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Bactericidal Activity of Ciprofloxacin against Amikacin- and Cefotaxime-Resistant Gram-Negative Bacilli and Methicillin-Resistant Staphylococci

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The MICs and MBCs of ciprofloxacin were determined for clinical isolates of antibiotic-resistant aerobic bacteria. Decreased susceptibility to ciprofloxacin of cefotaxime- and amikacin-resistant *Serratia marcescens* and amikacin-resistant *Pseudomonas aeruginosa* strains were noted. The data suggest that ciprofloxacin susceptibility should be carefully monitored in treating patients with hospital-acquired bacterial infections.

Ciprofloxacin is highly active against most aerobic gram-positive and -negative bacteria in vitro (1, 3, 5, 7, 21). Primary resistance to ciprofloxacin among aerobic pathogens is rare (6, 12). However, resistant strains may be isolated after treatment (4, 12, 14), and mutants may be selected in the laboratory by exposure to this agent (16). In this study, we report a significantly decreased susceptibility to ciprofloxacin among cefotaxime- and amikacin-resistant nosocomial pathogens not previously exposed to this class of antibiotic.

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Organisms. The organisms used in this study were clinical isolates that were recovered and identified by standard techniques in the Clinical Microbiology Laboratory of the New York Veterans Administration Medical Center. The isolates were obtained before clinical use of ciprofloxacin in this hospital, and they were frozen and stored at −70°C from identification until use in this study.

Antibiotics. Ciprofloxacin was obtained from Miles Pharmaceuticals, West Haven, Conn. Cefotaxime was obtained from Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J., and amikacin was obtained from Bristol Laboratories, Syracuse, N.Y. The antibiotics were dissolved in sterile water and diluted in sterile medium for each experiment.

Susceptibility tests. Antibiotic susceptibilites were determined by macrotube dilution as described by the National Committee for Clinical Laboratory Standards (20). Serial twofold dilutions of the antibiotics were prepared in Mueller-Hinton Broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium and magnesium (50 and 25 mg/liter, respectively). Samples of 10^5 bacteria were added to a final volume of 1 ml to aliquots of the antibiotic broth solutions in glass tubes, and after gentle agitation, these were incubated at 35°C. The MIC of the antibiotic was defined as the lowest concentration which inhibited visible growth (turbidity) in the broth mixtures after overnight incubation. Clear tubes were subcultured onto antibiotic-free Mueller-Hinton agar (Difco) with a calibrated 0.01-ml sterile loop to determine the MBC of the antibiotic. This was defined as the lowest concentration of the antibiotic that killed 99.9% of the inoculum. Susceptibilities of gram-negative bacteria to ciprofloxacin, cefotaxime, and amikacin were tested simultaneously. Criteria for determining susceptibility and resistance to amikacin (MIC ≥ 64 μg/ml) and cefotaxime (MIC ≥ 64 μg/ml) were as previously described (20). A reference strain of *Pseudomonas aeruginosa* (ATCC 27853) or *Escherichia coli* (ATCC 25922) was included in each run with the

TABLE 1. Ciprofloxacin susceptibilities of amikacin-susceptide and -resistant gram-negative bacilli

Species	Amikacin	No. of strains tested	MIC (μg/ml)		P	MBC (μg/ml)		P
			Range	Mean (SD)		Range	Mean (SD)	
K. pneumoniae	Susceptible or moderate[a]	9	0.03–0.25	0.086 (0.07)		0.06–0.25	0.095 (0.06)	
	Resistant[b]	11	0.06–1	0.096 (0.64)	0.293	0.06–1	0.210 (0.27)	0.225
S. marcescens	Susceptible or moderate	10	0.06–0.5	0.318 (0.26)		0.125–0.5	0.462 (0.32)	
	Resistant	10	0.5–2	1.1 (0.52)	<0.001	0.5–2	1.5 (0.67)	<0.001
P. aeruginosa	Susceptible or moderate	8	0.25–4	0.875 (1.28)		0.5–8	1.938 (2.48)	
	Resistant	12	0.5–16	8.625 (6.10)	0.0025	2–32	15.0 (9.63)	0.0015

[a] Susceptible or moderately susceptible to amikacin, MIC ≤ 32 μg/ml.
[b] Resistant to amikacin, MIC > 32 μg/ml.

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TABLE 2. Ciprofloxacin susceptibilities of cefotaxime-susceptible and resistant gram-negative bacilli

Species	Cefotaxime	No. of strains tested	MIC (μg/ml)		P	MBC (μg/ml)		P
			Range	Mean (SD)		Range	Mean (SD)	
S. marcescens	Susceptible or moderate[a]	11	0.06–0.5	0.290 (0.15)		0.125–0.5	0.42 (0.14)	
	Resistant[b]	9	1–2	1.222 (0.44)	<0.001	0.5–2	1.667 (0.5)	<0.001
P. aeruginosa	Susceptible or moderate	6	0.25–8	3.5 (3.76)		1–16	7.333 (7.2)	
	Resistant	14	0.25–16	6.392 (6.81)	0.345	0.5–32	10.821 (11.0)	0.487

[a] Susceptible or moderately susceptible to cefotaxime, MIC ≤ 32 μg/ml.
[b] Resistant to cefotaxime, MIC > 32 μg/ml.

gram-negative bacteria, and *Staphylococcus aureus* (ATCC 29213) or *Streptococcus faecalis* (ATCC 29212) was used during tests with gram-positive bacteria. The MICs of the test antibiotics for the reference strains were within accepted standards (20) for each experiment. All statistical comparisons were by Student's *t* test.

In preliminary studies, we found that most of the clinical isolates of aerobic gram-negative bacteria tested were susceptible to amikacin and cefotaxime, and that these organisms were also exquisitely susceptible to ciprofloxacin (data not shown). However, a substantial number of the *Klebsiella pneumoniae*, *Serratia marcescens*, and *P. aeruginosa* isolates tested were resistant to amikacin. There was no difference in the ciprofloxacin susceptibilities of the amikacin-susceptible and -resistant *K. pneumoniae* strains (Table 1). However, amikacin-resistant *S. marcescens* and *P. aeruginosa* isolates were significantly more resistant to ciprofloxacin than were the amikacin-susceptible organisms.

Of the *P. aeruginosa* strains, 70% were resistant to cefotaxime, and there was no difference in ciprofloxacin susceptibility among these organisms (Table 2). However, *S. marcescens* strains that were resistant to cefotaxime were more resistant to ciprofloxacin than were the cefotaxime-susceptible isolates. Of the 20 *S. marcescens* strains tested, 9 were susceptible to cefotaxime and amikacin, and 8 were resistant to both. The mean (± standard deviation) MIC of ciprofloxacin for the susceptible organisms was 0.243 (±0.12) μg/ml (range, 0.06 to 0.5 μg/ml), and the mean (± standard deviation) MIC for resistant strains was 1.25 (±0.46) μg/ml (range 1 to 2 μg/ml) ($P \leq 0.001$).

The activity of ciprofloxacin against gram-positive bacteria was recorded (Table 3). Ciprofloxacin was active against methicillin-susceptible and -resistant *S. aureus* and *Staphylococcus epidermidis*, penicillin-susceptible and -resistant *Streptococcus pneumoniae* strains, and enterococci.

Ciprofloxacin has remarkably broad-spectrum activity against aerobic bacteria in vitro (1, 3, 5, 7, 21), and it is effective in treating experimental (9, 17, 19) and clinical infections (6, 12). However, Sanders et al. have selected resistant mutants by incubation of organisms of the *Enterobacteriaceae* family or *P. aeruginosa* with quinolone, aminoglycoside, or β-lactam antibiotics in the laboratory (16). Some of these mutants were cross-resistant to different classes of antibiotics. Other workers have isolated mutants that are resistant to nalidixic acid and other antibiotics because of outer membrane protein alterations (10, 13). Recently, Sanders and Watanakunakorn reported serial isolates of *S. marcescens* from a patient treated with ticarcillin and tobramycin followed by cefazolin and gentamicin. Posttherapy isolates were resistant to ciprofloxacin as well as to cefotaxime and aminoglycosides, including amikacin. They found evidence of altered outer membrane proteins and an aminoglycoside acetylating enzyme (AAC 6′) in the resistant isolate (15).

At the time that our study was performed, the newer quinolone antibiotics had not been used in this hospital. We have previously noted a high level of amikacin resistance among nosocomial pathogens studied, including *K. pneumoniae*, *S. marcescens*, and *P. aeruginosa* (11). Our data suggest that some cross-resistance to ciprofloxacin occurred among these organisms, particularly *P. aeruginosa* strains. The cefotaxime- and amikacin-resistant *S. marcescens* strains were less susceptible to ciprofloxacin than the susceptible strains were, but most were inhibited by clinically achievable concentrations of the antibiotic (2, 6, 8, 12).

The decreased susceptibility to ciprofloxacin among amikacin-resistant *P. aeruginosa* strains and cefotaxime- and amikacin-resistant *S. marcescens* strains suggests that this antibiotic should be used with caution in institutions where resistance to the newer cephalosporins and amikacin exists.

TABLE 3. Ciprofloxacin susceptibilities of methicillin- and pencillin-susceptible and -resistant gram-positive bacteria

Organism	No. of strains tested	MIC (μg/ml)			MBC (μg/ml)		
		Range	50%	90%	Range	50%	90%
S. aureus (methicillin susceptible)[a]	20	0.25–0.5	0.25	0.5	0.5–8	0.5	2
S. aureus (methicillin resistant)	20	0.125–2	0.25	0.5	0.25–8	1	4
S. epidermidis (methicillin susceptible)	10	0.125–0.25	0.25	0.25	0.125–1	0.5	0.5
S. epidermidis (methicillin resistant)	10	0.125–0.25	0.125	0.25	0.125–0.5	0.5	0.5
Enterococci	10	0.125–1	1	1	0.25–2	1	2
S. pneumoniae (penicillin susceptible)[b]	12	0.5–2	1	1	1–16	2	8
S. pneumoniae (penicillin resistant)	13	0.25–2	1	1	2–8	4	8

[a] Methicillin susceptibility was determined by zone size with a l-μg oxacillin disk. A zone size of >13 mm indicated susceptibility; a zone size of ≤10 μg indicated resistance.
[b] Penicillin susceptibility was determined by tube and agar dilution (18). An MIC of <0.1 μg/ml indicated susceptibility; an MIC of ≥0.1 μg/ml indicated resistance.

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