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In Vitro Activities of Five Fluoroquinolone Compounds against Strains of *Streptococcus pneumoniae* with Resistance to Other Antimicrobial Agents

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Ciprofloxacin, clinafloxacin, PD 131628, sparfloxacin, and trovafloxacin were tested against 236 strains of *Streptococcus pneumoniae*, most of which were resistant to other agents. Resistance to multiple antibiotics did not affect the organism's susceptibility to the fluoroquinolones. The fluoroquinolones with in vitro antipneumococcal activity might be particularly useful against strains that are resistant to the more traditional therapeutic agents.

The currently marketed fluoroquinolone compounds have relatively poor in vitro activity against most gram-positive cocci, including *Streptococcus pneumoniae*. Consequently, they have limited value for treating pneumococcal infections (12). Recently, there has been a dramatic increase in the prevalence of pneumococci that are no longer susceptible to drugs that have been used in the past, and alternative therapeutic agents are needed (1, 4). Several fluoroquinolones with improved activities against gram-positive cocci, including *S. pneumoniae*, are currently under investigation. We have challenged four of the pneumococcus-active fluoroquinolones to document their in vitro potencies against 236 isolates that were selected to include a predominance of drug-resistant strains. Included were 201 clinical isolates of *S. pneumoniae* that were originally recovered between 1993 and 1995 from a variety of patients throughout the United States. A few (35) stock cultures were added to increase the number of antibiotic-resistant strains. The data were examined to determine whether resistance to one or more unrelated antimicrobial agents might affect an organism's susceptibility to the fluoroquinolones.

Broth microdilution susceptibility tests were performed according to the procedure outlined by the National Committee for Clinical Laboratory Standards (7). Cation-adjusted Mueller-Hinton broth was supplemented with lysed horse blood (2 to 3%), and MICs were recorded after incubation for 20 to 24 h at 35°C without added CO_2. The few strains that failed to grow under those conditions were not included in this series. The inocula consisted of approximately 5×10^5 CFU/ml as confirmed by periodic colony counts.

Fluoroquinolones included in this study were obtained from the following manufacturers: Bayer Corp., West Haven, Conn. (ciprofloxacin); Parke-Davis Pharmaceuticals, Ann Arbor, Mich. (clinafloxacin and PD131628); Rhone-Poulenc Rorer Central Research, Collegeville, Pa. (sparfloxacin); and Pfizer Central Research, Groton, Conn. (trovafloxacin). The concentrations of the serially diluted agents ranged from 0.03 to 16 μg/ml for ciprofloxacin and from 0.016 to 8.0 μg/ml for the other fluoroquinolones. Microdilution test panels also contained breakpoint concentrations of nonquinolone antimicrobial agents to permit categorical classification of each strain with seven different nonquinolone compounds. The antimicrobial agents and breakpoint concentrations that were tested are shown in Table 1. Agar dilution and disk diffusion methods were used to confirm chloramphenicol resistance among the 50 strains that grew in the presence of that drug at a concentration of 4.0 μg/ml in the microdilution trays. The agar dilution MICs were 8.0 μg/ml (11 strains) or 16 μg/ml (39 strains), and all strains gave zones of ≤18 mm in diameter around a 30-μg chloramphenicol disk.

Antibiotic susceptibility and resistance results for the isolates that were selected for this study are described in Table 1. Erythromycin resistance occurred among 34% of the 236 isolates. All clindamycin-resistant strains (15%) were also resistant to erythromycin. All 50 chloramphenicol-resistant strains

TABLE 1. In vitro susceptibilities of a challenge set of *S. pneumoniae* isolates to 12 antimicrobial agents

Antimicrobial agent	% of 236 isolates that are[a]:			Range of 236 MICs (μg/ml)[b]	
	Susceptible	Intermediate	Resistant	Minimum	Maximum
Nonquinolones					
Penicillin	25 (≤0.06)	27 (0.12–1.0)	48 (≥2.0)		
Cefotaxime	52 (≤0.5)	28 (1.0)	20 (≥2.0)		
Erythromycin	65 (≤0.25)	1 (0.5)	34 (≥1.0)		
Clindamycin	84 (≤0.25)	1 (0.5)	15 (≥1.0)		
Trimethoprim-sulfamethoxazole[c]	36 (≤0.5)	19 (1–2)	45 (≥4.0)		
Tetracycline	66 (≤2.0)	0 (4.0)	34 (≥8.0)		
Chloramphenicol	79 (≤4.0)	NA[d]	21 (≥8.0)		
Fluoroquinolones					
Ciprofloxacin				0.25	4
Sparfloxacin				0.12	1
PD 131628				0.06	1
Trovafloxacin				0.03	0.5
Clinafloxacin				0.03	0.25

[a] MIC breakpoints that were used for this study (in micrograms per milliliter) are noted in parentheses.
[b] MIC ranges are described for fluoroquinolones since there are no MIC breakpoints for these compounds.
[c] Trimethoprim-sulfamethoxazole ratio, 1:19; MICs are expressed as trimethoprim concentrations.
[d] NA, not applicable; i.e., no intermediate category has been assigned.

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TABLE 2. Susceptibility of penicillin-susceptible, -intermediate, and -resistant pneumococci to six different antimicrobial agents

Penicillin category[a]	Antimicrobial agent	Percentage of isolates that were[b]:		
		Susceptible	Intermediate	Resistant
Pen-S	Cefotaxime	100	0	0
Pen-I	Cefotaxime	83	11	6
Pen-R	Cefotaxime	9	53	37
Pen-S	Erythromycin	82	1	17
Pen-I	Erythromycin	75	0	25
Pen-R	Erythromycin	51	0	49
Pen-S	Clindamycin	98	0	2
Pen-I	Clindamycin	91	0	9
Pen-R	Clindamycin	72	2	26
Pen-S	Trimethoprim-sulfamethoxazole	77	13	10
Pen-I	Trimethoprim-sulfamethoxazole	42	19	39
Pen-R	Trimethoprim-sulfamethoxazole	12	21	67
Pen-S	Tetracycline	92	0	8
Pen-I	Tetracycline	63	0	37
Pen-R	Tetracycline	54	0	46
Pen-S	Chloramphenicol	97	NA[c]	3
Pen-I	Chloramphenicol	83	NA	17
Pen-R	Chloramphenicol	67	NA	33

[a] Sixty penicillin-susceptible (Pen-S), 64 penicillin-intermediate (Pen-I), and 112 penicillin-resistant (Pen-R) strains of *S. pneumoniae* were tested against the six drugs that are listed.
[b] MIC breakpoints defined in Table 1 were used to assign each of the six drugs listed to interpretive categories.
[c] NA, not applicable (no intermediate category defined).

were also resistant to tetracycline. For ciprofloxacin, 90% of the strains were inhibited by ≤1.0 μg/ml, 9% required MICs of 2.0 μg/ml, and 1% required MICs of 4.0 μg/ml. Ciprofloxacin MICs of ≥8.0 μg/ml were not observed in this challenge set of pneumococci. The four other fluoroquinolones inhibited all 236 isolates at concentrations of ≤1.0 μg/ml (see Table 1 for MIC ranges).

The antibiotic-resistant strains were predominantly penicillin resistant or intermediate, whereas penicillin-susceptible strains were rarely resistant to the other compounds (Table 2). Most (44 of 60) penicillin-susceptible strains were susceptible to the six other nonquinolone drugs, but only 12 of 64 penicillin-intermediate strains and 1 of 112 penicillin-resistant strains were susceptible to all the other drugs. Eighteen strains (17 penicillin resistant) were resistant or intermediate to all seven nonquinolone drugs, and 14 additional strains were susceptible to no more than one of the agents.

Table 3 defines the in vitro potencies of all of the fluoroquinolones as geometric mean MICs; the newer fluoroquinolones were approximately four to eight times more active than ciprofloxacin. For pneumococci that were susceptible, intermediate, or resistant to each of seven nonquinolone antibiotics, geometric mean MICs of the fluoroquinolones did not change substantially from those calculated for all isolates combined. We concluded that resistance to any one of the seven nonquinolone drugs does not affect the potency of the fluoroquinolones to any important extent.

Penicillin-intermediate and penicillin-resistant strains of *S. pneumoniae* have now spread throughout the world. In 1994 and 1995, only 76% of pneumococci in the United States were susceptible to penicillin and 9.5% were resistant (MIC, ≥2.0 μg/ml) (3). Relative resistance to penicillin results from alterations in penicillin-binding proteins produced by some pneumococci, and those alterations also reduce the susceptibility to many other β-lactam drugs (6). Resistance to non-β-lactam drugs is also more common among penicillin-resistant strains (3, 8, 10, 11). For that reason, there is an increased interest in alternative orally administered antimicrobial agents that might be useful for treating pneumococcal infections of the respiratory tract.

Erythromycin and the newer macrolides and azalides are likely candidates for empiric therapy of respiratory tract infections caused by *S. pneumoniae*. Unfortunately, most macrolide-resistant pneumococci are also resistant to penicillin and many other β-lactams (3, 10, 11). The same observation can be made for trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol.

Ciprofloxacin has not proven to be consistently reliable for treating pneumococcal disease (2, 5, 9). This report describes the in vitro potencies of four other fluoroquinolones against multiply resistant strains of *S. pneumoniae*. The relative antipneumococcal activity of these agents was clinafloxacin > trovafloxacin > PD 131628 > sparfloxacin > ciprofloxacin.

TABLE 3. In vitro activities of five fluoroquinolones in relation to susceptibility or resistance to other classes of antimicrobial agents

Interpretive category[a]	No. of strains	Geometric mean MIC (μg/ml) of:				
		Ciprofloxacin	Sparfloxacin	PD 131628	Trovafloxacin	Clinafloxacin
All isolates	236	0.83	0.23	0.20	0.09	0.07
Penicillin						
Susceptible	60	0.77	0.24	0.20	0.10	0.07
Intermediate	64	0.89	0.23	0.21	0.10	0.07
Resistant	112	0.83	0.23	0.19	0.09	0.07
Cefotaxime						
Susceptible	123	0.84	0.23	0.21	0.10	0.07
Intermediate	66	0.84	0.23	0.20	0.09	0.07
Resistant	47	0.79	0.22	0.17	0.08	0.07
Erythromycin[b]						
Susceptible	154	0.81	0.23	0.20	0.09	0.07
Resistant	81	0.87	0.23	0.19	0.09	0.07
Clindamycin[b]						
Susceptible	198	0.83	0.23	0.20	0.09	0.07
Resistant	36	0.82	0.23	0.21	0.09	0.07
Trimethoprim-sulfamethoxazole						
Susceptible	86	0.86	0.25	0.22	0.10	0.07
Intermediate	44	0.72	0.20	0.18	0.08	0.07
Resistant	106	0.85	0.23	0.19	0.09	0.07
Tetracycline[b]						
Susceptible	155	0.81	0.24	0.20	0.10	0.07
Resistant	81	0.86	0.22	0.20	0.09	0.07
Chloramphenicol[b]						
Susceptible	186	0.82	0.24	0.20	0.09	0.07
Resistant	50	0.87	0.20	0.19	0.09	0.07

[a] Interpretive categories were assigned by applying MIC breakpoints that are defined in Table 1.
[b] Strains with intermediate MICs were excluded if there were less than 10 strains that fell within that category (see Table 1) or if no intermediate category has been defined (chloramphenicol).

Whether the differences in in vitro potency offer any important clinical advantage remains to be seen. The important conclusion drawn from our data is that the potency of the fluoroquinolones is not influenced by resistance to other agents. This observation may provide the rationale for alternative oral therapy that can be used in patient populations that are likely to be colonized with multiply resistant pneumococci. Of course, the newer fluoroquinolones must first be shown to be clinically effective in treating pneumococcal respiratory tract infections. In vitro activity is only one aspect that must be considered when addressing the question of how these drugs will be used in medical practice.

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