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Cross-Resistance Among Cinoxacin, Ciprofloxacin, DJ-6783, Enoxacin, Nalidixic Acid, Norfloxacin, and Oxolinic Acid After In Vitro Selection of Resistant Populations

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Six different gram-negative bacilli were serially transferred through subinhibitory concentrations of seven quinolone derivatives or related organic acids. A gradual, stepwise decrease in susceptibility was noted with all seven drugs, and the resistant cultures demonstrated a concomitant cross-resistance to the other drugs.

Rapid emergence of resistance in the presence of nalidixic acid and cinoxacin has been well documented (6, 10). A variety of structurally related quinolone derivatives have been developed recently for treatment of urinary tract or systemic infections, including ciprofloxacin (3, 13) (BAY o 9867; Miles Laboratories, West Haven, Conn.), DJ-6783 (9) (a new quinolone derivative in early phases of development by Marion Laboratories, Kansas City, Mo.) and enoxacin (1, 11) (CI-919 or AT-2266; Parke-Davis, Div. of Warner-Lambert Co., Ann Arbor, Mich.). Norfloxacin (Merck Institute for Therapeutic Research, Rahway, N.J.) was one of the first quinolone derivatives to be investigated (5, 8). Tenney et al. (12) documented a rapid stepwise emergence of resistance to norfloxacin, cinoxacin, and nalidixic acid by serial transfers on agar plates containing subinhibitory concentrations of each of the three drugs. The resistant cultures were resistant to all three drugs, regardless of the drug used for selection. The latter study was limited to tests with two standard quality control strains (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). The *P. aeruginosa* strain developed high-level resistance to norfloxacin (MIC > 256 μg/ml), but the *E. coli* strain never exceeded 16 μg/ml. With the *E. coli* strain, there was a concomitant rise in MICs for both cinoxacin and nalidixic acid (from 2.0 to 256 μg/ml).

In the present report, we describe nearly complete cross-resistance among three new agents, ciprofloxacin, DJ-6783, and enoxacin, as well as cinoxacin, nalidixic acid, norfloxacin, and oxolinic acid. Seven different clinical isolates were tested by serial transfers through broth containing separate subinhibitory concentrations of each drug. In the first experiment, standardized microdilution susceptibility tests (7) were used to determine MICs for the last six drugs listed. The trays were inoculated with about 5×10^5 CFU/ml, and MICs were recorded after 16 to 18 h at 35°C. All of the broth (0.1 ml) in those wells that contained the highest concentration with visible growth was transferred to 5 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) After 5 to 8 h of incubation, the broth subcultures were adjusted to match the turbidity of a McFarland 0.5 standard. That adjusted suspension was then used to inoculate another microdilution panel, and the whole process was repeated for 7 consecutive days. In the first experiment, serial passage through each of six study drugs (excluding ciprofloxacin) was accomplished, and then MICs for the five other drugs were determined. Ciprofloxacin was tested in a second experiment, and thus ciprofloxacin MICs were not determined after passage through the other compounds, but concomitant resistance to all seven drugs was documented.

Table 1 describes the result of these experiments. All seven drugs demonstrated a stepwise increase in MICs of the drug that the cultures were being passed through. At the same time, MICs for the other drugs were concomitantly increased. With all six gram-negative bacilli, development of resistance and concomitant cross-resistance was demonstrated. Norfloxacin MICs > 16 μg/ml were obtained only with *Klebsiella pneumoniae*, and MICs ≥ 8.0 μg/ml were obtained with both *Enterobacter cloacae* strains and with one strain of *P. aeruginosa*. Enoxacin MICs ≥ 8.0 μg/ml were obtained with all three *Enterobacter* strains and one of the *P. aeruginosa* strains. DJ-6783 MICs ≥ 8.0 μg/ml were obtained with all six isolates. Ciprofloxacin MICs never exceeded 1.0 μg/ml, but 32-fold increases were seen. Resistance to DJ-6783, enoxacin, or norfloxacin could be developed by passage through nalidixic acid or cinoxacin.

Our observations with norfloxacin confirm and expand the results reported by Tenney et al. (12). These investigators utilized an agar dilution technique with an inoculum significantly greater than that used in our broth dilution testing system. Chin and Neu (1) determined that the frequency of resistant variants for enoxacin and norfloxacin was generally $<10^{-9}$ within different cultures. Consequently, a very heavy inoculum would be needed to reliably select resistant cells within a population. With a lighter inoculum, random selection could require several passages before resistant cells were detected. Even with a lighter inoculum of ca. 5×10^4 CFU per microdilution well (5×10^5 CFU/ml), we were able to observe a gradual stepwise increase in MICs for all seven quinolone derivatives or organic acids. Serial transfers through subinhibitory concentrations of ciprofloxacin, DJ-6783, enoxacin, or norfloxacin did result in increased MICs, but multiple transfers were required. Cinoxacin and nalidixic acid developed resistance more rapidly. The rate of resistance development cannot be measured quantitatively with the methods used in this study.

In our studies, resistance to the newer quinolone derivatives was rarely of obvious clinical significance; presumably, higher levels of resistance could have been achieved if other laboratory methods had been utilized (1). Since ciprofloxacin is intended for systemic therapy, moderate increases in MICs may be much more significant, because lower concentrations could be achieved at the site of infection compared with levels obtained in the urinary tract. Crump et al. (2) observed peak serum levels of 2.4 μg/ml after dosage with a

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TABLE 1. Decreased susceptibility to seven quinolone derivatives or organic acids after seven daily passages through separate subinhibitory concentrations of each drug

Organism	MIC (μg/ml) for:						
	Nalidixic acid	Cinoxacin	Oxolinic acid	Norfloxacin	Enoxacin	DJ-6783	Ciprofloxacin[a]
K. pneumoniae							
Before passage	4.0	4.0	5.0	0.12	0.25	0.25	0.03
After passage in:							
Nalidixic acid	>128	64	128	>128	64	>128	NT
Cinoxacin	128	>128	128	>128	64	>128	NT
Oxolinic acid	16	8.0	16	>32	8.0	>32	NT
Norfloxacin	4.0	2.0	4.0	32	4.0	16	NT
Enoxacin	8.0	4.0	4.0	>32	2.0	32	NT
DJ-6783	8.0	4.0	8.0	>32	4.0	>32	NT
Ciprofloxacin	32	32	8.0	2.0	2.0	2.0	0.25
E. aerogenes							
Before passage	4.0	8.0	0.5	0.12	0.25	0.25	0.015
After passage in:							
Nalidixic acid	64	64	8.0	1.0	2.0	4.0	NT
Cinoxacin	16	32	2.0	0.25	1.0	2.0	NT
Oxolinic acid	32	32	4.0	1.0	2.0	2.0	NT
Norfloxacin	64	64	16	2.0	4.0	4.0	NT
Enoxacin	>128	>128	16	2.0	16	16	NT
DJ-6783	>128	>128	32	4.0	8.0	16	NT
Ciprofloxacin	>128	>128	16	1.0	2.0	2.0	0.25
E. cloacae							
Before passage	4.0	8.0	0.5	0.12	0.12	0.25	0.008
After passage in:							
Nalidixic acid	128	>128	>32	8.0	8.0	16	NT
Cinoxacin	128	>128	16	2.0	2.0	4.0	NT
Oxolinic acid	128	>128	16	4.0	4.0	16	NT
Norfloxacin	>128	>128	32	8.0	8.0	16	NT
Enoxacin	>128	>128	>32	16	32	>32	NT
DJ-6783	128	128	32	8.0	16	16	NT
Ciprofloxacin	64	64	8.0	2.0	2.0	2.0	0.25
E. cloacae							
Before passage	4.0	8.0	0.5	0.12	0.25	0.25	0.015
After passage in:							
Nalidixic acid	128	>128	16	4.0	8.0	8.0	NT
Cinoxacin	32	>128	2.0	0.5	0.5	2.0	NT
Oxolinic acid	64	>128	8.0	2.0	4.0	4.0	NT
Norfloxacin	128	>128	16	8.0	8.0	8.0	NT
Enoxacin	>128	>128	32	8.0	8.0	16	NT
DJ-6783	128	>128	16	4.0	4.0	8.0	NT
Ciprofloxacin	64	>128	16	2.0	4.0	2.0	0.25
P. aeruginosa							
Before passage	>128	>128	4.0	0.25	0.5	1.0	0.03
After passage in:							
Oxolinic acid	>128	>128	16	0.25	1.0	4.0	NT
Norfloxacin	>128	>128	>32	2.0	4.0	>32	NT
Enoxacin	>128	>128	32	2.0	4.0	32	NT
DJ-6783	>128	>128	>32	2.0	4.0	>32	NT
Ciprofloxacin	>128	>128	>32	4.0	4.0	4.0	1.0
P. aeruginosa							
Before passage	>128	>128	4.0	0.25	0.5	1.0	0.03
After passage in:							
Oxolinic acid	>128	>128	>32	4.0	8.0	>32	NT
Norfloxacin	>128	>128	8.0	8.0	16	16	NT
Enoxacin	>128	>128	>32	8.0	16	>32	NT
DJ-6783	>128	>128	>32	8.0	16	>32	NT
Ciprofloxacin	>128	>128	>32	4.0	4.0	16	1.0

[a] NT, Not tested. Ciprofloxacin passages were carried out in a separate experiment; consequently, ciprofloxacin MICs were determined only before and after serial exposures to ciprofloxacin.

500-mg ciprofloxacin tablet, and the serum half-life was 3.9 h. Consequently, 5 to 6 h after dosing, the serum level may be below 1.0 μg/ml. They also determined that 57% of the drug in the serum penetrated into blister fluid. Consequently, the concentration at the site of infection could very well be below 1.0 μg/ml, which approaches the maximal MIC that we observed with *P. aeruginosa*. Enoxacin has similar pharmacokinetic properties, but higher serum levels can be achieved. Chin and Neu (1) expect serum levels in excess of 4.0 μg/ml for 6 to 8 h after dosing. After our strains were exposed to enoxacin, we observed MICs as high as 32 μg/ml, and that could be clinically significant.

Our primary goal was to evaluate cross-resistance between the quinolone derivatives and related organic acids. Jones and Barry (5) demonstrated that *Enterobacteriaceae* with elevated MICs for cinoxacin, nalidixic acid, or oxolinic acid tended to be less susceptible to norfloxacin. Fass (3) observed a similar relationship between ciprofloxacin and norfloxacin. In the current study, we observed that resistance to one compound can be developed by serial transfers through subinhibitory concentrations of a different quinolone derivative or related organic acid. Presumably, the mechanism responsible for development of resistance affects all of the compounds that we have studied, although with the newer, more potent quinolone derivations, significant levels of resistance develop much more slowly. The mechanism of resistance may be analogous to that which has been defined for nalidixic acid, i.e., a change in the configuration of bacterial DNA gyrase (4).

Extremely high MICs for cinoxacin and nalidixic acid are obtained after a few serial transfers through subinhibitory concentrations of either drug (6, 10, 12). Clinically significant resistance does occur during therapy with either drug (6, 10). Our data indicate that selection of resistant populations by widespread use of cinoxacin or nalidixic acid might seriously diminish the efficacy of the newer quinolone derivatives, especially those intended for systemic therapy.

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