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Synergistic Interactions of Ciprofloxacin and Extended-Spectrum β-Lactams or Aminoglycosides against Multiply Drug-Resistant *Pseudomonas maltophilia*

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The susceptibility of 28 clinical isolates of *Pseudomonas maltophilia* to 16 antimicrobial agents was determined in vitro by a standard agar dilution method with inoculum sizes of 10^4 and 10^6 CFU. All isolates exhibited multiple drug resistance. Nine isolates were selected for studies of combinations of ciprofloxacin with seven antipseudomonal β-lactams and three aminoglycosides by a checkerboard agar dilution technique. Synergistic or additive combinations of ciprofloxacin in clinically achievable concentrations were most frequent with mezlocillin (89%), followed by cefoperazone (67%), piperacillin (56%), cefsulodin (56%), and ceftazidime (33%), and were infrequent with aztreonam (11%), the aminoglycosides (0 to 14%), or imipenem (0%). Antagonism was not observed in any combination. These data suggest that combinations of ciprofloxacin with these agents may be useful for some nosocomial multiply drug-resistant *P. maltophilia* infections.

Pseudomonas maltophilia is an important nosocomial pathogen (12) which presents a unique therapeutic challenge because of its tendency to exhibit multiple resistance to aminoglycosides and β-lactams (3, 6, 9, 13). Furthermore, synergistic activity of aminoglycoside–β-lactam combinations is infrequently observed (3, 9). We examined the in vitro susceptibility of 28 clinical isolates of *P. maltophilia* to ciprofloxacin and three other quinolones (norfloxacin, difloxacin, and A-56620), as well as to nine extended-spectrum β-lactams (carbenicillin, ticarcillin, piperacillin, imipenem, SCH-34343, aztreonam, ceftazidime, cefoperazone, and cefsulodin) and three aminoglycosides (gentamicin, tobramycin, and amikacin), by an agar dilution method. Selected strains were used to test for synergy of ciprofloxacin in combination with other agents by a checkerboard agar dilution technique. Demonstration of synergistic activity in vitro with these antimicrobial combinations may suggest useful therapeutic regimens for these difficult infections in the clinical setting.

A total of 28 *P. maltophilia* isolates obtained from patients in Vancouver General Hospital were available for study. Sources included sputum (7 isolates), wounds (7 isolates), urine (2 isolates), cerebro spinal fluid (1 isolate), and miscellaneous sites (11 isolates). In vitro susceptibility to each antibiotic was determined by the agar dilution method (1). A Steers replicator was used to deliver a 0.0025-ml inoculum onto the surface of Mueller-Hinton agar (BBL Microbiology Systems) supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) and containing twofold serial dilutions of the test antibiotics. Unsupplemented Mueller-Hinton agar contained 1.5 mM calcium and 0.7 mM magnesium, as determined by atomic absorption spectrophotometry. The final cation concentrations in the supplemented medium were 2.7 mM calcium and 1.7 mM magnesium. The effect of inoculum was assessed by testing both inoculum sizes, 10^4 and 10^6 CFU per spot, for each organism. Plates without antibiotics served as controls. All plates were incubated for 24 h at 37°C. The MIC was the lowest concentration of antibiotic which permitted no visible growth. Three reference strains, *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923, were included in each experiment for quality control. The MICs of each antibiotic for these strains were all within the expected ranges (7, 8) and did not vary more than 1 twofold dilution in 10 separate experiments (95% confidence interval).

Nine isolates with clear resistance to ciprofloxacin (MIC, ≥4 μg/ml) were selected for the combination studies. Combinations of ciprofloxacin with seven extended-spectrum β-lactams (imipenem, aztreonam, mezlocillin, piperacillin, ceftazidime, cefoperazone, and cefsulodin) and three aminoglycosides (gentamicin, tobramycin, and amikacin) were examined by a two-dimensional checkerboard agar dilution technique. Twofold serial dilutions of antibiotics were prepared to give initial concentrations four times the MICs of the respective antibiotics alone as determined in individual susceptibility tests. Combinations of antibiotics were added with one drug diluted along the x axis and the other drug diluted along the y axis. Thus, for a given range of dilutions every possible combination of drug concentrations was achieved. Plates were inoculated with 10^4 CFU per inoculum. In evaluating the combination effects, the ratio of the MIC of one antibiotic in the combination to the MIC of that antibiotic used alone, termed the fractional inhibitory concentration (FIC), was calculated for each antibiotic in each combination, and the FICs were then summated (ΣFIC). Synergy and antagonism were defined as a minimum ΣFIC of ≤0.5 and a maximum ΣFIC of ≥4.0, respectively. Additive or indifferent interactions were defined as ΣFICs in between these two parameters. Interaction indices were noted particularly if they occurred at antibiotic concentrations that can be readily achieved in serum clinically.

The 28 *P. maltophilia* strains were highly resistant to all β-lactams and aminoglycosides, regardless of the inoculum size (Table 1). They were also resistant to cipofloxacin, difloxacin, and A-56620 (MIC for 50% of isolates, 2 to 4 μg/ml; MIC for 90% of isolates, 16 μg/ml; range, 0.25 to 16 μg/ml). With a larger inoculum (10^6 CFU), the MICs of these

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TABLE 1. Susceptibilities of *P. maltophilia* to ciprofloxacin and other antibiotics, singly and in combination[a]

Antibiotic	Single-agent (n = 28) MIC (μg/ml)[b]			Minimum ΣFIC of agent in combination with ciprofloxacin		No. of isolates for which ΣFIC was ≤0.5	Maximum ΣFIC of agent in combination with ciprofloxacin		No. of isolates for which ΣFIC was >2.0
	50%	90%	Range	Median	Range		Median	Range	
Ciprofloxacin	4	16	0.5–16						
Norfloxacin	32	≥128	0.25–≥128						
Difloxacin	4	16	0.5–16						
A-56620	2	16	0.25–16						
Carbenicillin	500	≥1,000	16–≥1000						
Ticarcillin	500	≥1,000	8–≥1000						
Piperacillin	250	≥1,000	8–≥1000	0.50	0.19–0.62	6	1.03	1.01–1.50	0
Mezlocillin	128	128	16–256	0.38	0.19–0.75	5	1.03	1.02–2.06	2
Imipenem	≥128	≥128	16–≥128	0.75	0.38–1.01	3	1.25	1.03–2.13	2
Sch 34343	64	≥128	16–≥128						
Aztreonam	≥128	≥128	8–≥128	0.50	0.19–1.01	6	1.02	0.63–2.02	1
Ceftazidime	32	≥128	2–≥128	0.38	0.16–0.50	9	1.03	1.02–2.06	4
Cefoperazone	64	≥128	8–≥128	0.38	0.27–1.0	6	1.02	0.63–1.06	0
Cefsulodin	≥128	≥128	16–≥128	0.27	0.19–0.56	6	1.01	0.51–1.13	0
Gentamicin	100	≥400	3.1–≥400	0.50	0.26–1.01	3[c]	1.25	1.00–2.06	1[c]
Tobramycin	200	≥400	0.8–≥400	0.53	0.50–1.01	3	1.13	1.00–2.06	2
Amikacin	200	≥400	0.8–≥400	0.51	0.19–1.01	2[c]	1.25	1.00–2.06	2[c]

[a] Nine isolates were used in combination tests, except as noted otherwise.
[b] 50 and 90%, MIC for 50 and 90% of isolates, respectively.
[c] Only seven isolates were tested.

agents for 50% of isolates were at least four times higher, while the MICs for 90% of isolates were at least two times higher (data not shown). All isolates were highly resistant to norfloxacin.

Nine isolates relatively resistant to ciprofloxacin (MIC, ≥4 μg/ml) were selected for combination studies with ciprofloxacin (Table 1). Although in vitro synergy (minimum ΣFIC, ≤0.5) was most frequent with ceftazidime (100% of isolates), followed by piperacillin, cefoperazone, cefsulodin, and aztreonam (67% each), mezlocillin plus ciprofloxacin was the most active combination at clinically achievable concentrations (either synergistic or additive for 89% of isolates), followed by cefoperazone (67%), piperacillin (56%), cefsulodin (56%), and ceftazidime (33%) plus ciprofloxacin (Table 2). For each antibiotic combination, the interaction indices at each antibiotic concentration for all isolates were tabulated. Synergistic interactions (ΣFIC, <0.5) were most frequently observed with cefsulodin (27% of total indices) and ceftazidime (23%), followed by mezlocillin (19%), aztreonam (18%), cefoperazone (14%), piperacillin (13%), the aminoglycosides (4 to 8%), and imipenem (4%). These were well above the 1% frequency which Haller considered as expected "background activity" due to methodological variations of the checkerboard assay (10). Antagonism (maximum ΣFIC, >4.0) was not observed in any combination, although several antibiotic combinations yielded maximum ΣFICs of >2.0 (Table 1).

The in vitro susceptibility of *P. maltophilia* recorded here was similar to that previously reported by Felegie et al. (9) and several others (3, 6, 13, 15), all of whom demonstrated the multiple resistance of these organisms to antipseudomonal β-lactams and aminoglycosides. Our data indicated that ciprofloxacin and the quinolones from Abbott Laboratories (difloxacin and A-56620) were also only moderately active. These results were similar to those of Kelley et al. (14) and several others (4–6, 13), even though different methods of susceptibility testing were used by these authors. It has been reported by several investigators that the cation concentration and type of medium used for susceptibility testing may greatly influence the in vitro activity of β-lactams, aminoglycosides, and quinolones (1, 2, 9, 11, 17). Although the National Committee for Clinical Laboratory Standards specifically states that Mueller-Hinton agar should not be supplemented with cations, we feel that it is prudent to routinely determine the calcium and magnesium concentrations of the medium to be used to appropriately assess the need for supplementation, since variations in media between lots and from different manufacturers do exist (1). The lot of Mueller-Hinton agar we used was low in calcium and magnesium and required supplementation to attain physiologic concentrations (calcium, 2.25 to 2.75 mM; magnesium, 0.75 to 1.25 mM).

Our finding that synergistic or additive activity against *P. maltophilia* was relatively frequent for combinations of ciprofloxacin with mezlocillin, cefoperazone, piperacillin, and cefsulodin at clinically achievable concentrations and, to a lesser extent, with ceftazidime, aztreonam, and aminoglycosides is of considerable interest. The mechanisms of

TABLE 2. Susceptibilities of *P. maltophilia* to ciprofloxacin in combination with other antibiotics

Antibiotic	Achievable concn (μg/ml)[a]	No. of strains tested	No. of isolates with:		
			ΣFIC ≤ 0.5 (Synergistic)[b]	1 ≥ ΣFIC > 0.5 (Additive)[b]	4 > ΣFIC > 1 (Indifferent)
Mezlocillin	64	9	5 (5)	4 (3)	0
Piperacillin	64	9	6 (3)	3 (2)	0
Imipenem	16	9	3 (0)	4 (0)	2
Aztreonam	16	9	6 (1)	2 (0)	1
Ceftazidime	32	9	9 (3)	0	0
Cefoperazone	32	9	6 (4)	3 (2)	0
Cefsoludin	32	9	6 (5)	3 (0)	0
Gentamicin	8	7	3 (1)	2 (0)	2
Tobramycin	8	9	3 (0)	4 (0)	2
Amikacin	32	7	2 (1)	4 (0)	1

[a] The concentration of ciprofloxacin readily achievable in serum was considered to be 2 μg/ml.
[b] Numbers in parentheses represent the numbers of isolates inhibited at clinically achievable concentrations of the antibiotic combinations.

synergy between these agents are unknown. Whether similar synergistic activity can also be demonstrated with the time-kill curve technique or with a larger inoculum will require further study. Several authors have pointed out the relative lack of agreement between the checkerboard and time-kill curve techniques in demonstrating antimicrobial synergy against pseudomonads (3, 9, 10). Apart from ciprofloxacin combinations demonstrated in the present study, the frequent occurrence of synergistic interactions against *P. maltophilia* has also been observed for combinations containing trimethoprim-sulfamethoxazole and carbencillin with or without rifampin (9, 16) and for triple combinations of gentamicin, carbenicillin, and rifampin (16). The clinical relevance of these in vitro interactions will need to be confirmed by in vivo investigations of therapeutic efficacy. Nevertheless, our data indicate that ciprofloxacin does not appear to antagonize the activity of several extended-spectrum β-lactams and aminoglycosides, and combinations of ciprofloxacin with these agents may prove useful for the management of difficult infections caused by multiply drug-resistant *P. maltophilia*.

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