

Plasmid-Mediated Quinolone Resistance in Non-Typhi Serotypes of *Salmonella enterica*

Kathryn Gay,^{1,2,a} Ari Robicsek,^{3,a} Jacob Strahilevitz,⁴ Chi Hye Park,⁴ George Jacoby,⁵ Timothy J. Barrett,² Felicity Medalla,^{1,2} Tom M. Chiller,² and David C. Hooper⁵

¹Atlanta Research and Education Foundation and ²Centers for Disease Control and Prevention, Atlanta, Georgia; ³Evanston Northwestern Healthcare, Evanston, Illinois; ⁴Massachusetts General Hospital, Boston; and ⁵Lahey Clinic, Burlington, Massachusetts

Background. Serious infections with *Salmonella* species are often treated with fluoroquinolones or extended-spectrum β -lactams. Increasingly recognized in *Enterobacteriaceae*, plasmid-mediated quinolone resistance is encoded by *qnr* genes. Here, we report the presence of *qnr* variants in human isolates of non-Typhi serotypes of *Salmonella enterica* (hereafter referred to as non-Typhi *Salmonella*) from the United States National Antimicrobial Resistance Monitoring System for Enteric Bacteria.

Methods. All non-Typhi *Salmonella* specimens from the United States National Antimicrobial Resistance Monitoring System for Enteric Bacteria collected from 1996 to 2003 with ciprofloxacin minimum inhibitory concentrations ≥ 0.06 $\mu\text{g/mL}$ (233 specimens) and a subset with minimum inhibitory concentrations ≤ 0.03 $\mu\text{g/mL}$ (102 specimens) were screened for all known *qnr* genes (A, B, and S) by polymerase chain reaction. For isolates with positive results, *qnr* and quinolone resistance-determining region sequences were determined. Plasmids containing *qnr* genes were characterized by conjugation or transformation.

Results. Conjugative plasmids harboring *qnrB* variants were detected in 7 *Salmonella enterica* serotype Berta isolates and 1 *Salmonella enterica* serotype Mbandaka isolate. The *S. Mbandaka* plasmid also had an extended-spectrum β -lactamase. Variants of *qnrS* on nonconjugative plasmids were detected in isolates of *Salmonella enterica* serotype Anatum and *Salmonella enterica* serotype Bovismorbificans.

Conclusions. Plasmid-mediated quinolone resistance appears to be widely distributed, though it is still uncommon in non-Typhi *Salmonella* isolates from the United States, including strains that are quinolone susceptible by the criteria of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards). The presence of this gene in non-Typhi *Salmonella* that causes infection in humans suggests potential for spread through the food supply, which is a public health concern.

Until recently, quinolone resistance was believed to arise solely from chromosomal mutations in genes encoding target enzymes or affecting drug accumulation [1]. In 1998, a novel means of quinolone resistance was described in the first confirmed report of horizontally transmissible quinolone resistance [2]. The locus responsible for this plasmid-mediated quinolone resistance, designated *qnrA*, was found on the conjugative plasmid of a clinical *Klebsiella pneumoniae* isolate. Encoding a pentapeptide repeat protein protecting type II

topoisomerases, *qnrA* was later found to reside within class 1 integrons harboring other antimicrobial resistance cassettes [3, 4]. The *qnrA* gene confers nalidixic acid and low-level fluoroquinolone (e.g., ciprofloxacin) resistance, and its presence has been shown to facilitate selection of chromosomal mutations that confer higher levels of resistance [2]. Subsequently, *qnrA* has been described in clinical isolates of *K. pneumoniae*, *Escherichia coli*, *Providencia stuartii*, and *Citrobacter freundii* [5, 6]. A recent study found that 17% of ceftazidime-resistant *Enterobacter* species in the United States harbored *qnrA* [7].

Within the last 2 years, other plasmid-mediated members of the pentapeptide repeat family, *QnrB*, and *QnrS*, have been identified in *Enterobacteriaceae* species [5]. *qnrB* was found in clinical isolates of *K. pneumoniae*, *Citrobacter koseri*, *Enterobacter cloacae*, and *E. coli* from India and the United States [8], and *qnrS* was discovered

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^a K.G. and A.R. contributed equally to this article.

Reprints or correspondence: Dr. Kathryn Gay, Mail Stop G-29, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333 (kgay@cdc.gov).

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in a strain of *Shigella flexneri* 2b from an outbreak of food poisoning in Japan [9]. *qnrB* and *qnrS* are also associated with β -lactamase genes on conjugative plasmids.

Most *Enterobacteriaceae* species in which *qnr* genes have been detected are regular constituents of human flora. Thus, the maintenance and spread of quinolone resistance in these organisms may be promoted by human exposure to quinolones. In contrast, non-Typhi serotypes of *Salmonella enterica* (hereafter referred to as non-Typhi *Salmonella*) are largely carried in the intestinal tract of food animals and are transmitted to humans through the food chain [10]. Therefore, quinolone use in agriculture may drive the dissemination of *qnr*-mediated resistance in these pathogens. Until recently, it was not known whether animal-associated infectious agents could harbor *qnr* genes. The report of a *qnrA* variant in an isolate of *Salmonella enterica* serotype Enteritidis in Hong Kong, however, indicates that this is possible [11].

An estimated 1.4 million people in the United States are infected with non-Typhi *Salmonella* annually, resulting in 15,000 hospitalizations and >400 deaths [12]. Fluoroquinolones are an important antimicrobial class in the treatment of severe *Salmonella* infection [13]. The prevalence of quinolone-resistant *Salmonella* has recently increased in the United States (J. Stevenson, F. Angulo, K.G., T.B., F.M., T.C.; unpublished data). The role of plasmid mediation in this form of quinolone resistance is not known. Therefore, we used strains collected by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS-EB) to determine the incidence of plasmid-mediated quinolone resistance among non-Typhi *Salmonella* serotypes in the United States.

METHODS

Bacteria and antimicrobial susceptibility testing. Starting in 1996, public health laboratories from 13 states submitted every 10th *Salmonella* specimen isolated from humans to the Centers for Disease Control and Prevention for susceptibility testing. The original sites were California (Alameda, Los Angeles, and San Francisco counties), Colorado, Connecticut, Florida, Georgia, Kansas, Massachusetts, Minnesota, New Jersey, New York (Bronx, Brooklyn, New York, Queens, and Richmond counties), Oregon, Washington, and West Virginia. Maryland began submitting isolates in 1997. In 1999, Tennessee was added, and statewide submissions from New York were accepted. Twenty-six states participated in 2002, with the addition of the following states: Arizona, Hawaii, Louisiana, Maine, Michigan, Montana, Nebraska, New Mexico, South Dakota, Texas, and Wisconsin. Also in 2002, surveillance in California extended statewide. In 2003, the study expanded nationwide, and each site submitted every 20th *Salmonella* isolate from humans. Further information on NARMS-EB surveillance can be found on the NARMS-EB Web site [14].

Antimicrobial susceptibility testing was performed at the Centers for Disease Control and Prevention (Atlanta, GA). MICs were determined by microbroth dilution using Sensititre (Trek Diagnostic Systems) for ampicillin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole. With the exception of ceftiofur and streptomycin, susceptibility interpretations for available antimicrobials were defined according to Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) breakpoints; ciprofloxacin and nalidixic acid resistances were defined as MICs ≥ 4 $\mu\text{g/mL}$ and ≥ 32 $\mu\text{g/mL}$, respectively [15]. Ceftiofur and streptomycin resistance breakpoints were defined as MICs ≥ 8 $\mu\text{g/mL}$ and ≥ 64 $\mu\text{g/mL}$, respectively. All available isolates with ciprofloxacin MICs ≥ 0.06 $\mu\text{g/mL}$ were selected for screening for *qnr* genes by PCR. A control group was selected as follows. For each *qnr*-positive isolate, 2 isolates were selected with ciprofloxacin MICs ≤ 0.03 matched for serotype and year and location of acquisition (20 isolates). An additional 82 isolates with ciprofloxacin MICs ≤ 0.03 were selected to represent the 5 most common *Salmonella* serotypes isolated from 1996 to 2003.

PCR screening. Screening was carried out by multiplex PCR amplification of *qnrA*, *qnrB*, and *qnrS*. Colonies were suspended in 50 μL of water in a microcentrifuge tube and boiled to prepare DNA templates for PCR. The primers used to amplify *qnrA* to give a 516-base pair (bp) product were 5'-ATTTCTCACGCCAGGATTTG and 5'-GATCGGCAAAGGTTAGG-TCA. The primers used to amplify *qnrA* to give a 469-bp product were 5'-GATCGTGAAAGCCAGAAAGG and 5'-ACG-ATGCCTGGTAGTTGTCC. The primers used to amplify *qnrS* to give a 417-bp product were 5'-ACGACATTCGTCAACTG-CAA and 5'-TAAATTGGCACCCTGTAGGC. All 6 primers were added to template and PCR Supermix High Fidelity (Invitrogen). PCR conditions were 94°C for 45 s, 53°C for 45 s, and 72°C for 60 s, cycled 32 times. Clinical isolates that had previous confirmation of the *qnr* gene by DNA sequencing were used as positive controls for *qnrA*, *qnrB*, and *qnrS*. Reaction mixes without a DNA template served as negative controls. All positive results were confirmed by direct sequencing of the PCR products.

Transfer of quinolone resistance and plasmid characterization. Conjugation experiments were carried out in Luria-Bertani broth with azide-resistant *E. coli* J53 as the recipient. Transconjugants were selected on trypticase soy agar plates containing ampicillin (100 $\mu\text{g/mL}$), gentamicin (10 $\mu\text{g/mL}$), tetracycline (20 $\mu\text{g/mL}$), or streptomycin (64 $\mu\text{g/mL}$)—but not a quinolone—to avoid the unintentional introduction of spontaneous quinolone-resistance mutations. Sodium azide (100 $\mu\text{g/mL}$) was used for counterselection. Transconjugants displaying

Table 1. Ciprofloxacin MICs of non-Typhi serotypes of *Salmonella enterica*, 1996–2003.

Ciprofloxacin MIC, $\mu\text{g/mL}$	Year								Total
	1996	1997	1998	1999	2000	2001	2002	2003	
≤ 0.03	1311	1282	1433	1478	1338 (1)	1379	1968 (1)	1821	12,010
0.06	8	4	6	4 (1)	7 (2)	6 (2)	4 (2)	5 (2)	44
0.12	0	6 (4)	10 (9)	3 (2)	12 (11)	19 (19)	20 (18)	14 (14)	84
0.25	2 (2)	6 (4)	7 (6)	8 (8)	12 (12)	6 (6)	11 (10)	13 (13)	65
0.5	3 (3)	3 (3)	3 (3)	3 (3)	2 (2)	5 (5)	4 (4)	7 (6)	30
1	0	0	0	1 (1)	1 (1)	1 (1)	0	1 (1)	4
2	0	0	0	0	0	0	0	1	1
≥ 4	0	0	1 (1)	1 (1)	5 (5)	3 (3)	1 (1)	3 (3)	14
Total	1324	1301	1460	1498	1377	1419	2008	1868	12253

NOTE. Data are no. of isolates (no. of nalidixic acid-resistant isolates).

reduced susceptibility to ciprofloxacin were tested against an antibiotic panel (nalidixic acid, ciprofloxacin, ampicillin, cefotaxime, chloramphenicol, gentamicin, sulfamethoxazole, and tetracycline) by agar dilution, in accordance with Clinical and Laboratory Standards Institute guidelines.

The *qnrS*-bearing plasmids could not be transferred by conjugation. For these, transformation was performed. Plasmid DNA was extracted from parent *Salmonella* strains using the Wizard Plus SV Miniprep (Promega) and introduced into electrocompetent *E. coli* DH10B (Invitrogen) by electroporation. Colonies were selected on ciprofloxacin 0.04 $\mu\text{g/mL}$. Plasmid DNA was extracted in the same way from these colonies, and the presence of *qnrS* was confirmed with Southern blot hybridization using a horseradish peroxidase-labeled *qnrS* PCR product obtained with the *qnrS* primers listed above.

Transformants selected in this manner on ciprofloxacin agar could have accumulated chromosomal ciprofloxacin-resistance mutations, invalidating MIC test results. Thus, to isolate transformants suitable for quinolone MIC testing (i.e., without the use of ciprofloxacin agar for selection), a Tn7-based transposon carrying a chloramphenicol-resistance gene was inserted into purified plasmids using the GPS-1 Genome Priming System (New England BioLabs). The resulting derivatives were introduced via electroporation into *E. coli* J53 and selected on chloramphenicol (20 $\mu\text{g/mL}$). Colonies were then individually tested for quinolone resistance. MICs for *E. coli* displaying reduced susceptibility to ciprofloxacin were tested as described above. For both *qnrS*-bearing plasmids, ~ 4 kilobases (kb) of DNA flanking the *qnr* gene was sequenced using a series of outward-facing primers. The *qnrS*-bearing plasmids were extracted for size estimation using the Qiagen Midi Kit (Qiagen), according to the manufacturer's instructions, and run on a 0.8% agarose gel accompanied by a supercoiled DNA ladder (Invitrogen). pMG305 was extracted similarly, and run on 0.4% Megabase Agarose (Bio-Rad Laboratories) alongside a BAC-Tracker supercoiled DNA ladder (Epicentre) and comparator large plasmids. The sequences for *qnrB5*, *qnrS1*, and *qnrS2*

were submitted to GenBank (GenBank accession numbers DQ303919, DQ485529, and DQ485530, respectively). The β -lactamase gene in plasmid pMG307 was characterized and sequenced as previously described [6].

Quinolone resistance-determining region (QRDR) sequencing. The QRDR of *qnr*-positive strains was sequenced as follows. Crude DNA was prepared by suspending 4 or 5 colonies in 100 μL of water. A 255-bp region covering the QRDR of *gyrA* (Met52 to Leu137) was amplified with primers *gyrA1* (5'-CATGAACGTATTGGGCAATG) and *gyrA2* (5'-AGATCGGCCATCAGTTCGTG). The QRDRs of *gyrB*, *parC*, and *parE* were amplified using previously described primers [16]. PCR reactions contained 1 μL of the crude DNA suspension, 0.4 μM of each primer, and AmpliTaq Gold PCR Master Mix (Applied Biosystems) in a final volume of 50 μL . Primers were synthesized at the Biotechnology Core Facility-Scientific Resources Program, National Center for Infectious Diseases at the Centers for Disease Control and Prevention (Atlanta, GA). PCR was carried out in a thermal cycler (MJ Research) programmed with an initial 5-min denaturing step at 95°C, followed by 30 s at 95°C, 1 min at 55°C, and 30 s at 72°C for 35 cycles. Amplicons were sequenced with the primers listed above using ABI Big-Dye 3.1 dye chemistry and ABI 3730XL automated DNA sequencers (PE Biosystems). Analysis was performed using BioEdit software, version 7.05 [17]. The QRDR DNA sequences of *gyrA*, *gyrB*, *parC*, and *parE* for each of the *qnr*-positive isolates were compared with the QRDR DNA sequences of *Salmonella enterica* serotype Typhimurium LT2 (GenBank accession numbers AE008801, AE008878, AE008846, and AE008846, respectively).

RESULTS

Prevalence of fluoroquinolone resistance. From January 1996 through December 2003, NARMS-EB received 12,253 non-Typhi *Salmonella* isolates (table 1). Fourteen isolates (0.1%) were ciprofloxacin resistant (MIC, ≥ 4 $\mu\text{g/mL}$). Of the 14 iso-

Table 2. Characteristics of *Salmonella* isolates with positive test results for the *qnr* gene.

Strain no.	Year of collection	<i>Salmonella enterica</i> serotype	Location	Patient age, sex	Isolate source	Resistance ^a	Ciprofloxacin MIC, $\mu\text{g/mL}$	Nalidixic acid MIC, $\mu\text{g/mL}$	<i>qnr</i> Type
7	1997	Berta	Los Angeles County, California	32/F	Stool	AMP, GEN, STR (KAN)	0.5	32	<i>qnrB5</i>
14	1998	Berta	Los Angeles County, California	8/M	Stool	AMP, GEN, KAN, STR (AMX-CLA, CEF)	0.5	32	<i>qnrB5</i>
25	1999	Berta	Los Angeles County, California	11/M	Stool	AMP, GEN, STR (AMX-CLA, KAN)	0.5	64	<i>qnrB5</i>
30	1999	Berta	Maryland	42/F	Stool	AMP, GEN, STR	0.25	32	<i>qnrB5</i>
40	2000	Berta	Los Angeles County, California	27/F	Stool	AMP, GEN, STR	0.25	32	<i>qnrB5</i>
42	2000	Berta	Los Angeles County, California	20/F	Stool	AMP, GEN, STR (KAN)	0.25	32	<i>qnrB5</i>
47	2000	Berta	Los Angeles County, California	5 months/F	Stool	AMP, GEN, STR (CEF, KAN)	0.25	32	<i>qnrB5</i>
56	2002	Bovismorbificans	Hawaii	38/M	Stool	AMP, SUL	0.5	32	<i>qnrS1</i>
60	2002	Mbandaka	New York state	37/F ^b	Urine	AMP, TIO, SUL, SXT (CHL)	0.25	16	<i>qnrB2</i>
77	2003	Anatum	Massachusetts	77/F	Stool	...	0.5	16	<i>qnrS2</i>

NOTE. AMP, ampicillin; AMX-CLA, amoxicillin-clavulanic acid; CEF, cephalothin; CHL, chloramphenicol; FOX, cefoxitin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; SUL, sulfamethoxazole, SXT, trimethoprim-sulfamethoxazole; TIO, ceftiofur.

^a Parentheses denote intermediate resistance, as defined by the Clinical and Laboratory Standards Institute.

^b This patient had HIV infection and was in prison.

Table 3. Profiles of transconjugants and/or transformants and quinolone resistance recipient *Escherichia coli* J53.

Strain or plasmid	<i>Salmonella enterica</i> source serotype	Conjugative	<i>qnr</i> Type	MIC, $\mu\text{g}/\text{mL}$						
				NAL	CIP	AMP	CTX	GEN	SUL	TET
<i>E. coli</i> J53	4	≤ 0.016	8	0.064	0.25	64	2
pMG305	Berta	Positive	<i>qnrB5</i>	32	1	>32	0.064	256	64	2
pMG306	Bovismorbificans	Negative	<i>qnrS1</i>	32	1	8	0.064	0.25	64	2
pMG307	Mbandaka	Positive	<i>qnrB2</i>	32	0.128	>32	0.25	0.25	>512	2
pMG308	Anatum	Negative	<i>qnrS2</i>	32	1	8	0.064	0.25	64	2

NOTE. AMP, ampicillin; CIP, ciprofloxacin; CTX, cefotaxime; GEN, gentamicin; NAL, nalidixic acid; SUL, sulfamethoxazole; TET, tetracycline.

lates, 9 were *Salmonella enterica* serotype Senftenberg, 3 were *Salmonella enterica* serotype Schwarzengrund, 1 was *Salmonella enterica* serotype Indiana, and 1 was *S. Typhimurium*. Low-level ciprofloxacin resistance (MIC, $\geq 0.25 \mu\text{g}/\text{mL}$ but $< 4 \mu\text{g}/\text{mL}$) was found in 100 (0.8%) isolates. The 3 most common serotypes among isolates with low-level ciprofloxacin resistance were *Salmonella enterica* serotype Enteritidis (18 isolates), *Salmonella enterica* serotype Paratyphi A (18 isolates), and *S. Typhimurium* (14 isolates). Low-level ciprofloxacin resistance was also found in 2 *Salmonella enterica* serotype Anatum isolates (2.3%), 7 *S. Berta* isolates (8.5%), 2 *Salmonella enterica* serotype Bovismorbificans isolates (8.3%), and 1 *S. Mbandaka* isolate (1.6%). Of the 242 isolates with ciprofloxacin MICs $\geq 0.06 \mu\text{g}/\text{mL}$, 233 (96.3%) were available for screening.

Screening for *qnr* genes. A PCR product corresponding in size to a positive control could be amplified from 10 (5%) of 233 tested isolates with ciprofloxacin MICs $\geq 0.06 \mu\text{g}/\text{mL}$. No product was detected in the 102 isolates with ciprofloxacin MICs $\leq 0.03 \mu\text{g}/\text{mL}$ that were screened. Sequencing confirmed all 10 PCR products to be *qnr* genes. Four serotypes were involved (table 2). Seven *S. Berta* isolates, collected from 1997 through 2000, carried *qnrB*. Of the isolates collected in 2002, 1 *S. Bovismorbificans* isolate carried *qnrB*, and 1 *S. Mbandaka* isolate carried *qnrS*. In 2003, 1 *S. Anatum* isolate found in a urine sample contained *qnrS*. All other *qnr*-positive isolates came from stool samples. All 10 *qnr*-positive isolates had low-level resistance to ciprofloxacin (MIC, $0.25 \mu\text{g}/\text{mL}$ – $0.5 \mu\text{g}/\text{mL}$). Eight of the 10 isolates were resistant to nalidixic acid, and 9 of the 10 were resistant to non-quinolone antimicrobials. The *qnr* gene was not found in any of the fully ciprofloxacin-resistant isolates in the collection.

Transfer of quinolone resistance. Quinolone resistance was transferred to *E. coli* J53 Azi^R by conjugation from *S. Berta* strains 7, 30, and 40 (chosen for their temporal and geographical diversity) and *S. Mbandaka* strain 60 and by transformation from *S. Bovismorbificans* strain 56 and *S. Anatum* strain 77. The 3 *S. Berta* strains transferred *qnrB*-bearing plasmids identical in size and resistance properties that are represented by

pMG305, a 95-kb plasmid that carries resistance to ampicillin and gentamicin, as well as to quinolones. The *qnrB*-bearing plasmid from the *S. Mbandaka* isolate (pMG307) carried resistance to ampicillin and sulfamethoxazole. It was found to harbor the extended-spectrum β -lactamase SHV-30. The non-conjugative *qnrS* plasmids in *S. Bovismorbificans* (pMG306) and *S. Anatum* (pMG308) were 10 kb and 8 kb in size, respectively, and neither appeared to carry other resistance genes (table 3).

Sequencing of *qnr* genes and flanking DNA. The *qnrB* allele from *S. Mbandaka* was identical to a variant (*qnrB2*) that is predominant among recent isolates of *Klebsiella* and *Enterobacter* species collected in the United States [8]. The *qnrB* allele found on pMG305 from *S. Berta* was novel, comprising a 678-bp open reading frame encoding a protein (QnrB5) with 95.6% amino acid identity to QnrB1 (figure 1). The *qnrS* allele (now designated *qnrS1*) located on pMG306 from *S. Bovismorbificans* showed complete sequence identity to that previously found on conjugative plasmid pAH0376 from a *Shigella* species strain isolated in Japan [9]. Sequencing of pMG306 from *S. Bovismorbificans* showed that *qnrS1* was located within a 1087-bp segment identical to the region including and surrounding *qnrS1* in pAH0376 (figure 2). This region was flanked by inverted repeat segments at least 1.2 kb in length that are related to a putative insertion sequence in a plasmid from a *Serratia* species isolate. (GenBank accession number AF135182). The *qnrS* allele detected on pMG308 from *S. Anatum* codes for a protein 91.3% identical in amino acid sequence to QnrS1 and has been designated *qnrS2* (figure 1). pMG308 had no similarity to pAH0376 in ~ 5 kb of flanking sequence, nor did this region appear to include resistance genes, transposable elements, or components of an integron. Open reading frames similar to *repC* and *repA*, genes involved in plasmid replication, were located immediately downstream of *qnrS2*. A third open reading frame, 606 bp in length, had no related sequence in GenBank.

QRDR sequencing. No *gyrA* mutations were detected in the *qnr*-positive *Salmonella* isolates. Strains 56 and 60, the *S.*

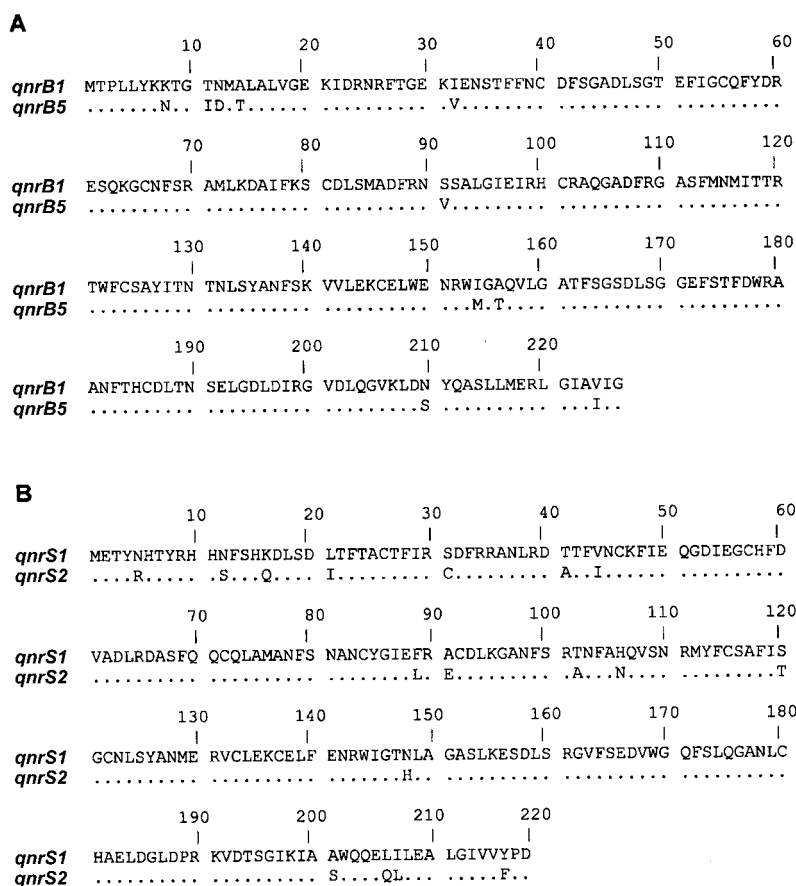


Figure 1. Sequence comparison of *qnr* alleles. A, *qnrB1* and *qnrB5*. B, *qnrS1* and *qnrS2*. The dots indicate identical amino acid residues.

Bovismorbificans and the *S. Mbandaka* isolates, had wild-type sequence for *gyrB* codons 443 and 444. The other isolates had *gyrB* point mutations with the resulting amino acid substitutions Ala443Val and Arg444Lys. In *parC*, the following substitutions were found: Thr57Ser alone (4 substitutions); Met49Ile, Ser50Ala, and Thr57Ser (3 substitutions); Met49Ile and Thr57Ser (2 substitutions); and Met49Ile and Ser50Ala (1 substitution). None of these *gyrB* and *parC* mutations have been linked to quinolone resistance.

DISCUSSION

This is the first report of plasmid-mediated quinolone resistance in *Salmonella* isolates from the United States. Ten of 335 tested isolates were positive for either *qnrB* or *qnrS*. The *Salmonella* plasmids bearing a *qnr* gene were neither an isolated nor a local phenomenon. We identified *qnr* genes in 4 non-Typhi serotypes: *S. Anatum*, *S. Berta*, *S. Bovismorbificans*, and *S. Mbandaka*. The geographical distribution of these strains was broad, with 3 strains collected from the east coast of the United States, 6 from the west coast, and 1 from Hawaii.

Seven of the 10 *qnr*-positive isolates in this study were *S. Berta*, a poultry-associated serotype. Although 6 of the *S. Berta*

isolates were found in specimens from residents of Los Angeles County, California, they were not associated with any known outbreak, and each isolate had a unique PFGE pattern (data not shown). In Hong Kong, plasmids containing a variant of *qnrA* were recently described in *S. Enteritidis*, another poultry-associated serotype [11].

Plasmid-mediated quinolone resistance in *Salmonella* has important public health implications. Fluoroquinolones, such as ciprofloxacin, are commonly used to treat adults with invasive illness or other serious infections due to *Salmonella* species. A number of studies indicate that patients infected with *Salmonella* with low-level fluoroquinolone resistance may respond poorly to fluoroquinolone treatment [18]. Low- and high-level fluoroquinolone resistance has become increasingly prevalent, especially in Asia [19, 20], but also in Europe and North America (J. Stevenson, F. Angulo, K.G., T.B., F.M., T.C.; unpublished data) [21]. The emergence and spread of *qnr* genes on plasmids would further promote the rapid spread of fluoroquinolone resistance. Furthermore, plasmid-mediated quinolone resistance appears often to be cotransmitted with resistance to broad-spectrum β -lactams, enabling the promulgation of simultaneous resistance to 2 classes of antimicrobial agents com-

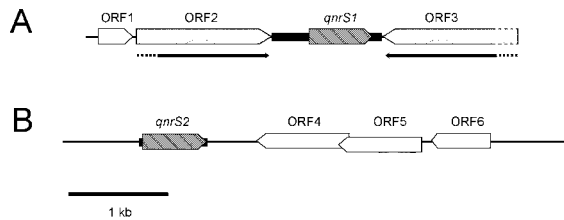


Figure 2. Regions containing the *qnrS* gene in the nonconjugative plasmids pMG306 and pMG308. *A*, In pMG306, ORF1 has 82% amino acid identity with YdaA of *Escherichia coli* (AAK09055), a putative resolvase. ORF2 and ORF3 each have >80% amino acid identity with both Sea14 (AAR13141) and Sea15 (AAR13142) of *Serratia entomophilla* and are within inverted repeats represented by arrows. Dashed lines represent unsequenced region. The bold line represents nucleotide sequence identical to pAH0376 of *Shigella flexneri* (AB187515) and contains *qnrS1*. *B*, In pMG308, ORF4 has 74% amino acid identity with RepC of a *Pasturella multocida* plasmid (YP_232871). ORF5 has 84% amino acid identity with RepA of an *Aeromonas salmonicida* plasmid (NP_387463). The bold line represents nucleotide sequence with 89% nucleotide identity to pAH0376.

monly used to treat serious *Salmonella* infection and coselection by use of either agent alone.

To determine whether *qnr* genes alone were the likely determinants of reduced ciprofloxacin susceptibility, we sequenced the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* in all 10 isolates. All isolates had wild-type *gyrA*. Point mutations were detected in *gyrB*, *parC*, and *parE*, but the significance of these mutations is unclear, because they have not been linked to reduced susceptibility to quinolones. All *qnr*-positive isolates, except for *S. Bovismorbificans*, had a *parC* Tyr57Ser substitution. This substitution was previously described in *Salmonella* species by Eaves et al. [16] as being associated with increased susceptibility to ciprofloxacin but not nalidixic acid. The elevated quinolone MICs of the *E. coli* transconjugants suggest that reduced ciprofloxacin susceptibility of these *Salmonella* isolates is attributable to the plasmid-encoded genes alone. The Clinical and Laboratory Standards Institute recommends that extraintestinal *Salmonella* isolates be screened for nalidixic acid resistance (MIC, ≥ 32 $\mu\text{g}/\text{mL}$) [15]. Our findings suggest that salmonellae in which the single mechanism of resistance is not a QRDR mutation, but a *qnr* gene, may have a nalidixic acid MIC as low as 16 $\mu\text{g}/\text{mL}$ and, hence, would be missed by the recommended clinical laboratory screening.

The ongoing sources of selective pressure driving quinolone resistance are not certain. In 1996, the veterinary fluoroquinolone, enrofloxacin, was approved by the Food and Drug Administration for the control of chicken mortality associated with *E. coli* and turkey mortality associated with *E. coli* and *Pasturella multocida*. The subsequent emergence of fluoroquinolone-resistant *Campylobacter* species and the associated public health hazard led to the recent withdrawal of enrofloxacin for use in poultry

in July 2005 [22]. Although this action should reduce selective pressure for the dissemination of quinolone resistance of *Salmonella* in poultry, fluoroquinolones continue to be used in the treatment of beef cattle in the United States. Also, imported foods and foreign travel remain threats to human health from quinolone use in food animals in other countries.

We have demonstrated that plasmid-mediated quinolone resistance exists among non-Typhi *Salmonella* isolates in the United States. The presence of multiple variants of *qnr* genes suggests they have a substantial evolutionary history, and their presence in several *Salmonella* serotypes, from widely separated states, suggests broad host and geographic distribution. The relatively low MIC of nalidixic acid found in these organisms raises the concern that these genes could spread insidiously, resulting in a substantial population of organisms on the threshold of high-level resistance that would continue to be exposed to quinolones. We identified *qnr* genes on 4 substantially different plasmids. The cotransmission of extended-spectrum β -lactamases on such plasmids is a particular threat to therapy. Characterization of *Salmonella* strains from animals and foods may clarify the sources and selective pressures leading to the emergence of these strains. Conjugative *qnr*-bearing plasmids in non-Typhi *Salmonella* demonstrate the sobering potential for quinolone resistance selected and maintained through antibiotic use in animals to take hold in human flora.

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