Antimicrobial Activity of Amikacin Combinations Against Enterobacteriaceae Moderately Susceptible to Third-Generation Cephalosporins

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Enterobacteriaceae strains having elevated minimal inhibitory concentrations (≥2.0 to ≤32 μg/ml) of cefoperazone, cefotaxime, ceftazidime, and moxalactam were synergistically inhibited by amikacin combinations (54.1 to 69.6% occurrence). Indifference was rare (8.1% for moxalactam), and true antagonistic interactions were not observed. Strains resistant or susceptible to these new cephalosporins were also synergistically inhibited by the addition of amikacin, reducing resistant cephalosporin minimal inhibitory concentrations to clinically achievable levels.

Amikacin has been recognized as the currently available aminoglycoside most refractory to bacterial enzyme inactivation (18). This stability has generally limited the use of amikacin to those clinical bacterial strains resistant to kanamycin, gentamicin, and tobramycin by enzyme plasmid-mediated mechanisms. Similarly, the recently introduced third-generation cephalosporins have substantial stability to bacterial β-lactamases and a potent antimicrobial activity (2, 3, 6–9, 15, 16). The bacteria most likely to harbor the higher minimal inhibitory concentrations (MICs) for these new β-lactams are the nonfermentative bacilli and the serogroup D Streptococcus spp.; however, a few strains of Enterobacteriaceae may have MICs of 2.0 to 32 μg/ml (3, 7–9). These latter organisms represent a very different population of enteric bacilli only moderately susceptible (MS) to these highly active new drugs. Very few Enterobacteriaceae are currently considered resistant (MICs, ≥64 μg/ml) to cefoperazone, cefotaxime, ceftazidime, or moxalactam.

To determine the possibility of using antimicrobial combinations to treat infections caused by these MS strains of Enterobacteriaceae (cefoperazone, cefotaxime, ceftazidime, or moxalactam MICs, 2.0 to 32 μg/ml), we screened over 6,000 organisms to find strains having elevated MICs as described above. These recent clinical isolates from The Cleveland Clinic Foundation (Cleveland, Ohio), St. Francis Hospital (Wichita, Kans.), St. Vincent Hospital and Medical Center (Portland, Oreg.), and Northwestern Memorial Hospital (Chicago, Ill.) were collected and then retested for their susceptibility to the new β-lactams singly and in combination with amikacin. Synergy analysis was determined by the checkerboard broth dilution technique, confirmed by a limited number of bactericidal concentration isobolograms and kill curves. Some additional organisms were tested, including several with resistance to one or more of the five broad-spectrum drugs to detect their possible conversion from resistant-to-susceptible MIC results by the addition of another antimicrobial agent.

MATERIALS AND METHODS

Antibiotics. The broad-spectrum drugs utilized in all phases of this study were supplied by the following manufacturers: amikacin, Bristol Laboratories, Syracuse, N.Y.; cefoperazone, Pfizer Inc., New York, N.Y.; cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; ceftazidime, Glaxo Inc., Research Triangle Park, N.C.; moxalactam, Eli Lilly & Co., Indianapolis, Ind.

Bacterial isolates. Seventy-five study strains were discovered during the screening phase and had MIC determinations confirmed at a second laboratory (Kaiser Foundation Laboratories, Clackamas, Oreg.). Several isolates with an in vitro resistance to amikacin (MIC, ≥32 μg/ml), cefoperazone (MIC, ≥64 μg/ml), ceftazidime (MIC, ≥64 μg/ml), or cefotaxime (MIC, ≥64 μg/ml) were also collected to provide organisms for other antimicrobial combination trials. The organisms tested that were MS to one or more of the new cephalosporins were Serratia spp. (21 strains), Escherichia coli (13 strains), Citrobacter freundii (10 strains), Enterobacter aerogenes (9 strains), Klebsiella spp. (7 strains), Enterobacter cloacae (5 strains), Citrobacter diversus (3 strains), Providencia stuartii (2 strains), Proteus vulgaris (2 strains), Morganella morganii (1 strain), Providencia rettgeri (1 strain), and Salmonella enteritidis (1 strain). Strains resistant to one or more of the study drugs were Serratia marcescens (10), C. freundii (8), Escherichia coli (5), Enterob-
bac ter aerogenes (3), and one strain each of Enterobacter cloacae, K. pneumoniae, M. morganii, Providencia stuartii, and Salmonella species. Thirty-three Enterobacteriaceae strains susceptible to very low levels of all β-lactam study drugs (≤1.0 μg/ml) were also tested for synergy, although enhanced inhibitory activity was technically difficult to determine on such isolates (2, 6, 15, 16).

Antibiotic susceptibility tests and synergy studies. The MIC screening phase and the retesting of strains were performed by dilution susceptibility tests, using methods described previously and published in the latest edition (tentative standard) of the National Committee for Clinical Laboratory Standards (NCCLS) dilution methods (3, 14). In the screening tests, two antimicrobial concentrations were used for each drug (1.0 and 32 μg/ml for cefotaxime, ceftaziidine, and moxalactam; 2.0 and 64 μg/ml for ceferazone), and those Enterobacteriaceae isolates ultimately having MICs of ≥2.0 and ≤32 μg/ml were selected for synergy studies at The Clinical Microbiology Institute, Tualatin, Oreg.

For the checkerboard technique, broth microdilution synergy trays were prepared in plastics (Dynatech Laboratories, Inc., Alexandria, Va.), in which amikacin was combined with each of the new β-lactams. Methods described previously, using divalent cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (6, 14), were employed. Each test strain was inoculated into the plastic trays at a final density of 5 × 10⁸ colony-forming units per ml and then incubated overnight (15 to 20 h), read as MICs, and plotted as isobolograms. Minimal bactericidal concentration isobolograms and kill curves were randomly performed to confirm (internal quality control) the interpretative validity of the use of inhibitory endpoints against Enterobacteriaceae (6). Approximately 10% of the well volumes (0.01 ml) was subcultured after tray agitation to drug-free 5% sheep blood agar plates. The minimal bactericidal concentration was defined as the lowest concentration resulting in a ≥99.9% reduction in the original inoculum concentration (≤5 × 10⁸ colony-forming units per ml) by the criteria of Pearson et al. (17). Kill curves (incoculum of 5 × 10⁸ colony-forming units per ml) were performed in 2 ml of Mueller-Hinton broth combining one-fourth of the MIC of amikacin and the MIC of the other drug. A synergistic bactericidal endpoint was defined as ≥99% superior reduction in colony-forming units at 24 h compared with the β-lactam concentration alone (10). Data confirmed the comparability of the previously documented methods of results to within one interpretative category (6).

A total of 216 drug synergy studies were performed in which amikacin was combined with β-lactams. The criteria for synergy were the graphic concavity of the isobologram plots and a fourfold or more decrease in the MIC of one antimicrobial agent when combined with a concentration equivalent to one-fourth of the MIC of the other. The additive interaction was defined as a fourfold or greater decrease in the MIC of one agent and a twofold reduction in the MIC of the other drug (sometimes called partial synergy) or as a twofold decrease in the MICs of both tested drugs. Indifference was defined as no significant decrease in the MIC of either compound or only a twofold decrease or increase in the MIC of one drug. Antagonism was defined as a fourfold or greater increase in the MICs of either or both antimicrobial agents (6). A clinically meaningful synergy would imply that each antimicrobial agent must have an MIC of, or reduced to, a drug level readily achievable in serum or tissue with the usual recommended dose. Synergy would also be considered of questionable significance if both drugs were highly active against a strain when tested singly.

The significance of the synergy results between the drug combinations were determined by the chi-square test. Data on the incidence of MICs of antimicrobial agents were from earlier, more extensive studies of Enterobacteriaceae (4,679 to 8,038 consecutive clinical isolates) from six geographically separated medical centers in comparative in vitro investigations of ceferazone, cefotaxime, ceftaziidine, and moxalactam (3, 7-9; see Table 1). Similar data were derived for amikacin from in vitro studies of fortimicin A (5).

RESULTS

The number of isolates found in the susceptibility screening phase of this study were examined for comparability with previous geographically dispersed clinical bacterial samples (Table 1). The frequency of strains having MICs of ≥2.0 to ≤32 μg/ml should be found in the following order: ceferazone > moxalactam > cefotaxime > ceftaziidine (3, 7-9). This result was identical to the findings among the 75 cephalosporin MS Enterobacteriaceae, i.e., ceferazone (56 strains) > moxalactam (37 strains) > cefotaxime (36 strains) > ceftaziidine (23 strains). The species distribution of MS strains for each drug was most similar for cefotaxime and moxalactam, for which the majority were from the genera Enterobacter, Citrobacter, and Serratia. Moderate susceptibility to ceferazone was most often detected among strains of Serratia marcescens and Escherichia coli and less often for Enterobacter and Citrobacter spp. Of the 23 strains MS to ceftaziidine, 16 were Citrobacter or Enterobacter spp. Overall, the Enterobacter spp. isolates had the greatest number (27.6%) of elevated but susceptible MICs to the newer cephalosporins, followed closely by Serratia marcescens (25.0%) and Citrobacter spp. (23.0%). Table 1 shows the rare occurrence of enteric bacilli MS to the new cephalosporins by the study criteria or by the recent interpretive criteria of the NCCLS (14). By the recommended NCCLS interpretation, only 0.5% (ceferazone) to 1.3% (moxalactam) of the MICs would have an MS interpretation. Moreover, 98.6 to 99.3% of strains were either susceptible or MS to the four new β-lactams.

The results of the synergy studies are shown in Table 2. A total of 152 studies were performed on the 75 MS Enterobacteriaceae. All of these drugs had very positive (enhanced killing) interactions when combined with amikacin. Cefoperazone produced the highest rate (69.6%) of synergy. The lowest rates of synergy for cefotaxime
were seen among the C. freundii and Enterobacter aerogenes strains; the lower rates of synergy for moxalactam were found for the C. freundii and Enterobacter cloacae isolates, and 3 of 8 non-synergy interactions with ceftazidime were found for Enterobacter aerogenes strains. Only moxalactam showed a lack of enhanced killing (indifference) for one strain each of Escherichia coli, Enterobacter aerogenes, and C. freundii. By the commonly used fractional inhibitory concentration index of \( \geq 2.0 \) for antagonistic definition, the three moxalactam-amikacin studies show antagonism (10). The vast majority of additive results met the criterion of partial synergy if this definition was applied.

The rates of synergy and additive interactions for each \( \beta \)-lactam-amikacin combination showed no statistically significant \( (P > 0.05) \) differences, although a lower rate was noted for the moxalactam-amikacin combination. Other synergy studies on 16 cefoperazone-resistant (MIC, \( \geq 64 \mu g/ml \)) strains demonstrated 100% additive or synergy results. All cefoperazone MICs were reduced to \( \leq 32 \mu g/ml \) by clinically achievable levels of amikacin (\( \leq 16 \mu g/ml \)). Four of five ceftazidime-resistant strains showed synergy with amikacin, and the remaining isolate had an additive interaction (ceftazidime MICs, all \( \leq 16 \mu g/ml \)). The only cefotaxime-resistant C. freundii strain was additive. All four amikacin-resistant (MIC, \( > 16 \mu g/ml \)) strains were synergistically inhibited by various susceptible levels of \( \beta \)-lactam combinations, reducing the amikacin MICs to \( \leq 8.0 \mu g/ml \). Strains susceptible (MICs, \( \leq 1.0 \mu g/ml \)) to all \( \beta \)-lactams were difficult to evaluate by drug interaction methods because of their very low MIC endpoints. However, most demonstrated favorable drug interactions, and no evidence of antagonism was detected (33 randomly selected strains). In summary, all isolates tested, regardless of the initial individual drug MIC, were shown to be susceptible or MS to combinations or to possess clinically meaningful synergy or drug interactions.

**DISCUSSION**

This study principally evaluated the in vitro combination effects of amikacin and four newer cephalosporin drugs against *Enterobacteriaceae*.

**TABLE 2. Comparative antimicrobial interaction of amikacin with four new \( \beta \)-lactam drugs on *Enterobacteriaceae* having elevated but susceptible MICs**

<table>
<thead>
<tr>
<th>Category of interaction</th>
<th>Cefoperazone</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Moxalactam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synergy</td>
<td>39 (69.6)</td>
<td>24 (66.7)</td>
<td>15 (65.2)</td>
<td>20 (54.1)</td>
</tr>
<tr>
<td>Additive</td>
<td>17 (30.4)</td>
<td>12 (33.3)</td>
<td>8 (34.8)</td>
<td>14 (37.8)</td>
</tr>
<tr>
<td>Indifferent</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>Total (% enhanced activity)</td>
<td>56 (100.0)</td>
<td>36 (100.0)</td>
<td>23 (100.0)</td>
<td>37 (91.9)</td>
</tr>
</tbody>
</table>

\(a\) MICs of 2.0 to 32 \( \mu g/ml \). A total of 75 strains were tested in 152 total synergy studies including *Serratia* spp. (21 strains), *Escherichia coli* (13 strains), *C. freundii* (10 strains), *Enterobacter aerogenes* (9 strains), *Klebsiella* spp. (7 strains), *Enterobacter cloacae* (5 strains), *C. diversus* (3 strains), *Providencia stuartii* (2 strains), *Proteus vulgaris* (2 strains), *M. morganii* (1 strain), *Providencia rettgeri* (1 strain) and *Salmonella enteritidis* (1 strain).

\(b\) Antagonism by fractional inhibitory concentration index (10).
strains that have elevated but susceptible β-lactam MICs. Each of the drugs evaluated in this study were rarely (<2.0%) ineffective against enteric bacilli. When resistant or MS isolates were found, their higher MICs seemed to be associated with an AAC (6')-4 acetylating enzyme, a permeability mutation, or a target site alteration for amikacin and a potent β-lactamase, a permeability mutation, an altered penicillin-binding protein, or a trapping enzyme for the new β-lactams (2, 5, 7-9, 15, 16, 18, 19). Amikacin has been used with the semisynthetic penicillins in vitro and in vivo, with excellent synergy results (1, 11, 20). Few studies have been reported in which aminoglycosides were used with cefotaxime, cefoperazone, ceftazidime, or moxalactam (2, 13, 15, 20). In most of these instances, amikacin was found to be superior to other aminoglycosides by producing clinically meaningful synergy, i.e., enhanced killing at achievable in vivo drug concentrations. Few synergy evaluations have addressed the MS or resistant Enterobacteriaceae, whereas modest rates of synergy have been reported for cefotaxime, cefoperazone, or moxalactam-aminoglycoside combinations against Pseudomonas aeruginosa (2, 13, 15, 20). Moreover, synergy has proven to be valuable for the treatment of some infected patient populations, especially those with leukopenia and compromised immune mechanisms (12, 20). It also seems critical to select the antimicrobial agents that are compatible in vitro and in vivo. Amikacin has proven to be among the least inactivated aminoglycosides by high β-lactam concentrations (4).

The results of this in vitro trial provide evidence that amikacin used in combination with either cefotaxime, cefoperazone, ceftazidime, or moxalactam will produce high rates of clinically meaningful synergy against those Enterobacteriaceae isolates most likely to require multiple drug therapy. These data confirm an earlier trend toward slightly lower synergy rates with moxalactam-aminoglycoside combinations and the fact that synergy rates are usually highest for the organism having elevated MICs for the individual antibiotics. Combining amikacin and third-generation cephalosporin provides the broadest-spectrum empirical coverage for serious clinical infections among currently available drugs. Comparable antimicrobial coverage and synergy might be expected substituting other aminoglycosides, but this possibility will require supporting studies against a similarly selected organism population (R. N. Jones, manuscript in preparation).

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LITERATURE CITED


