In Vitro Susceptibility of Cephalothin-Resistant Enterobacteriaceae and Pseudomonas aeruginosa to Amikacin and Selected New β-Lactam Agents

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Amikacin was evaluated in vitro by agar dilution testing against 148 different clinical isolates of cephalothin-resistant Enterobacteriaceae and Pseudomonas aeruginosa in parallel with cephalothin, cefoxitin, moxalactam, N-formimidoyl thienamycin, ceftriaxone, and cefmenoxime. Cefsludin was also evaluated against 39 isolates of P. aeruginosa. More than 80% of all isolates tested were also gentamicin resistant, as determined by disk testing. Moxalactam and amikacin had comparable high activities against Proteus species, Escherichia coli, Serratia species, and Providencia species, and both amikacin and N-formimidoyl thienamycin had comparably high activities against the Klebsiella-Enterobacter group. N-Formimidoyl thienamycin was the most active agent against P. aeruginosa, followed by cefsludin and amikacin.

It is well known that the patterns of resistance in nosocomial bacterial pathogens change (7). Clearly, members of the Enterobacteriaceae and Pseudomonas aeruginosa appear to show increasing resistance to gentamicin and other agents (8, 13, 16, 17, 30); amikacin has been shown to be the most effective alternative aminoglycoside in vitro (11, 15). Recently, a plethora of new antimicrobial agents has been investigated in order to meet the challenge of increasing resistance and to offer agents with less potential for nephrotoxicity and ototoxicity. Moxalactam, which is a 1-oxa cephalosporin (18) with a broad spectrum of activity against most gram-positive and gram-negative bacteria, including staphylococci and anaerobic organisms (2, 6, 14, 18, 24, 29), has recently been introduced for clinical use.

Agents which have been investigated include the carbapenem N-formimidoyl thienamycin (MK0787), which differs from cephalosporins by its 6-a-hydroxethyl side chain and by the absence of sulfur on the secondary ring (9). N-Formimidoyl thienamycin has demonstrated competitive inhibition against many β-lactamases (23) and in initial studies seemed very active against most gram-positive and gram-negative organisms (11, 22, 28). Ceftriaxone (Ro 13,9904) and cefmenoxime (SCE-1365) are amnithiazolyl cephalosporins. Ceftriaxone demonstrates good activity against Haemophilus sp., Neisseria gonorrhoea, and Neisseria meningitidis and is generally as active as cefotaxime and moxalactam against members of the Enterobacteriaceae (4, 19). Broad in vitro activity has also been observed with cefmenoxime (25, 27). Cefsludin was first introduced as an antipseudomonal cephalosporin (26) and, compared with other cephalosporins, is less active than cefazidime but more active than cefoperazone or cefmenoxime against P. aeruginosa (10, 26, 27).

The aim of the present study was to compare four new β-lactam agents with moxalactam, cefoxitin, and amikacin by using multiply drug-resistant nosocomial gram-negative bacilli.

MATERIALS AND METHODS

The clinical isolates were selected from a pool of Enterobacteriaceae and P. aeruginosa isolates collected from the Wadsworth Veterans Administration Medical Center from 1975 through August 1981 and found to be resistant to cephalothin by standardized disk testing (3). Each isolate was from a different patient and was identified by standard criteria (12). Serratia marcescens was identified to species by the fermentation of arabinose. Isolates demonstrating zone sizes of <14 mm with 30-μg cephalothin disks on repeated testing (3) were tested by the agar plate dilution method recommended by the International Collaborative Study of The World Health Organization (5). In addition, 82% of the isolates had zone sizes of <12 mm with 10-μg gentamicin disks. Approximately 10° organisms grown overnight at 37°C in Mueller-Hinton broth were inoculated with a replicating device (21) onto Mueller-Hinton broth solidified with 1.5% agar (Difco Laboratories) and 5% defibrinated sheep blood and prepared to contain one of the antibiotics being tested. Amikacin, cephalothin, cefoxitin, moxalactam,
N-formimidoyl thienamycin, ceftriaxone, cefmenoxime, and cefsulodin were added to the medium in twofold dilutions from 128 to 1 μg/ml. Plates with and without sheep blood and lacking antibiotic were used as controls.

Amikacin was supplied by Bristol Laboratories, cephalothin and moxalactam were supplied by Eli Lilly & Co., cefoxitin and N-formimidoyl thienamycin were supplied by Merck Sharp & Dohme, ceftriaxone was supplied by Hoffmann-La Roche Inc., and cefmenoxime and cefsulodin were supplied by Abbott Laboratories.

The magnesium content of the liquid obtained by freeze-thaw extraction of the media (5) was 1.56 to 1.95 mg/dl (mean ± standard deviation, 1.75 ± 0.10 mg/dl), as measured by atomic absorption spectrophotometry. As determined by a similar analysis, the calcium content was 2.89 ± 0.30 mg/dl (range, 2.42 to 3.83 mg/dl).

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic showing only a haze, one colony, or no growth after 18 h of incubation (5). Only organisms with a cephalothin MIC of ≥32 μg/ml as determined by agar dilution testing were included (1). A total of 148 isolates fulfilled these criteria. Reference strains of Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 29259) were included in parallel tests; these strains had amikacin MICs of 1 and 2 μg/ml, respectively, and cephalothin MICs of 16 and <1 μg/ml, respectively.

Susceptibility to an antibiotic was based upon inhibition of an isolate at or below the peak serum levels of the antibiotic achieved in clinical use or in preclinical studies with volunteers. The following MICs in agar were considered as showing susceptibility: amikacin, ≤16 μg/ml; cephalothin, ≤16 μg/ml (1); cefoxitin, ≤32 μg/ml; moxalactam, ≤32 μg/ml; N-formimidoyl thienamycin, ≤32 μg/ml; ceftriaxone, ≤32 μg/ml; cefmenoxime, ≤32 μg/ml; cefsulodin, ≥32 μg/ml.

RESULTS

P. aeruginosa. N-Formimidoyl thienamycin showed the greatest degree of in vitro activity. A total of 97% of the strains were inhibited by a concentration of 32 μg/ml; this included several isolates that were resistant to amikacin. The anti-pseudomonal cephalosporin cefsulodin showed somewhat less activity, with 56% of the strains susceptible at a concentration of 16 μg/ml and 72% susceptible at a concentration of 32 μg/ml. In comparison, amikacin inhibited only 50% of the isolates at a concentration of 16 μg/ml, whereas 32 μg of cefmenoxime inhibited 50% of the isolates. Only 36% of the isolates were inhibited by moxalactam, and 26% were inhibited by 32 μg of ceftriaxone per ml (Table 1).

Serratia species. Amikacin and moxalactam demonstrated considerable activity, with 100% of the isolates susceptible to 16 μg of amikacin per ml and 97.5% susceptible to 32 μg of moxalactam per ml. Cefmenoxime, N-formimidoyl thienamycin, cefoxitin, and ceftriaxone showed less activity; 40% of the isolates were susceptible at concentrations of 32 μg/ml.

Klebsiella-Enterobacter group. Amikacin showed the most activity, closely paralleled by N-formimidoyl thienamycin. Cefmenoxime, ceftriaxone, and moxalactam inhibited 40 to 60% of the isolates at concentrations of 32 μg/ml, and cefoxitin showed poor activity.

Providencia sp. Moxalactam and amikacin were the most active antibiotics in vitro; both of these drugs suppressed 100% of the strains at concentrations of 16 μg/ml. However, amikacin was more active at lower concentrations; more than 50% of the isolates were inhibited at a concentration of 1 μg/ml. Ceftriaxone and N-formimidoyl thienamycin were equally effective, with 100% of the isolates inhibited by concentrations of 32 μg/ml, and cefoxitin did almost as well, with 84% of the isolates inhibited by a concentration of 32 μg/ml. Cefmenoxime did poorly.

Proteus species. Moxalactam demonstrated the most activity, with 90% of the isolates inhibited at a concentration of 16 μg/ml. Amikacin showed 90% inhibition at a concentration of 8 μg/ml. Cefmenoxime, cefoxitin, and ceftriaxone inhibited 80% of the isolates, and 32 μg of N-formimidoyl thienamycin per ml inhibited 70% of the isolates.

E. coli. Moxalactam again showed the most in vitro activity, with 100% of the strains inhibited at a concentration of 4 μg/ml. Amikacin, cefoxitin, cefmenoxime, and N-formimidoyl thienamycin were similar and inhibited 100% of the isolates at concentrations of 16 μg/ml. Ceftriaxone was less active, showing 80% inhibition at a concentration of 32 μg/ml.

DISCUSSION

Amikacin demonstrated appreciable activity against most of the cephalothin-resistant Enterobacteriaceae and P. aeruginosa strains studied, inhibiting 121 of the 148 (80%) clinical isolates tested. Because of the rather consistent activity of amikacin against these organisms, it has been used as the initial drug of choice in our institution and others for initial therapy of serious nosocomial gram-negative infections when gentamicin resistance may be encountered (14, 15, 16, 30). However, newer agents have significant activity against multiply drug-resistant Enterobacteriaceae, and some even have activity against P. aeruginosa.

Moxalactam did as well in vitro as amikacin against most Serratia species isolates and had considerable activity against Providencia species, E. coli, and Proteus species. N-Formimidoyl thienamycin was very active against P. aeruginosa, was as active as amikacin against the Klebsiella-Enterobacter group, and was very
active against Providencia, Proteus, and E. coli. Cefsulodin showed moderate activity against P. aeruginosa. Both ceftiraxone and cefmenoxime (aminothiazolyl synthetic cephalosporins) have demonstrated β-lactamase resistance, apparently due to their aminothiazolyl side chains (25). Cefsulodin is also resistant to β-lactamases. Ceftiraxone has shown as good activity against Enterobacteriaceae as cefotaxime and moxalactam (4, 19). Cefmenoxime also has demonstrated broad activity against members of the Enterobacteriaceae and has done well in a mouse model for Klebsiella pneumoniae and Proteus mirabilis urinary infections (25). There may be synergy between cefmenoxime and gentamicin, as with other β-lactam agents and aminoglycosides against Enterobacter or P. aeruginosa (20). Cefsulodin, which is very promising as an agent against P. aeruginosa (26, 27), showed less activity than ceftazidime in one study (27) and less activity than N-formimidoyl thienamycin in this study. Moxalactam, which has a methoxyl group at the 7-α position of the cepham nucleus (like cefoxitin) and an oxygen moi-
thienamycin, may also, in aminoglycosides, inhibited mycin appeared bers of cephalosporin, active ginosa (11, 22, 28).

In this study, N-formimidoyl thienamycin was unequivocally the most active agent against \textit{P. aeruginosa} and had activity similar to the activities of other newer cephalosporins against members of the \textit{Enterobacteriaceae}. Even though a specific new cephalosporin may show somewhat better activity against a specific organism than another cephalosporin, N-formimidoyl thienamycin appeared to have the most reliable in vitro activity against all nosocomial organisms tested in this study. Overall, moxalactam was the next most active \textit{b-lactam} agent. When N-formimidoyl thienamycin was not active in vitro amikacin inhibited the isolate. Thus, N-formimidoyl thienamycin, which is now entering clinical trials, may become very useful as an adjunct to aminoglycosides in empiric therapy or in use by itself.

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LITERATURE CITED


