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Prevalence of Plasmid-Mediated Quinolone Resistance

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Quinolone resistance encoded by the *qnr* gene and mediated by plasmid pMG252 was discovered in a clinical strain of *Klebsiella pneumoniae* that was isolated in 1994 at the University of Alabama at Birmingham Medical Center. The gene codes for a protein that protects DNA gyrase from quinolone inhibition and that belongs to the pentapeptide repeat family of proteins. The prevalence of the gene has been investigated by using PCR with *qnr*-specific primers with a sample of more than 350 gram-negative strains that originated in 18 countries and 24 states in the United States and that included many strains with plasmid-mediated AmpC or extended spectrum β-lactamase enzymes. *qnr* was found in isolates from the University of Alabama at Birmingham only during 6 months in 1994, despite the persistence of the gene for FOX-5 β-lactamase, which is linked to *qnr* on pMG252. Isolates from other locations were negative for *qnr*. The prevalence of *mcbG* in the same sample was also examined. *mcbG* encodes another member of the pentapeptide repeat family and is involved in immunity to microcin B17, which, like quinolones, targets DNA gyrase. A single clinical isolate contained *mcbG* on a transmissible R plasmid. This plasmid and one carrying the complete microcin B17 operon slightly decreased sparfloxacin susceptibility but had a much less protective effect than pMG252. Plasmid-mediated quinolone resistance was thus rare in the sample examined.

Plasmid pMG252, which mediates low-level quinolone resistance, was discovered in a *Klebsiella pneumoniae* strain that was isolated in July 1994 from the urine of a patient at the University of Alabama at Birmingham Medical Center (UAB) (9). The plasmid increased resistance to both nalidixic acid and fluoroquinolones, had a broad host range, and belonged to the plasmid incompatibility group IncC (9). The only previous claim of naturally occurring, transmissible quinolone resistance (11) involved a plasmid from a *Shigella dysenteriae* isolate from Bangladesh, but the claim was later withdrawn (1).

The quinolone resistance gene (*qnr*) has been cloned and sequenced (16). Purified Qnr blocks ciprofloxacin inhibition of DNA gyrase and belongs to the pentapeptide repeat family of proteins (2) that also includes McbG, which with McbE and McbF protects a microcin B17-producing strain from self- inhibition (3). Microcins are a class of small inhibitory proteins (less than 10 kDa) that are encoded by bacterial plasmids and differ in their mechanisms of action. Microcin B17, like quinolones, targets DNA gyrase, and McbEFG has been reported to produce low-level resistance to quinolones such as sparfloxacin (8).

Plasmid pMG252 also encodes the AmpC-type β-lactamase FOX-5, which has been found at other sites in the United States (15). Quinolone resistance is unexpectedly high (18 to 56%) in extended-spectrum β-lactamase-producing (ESBL) isolates (5, 7, 14). The prevalence of *qnr* and *mcbG* in clinical isolates is not known. We have used PCR to determine the frequencies of both genes in a sample of more than 350 strains, including many with plasmid-mediated AmpC or ESBL enzymes, and have also traced the fate of *qnr* in resistant isolates from the UAB, where it was discovered.

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MATERIALS AND METHODS

Strains. *K. pneumoniae* UAB1 containing pMG252 (9) served as a positive control for the presence of *qnr*. *Escherichia coli* RYC1000(pMM39), a pBR322 derivative carrying *mcbABCDEFG* (3), came from Rosario Baquero and provided a positive control for *mcbG*. *S. dysenteriae* containing plasmid pICD01 (1), initially reported as conferring quinolone resistance (11), was provided by David Sack. *E. coli* J53 Azi^r (azide resistant) (6) was a common host for many plasmids.

In the UAB clinical laboratory, consecutive oxyimino-β-lactam-resistant, gram-negative strains were collected between June 1994 and June 1995 (33 isolates) and from September 2000 to April 2001 (26 isolates). Each strain came from a unique patient. Strains were identified and antimicrobial susceptibility was determined according to routine laboratory protocols by using WalkAway 96 Gram-Negative MIC Combo Panels (Dade Microscan, West Sacramento, Calif.). By this evaluation, 24 of the UAB strains were ofloxacin resistant, 2 were intermediate, and 33 were susceptible. Other test strains were chosen to represent different genera of gram-negative bacilli and diverse geographical origins. The test strains came from Argentina, Bangladesh, Belgium, Brazil, Bulgaria, Canada, England, France, Germany, Greece, India, Italy, Japan, Korea, Mexico, Spain, Switzerland, Tunisia, and 24 states in the United States. Most were collected in the 1990s. In addition to 191 *Klebsiella pneumoniae* strains and 91 *E. coli* strains, the sample contained isolates of the following organisms (with numbers of isolates shown in parentheses): *Achromobacter xylosoxydans* (1), *Acinetobacter baumannii* (1), *Acinetobacter calcoaceticus* (1), *Citrobacter koseri* (1), *Enterobacter cloacae* (5), *Klebsiella oxytoca* (10), *Kluyvera ascorbata* (2), *Morganella morganii* (2), *Proteus mirabilis* (5), *Providencia rettgeri* (1), *Providencia stuartii* (1), *Pseudomonas aeruginosa* (20), *Salmonella* spp. (5), and *Serratia marcescens* (1). Sixteen stock *E. coli* strains carrying IncC plasmids R16a, R40a, R40b, R55, R57b, R57b-1, R64, JR211, R692, R707, R715b, R742, R816, plac, pMG202, and pMG203 were included. To ensure a diversity of plasmid types and to test for an association of *qnr* with extended-spectrum or AmpC-type β-lactamase genes, the sample incorporated 68 stock plasmid-containing strains producing the following β-lactamases: ACC-1; ACT-1; CARB-4; CMY-2 and -4; CTX-M-14; DHA-1; FOX-1, -3, -4, and-5; HMS-1; LAT-1; LCR-1; LXA-1; MIR-1; MOX-1 and -2; OHIO-1; OXA-1, -2, -3, -4, -5, -6, -7, and -10; PSE-1 and -3; SAR-1; SHV-1, -2, -3, -4, -5, -6, -7, -8, -9, -10, -11, -12, and -18; TEM-1, -2, -3, -4, -5, -6, -7, -8, -9,- 10, -11, -12,-15, -16, -19, -20, -21, -22, -24,-25, -26, -52, -61, -71, and -88.

PCR detection of *qnr* and *mcbG*. *qnr* (length, 657 bp; GenBank accession number AY070235) was amplified by using primers QP1 (5′-GATAAAGTTTT TCAGCAAGAGG; starting at the 12th *qnr* nucleotide) and QP2 (5′-ATCCAG ATCGGCAAAGGTTA) to produce a 593-bp product. PCR conditions were 94°C for 1 min, 57°C for 30 s, and 72°C for 1 min for 30 cycles. *mcbG* (length, 564

TABLE 1. Strain properties

Isolate	Identity	Source	Isolation date (mo/yr)	PCR positive, for:	MIC (μg/ml) of indicated drug for isolate			MIC (μg/ml) of indicated drug for J53 transconjugant		
					Nalidixic acid	Ciprofloxacin	Sparfloxacin	Nalidixic acid	Ciprofloxacin	Sparfloxacin
UAB1	*K. pneumoniae*	Urine	7/94	*qnr*	256	8	16	32	0.5	0.5
UAB2	*Klebsiella* sp.	Urine	7/94	*qnr*	≥512	16	64	32	0.25	0.5
UAB4	*E. coli*	Urine	8/94	*qnr*	16	0.5	0.5	32	0.25	0.5
UAB24	*K. pneumoniae*	Sputum	10/94	*qnr*	16	0.5	1	32	0.5	0.5
UAB33	*K. pneumoniae*	Urine	12/94	*qnr*	16	0.5	1	32	0.5	0.5
UAB38	*K. pneumoniae*	Urine	12/94	*qnr*	512	16	32	32	0.25	0.5
UAB45	*E. coli*	Wound	2/95	*mcbG*	1	0.008	≤0.004	2	0.008	0.008
J53 Azi^r	*E. coli*							2	0.008	≤0.004

bp; GenBank accession number X07875) was amplified by using primers McbG1 (5′-GAAAAAAGAATCACAAAACGACACC; starting at the 12th *mcbG* nucleotide) and McbG2 (5′-CCACTCCCATTCGTTTAAGTATTTT) to produce a 538-bp product. PCR conditions were 94°C for 1 min, 61°C for 30 s, and 72°C for 1 min. Products were detected by electrophoresis on a 1% agarose gel by using Tris-borate-EDTA buffer (Sigma, St. Louis, Mo.), stained with 0.5 mg of ethidium bromide/ml and photographed under UV light. Strains positive and negative for *qnr* and *mcbG* were included in each batch of strains tested. The amplification product obtained from pMG277 was analyzed further by using primers McbG1 and McbG2 via cycle sequencing (Perkin-Elmer Cetus, Norwalk, Conn.).

β-Lactamase characterization. Unknown β-lactamases were initially characterized, usually in *E. coli* J53 Azi^r transconjugants, by isoelectric focusing on polyacrylamide gels incorporating a pH 3.0 to 9.0 gradient with the PhastSystem (Pharmacia Biotech, Uppsala, Sweden) as described by Huovinen (4). The FOX-5 β-lactamase of pMG252 was identified by cloning and nucleotide sequencing. Strains making AmpC-type enzymes with similar isoelectric points and producing a characteristic PCR product with FOX-specific primers came from Dallas, Fort Worth, and San Antonio, Tex.; Coral Gables, Fla., and northern Florida; Greensboro, N.C.; and Rochester, N. Y. (G. Jacoby, J. Tran, and M. Alvarez, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1481, 1999).

Other procedures. Plasmids were transferred to *E. coli* J53 Azi^r by transformation or conjugation by using 200 μg of sodium azide per ml for counterselection. Antimicrobial agents other than quinolones were used in mating experiments to avoid selection of quinolone resistance chromosomal mutation. Strains were tested quantitatively for quinolone susceptibility by agar dilution by using Mueller-Hinton medium, ~10^4 organisms per spot, and overnight incubation at 37°C (12). *E. coli* J53 Azi^r and ATCC 25922 were used for quality control. Ciprofloxacin was obtained from Bayer Corporation (West Haven, Conn.), nalidixic acid was from Sigma, and sparfloxacin was from Rhône-Poulenc Rorer. Susceptibility to other antimicrobial agents was determined by disk testing (13). Sparfloxacin-resistant mutants were selected on Mueller-Hinton agar plates containing graded concentrations of the antibiotic spread with 5×10^7 to 10×10^7 bacteria.

RESULTS

The *qnr* gene was sought by PCR by using specific primers in over 350 strains that included 13 genera of gram-negative bacilli originating in 24 states in the United States and 18 other countries. Since strains carrying *qnr* may test as quinolone susceptible, the sample included both quinolone-susceptible and -resistant isolates. The strains were, however, known to make at least 68 different β-lactamases, including many ESBL and plasmid-mediated AmpC types. The sample also incorporated strains with 16 plasmids that, like pMG252, belonged to the IncC group and 59 oxyimino-β-lactam-resistant isolates of *E. coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, and *P. aeruginosa* collected between June 1994 and April 2001 at the UAB.

At the UAB, *qnr* could be detected in isolates collected between July and December 1994 (Table 1). Positive isolates included one *E. coli* isolate, four *Klebsiella pneumoniae* isolates, and one *Klebsiella* sp. that came from cultures of urine and sputum. Each was isolated from a different patient. Three of the six strains were resistant to ciprofloxacin, nalidixic acid, and sparfloxacin. All could transfer the same set of antibiotic resistances to *E. coli* strain J53 Azi^r on plasmids of similar sizes. Quinolone resistance was, however, somewhat unstable on transfer. With strain UAB1 as the donor, 12 of 100 ceftazidime-resistant J53 transconjugants lost resistance to nalidixic acid, including three that also lost resistance to streptomycin and trimethoprim. J53 derivatives carrying *qnr*-bearing plasmids from each positive strain were more quinolone resistant than J53 without R plasmid (R^-), but only the MIC of nalidixic acid was in the resistant range (Table 1). Ciprofloxacin MICs rose from 0.008 to 0.25 or 0.5 μg/ml, and sparfloxacin MICs increased from ≤0.004 to 0.5 μg/ml. The PCR-positive isolates and their transconjugants produced FOX-5 β-lactamase, but FOX-5- producing clinical isolates at the UAB from January and April 1995 were *qnr* negative, and none of the 26 UAB specimens collected between September 2000 and April 2001 contained *qnr* even though FOX-5 was still present in *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates. Strains with plasmid-mediated FOX-5 from Florida, North Carolina, New York, and Texas also failed to amplify with *qnr* primers.

The nalidixic acid-resistant strain from Bangladesh that was initially reported (11) to carry plasmid-mediated quinolone resistance was negative for *qnr* and transferred a plasmid carrying trimethoprim resistance only. The *qnr* gene was not detected in almost 300 other strains, including many with plasmid-mediated ESBL and AmpC enzymes. The gene was not found in the genomes of 14 genera of gram-negative organisms or in strains with 16 IncC plasmids.

The complete set of strains was also tested for the presence of *mcbG* by PCR. A single strain gave a positive result that was confirmed by sequencing the amplification product. *mcbG* was present on a transmissible plasmid (pMG277) mediating resistance to gentamicin, streptomycin, and tetracycline. Curiously, the *mcbG*-positive strain, *E. coli* UAB45, also came from the UAB. In J53 Azi^r, plasmid pMG277 produced a slight increment in resistance to sparfloxacin but did not affect susceptibility to ciprofloxacin or nalidixic acid (Table 1).

To test for the ability of plasmid-containing strains to facil-

TABLE 2. Colonies per Mueller-Hinton agar plate containing increasing concentrations of sparfloxacin spread with 5×10^7 to 10^8 CFU of the indicated strains

Sparfloxacin concn (μg/ml)	No. of colonies/plate				
	J53	J53 (pMM39) *mcbABCDEFG*	J53 (pMG277)	J53 (pICD01) (Bangladesh)	J53 (pMG252) *qnr*
0.03	Confluent	Confluent	Confluent	Confluent	Confluent
0.06	400	Confluent	$>10^3$	165	Confluent
0.125	0	131	17	0	Confluent
0.25	0	0	0	0	Confluent
0.50	0	0	0	0	Confluent
1.0	0	0	0	0	Confluent
2.0	0	0	0	0	133
4.0	0	0	0	0	7

itate selection of higher-level quinolone resistance mutants, plasmids pMG277, positive for *mcbG*, pMM39, carrying the *mcbABCDEFG* cluster, pICD01, originally thought to determine quinolone resistance and reported to facilitate nalidixic acid resistance mutations (1), and pMG252, with *qnr*, were transferred to *E. coli* J53 as a common host and the strains were spread on plates containing increasing concentrations of sparfloxacin (Table 2). Plasmid pICD01 did not enhance the selection of sparfloxacin-resistant mutants. J53 with pMG277 or pMM39 gave rise to mutants resistant to sparfloxacin at a concentration that was 2-fold higher than that for J53 R^-, but their effect was much less dramatic than that of J53 with pMG252, which allowed the selection of mutants at a 32-fold-higher concentration.

DISCUSSION

By using a gene-specific technique, *qnr* has been found only in strains from the UAB and only in samples collected over a 6-month period in 1994. Isolates from elsewhere, even those encoding the FOX-5 β-lactamase that is linked to *qnr* on plasmid pMG252, were nonreactive by PCR. Quinolone resistance mediated by *qnr* thus appears to be rare, although amplification by PCR requires close sequence homology so that plasmids utilizing the same DNA gyrase-protective mechanism but with genes differing in a few nucleotides from primers QP1 and QP2 could have been overlooked.

Other plasmid-determined genes besides *qnr* may influence quinolone susceptibility. Plasmid pMM39 carrying *mcbABC DEFG* decreased sparfloxacin susceptibility two-fold (Table 2). Lomovskaya et al. first described this effect, reporting that the *mcbEFG* genes were sufficient to reduce sparfloxacin susceptibility, and attributed protection to the pump genes *mcbE* and *mcbF* (8). However, *mcbG* by itself is protective (D. Hooper and J.-L. Yu, personal communication) and encodes a protein that, like Qnr, belongs to the pentapeptide repeat family. PCR with *mcbG*-specific primers detected in more than 350 tests one strain that carried this gene on transmissible plasmid pMG277, which, like pMM39, decreased sparfloxacin susceptibility (Table 2). Protection was slight, however, and the *E. coli* clinical isolate in which pMG277 was found tested quinolone susceptible, although a J53(pMG277) transconjugant showed a small increase in sparfloxacin resistance (Table 1). A recently described protein from *Mycobacterium smegmatis* belongs to the pentapeptide repeat family and causes a four- to eight-fold decrease in sparfloxacin susceptibility (10). Similar proteins are found in *Mycobacterium tuberculosis* and *Mycobacterium avium*. All of these proteins are presumably determined by chromosomal genes, but their acquisition by an integron would provide one route for the evolution of plasmid-mediated quinolone resistance. So far the search for a *qnr* progenitor in the genomes of gram-negative bacteria has been unrevealing, but mycobacteria and organisms such as cyanobacteria, which produce many proteins in the pentapeptide repeat family, have not yet been examined.

Although *qnr* confers only low-level quinolone resistance and currently seems to be uncommon, its clinical importance lies in its ability to supplement resistance due to DNA gyrase, topoisomerase IV, porin, or efflux mutations (L. Martínez-Martínez, A. Pascual, I. García, J. Tran, and G. A. Jacoby, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-99, 1998) and to allow selection of such chromosomal mutations at concentrations of quinolone that would be lethal in its absence. Thus, while the sparfloxacin MIC for *E. coli* J53 was ≤0.004 μg/ml (Table 1), mutants of J53 carrying pMG252 could grow at a sparfloxacin concentration of 4 μg/ml (Table 2); in addition, in a clinical isolate of *Klebsiella pneumoniae* with porin mutations and undoubtedly others, pMG252 can raise the sparfloxacin MIC from 4 to 64 μg/ml and those of other fluoroquinolones to even higher levels (9).

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REFERENCES

1. **Ashraf, M. M., Z. U. Ahmed, and D. A. Sack.** 1991. Unusual association of a plasmid with nalidixic acid resistance in an epidemic strain of *Shigella dysenteriae* type 1 from Asia. Can. J. Microbiol. **37:**59–63.
2. **Bateman, A., A. G. Murzin, and S. A. Teichmann.** 1998. Structure and distribution of pentapeptide repeats in bacteria. Protein Sci. **7:**1477–1480.
3. **Garrido, M. C., M. Herrero, R. Kolter, and F. Moreno.** 1988. The export of the DNA replication inhibitor microcin B17 provides immunity for the host cell. EMBO J. **7:**1853–1862.
4. **Huovinen, S.** 1988. Rapid isoelectric focusing of plasmid-mediated β-lactamases with Pharmacia PhastSyst. Antimicrob. Agents Chemother. **32:**1730–1732.
5. **Itokazu, G. S., J. P. Quinn, C. Bell-Dixon, F. M. Kahan, and R. A. Weinstein.** 1996. Antimicrobial resistance rates among aerobic gram-negative bacilli recovered from patients in intensive care units: evaluation of a national postmarketing surveillance program. Clin. Infect. Dis. **23:**779–784.
6. **Jacoby, G. A., and P. Han.** 1996. Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. J. Clin. Microbiol. **34:**908–911.
7. **Lautenbach, E., B. L. Strom, W. B. Bilker, J. B. Patel, P. H. Edelstein, and N. O. Fishman.** 2001. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. Clin. Infect. Dis. **33:**1288–1294.
8. **Lomovskaya, O., F. Kawai, and A. Matin.** 1996. Differential regulation of the *mcb* and *emr* operons of *Escherichia coli*: role of *mcb* in multidrug resistance. Antimicrob. Agents Chemother. **40:**1050–1052.
9. **Martínez-Martínez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
10. **Montero, C., G. Mateu, R. Rodriguez, and H. Takiff.** 2001. Intrinsic resistance of *Mycobacterium smegmatis* to fluoroquinolones may be influenced by new pentapeptide protein MfpA. Antimicrob. Agents Chemother. **45:**3387–3392.
11. **Munshi, M. H., D. A. Sack, K. Haider, Z. U. Ahmed, M. M. Rahaman, and M. G. Morshed.** 1987. Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1. Lancet **ii:**419–421.
12. **National Committee for Clinical Laboratory Standards.** 1997. Methods for

dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.

13. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests, 6th ed. Approved standard. NCCLS document M2-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
14. **Paterson, D. L., L. Mulazimoglu, J. M. Casellas, W. C. Ko, H. Goossens, A. Von Gottberg, S. Mohapatra, G. M. Trenholme, K. P. Klugman, J. G. McCormack, and V. L. Yu.** 2000. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum β-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. Clin. Infect. Dis. **30:**473–478.
15. **Queenan, A. M., S. Jenkins, and K. Bush.** 2001. Cloning and biochemical characterization of FOX-5, an AmpC-type plasmid-encoded β-lactamase from a New York City *Klebsiella pneumoniae* clinical isolate. Antimicrob. Agents Chemother. **45:**3189–3194.
16. **Tran, J. H., and G. A. Jacoby.** 2002. Mechanism of plasmid-mediated quinolone resistance. Proc. Natl. Acad. Sci. USA **99:**5638–5642.