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In Vitro Activity of Ciprofloxacin Combined with Azlocillin

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A ciprofloxacin plus azlocillin broth microdilution checkerboard was evaluated against 125 aerobic gram-negative and gram-positive bacteria. Synergism (ΣFIC ≤ 0.5) occurred among 56% of *Pseudomonas aeruginosa*, 30% of *Acinetobacter* species, and 40% of *Staphylococcus aureus* studied. Antagonism (ΣFIC ≥ 2) was present in <1% of the organisms.

Ciprofloxacin is a broad-spectrum quinolone derivative that has been the subject of several papers on in vitro susceptibility (1–4, 6, 13). The purpose of this study was to investigate the potential in vitro interactions of ciprofloxacin combined with azlocillin against 125 aerobic gram-negative bacilli and gram-positive cocci. Azlocillin and ciprofloxacin standard antibiotic powders were obtained from Miles Pharmaceuticals, West Haven, Conn. The organisms studied were clinical isolates with a high rate of aminoglycoside resistance. Among the gram-negative bacilli, 55% were resistant to gentamicin (≥8 μg/ml), 46% to tobramycin (≥8 μg/ml), and 9% to amikacin (≥32 μg/ml) as defined by the National Committee for Clinical Laboratory Standards (10).

TABLE 1. Single-agent susceptibility (in μg/ml) and cumulative interaction indices between ciprofloxacin and azlocillin

Organism (no. tested)	MIC			Index	Cumulative interaction indices			
	Ciprofloxacin		Azlocillin					
	Range	90%	90%		Synergism	Indifference	Antagonism	ND[a]
Staphylococcus aureus (penicillin resistant) (10)	0.25–1	1	64	ΣFIC	4/10	6/10		
Streptococcus pneumoniae (1 strain penicillin resistant) (10)	0.5–1	1	0.125	ΣFIC		10/10		
Enterococcus (10)								
Streptococcus faecalis (8)	1–2	2	2	ΣFIC		8/8		
Streptococcus avium (2)	0.5–1	1	64	ΣFIC		2/2		
Pseudomonas aeruginosa (25)	0.125–2	2	128	ΣFIC	14/25	11/25		
Acinetobacter calcoaceticus var. *anitratus* (10)	0.125–2	1	128	ΣFIC	3/10	7/10		
Escherichia coli (10)	≤0.015–0.03	≤0.015	512	ΣFIC		8/10		2/10
Klebsiella pneumoniae (12)	≤0.015–4	0.06	>512	ΣFIC	1/12	2/12		9/12
Enterobacter aerogenes (8)	≤0.015–0.5	0.5	>512	ΣFIC	1/8	3/8		4/8
Serratia marcescens (30)	0.03–1	0.125	>512	ΣFIC	1/30	16/30	1/30	12/30

[a] ND, Interaction not determinable, azlocillin MIC not defined with test system.

All isolates were stored at −80°C on glass beads in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% glycerol until subcultured to tryptic soy agar with 5% sheep blood agar (GIBCO Diagnostics, Madison, Wis.) (5).

A two-panel broth microdilution checkerboard consisted of serial twofold dilutions of ciprofloxacin (16 to 0.015 μg/ml) and azlocillin (512 to 0.06 μg/ml) produced with an MIC-2000 96-channel dispenser (Dynatech Laboratories, Inc., Alexandria, Va.) (8). Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was supplemented to contain 52 μg of calcium and 25 μg of magnesium per ml as previously described (9). Prepared panels were stored at −80°C untilused. All organisms, except *Streptococcus pneumoniae*, were suspended in broth and diluted in sterile water. The *S. pneumoniae* inoculum was prepared by a modification of the Thornsberry and Swenson method (12) in which the *S. pneumoniae* was suspended and diluted in Trypticase soy broth to match a McFarland 0.5 barium sulfate standard from an overnight sheep blood agar growth in 5% CO_2. A 20-μl sample was then added to 9 parts of supplemented Mueller-Hinton broth and 1 part of fresh defibrinated horse blood (Wilfer Laboratories, Stillwater, Minn.). Portions (0.1 ml) were dispensed into thawed microtiter panels, halving the previously stated checkerboard concentrations. All panels contained a final organism inoculum density of 5×10^4 to 1×10^5 CFU/ml and were incubated overnight (20 to 22 h) at 35°C and read as MICs. Standard reference organisms were tested biweekly for the control of dilution tests.

Fractional inhibitory concentrations (ΣFIC) were calculated as described by Hallander et al. (7). The interaction index (I) was defined as synergism if I ≤ 0.5, as antagonism if I ≥ 2.0, and as indifferent if 0.5 < I < 2.0.

Ciprofloxacin ranges and the MIC at which 90% of the strains were inhibited (MIC_{90}), azlocillin MIC_{90} determinations, and ciprofloxacin plus azlocillin interaction indices

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TABLE 2. Comparison of single-agent susceptibility and combination interactions

Organism (no.) and index	Interaction	Ciprofloxacin		Azlocillin	
		No. with MIC <2 μg/ml	No. with MIC ≥2 μg/ml	No. with MIC <128 μg/ml	No. with MIC ≥128 μg/ml
Non-pseudomonas (100) ΣFIC	Synergism	8	2	6	4
	Indifference	58	4	38	24
	Antagonism	1	0	0	1
	Not evaluable	27	0	2	25
Pseudomonas aeruginosa (25) ΣFIC	Synergism	9	5	11	3
	Indifference	8	3	11	0
	Antagonism	0	0	0	0

(ΣFIC) are shown in Table 1. Among all isolates studied, ciprofloxacin resistance (>2 μg/ml) was <1%, and azlocillin resistance (≥128 μg/ml) was 46%.

ΣFIC synergism was most evident among *Pseudomonas aeruginosa* (56%), *Staphylococcus aureus* (40%), and *Acinetobacter calcoaceticus* var. *anitratus* (30%). Rudin et al. (11) have also reported ciprofloxacin plus azlocillin synergism in 60% of ten *P. aeruginosa* studied. In our study, organisms which could not be evaluated for an interaction index exhibited undefined azlocillin endpoints in the test system. Most organisms (58%) were indifferent to the combination by ΣFIC, and <1% of the organisms displayed ΣFIC antagonism.

Initial single-agent resistance or sensitivity was not predictive of ciprofloxacin plus azlocillin combination interactions. As shown in Table 2, all organisms including *P. aeruginosa* lacked predictability, i.e., sensitive organisms exhibited synergism and resistant organisms exhibited antagonism or vice versa. Among all the organisms tested, sensitivity to both drugs or resistance to both antibiotics was not predictive of the combination interaction. Among the organisms susceptible to both ciprofloxacin and azlocillin (46%), 26% showed synergism, 74% exhibited indifference, and none showed antagonism by ΣFIC. There were too few organisms resistant to both antibiotics to evaluate this group.

Future investigations to evaluate ciprofloxacin in combination with other agents should be designed to evaluate clinical or treatment efficacy and to determine the implications of the potential synergism or antagonism observed with the checkerboard method in vitro.

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