Identification and Characterization of *Salmonella enterica* Serotype Newport Isolates with Decreased Susceptibility to Ciprofloxacin in the United States

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ABSTRACT Nontyphoidal *Salmonella* (NTS) causes an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the United States. Decreased susceptibility to ciprofloxacin (DSC) has historically been associated with chromosomal mutations of the quinolone resistance determining region (QRDR), but plasmid-mediated quinolone resistance (PMQR) genes are increasing. To investigate DSC among *Salmonella enterica* serotype Newport strains, we examined 40 isolates from 1996 to 2016 with DSC. Thirty isolates (71%) contained the PMQR gene *qnrB* and eight isolates (19%) contained a QRDR.

KEYWORDS *Salmonella*, antibiotic resistance

Nontyphoidal *Salmonella* (NTS) strains cause an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the United States and are transmitted commonly through food (1). Resistance to fluoroquinolones (e.g., ciprofloxacin), which are used to treat severe NTS infections in adults, has historically been associated with chromosomal mutations in quinolone resistance determining regions (QRDRs) of *gyrA* and *parC*, but plasmid-mediated quinolone resistance (PMQR) genes appear to be increasing in frequency (2). The PMQR genes include *qnr* genes, *aac(6′)-Ib-cr*, *qepA*, and *oqxAB*. Isolates with PMQR can have decreased susceptibility (intermediate or resistant) to ciprofloxacin (DSC; MIC, ≥ 0.12 mg/liter), but unlike those with a QRDR, may not be resistant to the quinolone nalidixic acid (MIC, ≥ 32 mg/liter). DSC can contribute to treatment failure in patients infected with *Salmonella* (3). PMQR genes may facilitate the acquisition of a secondary chromosomal mutation, resulting in higher levels of resistance to fluoroquinolones (4, 5, 6).

Since 1996, the National Antimicrobial Resistance Monitoring System (NARMS) at the Centers for Disease Control and Prevention (CDC) has conducted susceptibility testing on *Salmonella* isolates from humans for up to 15 antimicrobials (7). Health departments send the CDC a frequency-based sample of *Salmonella* isolates received from clinical laboratories in the United States for routine surveillance. They also send isolates from outbreaks. MICs were determined using broth microdilution (Sensititre; Thermo Fisher Scientific, Waltham, MA) with interpretive criteria from the Clinical and Laboratory Standards Institute (CLSI) when available (7, 8). DSC increased from 0.4% (out of 1,325 isolates) in 1996 to 4.3% (out of 2,127 isolates) in 2014 in NTS surveillance isolates (7). Approximately 20% of NTS surveillance isolates that showed DSC were nalidixic acid susceptible, suggesting that they may have PMQR, which usually confers decreased susceptibility to fluoroquinolones but has less effect on nalidixic acid; *S. Newport* was the second most common serotype among these isolates. To investigate DSC in *Salmonella enterica* serotype Newport, we examined all 40 isolates with DSC (out of 4,545 isolates; 0.9%), including 39 patient isolates (26 from routine surveillance and 13

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from outbreaks) and one food (tomato) isolate (from an outbreak investigation), collected from 1996 through 2016.

To identify the presence of PMQR genes and QRDRs, whole-genome sequence (WGS) analysis and/or PCR analysis was performed. Genomic DNA was purified using the DNeasy blood and tissue kit (Qiagen Inc.) according to the manufacturer's protocol. Libraries were constructed for the genome, inclusive of plasmids, using NexteraXT (Illumina, San Diego, CA) or NEBNext (New England BioLabs, Ipswich, MA) library kits, and paired-end base pair reads were generated on a MiSeq or HiSeq instrument (Illumina, San Diego, CA).

Raw reads were assembled *de novo* using CLC Genomics Workbench 7 (Qiagen Inc.). The whole-genome sequence was analyzed using ResFinder 2.1 (90% threshold for percent identity and 60% minimum length) (<http://cge.cbs.dtu.dk/services/>) to identify PMQR genes and using an in-house workflow to identify QRDRs. PCR analysis and sequencing (to identify mutations in QRDRs) was used to identify the following PMQR genes and QRDRs: *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, *oqxAB*, *aac(6')-Ib-cr*, *gyrA*, and *parC*. A DNA template for PCR was obtained using the boil-prep method (9). PCR mixtures contained 2× HotStart PCR master mix (Qiagen Inc., Valencia, CA), 0.4 μM each primer, 5 μl of template DNA, and sterile PCR water to a final volume of 50 μl. Thermal cycling was performed as previously described (10, 11, 12, 13, 14, 15, 16). XbaI restriction enzyme pulsed-field gel electrophoresis (PFGE) was performed on 35 of the 40 isolates according to the CDC PulseNet *Salmonella* protocol, and all generated PFGE profiles were submitted to the PulseNet National Database (17).

Thirty of 40 (75%) *S. Newport* isolates contained the PMQR gene, *qnrB* (Table 1). Six of these *qnrB*-positive isolates had WGS data, which allowed us to identify five alleles as *qnrB19* and one (AM42797) as *qnrB2*. No additional PMQR genes were identified. Eight (20%) isolates contained a point mutation in the QRDR, and all of these were common *gyrA* mutations, including S83P, S83Y, and D87G. Two (5%) isolates had no PMQR genes or QRDR detected. Unfortunately, isolate AM18182 was lost and could not be retested. However, isolate 2013AM-1610 was confirmed as DSC and nalidixic acid susceptible, suggesting that it may contain a novel PMQR mechanism. Several plasmid alleles, including ColE, IncP, Inc11, and IncA/C, were identified among *qnrB*-positive isolates (data not shown). Ten different pulsed-field gel electrophoresis (PFGE) patterns were identified among 30 isolates that contained a *qnrB* gene, suggesting that clonal expansion alone is not responsible for the observed DSC (data not shown). Most of the isolates with QRDR resistance mechanisms were from earliest study period years.

The data suggest that the DSC among *Salmonella enterica* serotype Newport isolates is due to multiple acquisitions of the PMQR gene *qnrB*. In two isolates with WGS data, the *qnrB19* gene was on the ColE plasmid, and this plasmid type was present in almost all other *qnrB*-positive isolates. This is consistent with our recent WGS analysis of all *Salmonella* serotypes, in which the majority of *qnrB19* genes are located on small ColE plasmids (data not shown). The *qnrB2* gene was not located on a ColE plasmid. Previous U.S. studies have identified PMQR genes, including *qnrA*, *qnrB*, and *qnrS*, among NTS isolates from humans (18, 19, 20). Globally, PMQR genes have been identified in *Salmonella* isolates from international travelers and from a wide range of foods and animals (21, 22, 23, 24). More recently, a number of *qnrB19*-positive nontyphoidal *Salmonella* isolates were found in U.S. swine cecal and pork chop samples collected from 2013 to 2015 by NARMS; the *qnrB19* genes were carried on ColE plasmids (25). This suggests that swine may be a source of *qnrB* genes in the United States; however, none of the *Salmonella* isolates in that study were *Salmonella enterica* serotype Newport. None of the *S. Newport* isolates in our study, whether routine or outbreak associated, were specifically linked to swine or pork products.

Additional studies are needed to better understand the spread of *qnrB* genes in the United States.

TABLE 1 PMQR genes and QRDRs identified among *Salmonella* Newport isolates with decreased susceptibility to ciprofloxacin^c

Isolate	Yr isolated	Ciprofloxacin MIC (μg/ml)	Ciprofloxacin interpretation	Nalidixic acid MIC (μg/ml)	Nalidixic acid conclusion	PMQR gene	QRDR mutation
AM03005 ^b	1998	0.25	I	16	S	<i>qnrB</i>	
AM15201 ^b	2002	0.12	I	>32	R		<i>gyrA</i> (S83P)
AM13780	2002	0.12	I	>32	R		<i>gyrA</i> (S83P)
AM18182	2003	0.12	I	>32	R		
AM21465 ^b	2004	0.12	I	>32	R		<i>gyrA</i> (S83P)
AM27596	2006	0.5	I	>32	R		<i>gyrA</i> (S83P)
AM27090	2006	0.5	I	>32	R		<i>gyrA</i> (S83P)
AM37895	2008	0.12	I	>32	R		<i>gyrA</i> (S83Y)
AM45366	2010	0.12	I	>32	R		<i>gyrA</i> (D87G)
AM42797 ^a	2010	0.25	I	8	S	<i>qnrB</i>	
AM48381	2011	0.5	I	32	R	<i>qnrB</i>	
AM51389	2012	0.5	I	16	S	<i>qnrB</i>	
2012AM-0810	2012	0.5	I	16	S	<i>qnrB</i>	
2012AM-0887	2012	0.5	I	16	S	<i>qnrB</i>	
2012K-0801	2012	0.5	I	16	S	<i>qnrB</i>	
2012K-0782	2012	0.5	I	16	S	<i>qnrB</i>	
2012K-0781	2012	0.5	I	16	S	<i>qnrB</i>	
AM51676	2012	0.5	I	16	S	<i>qnrB</i>	
2012AM-0809	2012	0.5	I	16	S	<i>qnrB</i>	
AM51387	2012	0.5	I	16	S	<i>qnrB</i>	
2012K-0939	2012	0.5	I	16	S	<i>qnrB</i>	
2012K-0940	2012	0.5	I	32	R	<i>qnrB</i>	
AM51675	2012	0.5	I	16	S	<i>qnrB</i>	
2012K-0938	2012	0.5	I	32	R	<i>qnrB</i>	
2012k-0783	2012	0.5	I	16	S	<i>qnrB</i>	
2013AM-1610	2013	0.12	I	16	S		
2013AM-0157	2013	0.5	I	16	S	<i>qnrB</i>	
2013K-1663	2013	0.5	I	16	S	<i>qnrB</i>	
2013AM-2500	2013	0.5	I	16	S	<i>qnrB</i>	
2013K-1664	2013	0.5	I	16	S	<i>qnrB</i>	
2013K-1665	2013	0.5	I	16	S	<i>qnrB</i>	
2014K-0127	2014	0.5	I	16	S	<i>qnrB</i>	
2014K-0126	2014	0.5	I	16	S	<i>qnrB</i>	
2014K-0125	2014	0.5	I	16	S	<i>qnrB</i>	
2014AM-3129 ^a	2014	0.5	I	16	S	<i>qnrB</i>	
2015AM-0402 ^a	2015	0.5	I	16	S	<i>qnrB</i>	
2015AM-1580 ^a	2015	0.5	I	16	S	<i>qnrB</i>	
2015AM-2492 ^a	2015	0.25	I	32	R		<i>gyrA</i> (S83P)
2016AM-0477 ^a	2016	0.5	I	16	S	<i>qnrB</i>	
2016AM-1098 ^a	2016	0.5	I	16	S	<i>qnrB</i>	

^aIsolates that have WGS data used for analysis (NCBI GenBank accession numbers [SRR1840729](https://doi.org/10.1093/aac.111101), [SRR6457029](https://doi.org/10.1093/aac.111102), [SRR5839938](https://doi.org/10.1093/aac.111103), [SRR5759622](https://doi.org/10.1093/aac.111104), [SRR6929568](https://doi.org/10.1093/aac.111105), [SRR6929569](https://doi.org/10.1093/aac.111106), and [SRR6929570](https://doi.org/10.1093/aac.111107)).

^bIsolates previously reported in reference 26.

^cPMQR, plasmid-mediated quinolone resistance; QRDR, quinolone resistance-determining region.

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