

## Activities of Newer Quinolones against *Escherichia coli* and *Klebsiella pneumoniae* Containing the Plasmid-Mediated Quinolone Resistance Determinant *qnr*

Mingui Wang,<sup>1,2</sup> Daniel F. Sahn,<sup>3</sup> George A. Jacoby,<sup>4</sup> Yingyuan Zhang,<sup>2</sup>  
and David C. Hooper<sup>1\*</sup>

Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts 02114<sup>1</sup>; Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, China<sup>2</sup>; Focus Technologies, Herndon, Virginia 20171<sup>3</sup>; and Infectious Disease Department, Lahey Clinic, Burlington, Massachusetts 01805<sup>4</sup>

Received 10 October 2003/Returned for modification 19 December 2003/Accepted 30 December 2003

**Seventeen *qnr*-containing transconjugants were constructed with azide-resistant *Escherichia coli* J53 as the recipient, and the MICs of 12 quinolones were tested by agar dilution methods. Sitafloracin, BAYy3118, and premarloxacin had higher activity in vitro than ciprofloxacin against transconjugants and donors containing *qnr*. The donors had higher quinolone MICs than the transconjugants.**

Plasmid-mediated quinolone resistance was discovered in a clinical isolate of *Klebsiella pneumoniae* from Birmingham, Ala. (6). The gene responsible, *qnr*, has since been detected in more than 20 clinical strains of *K. pneumoniae* and *Escherichia coli* isolated in the United States and China. *qnr* confers low-level ciprofloxacin resistance (4, 6, 9, 11; M. Wang, D. F. Sahn, G. A. Jacoby, and D. C. Hooper, unpublished observations). Newer quinolones have enhanced potency against many resistant strains. Some newer quinolones have the same or only slightly higher MICs for DNA gyrase or topoisomerase IV mutants or mutants with efflux pump overexpression (1, 2, 8). They have not yet been studied for the protective effects of *qnr*. We constructed transconjugants containing different *qnr* plasmids and determined the activity of newer quinolones against both transconjugants and donor strains.

**Construction of *qnr*-containing transconjugants.** Seventeen transconjugants were obtained by conjugation with azide-resistant *E. coli* J53Az<sup>R</sup> as the recipient from 14 unique *qnr*-containing clinical strains, six *E. coli* and eight *K. pneumoniae*, which were screened in former studies (11; Wang et al., unpublished observations). Nine transconjugants were from six *E. coli* donors (transconjugants of different phenotypes were selected from each of three donors), seven from *K. pneumoniae* donors, and one from UAB1 (the original *K. pneumoniae* strain found to contain plasmid pMG252 carrying *qnr*).

**Activity of newer quinolones against transconjugants.** The MICs of 12 fluoroquinolones were tested by agar dilution (7). The fluoroquinolones tested included AM-1121 (Bristol-Myers Squibb, Princeton, N.J.), BAYy3118 and ciprofloxacin (Bayer Corporation, West Haven, Conn.), garenoxacin and gatifloxacin (Bristol-Myers Squibb), gemifloxacin (GlaxoSmithKline, West Sussex, United Kingdom), levofloxacin (Ortho/McNeil Pharmaceuticals, Raritan, N.J.), moxifloxacin (Bayer Corporation), norfloxacin (Sigma Chemical Co., St. Louis, Mo.), pre-

marloxacin, which was previously under development for veterinary use (Pharmacia & Upjohn, Kalamazoo, Mich.), sitafloracin (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), and sparfloracin (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan).

Sitafloracin, BAYy3118, and premarloxacin had four- to eightfold higher in vitro activity (MIC for 90% of strains, 0.125 to 0.25  $\mu\text{g/ml}$ ) than did ciprofloxacin (MIC for 90% of strains, 1  $\mu\text{g/ml}$ ) against transconjugants containing *qnr*. Compared to plasmid-free J53, the MIC of sitafloracin increased 15-fold, that of BAYy3118 increased 32-fold, and that of ciprofloxacin increased 125-fold. These three newer quinolones were also more active than ciprofloxacin against the donor strains which contained *qnr* and other resistance mechanisms. The activities of gatifloxacin, levofloxacin, AM-1121, gemifloxacin, and moxifloxacin were similar to that of ciprofloxacin. All quinolone MICs of the donors were all higher than those of the transconjugants, indicating the occurrence of additional resistance mechanisms in the donor strains that acted in concert with *qnr* (Table 1).

The MICs of each quinolone against eight transconjugants constructed from donor strains of *K. pneumoniae* isolated in the United States were the same or differed by no more than twofold. The quinolone MICs for nine transconjugants constructed from donor strains of *E. coli* isolated in Shanghai, China, exhibited substantial differences in susceptibility (ciprofloxacin MICs ranged from 0.125 to 2  $\mu\text{g/ml}$ ). The MICs of the three newer quinolones sitafloracin, BAYy3118, and premarloxacin were substantially lower than that of ciprofloxacin. All *E. coli* donors were highly resistant to ciprofloxacin (MIC, 64 to  $\geq 256$   $\mu\text{g/ml}$ ), while MICs of ciprofloxacin for *K. pneumoniae* donors were lower, 2 to 16  $\mu\text{g/ml}$  (Table 2).

Several newer quinolones appear to have greater and more closely balanced activity against DNA gyrase and topoisomerase IV (1, 3). Purified Qnr has been shown to block ciprofloxacin inhibition of both DNA gyrase (10) and topoisomerase IV (J. Tran, G. Jacoby, and D. Hooper, unpublished observations) and to have additive effects with *gyrA* mutations in intact cells (5). In this study we showed that some newer quinolones have

\* Corresponding author. Mailing address: Division of Infectious Diseases, Massachusetts General Hospital, 55 Fruit St., Boston, MA 02114-2696. Phone: (617) 726-3812. Fax: (617) 726-7416. E-mail: dhooper@partners.org.

TABLE 1. In vitro activity of newer quinolones against transconjugants containing *qnr* and donor strains<sup>a</sup>

Agent	<i>E. coli</i> J53	MIC (µg/ml)					
		Transconjugants (n = 17)			Donors (n = 15)		
		MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>R</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>R</sub>
AM-1121	0.008	0.5	0.5	0.125–1	16	≥64	2–≥64
BAYy3118	0.004	0.125	0.125	0.06–0.25	4	16	0.5–32
Ciprofloxacin	0.008	0.25	1	0.125–2	16	128	2–≥256
Garenoxacin	0.008	1	2	0.5–2	32	≥64	8–≥64
Gatifloxacin	0.008	0.25	0.5	0.25–1	16	≥32	2–≥32
Gemifloxacin	0.004	0.5	1	0.25–1	16	≥32	2–≥32
Levofloxacin	0.015	0.5	0.5	0.25–1	32	≥32	2–≥32
Moxifloxacin	0.03	0.5	1	0.5–1	32	≥64	2–≥64
Nalidixic acid	4	16	32	8–32	≥256	≥256	32–≥256
Premafloxacin	0.03	0.25	0.25	0.25–0.5	16	≥64	2–≥64
Sitafloxacin	0.008	0.125	0.125	0.06–0.25	4	8	0.5–16
Sparfloxacin	0.008	1	1	0.25–1	32	≥64	2–≥64

<sup>a</sup> MIC<sub>50</sub>, MIC for 50% of strains; MIC<sub>90</sub>, MIC for 90% of strains; MIC<sub>R</sub>, range of MICs.

enhanced in vitro activity against transconjugants carrying *qnr* on a plasmid, indicating that their increased potency extends to the new *qnr*-mediated resistance mechanism. Sitafloxacin, BAYy3118, and premafloxacin were the most potent of the quinolones studied against the *qnr*-containing transconjugants, exceeding even the potency of ciprofloxacin, heretofore one of the most active quinolones against gram-negative bacteria.

The MICs of each quinolone against eight transconjugants constructed from clinical strains of *K. pneumoniae* were similar or identical despite differences in the plasmids carrying *qnr* (Wang et al., unpublished data). In contrast, there were differences in the level of resistance in transconjugants constructed from *E. coli* donor strains isolated in Shanghai (11). These differences suggest differences in the levels of expression of *qnr*. Levels of *qnr* expression and the molecular basis for the observed differences are under investigation.

*qnr* can supplement resistance via altered quinolone target

enzymes, efflux pump activation, or deficiencies in outer membrane porin channels (5). The higher resistance to all quinolones tested in donor compared to transconjugant strains reflects such additional chromosomal resistance mutations.

This work was supported by grants AI43312 (to G.A.J. and D.C.H.) from the National Institutes of Health, U.S. Public Health Service, and grants from Daiichi Pharmaceutical Co., Ltd., and Kyorin Pharmaceuticals (to D.C.H.).

REFERENCES

1. Hooper, D. C. 2000. Mechanisms of action and resistance of older and newer fluoroquinolones. Clin. Infect. Dis. 31:S24–S28.
2. Ince, D., X. Zhang, and D. C. Hooper. 2003. Activity of and resistance to moxifloxacin in *Staphylococcus aureus*. Antimicrob. Agents Chemother. 47:1410–1415.
3. Ince, D., X. M. Zhang, L. C. Silver, and D. C. Hooper. 2002. Dual targeting of DNA gyrase and topoisomerase IV: target interactions of garenoxacin (BMS-284756, T-3811ME), a new desfluoroquinolone. Antimicrob. Agents Chemother. 46:3370–3380.
4. Jacoby, G. A., N. Chow, and K. B. Waites. 2003. Prevalence of plasmid-mediated quinolone resistance. Antimicrob. Agents Chemother. 47:559–562.
5. Martínez-Martínez, L., A. Pascual, I. García, J. Tran, and G. A. Jacoby. 2003. Interaction of plasmid and host quinolone resistance. J. Antimicrob. Chemother. 51:1037–1039.
6. Martínez-Martínez, L., A. Pascual, and G. A. Jacoby. 1998. Quinolone resistance from a transferable plasmid. Lancet 351:797–799.
7. National Committee for Clinical Laboratory Standards. 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, p. 1. Approved standard M7–A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. Piddock, L. J., M. Johnson, V. Ricci, and S. L. Hill. 1998. Activities of new fluoroquinolones against fluoroquinolone-resistant pathogens of the lower respiratory tract. Antimicrob. Agents Chemother. 42:2956–2960.
9. Rodríguez-Martínez, J. M., A. Pascual, I. García, and L. Martínez-Martínez. 2003. Detection of the plasmid-mediated quinolone resistance determinant *qnr* among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type β-lactamase. J. Antimicrob. Chemother. 52:703–706.
10. Tran, J. H., and G. A. Jacoby. 2002. Mechanism of plasmid-mediated quinolone resistance. Proc. Natl. Acad. Sci. USA 99:5638–5642.
11. Wang, M., J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, and D. C. Hooper. 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob. Agents Chemother. 47:2242–2248.

TABLE 2. Comparison of the MICs of transconjugants of *E. coli* with transconjugants of *K. pneumoniae* and of MICs of the donors

Agent	MIC (µg/ml)				
	<i>E. coli</i> J53	Transconjugants		Donors	
		<i>E. coli</i> (n = 9)	<i>K. pneumoniae</i> (n = 7)	<i>E. coli</i> (n = 6)	<i>K. pneumoniae</i> (n = 8)
AM-1121	0.008	0.125–1	0.25–0.5	32–≥64	2–16
BAYy3118	0.004	0.06–0.25	0.125	4–32	0.5–8
Ciprofloxacin	0.008	0.125–2	0.25–0.5	64–≥256	2–16
Garenoxacin	0.008	0.5–2	1–2	16–≥64	8–64
Gatifloxacin	0.008	0.25–1	0.25–0.5	16–≥32	2–32
Gemifloxacin	0.004	0.25–1	0.25–0.5	16–≥32	2–32
Levofloxacin	0.015	0.25–1	0.25–0.5	≥32	2–32
Moxifloxacin	0.03	0.5–1	0.5–1	32–≥64	2–32
Nalidixic acid	4	8–32	16–32	≥256	32–≥256
Premafloxacin	0.03	0.25–0.5	0.25	16–≥64	2–16
Sitafloxacin	0.008	0.06–0.25	0.125	4–16	0.5–8
Sparfloxacin	0.008	0.25–2	0.5–1	16–≥64	2–64