other hand, 35 of the 38

FIG. 1. *Comparison of MBCs of gentamicin with MBCs of amikacin (A), netilmicin (Sch 20569) (B), and carbenicillin (C) for each of the 55 isolates of S. marcescens. The solid line indicates equal susceptibility to the aminoglycosides on a weight basis. The dashed line in (A) compares the susceptibility to gentamicin with the susceptibility to amikacin at an equivalent fourfold higher MBC (see text).*

strains that were either susceptible or moderately resistant to gentamicin (MBC ≤25 μg/ml) were resistant to netilmicin (MBC ≥12.5 μg/ml).

An analysis of the MBCs of gentamicin and carbenicillin for each isolate (Fig. 1C) indicated that all 17 isolates (from at least six different strains) that were highly resistant to gentamicin (MBC ≥50 μg/ml) were also highly resistant to carbenicillin (MBC ≥8,000 μg/ml). Of the four additional isolates that were highly resistant to carbenicillin, two had MBCs of gentamicin at 25 μg/ml and the other two were susceptible (MBC ≤6.25 μg/ml). Of this total of 21 isolates with MBCs of carbenicillin ≥8,000 μg/ml (represented by closed circles in Fig. 2A–C), 18 had MBCs of netilmicin ≤6.25 μg/ml, whereas 15 had MBCs of amikacin ≤25 μg/ml. Of the remaining 34 isolates that were more susceptible to carbenicillin, 33 had MBCs of gentamicin ≤12.5 μg/ml, and 31 MBCs of amikacin ≤25 μg/ml. On the other hand, only 2 of these 34 isolates showed MBCs of netilmicin ≤12.5 μg/ml.

Synergism studies. Figure 2 compares the aminoglycoside MBCs of each *S. marcescens* isolate with its MBCs in the presence of sublethal concentrations of carbenicillin. All 34 isolates (from at least 17 strains) with MBCs of carbenicillin ≤2,000 μg/ml were susceptible to enhancement of the bactericidal effect of these aminoglycosides by the addition of carbenicillin at one-fourth of its MBC (or at 125 μg/ml for the two isolates whose MBC of carbencillin was 2,000 μg/ml). In particular, the MBCs of the three aminoglycosides for these 34 isolates were reduced synergistically in all but eight instances. (In these marginal cases, which involved seven of the isolates, there was reduction of an aminoglycoside MBC to one-half rather than one-fourth.) With the addition of carbenicillin at concentrations used to test for synergism, 33 of these 34 isolates with MBCs of carbenicillin ≤2,000 μg/ml were killed by only 1.56 μg of gentamicin per ml, whereas only four were killed by this concentration of gentamicin alone. Thirty-three of these thirty-four isolates were killed by 6.25 μg of amikacin per ml in combination with carbenicillin; six were killed by this concentration of amikacin alone. Netilmicin at 3.12 μg/ml killed 31 of these isolates in the presence of the sublethal concentrations of carbenicillin, whereas none was killed by this concentration of netilmicin without the addition of carbenicillin.

For the remaining 21 isolates (from at least nine different strains), there was no reduction of MBCs of any of the three aminoglycosides in the presence of carbenicillin at either 125 μg/ml or 500 μg/ml (Fig. 2A–C). These were all the isolates which were not killed, or even inhibited, by 2,000 μg of carbenicillin per ml; their MBCs were all ≥8,000 μg/ml.

DISCUSSION

The range of susceptibility to gentamicin in this series of isolates (Fig. 1) appeared to be shifted toward greater levels of resistance than has usually been seen for *S. marcescens* (4, 8, 11, 20, 38, 45–47), with MBCs up to 400 μg/ml and none less than 3.12 μg/ml. Clinically important resistance of gentamicin (MBC ≥12.5 μg/ml) was present in 51% of the isolates in the series, in agreement with disk susceptibility testing. Such a high incidence of gentamicin resistance as was shown among recent isolates of *S. marcescens* in our hospital has not been reported (4, 8, 11, 20, 38, 45–47) until very recently (37). This decrease in susceptibility to gentamicin exhibited by the diverse strains in this series provided an opportunity to test alternative antibiotic regimens in vitro.

Amikacin was more active than gentamicin against a majority of isolates when its MBCs were compared with those of gentamicin in a therapeutically equivalent ratio of 4:1. Although there was an absence of high-level resistance to amikacin, some gentamicin-resistant isolates, as well as some susceptible isolates, exhibited low-level resistance to amikacin.

While Rahal et al. (26) have reported that netilmicin has activity against many gentamicin-resistant gram-negative bacilli, they found that all of their six gentamicin-resistant *Serratia* isolates were resistant to netilmicin. However, all isolates in our series that were highly resistant to gentamicin showed susceptibility to netilmicin. Since blood levels of netilmicin used in animals were comparable to those of gentamicin (31), netilmicin may be a useful antibiotic for infections caused by gentamicin-resistant *Serratia*.

The results of the studies on synergism against this series showed that the addition of subinhibitory concentrations of carbenicillin yielded synergistic killing with aminoglycosides for all isolates with MBCs of carbenicillin ≤2,000 μg/ml. Although most of these isolates exhibited MBCs of carbenicillin well within the range of concentrations that are achievable clinically in the serum, it is likely that the addition of relatively low levels of aminoglycosides would ensure more rapid bactericidal effect than the use of carbenicillin alone (1, 40).

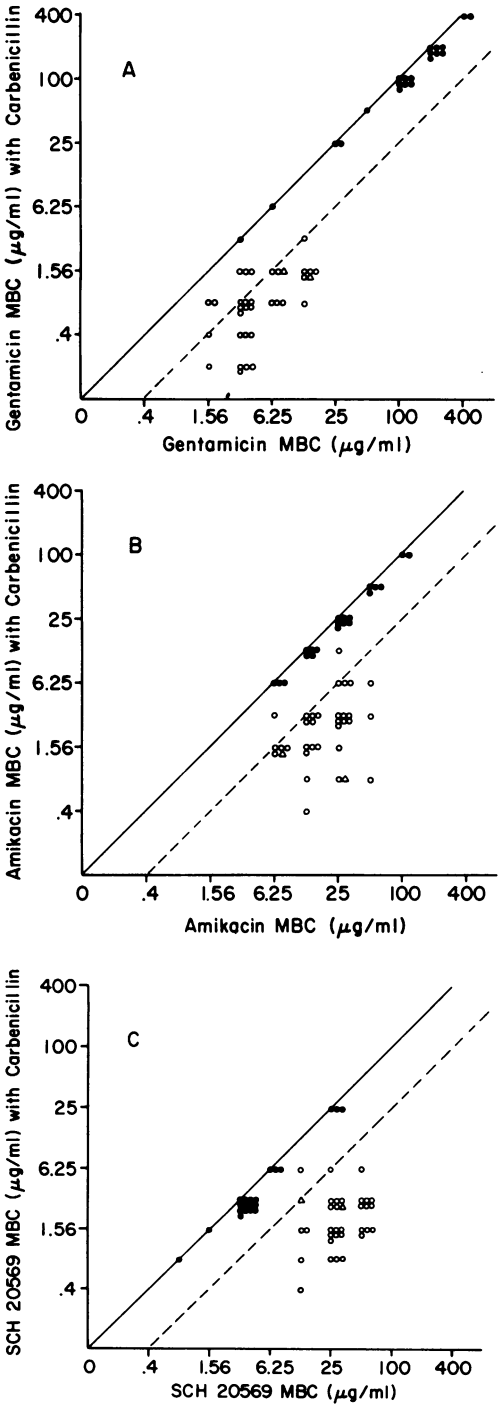


FIG. 2. *Killing of each of the 55 S. marcescens isolates by the combination of carbenicillin with gentamicin (A), amikacin (B), and netilmicin (Sch 20569) (C). For each isolate, the MBC of the aminoglycoside alone is compared with the MBC of the aminoglycoside in the presence of carbenicillin at the following concentrations: at one-fourth the MBC of carbenicillin for isolates with MBCs ≤500 μg/ml (○), at 125 μg/ml for isolates with MBCs at 2,000 μg/ml (△), and at 500 μg/ml for isolates with MBCs >2,000 μg/ml (●). The solid line indicates equal susceptibility to the aminoglycoside with and without carbenicillin. The dashed line indicates a synergistic reduction of the MBC of the aminoglycoside to one-fourth by the addition of carbenicillin.*

The only isolates that were not susceptible to synergistic lowering of aminoglycoside MBCs in the presence of carbenicillin at 125 μg/ml (or even at 500 μg/ml) were those with MBCs of carbenicillin ≥8,000 μg/ml. This group of isolates included two that were susceptible to gentamicin and many that were susceptible to amikacin and/or netilmicin. Therefore, in our series of isolates, lack of susceptibility to synergism with concentrations of carbenicillin that are clinically attainable in serum seems to correlate with high-level resistance to carbenicillin.

It thus appears that a synergistic combination of amikacin or gentamicin with carbenicillin may provide a more effective treatment regimen than the use of gentamicin alone for infections caused by *Serratia* isolates that are susceptible or only moderately resistant to gentamicin (especially if they are susceptible to killing by ≤2,000 μg of carbenicillin per ml). For isolates which are highly resistant to gentamicin (and generally highly resistant to carbenicillin as well), netilmicin or, in some cases, amikacin may be adequately bactericidal alone. Further clinical evaluation of amikacin, netilmicin, and combinations of carbenicillin with aminoglycosides are needed for the treatment of *Serratia* infections.

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