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Susceptibility of Cephalothin-Resistant Gram-Negative Bacilli to Piperacillin, Cefuroxime, and Other Selected Antibiotics

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The in vitro antibacterial activity of piperacillin and cefuroxime against 180 isolates of cephalothin-resistant *Enterobacteriaceae* and of piperacillin against 46 isolates of *Pseudomonas aeruginosa* was determined. Amikacin, gentamicin, carbenicillin, cefoxitin, and cefamandole were included for comparison. The activities of piperacillin and carbenicillin against *Enterobacteriaceae* were comparable. Piperacillin was appreciably more active against *Pseudomonas* than carbenicillin and was equivalent in activity to amikacin on a weight basis. The following beta-lactam agents were the most active against the indicated organisms (in parentheses): cefoxitin (indole-positive *Proteus* spp.), cefuroxime and cefoxitin, (*Klebsiella* spp.), piperacillin (*Enterobacter* spp.), cefuroxime and cefoxitin (*E. coli*), piperacillin and cefoxitin (*Serratia* spp.), and cefoxitin (*Providencia* spp.). Amikacin inhibited 98% of *Enterobacteriaceae* at clinically achievable serum levels.

Infections due to multiply drug-resistant gram-negative bacilli constitute a major problem in medical therapeutics. Gentamicin has been a mainstay of therapy in cases of cephalothin-resistant gram-negative bacillary infection, but its widespread use has resulted in increasing bacterial resistance (7).

The occurrence in our hospital of *Pseudomonas aeruginosa* and *Enterobacteriaceae* resistant to gentamicin or various beta-lactam antibiotics, or both, prompted this study of the in vitro susceptibility of multiply drug-resistant, gram-negative bacilli to piperacillin, cefuroxime, carbenicillin, cefoxitin, cefamandole, gentamicin and amikacin.

Piperacillin (T-1220), a new aminobenzyl penicillin, has in vitro activity against many *Enterobacteriaceae* and *P. aeruginosa* (10). Cefuroxime, a semisynthetic cephalosporin, is a new agent that is active against many cephalothin-resistant, gram-negative bacilli (8, 11).

MATERIALS AND METHODS

From 1974 to 1977, 180 different clinical isolates of *Enterobacteriaceae* resistant to cephalothin by standardized disk testing (zone size, ≤14 mm around a 30-μg cephalothin disk) and 46 isolates of *P. aeruginosa* were collected from the microbiology laboratory of Wadsworth Hospital Center. Susceptibility patterns demonstrated in this study do not reflect the general incidence of antimicrobial resistance at Wadsworth Hospital Center. The organisms were tested by the agar plate dilution method recommended by the International Collaborative Study of the World Health Organization (2). Approximately 10^4 organisms grown overnight at 37°C in Mueller-Hinton broth culture were inoculated by Steers replicator (9) onto media containing Mueller-Hinton agar and 5% defibrinated sheep blood prepared to contain either no antibiotic (control); cefoxitin, cefamandole, amikacin, cefuroxime, or piperacillin in twofold dilutions from 128 to 1 μg/ml; carbenicillin in twofold dilutions from 512 to 32 μg/ml; or gentamicin from 32 to 1 μg/ml. The following drugs were donated (donors in parentheses): cefuroxime (J. D. Price of Glaxo Holdings Ltd.), cefamandole (R. S. Griffith of Eli Lilly & Co.), amikacin (Edward Yevak of Bristol Laboratories), cefoxitin (C. Martin of Merck, Sharp and Dohme Co.), piperacillin (C. J. Tarrant of Lederle Laboratories), gentamicin sulfate (George Hough of the Schering Corp.), and carbenicillin (R. Donnegan of the Roerig Division of Pfizer Laboratories). Reference strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *P. aeruginosa* ATCC 27853 were included in parallel tests.

The minimal inhibitory concentration was recorded as the lowest concentration of antibiotic showing only a haze, one colony, or no growth after overnight incubation at 37°C (2).

Susceptibility to gentamicin, amikacin, and carbenicillin was based on the inhibition of isolates at or below peak serum levels reliably achieved in clinical use. The following minimum inhibitory concentrations in agar were considered to indicate susceptibility: gentamicin, ≤8 μg/ml; amikacin, ≤16 μg/ml; and carbenicillin, ≤128 μg/ml.

Reliably achievable peak serum levels for the inves-

tigational agents have not been established. A minimum inhibitory concentration of ≤32 μg/ml was chosen to indicate susceptibility because peak serum levels of 32 μg/ml have been achieved in clinical investigation or preclinical trials with cefuroxime (3), cefoxitin (5), and cefamandole (4). A minimum inhibitory concentration of ≤128 μg/ml was chosen to indicate susceptibility to piperacillin (1).

All determinations were made in duplicate or triplicate, and the minimum inhibitory concentrations were expressed as geometric averages. Solidified agar with 5% defibrinated sheep blood was subjected to repetitive freeze-thawing, and the extracted fluid was analyzed by atomic absorption spectrophotometry to determine divalent cation concentration. Calcium concentration ranged from 4.06 to 5.88 mg/dl and magnesium concentration ranged from 0.73 to 1.97 mg/dl.

RESULTS

Seventy-eight of 180 isolates of *Enterobacteriaceae* and 18 of 46 *Pseudomonas aeruginosa* were susceptible to 8 μg or less of gentamicin per ml. The activities of piperacillin and carbenicillin against *Enterobacteriaceae* were equivalent (Tables 1 and 3). More than three-fourths of gentamicin-susceptible *Enterobacter* spp., *Serratia* spp., and *E. coli* were susceptible to piperacillin and carbenicillin at concentrations of ≤128 μg/ml. All gentamicin-resistant *Klebsiella* spp., *Enterobacter* spp., *Providencia* spp., and *E. coli* and more than three-fourths of gentamicin-resistant *Serratia* spp. were resistant to 128 μg of piperacillin and carbenicillin per ml.

The activity of cefoxitin was equal to or greater than that of cefuroxime and cefamandole against all genera of *Enterobacteriaceae* tested except *Enterobacter* spp. (Tables 2 and 4). The activity of cefuroxime was equal to or greater than that of cefamandole against all *Enterobacteriaceae* tested except indole-positive *Proteus* spp. Amikacin inhibited 98% of all *Enterobacteriaceae* at clinically achievable concentrations.

On a weight basis, the activities of piperacillin and amikacin were similar against *Pseudomonas*. The minimum inhibitory concentrations of carbenicillin for *Pseudomonas* were two- to

TABLE 1. *Susceptibility of cephalothin-resistant, gentamicin-susceptible, gram-negative bacilli to piperacillin, carbenicillin, and amikacin*

Organism and antibiotic	Susceptibility[a] to antibiotic at concn (μg/ml) of:									
	1	2	4	8	16	32	64	128	256	512
Indole-positive *Proteus* spp. (6)[b]										
Piperacillin	17	17	50	50	50	50	50	50	NT[c]	NT
Carbenicillin	NT	NT	NT	NT	NT	50	50	50	50	50
Amikacin	33	83	100	100	100	100	100	100	NT	NT
Klebsiella spp. (6)										
Piperacillin	0	17	17	17	33	33	33	33	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	17	17	17	17	33
Amikacin	33	83	100	100	100	100	100	100	NT	NT
Enterobacter spp. (36)										
Piperacillin	6	50	75	81	86	92	94	94	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	75	89	92	92	100
Amikacin	31	92	97	97	100	100	100	100	NT	NT
E. coli (13)										
Piperacillin	8	31	62	69	77	77	77	77	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	77	77	77	77	77
Amikacin	0	69	85	92	92	92	100	100	NT	NT
Serratia spp. (17)										
Piperacillin	24	47	71	71	82	88	88	88	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	71	71	76	76	76
Amikacin	6	47	76	88	100	100	100	100	NT	NT
P. aeruginosa (18)										
Piperacillin	0	0	6	50	89	94	100	100	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	0	44	83	83	100
Amikacin	0	0	22	72	89	94	100	100	NT	NT

[a] Expressed as cumulative percentage.
[b] Number of isolates tested in parentheses.
[c] NT, Not tested.

TABLE 2. *Susceptibility of cephalothin-resistant, gentamicin-susceptible, gram-negative bacilli to cefuroxime, cefoxitin, and cefamandole*

Organism and antibiotic	Susceptibility[a] to antibiotic at concn (μg/ml) of:							
	1	2	4	8	16	32	64	128
Indole-positive *Proteus* spp. (6)[b]								
Cefuroxime	0	17	17	33	33	33	67	83
Cefoxitin	0	17	50	67	100	100	100	100
Cefamandole	17	17	50	67	67	67	83	83
Klebsiella spp. (6)								
Cefuroxime	0	0	83	83	83	83	83	83
Cefoxitin	0	17	67	83	83	83	83	100
Cefamandole	0	33	33	33	33	33	33	33
Enterobacter spp. (36)								
Cefuroxime	3	3	19	33	61	61	69	72
Cefoxitin	3	6	6	6	6	6	6	22
Cefamandole	8	28	33	55	67	69	69	72
E. coli (13)								
Cefuroxime	0	0	15	54	85	100	100	100
Cefoxitin	0	8	23	54	77	100	100	100
Cefamandole	8	31	54	85	92	100	100	100
Serratia spp. (17)								
Cefuroxime	0	0	0	0	0	0	18	24
Cefoxitin	0	0	0	6	35	76	76	88
Cefamandole	0	0	0	0	12	12	18	35

[a] Expressed as cumulative percentage.
[b] Number of isolates tested in parentheses.

fourfold higher than for either piperacillin or amikacin.

DISCUSSION

Cefuroxime, cefoxitin, and cefamandole exhibited appreciable activity against the cephalothin-resistant *Enterobacteriaceae* tested in this study (Tables 2 and 4). Cefoxitin was the most active of these three agents. However, cefuroxime and cefamandole were superior on a weight basis to cefoxitin against *Enterobacter* spp.; cefuroxime and cefoxitin were equivalent in activity against *Klebsiella* spp. and *E. coli*. Cefamandole was generally less active against gentamicin-resistant *Enterobacteriaceae* than either cefuroxime or cefoxitin. Susceptibility to one of these three antibiotics, therefore, did not necessarily predict susceptibility to either of the other two agents (Tables 2 and 4). The activity of cefuroxime against cephalothin-resistant *Klebsiella* spp., *Enterobacter* spp., *Providencia* spp., and *E. coli* is significant; in vivo studies and additional in vitro investigation seem indicated.

Amikacin inhibited 176 of 180 *Enterobacteriaceae* tested at clinically achievable serum levels and is currently the drug of choice in our institution for initial therapy of serious nosocomial gram-negative bacillary infections. The above data, however, indicate that some of the newer beta-lactam antibiotics also have significant activity against multiply drug-resistant gram-negative bacilli. The use of one of these agents rather than an aminoglycoside for the treatment of such infections would be expected to reduce the incidence of drug-related nephrotoxicity and to eliminate the risk of ototoxicity.

If cefuroxime, cefoxitin, or cefamandole were released for clinical use, a change in hospital laboratory susceptibility testing might become necessary because of the different degrees of activity of each of these agents against isolates of various genera of *Enterobacteriaceae* noted in this and other studies (8, 12).

Piperacillin was active against many of the cephalothin-resistant, gentamicin-susceptible *Enterobacteriaceae*. Clinical trials will be necessary to determine whether the development of resistance in vivo, as occurs with carbenicillin, will be a significant problem with the use of piperacillin as a single therapeutic agent.

Multiply drug-resistant *P. aeruginosa* currently represent a serious clinical problem in our hospital (6). In this study, 32% of selected gentamicin-resistant *P. aeruginosa* were resistant to amikacin, and 46% were resistant to carbeni-

TABLE 3. *Susceptibility of cephalothin-resistant, gentamicin-resistant, gram-negative bacilli to piperacillin, carbenicillin, and amikacin*

Organism and antibiotic	Susceptibility[a] to antibiotic at concn (μg/ml) of:									
	1	2	4	8	16	32	64	128	256	512
Indole-positive *Proteus* spp. (6)[b]										
Piperacillin	17	17	50	50	67	67	67	67	NT[c]	NT
Carbenicillin	NT	NT	NT	NT	NT	50	50	50	50	50
Amikacin	17	50	100	100	100	100	100	100	NT	NT
Klebsiella spp. (17)										
Piperacillin	0	0	0	0	0	0	0	0	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	0	0	0	0	0
Amikacin	41	100	100	100	100	100	100	100	NT	NT
Enterobacter spp. (4)										
Piperacillin	0	0	0	0	0	0	0	0	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	0	0	0	0	0
Amikacin	0	50	100	100	100	100	100	100	NT	NT
E. coli (7)										
Piperacillin	0	0	0	0	0	0	0	0	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	0	0	0	0	0
Amikacin	0	100	100	100	100	100	100	100	NT	NT
Serratia spp. (50)										
Piperacillin	2	6	8	14	16	20	22	22	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	8	8	10	12	12
Amikacin	4	36	70	86	94	98	98	100	NT	NT
Providencia spp. (18)										
Piperacillin	0	0	0	0	0	0	0	0	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	0	0	0	0	0
Amikacin	17	72	100	100	100	100	100	100	NT	NT
P. aeruginosa (28)										
Piperacillin	0	0	4	36	71	82	86	89	NT	NT
Carbenicillin	NT	NT	NT	NT	NT	0	43	54	75	89
Amikacin	0	4	21	43	68	96	100	NT	NT	NT

[a] Expressed as cumulative percentage.
[b] Number of isolates tested in parentheses.
[c] NT, Not tested.

TABLE 4. *Susceptibility of cephalothin-resistant, gentamicin-resistant, gram-negative bacilli to cefuroxime, cefoxitin, and cefamandole*

Organism and antibiotic	Susceptibility[a] to antibiotic at concn (μg/ml) of:							
	1	2	4	8	16	32	64	128
Indole-positive *Proteus* spp. (6)[b]								
Cefuroxime	0	0	17	33	33	50	100	100
Cefoxitin	0	33	50	83	100	100	100	100
Cefamandole	0	17	17	17	50	100	100	100
Klebsiella spp. (17)								
Cefuroxime	0	24	65	88	94	94	94	94
Cefoxitin	0	29	71	88	94	100	100	100
Cefamandole	0	0	0	0	0	6	6	18
Enterobacter spp. (4)								
Cefuroxime	0	0	0	100	100	100	100	100
Cefoxitin	0	0	25	25	25	25	25	25
Cefamandole	0	0	0	0	0	0	25	25
E. coli (7)								
Cefuroxime	0	29	71	100	100	100	100	100
Cefoxitin	14	43	100	100	100	100	100	100
Cefamandole	0	0	0	0	14	14	43	57
Serratia spp. (50)								
Cefuroxime	0	0	0	2	2	6	10	14
Cefoxitin	0	2	2	6	36	56	74	92
Cefamandole	0	0	2	2	6	8	12	14
Providencia spp. (18)								
Cefuroxime	0	0	6	44	100	100	100	100
Cefoxitin	6	78	100	100	100	100	100	100
Cefamandole	0	0	11	50	78	89	89	89

[a] Expressed as cumulative percentage.
[b] Number of isolates tested in parentheses.

cillin at clinically achievable levels; a previous study demonstrated that 81% of gentamicin-resistant *P. aeruginosa* in our institution were also resistant to tobramycin (6). Only 11% of gentamicin-resistant *P. aeruginosa* were resistant to piperacillin at clinically achievable serum concentrations.

In vitro and in vivo studies of piperacillin, in combination with an aminoglycoside and as a single agent, should be done to evaluate the activity of this new antibiotic, particularly against multiply drug-resistant *P. aeruginosa*.

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