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Comparative Susceptibilities of Clinical Isolates of *Serratia marcescens* to Newer Cephalosporins, Alone and in Combination with Various Aminoglycosides

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We examined 100 clinically significant isolates of *Serratia marcescens* for susceptibility to newer cephalosporin and cephamycin antibiotics, alone and in combination with various aminoglycosides. Moxalactam and cefotaxime were the most effective agents; all isolates were inhibited by 25 and 50 μg/ml, respectively. All strains were susceptible to amikacin at concentrations safely achievable in serum, whereas gentamicin, netilmicin, and tobramycin inhibited 63, 63, and 16% of the isolates, respectively. Moxalactam, cefotaxime, and amikacin were active against gentamicin-susceptible and gentamicin-resistant strains. Studies of synergy revealed that moxalactam and cefotaxime, in combination with netilmicin or amikacin, were often synergistic and infrequently antagonistic against cephalothin- and gentamicin-resistant strains. These results suggest that moxalactam and cefotaxime, alone or in combination, may be efficacious in treating infections due to multiply antibiotic-resistant *S. marcescens*.

Over the past decade, *Serratia marcescens* has achieved preeminence as a cause of serious nosocomial infections (6, 21, 23, 30). Susceptibility to aminoglycoside antibiotics was, at one time, taken for granted, but the emergence of multiply antibiotic-resistant strains has been a cause for concern (23, 30).

Cephalosporin antibiotics have been notoriously ineffective against *S. marcescens*. However, newer generations of cephalosporins, most notably moxalactam (LY127935) and cefotaxime (HR756), have been shown to be extremely active in vitro against both cephalothin-susceptible and cephalothin-resistant strains and some gentamicin-resistant organisms (7, 8, 10, 13, 24, 27). The potential for synergy has not been extensively evaluated. In the present study, the activity of cephalosporin and other β-lactam antibiotics, alone and in combination with various aminoglycosides, against *S. marcescens* was compared.

MATERIALS AND METHODS

Antibiotics. Moxalactam, cefamandole, cephalothin, and tobramycin were supplied by Eli Lilly & Co., Indianapolis, Ind. Cefotaxime was supplied by Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J. Gentamicin and netilmicin were furnished by the Schering Corp., Bloomfield, N.J. Carbenicillin and ticarcillin were obtained from Beecham Laboratories, Bristol, Tenn. Cefoxitin was furnished by Merck & Co., Inc., Rahway, N.J. Amikacin, cephapirin, and ceforanide (BL-S786) were supplied by Bristol Laboratories, Syracuse, N.Y. All drugs, except tobramycin, were supplied as standard powders. Antibiotic solutions were diluted to the appropriate concentrations and used within 24 h.

Bacterial isolates. All strains of *S. marcescens* were isolated by the Clinical Bacteriology Laboratory at the Medical College of Virginia and identified by standard criteria. Only strains judged to be involved in clinically significant infections were studied, including: (i) urinary isolates (36 strains) at more than 100,000 organisms per ml of urine on two consecutive daily urine cultures; (ii) wound isolates (21 strains) when *S. marcescens* was the only organism cultured from an infected area; (iii) sputum isolates (23 strains) when *S. marcescens* was consistently isolated in pure culture from a patient with purulent sputum, fever, and pulmonary infiltrates; (iv) blood (17 strains); (v) cerebrospinal fluid (2 strains); and (vi) synovial fluid (1 strain). Strains were stored in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with agar to 1% and frozen over sterile glass beads to −70°C.

Susceptibility testing. The antimicrobial susceptibilities of the 100 isolates of *S. marcescens* were determined in triplicate by microtiter broth dilution in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (16). Serial twofold dilutions of antibiotic (200 to 0.2 μg/ml) were added with a 50-μl calibrated microdiluter to the appropriate wells of a sterile plastic multiwell microtiter plate (Linbro/Titertek, Linbro Scientific, Inc., Hamden, Conn.). The inoculum was prepared from an overnight Trypticase soy broth culture and diluted to contain approximately 2.5×10^5 or 2.5×10^7 colony-forming units per ml. The minimal inhibitory concentration (MIC) was the lowest concentration of antibiotic giving no visible growth after 18 h of incubation at 37°C. Isolates were considered cephalothin- and gentamicin-resistant if they

were not inhibited by 25 μg or less of cephalothin per ml and 6.25 μg or less of gentamicin per ml. Appropriate wells with and without antibiotics served as sterility and growth controls, respectively.

Synergy studies. Cephalosporin and aminoglycoside synergy was evaluated for selected strains by microtiter broth dilution in Trypticase soy broth by the checkerboard pattern method (2). An inoculum containing 2.5×10^5 colony-forming units per ml was added to serial twofold dilutions of one antibiotic in combination with serial twofold dilutions of another. The lowest concentrations of both antibiotics inhibiting visible growth after 18 h of incubation at 37°C were the MICs. Synergism was present by this method when the MIC of each antibiotic in combination was one-fourth or less the MIC of each antibiotic tested alone, whereas a fourfold increase in the MIC of either or both antibiotics in combination was defined as antagonism. All other alterations of MICs were considered nonsynergistic. Determinations were done in duplicate. Isobolograms were constructed with the results of the checkerboard studies. Combinations of antibiotics were synergistic if the curve was concave and antagonistic if the curve bowed upward. All other contours were considered nonsynergistic. Finally, fractional inhibitory concentrations were calculated by the scheme recently outlined (4). Sums of <1 indicated synergism, sums of >1 indicated antagonism, and sums of 1 or varying with the checkerboard determinations employed indicated nonsynergism.

RESULTS

The cumulative percentage of isolates of *S. marcescens* inhibited by various antibiotics is shown in Table 1. Moxalactam and cefotaxime were the most active β-lactam antibiotics, with all isolates being inhibited by 25 μg or less of moxalactam per ml and 50 μg or less of cefotaxime per ml. Cefoxitin and cefamandole were considerably less active, whereas cephalothin, ceforanide, and cephapirin were without effect. Amikacin was the most effective aminoglycoside, with 100% of strains inhibited at a concentration of 25 μg or less per ml. Gentamicin inhibited 63% of strains at 6.25 μg or less per ml. Gentamicin-resistant strains required at least a 16-fold increase in antibiotic concentration to inhibit most of the remaining isolates. Netilmicin was as effective as gentamicin at safely achievable serum concentrations; in contrast, however, netilmicin-resistant isolates required a manyfold-less increase in antibiotic concentration for inhibition. Tobramycin was the least effective aminoglycoside.

Comparative susceptibilities of gentamicin-susceptible and gentamicin-resistant isolates of *S. marcescens* are shown in Table 2. The MICs of moxalactam and cefotaxime required to inhibit 50 and 90% of gentamicin-susceptible and gentamicin-resistant strains were virtually identical. The MICs of moxalactam and cefotaxime were two- to fourfold higher for gentamicin-resistant isolates when compared with the MICs for gentamicin-susceptible isolates. Cefoxitin and cefamandole were less effective against both groups of isolates, but particularly against the gentamicin-resistant organisms. Gentamicin susceptibility had no effect on the MICs of amikacin, with all strains remaining susceptible to the latter drug. The activity of netilmicin tended to parallel that of gentamicin for both groups of isolates. Tobramycin was, quantitatively, much less active. Most strains were not inhibited by either carbenicillin or ticarcillin.

The effect of inoculum size on the concentrations of moxalactam and cefotaxime required to inhibit 90% of isolates is shown in Table 3. MICs were twofold higher for both antibiotics at an inoculum of 10^6 colony-forming units. Such in-

TABLE 1. *Comparative susceptibilities[a] in vitro of 100 clinically significant strains of S. marcescens*

Antimicrobial agent	Cumulative % susceptible to antibiotic at the following concn (μg/ml):											% Susceptible
	≤0.2	0.4	0.8	1.6	3.12	6.25	12.5	25	50	100	≥200	
Moxalactam	14	23	47	65	72	84	91	100				100
Cefotaxime	9	14	26	44	58	72	86	93	100			100
Cefoxitin						2	19	49	70	79	100	49
Cefamandole							9	23	42	51	100	23
Cephalothin											100	0
Ceforanide											100	0
Cephapirin											100	0
Gentamicin	7	12	14	30	58	63	63	63	86	98	100	63
Tobramycin					5	16	35	41	67	91	100	16
Amikacin					21	67	93	100				100
Netilmicin					16	63	88	98	100			63
Carbenicillin				2	2	19	30	35	35	35	100	35
Ticarcillin					7	21	33	33	35	35	100	35

[a] Organisms were susceptible if the MIC was ≤50 μg of moxalactam and cefotaxime per ml, ≤25 μg of cefoxitin, cefamandole, cephalothin, ceforanide, and cephapirin per ml, ≤6.25 μg of gentamicin, tobramycin, and netilmicin per ml, ≤25 μg of amikacin per ml, and ≤100 μg of carbenicillin and ticarcillin per ml.

TABLE 2. *Comparison of antibiotic susceptibilities of gentamicin-resistant and gentamicin-susceptible strains of S. marcescens*

Antimicrobial agent	Gentamicin-susceptible strains[a]		Gentamicin-resistant strains[b]	
	MIC_{50}[c] (μg/ml)	MIC_{90}[d] (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Moxalactam	0.8	6.25	1.6	12.5
Cefotaxime	0.8	6.25	1.6	25
Cefoxitin	25	100	50	≥200
Cefamandole	50	≥200	≥200	≥200
Tobramycin	12.5	50	100	≥200
Amikacin	6.25	12.5	6.25	12.5
Netilmicin	6.25	12.5	6.25	25
Carbenicillin	12.5	≥200	≥200	≥200
Ticarcillin	12.5	≥200	≥200	≥200

[a] Strains inhibited by ≤6.25 μg of gentamicin per ml; number of strains, 33.
[b] Strains inhibited by >6.25 μg of gentamicin per ml; number of strains, 37.
[c] Concentration required to inhibit 50% of strains.
[d] Concentration required to inhibit 90% of strains.

creases were seen uniformly with most strains, although for a few strains the MICs increased four- to eightfold (data not shown).

The effects of moxalactam and cefotaxime in combination with various aminoglycoside antibiotics against 22 cephalothin- and gentamicin-resistant isolates of *S. marcescens* are shown in Table 4. Strains employed were susceptible to moxalactam and cefotaxime. Combinations including netilmicin or amikacin were more often synergistic than were combinations including other aminoglycosides. Moxalactam and netilmicin were synergistic against 82% (18 of 22) of strains, whereas cefotaxime and netilmicin were synergistic against 23% (5 of 22). Synergism was demonstrated for netilmicin-susceptible and netilmicin-resistant organisms. Antagonism was not seen with the moxalactam and netilmicin combination, but was seen with 18% (4 of 22) of strains tested with cefotaxime and netilmicin. Combinations including amikacin were synergistic against 64% (14 of 22) of strains with moxalactam and 18% (4 of 22) of strains with cefotaxime. Thus, the frequency of synergism and antagonism was roughly comparable with both the netilmicin and the amikacin combinations. Combinations including tobramycin were the least effective. The efficacy of gentamicin was roughly equivalent to that seen with tobramycin in terms of synergism and antagonism. However, when synergism was demonstrated, the MIC of gentamicin in combination was lowered to clinically achievable serum concentrations for three of six strains tested with moxalactam and one of two strains tested with cefotaxime, an effect not seen with tobramycin.

TABLE 3. *Effect of inoculum size on the MICs of moxalactam and cefotaxime against 100 strains of S. marcescens*

Antimicrobial agent	MIC_{90}[a] (μg/ml) at an inoculum of:	
	10^4 CFU[b]	10^6 CFU
Moxalactam	6.25	12.5
Cefotaxime	12.5	25

[a] Concentration required to inhibit 90% of strains.
[b] CFU, Colony-forming units.

TABLE 4. *Comparative synergistic activity of newer cephalosporins and aminoglycosides against 22 selected cephalothin- and gentamicin-resistant[a] strains of S. marcescens*

Antimicrobial combination	Synergy[b] (%)	No synergy (%)	Antagonism (%)
Moxalactam plus:			
Gentamicin	27 (6)[c]	46 (10)	27 (6)
Amikacin	64 (14)	36 (8)	0
Netilmicin	82 (18)	18 (4)	0
Tobramycin	18 (4)	59 (13)	23 (5)
Cefotaxime plus:			
Gentamicin	9 (2)	91 (22)	0
Amikacin	18 (4)	64 (14)	18 (4)
Netilmicin	23 (5)	59 (13)	18 (4)
Tobramycin	9 (2)	64 (14)	27 (6)

[a] Resistant to >25 μg of cephalothin per ml and >6.25 μg of gentamicin per ml.
[b] Combinations of antibiotics were synergistic only when they met criteria for synergism by all three methods described in the text.
[c] Number of strains is given within parentheses.

DISCUSSION

This study confirms observations reported by others that gentamicin-resistant *S. marcescens* isolates are frequent, approaching 40% of strains isolated (6, 30). This has stimulated a search for more effective antimicrobial regimens for treating infections due to *S. marcescens*. By the microtiter broth dilution method, amikacin proved to be the most effective aminoglycoside

tested, an experience shared by others (1, 14, 31). Gentamicin, netilmicin, and tobramycin were less effective. A total of 84% of the isolates were resistant to tobramycin, a frequency higher than that previously reported (6, 15, 18).

In general, *S. marcescens* is resistant to cephalosporins. However, moxalactam and cefotaxime were extremely effective in vitro. The activity of moxalactam against *S. marcescens* has been evaluated previously (3, 7, 8, 10, 13, 27, 28). The MIC_{90} has varied from 0.25 to 16 μg/ml, but, in general, it has been comparable to the MIC_{90} found in this study. Similar results have been reported for cefotaxime (19, 24). The effect of increased inoculum size on the activity of older cephalosporin antibiotics has been shown repeatedly. Most recent studies have reported less effect on the activity of moxalactam and cefotaxime, particularly with inocula of $\leq 10^6$ colony-forming units per ml (3, 8). Thus, moxalactam and cefotaxime appear to be the most active β-lactam antibiotics against *S. marcescens* reported to date.

Trager et al. compared the activity of moxalactam against gentamicin-susceptible and gentamicin-resistant strains of *S. marcescens* and found that the MIC_{90} for gentamicin-resistant strains was eightfold higher than the MIC_{90} for gentamicin-susceptible strains (27). Others have found little difference with both moxalactam and cefotaxime (8, 13). We found a one-dilution difference with moxalactam when the MIC_{50} and MIC_{90} for gentamicin-susceptible and gentamicin-resistant strains were compared, which was comparable to the findings of Hall et al. (13).

Despite several limitations (20), tests designed to assess synergism may be necessary to find more effective therapy for multiply antibiotic-resistant organisms such as *S. marcescens*. Many combinations of antibiotics have been shown to be synergistic to various degrees (9, 11, 12, 15, 18, 22, 26, 29). Few studies have evaluated the activities of moxalactam and cefotaxime in combination with other agents against *S. marcescens* and other bacteria (19). The combinations of moxalactam and netilmicin or amikacin showed synergistic activity against most cephalothin- and gentamicin-resistant isolates studied, with no antagonism. Netilmicin synergy has been described with other β-lactam antibiotics (9, 11) and could be due to increased efficacy of netilmicin against *S. marcescens*, including gentamicin-resistant isolates (5, 17, 25). Interestingly, this synergistic effect extended to netilmicin-resistant organisms, with a fall in the MIC of netilmicin to levels achievable in serum. Although all isolates were resistant to gentamicin and tobramycin, synergism was demonstrated for some strains. Whereas the MIC of gentamicin in combination was reduced to therapeutic levels, that of tobramycin was not, and both combinations demonstrated frequent antagonism. Thus, these combinations would seem to be less desirable therapeutic alternatives for infections due to *S. marcescens*.

Moxalactam and cefotaxime are new semisynthetic cephalosporin antibiotics with a wide spectrum of activity (3, 7, 8, 10, 13, 19, 24, 27, 28, 32). This spectrum includes organisms such as *S. marcescens*, which are notoriously resistant to older cephalosporins. Moxalactam and, to a lesser extent, cefotaxime are markedly active in vitro against *S. marcescens*, including gentamicin-resistant isolates (8, 13, 27). Not previously demonstrated, however, is the fact that moxalactam in combination with netilmicin and amikacin showed synergistic activity against most strains of cephalothin- and gentamicin-resistant *S. marcescens* and some strains of netilmicin-resistant organisms.

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