

## Decreased Susceptibility to Ciprofloxacin among *Shigella* Isolates in the United States, 2006 to 2009<sup>∇</sup>

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**We characterized 20 *Shigella* isolates with decreased susceptibility to fluoroquinolones. Most patients (80%) from whom a travel history was obtained reported travel to South or Southeast Asia. Mutations within the quinolone resistance determining regions of *gyrA* and *parC* and plasmid-mediated resistance determinants (*qnrB*, *qnrS*, and *aac(6′)-Ib-cr*) were identified. The rise in antimicrobial resistance among *Shigella* isolates may necessitate the increased use of extended-spectrum cephalosporins or macrolides in some patients.**

Shigellosis is a major source of gastroenteritis throughout the world, and severe infections may require antimicrobial treatment (16). Antimicrobial treatment can shorten the duration and severity of illness (12). However, the emergence of multidrug resistance has made the selection of effective antimicrobial therapy more difficult (1). Ampicillin and trimethoprim-sulfamethoxazole resistance is highly prevalent among *Shigella* strains in the United States and may necessitate the use of extended-spectrum cephalosporins or fluoroquinolones for the treatment of shigellosis (20). Recently, extended-spectrum cephalosporin resistance in *Shigella* has begun to emerge in the United States, threatening to leave fluoroquinolones as the mainstay of treatment. Importantly, fluoroquinolone resistance has been rising among *Shigella* strains, especially in Asia (4, 15). In this study, we characterize *Shigella* isolates collected in the United States with decreased susceptibility to ciprofloxacin.

State public health laboratories that participate in the National Antimicrobial Resistance Monitoring System (NARMS) submit every 20th *Shigella* isolate to the CDC for susceptibility testing. Broth microdilution (Sensititre; Trek Diagnostics, Westlake, OH) is used to determine the MIC for a panel of 15 antimicrobials. From 2006 to 2009, 2,026 routine surveillance *Shigella* isolates were submitted. Twenty isolates displayed decreased susceptibility (MIC  $\geq$  0.25  $\mu$ g/ml) to the fluoroquinolone ciprofloxacin. Fifteen of these isolates (75%) were resistant (MIC  $\geq$  32  $\mu$ g/ml) to the quinolone nalidixic acid (Table 1). Among the isolates, 10 were *Shigella flexneri*, six *S. sonnei*, three *S. boydii*, and one *S. dysenteriae*. The patients included 13 males, 5 females, and 2 for whom the sex was unknown; patients resided in 15 different states. The ages ranged from 1 to 76 years; the median age was 30.5 years. Additional information was available for 10 patients. Eight patients (80%) re-

ported recent travel to India or Thailand. Nine patients reported diarrhea, and four reported fever and vomiting. None of the isolates were part of any recognized *Shigella* outbreaks.

Decreased susceptibility to ciprofloxacin among the *Enterobacteriaceae* is usually due to the acquisition of mutations in the quinolone resistance determining regions (QRDR) of the DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) genes and/or plasmid-mediated quinolone resistance (PMQR) determinants, such as target protection genes (*qnr*), aminoglycoside acetyltransferase variants [*aac(6′)-Ib-cr*], or efflux genes (*qepA*). PCR was used to amplify the *gyrA* and *parC* genes from each isolate, and the QRDR region was sequenced (5). Fifteen isolates contained QRDR mutations (Table 1). All 15 had the common first-step *gyrA* mutation (Ser83Leu), 8 had a second *gyrA* mutation (Asp87Asn/Gly), and 13 had the common second-step *parC* mutation (Ser80Ile). The isolates containing QRDR mutations were nalidixic acid resistant, and nine were resistant to ciprofloxacin ( $\geq$ 4  $\mu$ g/ml). PCR analysis was used to screen all the isolates for PMQRs [*qnrA*, *qnrB*, *qnrD*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA*] (3, 14, 15, 19). Six isolates were PCR positive for *qnr* genes (four *qnrS* genes and two *qnrB* genes), and one *qnrB*-positive isolate was also positive for the *aac(6′)-Ib* gene (Table 2). Sequencing analysis determined that all four *qnrS* genes were *qnrSI*, while isolate AM29213 contained the *qnrB19* gene. Isolate AM38988 contained the *qnrB6* gene and the *aac(6′)-Ib-cr* variant (7, 18).

To determine the location of the PMQR genes, plasmids were purified from the six isolates and transferred by electroporation into *Escherichia coli* DH10B using 0.2  $\mu$ g/ml of ciprofloxacin for selection (6). PCR analysis confirmed that all PMQRs transferred and the *qnrB6* gene was located on the same plasmid as the *aac(6′)-Ib-cr* gene. The transformants demonstrated higher MICs to nalidixic acid and ciprofloxacin than untransformed DH10B, and most of the plasmids conferred resistance to additional antimicrobial drugs, including ampicillin, sulfisoxazole, trimethoprim-sulfamethoxazole, and tetracycline (Table 2).

PCR-based inc/rep typing (PBRT) and plasmid sizing determined that the *qnrB19* plasmid was a 7-kb ColE<sub>PB</sub> plasmid, while the *qnrB6* plus *aac(6′)-Ib-cr* plasmid was a 60-kb IncN

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TABLE 1. Characteristics of *Shigella* isolates with decreased susceptibility to ciprofloxacin<sup>a</sup>

Isolate <sup>b</sup>	Yr	Species	MIC (µg/ml)		PMQR determinant	Amino acid in QRDR <sup>d</sup>		
			NAL	CIP		<i>gyrA</i>		<i>parC</i> , Ser80
						Ser83	Asp87	
<b>AM25973</b>	2006	<i>S. flexneri</i>	>32	4	— <sup>c</sup>	Leu	Asn	Ile
AM26336	2006	<i>S. flexneri</i>	>32	0.5	—	Leu	wt	Ile
AM28928	2006	<i>S. flexneri</i>	>32	0.5	—	Leu	wt	Ile
AM29213	2006	<i>S. sonnei</i>	8	0.25	<i>qnrB19</i>	wt	wt	wt
AM29884	2006	<i>S. flexneri</i>	>32	0.5	—	Leu	wt	Ile
<b>AM31384</b>	2007	<i>S. flexneri</i>	8	0.25	<i>qnrS1</i>	wt	wt	wt
AM33008	2007	<i>S. flexneri</i>	>32	4	—	Leu	Asn	Ile
<b>AM34240</b>	2007	<i>S. flexneri</i>	>32	0.5	—	Leu	wt	Ile
AM35196	2008	<i>S. sonnei</i>	>32	4	—	Leu	wt	Ile
<b>AM36602</b>	2008	<i>S. sonnei</i>	>32	4	—	Leu	Gly	Ile
AM37173	2008	<i>S. flexneri</i>	>32	>4	—	Leu	Gly	Ile
<b>AM37916</b>	2008	<i>S. sonnei</i>	>32	4	—	Leu	Gly	Ile
AM38397	2008	<i>S. dysenteriae</i>	4	0.25	<i>qnrS1</i>	wt	wt	wt
<b>AM38786</b>	2008	<i>S. boydii</i>	8	0.25	<i>qnrS1</i>	wt	wt	wt
<b>AM38301</b>	2009	<i>S. boydii</i>	2	0.25	<i>qnrS1</i>	wt	wt	wt
AM38988	2009	<i>S. flexneri</i>	>32	>4	<i>qnrB6 + aac(6′)-Ib-cr</i>	Leu	wt	Ile
AM39146	2009	<i>S. flexneri</i>	>32	4	—	Leu	Asn	Ile
<b>AM39973</b>	2009	<i>S. sonnei</i>	>32	0.25	—	Leu	Asn	wt
AM41657	2009	<i>S. boydii</i>	>32	4	—	Leu	Asn	Ile
AM41735	2009	<i>S. sonnei</i>	>32	0.25	—	Leu	wt	wt

<sup>a</sup> CIP, ciprofloxacin; NAL, nalidixic acid; PMQR, plasmid-mediated quinolone resistance; Asn, asparagine; Asp, aspartic acid; Leu, leucine; Ile, isoleucine; Ser, serine.

<sup>b</sup> Isolates in bold were from patients who reported recent travel to India or Thailand.

<sup>c</sup> —, none identified.

<sup>d</sup> The number indicates the position of the amino acid change. “wt” indicates the wild-type amino acid.

and ColE-type plasmid (2, 7, 13). Of the four remaining plasmids, two were 60 kb and two were 80 kb in size, and all were untypeable for the replicons currently included in the PBRT scheme. Interestingly, *qnrB19*-ColE plasmids, similar to those identified in isolate AM29213, were previously identified in *Salmonella enterica* Typhimurium isolated in the Netherlands and in commensal *E. coli* isolated from children living in different urban areas of Peru and Bolivia (9, 13). These plasmids apparently play a major role in the widespread dissemination of *qnrB19* genes observed in both pathogenic and commensal enterobacteria.

Few studies have examined quinolone resistance among *Shigella* isolates in the United States and the resistance mechanisms involved (17, 20, 21). In this study, we have identified *Shigella* isolates with decreased susceptibility to ciprofloxacin. Isolates containing QRDR mutations (*n* = 15) displayed nalidixic resistance (MIC ≥ 32 µg/ml) and ciprofloxacin suscepti-

bility of ≥0.25 µg/ml, while those containing PMQR determinants in the absence of QRDR mutations displayed nalidixic acid susceptibility (MIC ≤ 8 µg/ml) and ciprofloxacin MICs of 0.25 µg/ml. All ciprofloxacin-resistant isolates contained multiple QRDR mutations. This supports the idea that QRDR mutations are largely responsible for nalidixic acid and ciprofloxacin resistance among *Shigella* isolates. However, PMQR determinants are located on mobile genetic elements (i.e., plasmids), which may allow for dissemination among *Shigella* and possibly additional members of the *Enterobacteriaceae*. Also, it is believed that the presence of PMQR may facilitate the selection of QRDR mutations, resulting in higher levels of quinolone resistance (10).

Most of the patients (80%) from whom a travel history was obtained reported recent travel to Asia. Several reports have described the increasing prevalence of ciprofloxacin resistance among sporadic and epidemic *Shigella* isolates from China and

TABLE 2. Characteristics of plasmids and transformants with decreased susceptibility to ciprofloxacin<sup>a</sup>

Transformant	MIC (µg/ml)		PMQR determinant	Additional resistance	Plasmid size (kb)	Incompatibility type
	NAL	CIP				
DH10B	1	<0.015	— <sup>b</sup>	STR	—	—
DH/29213	4	0.0625	<i>qnrB19</i>	STR	7	ColE <sub>PB</sub>
DH/31384	4	0.125	<i>qnrS1</i>	STR	60	Unknown
DH/38397	4	0.125	<i>qnrS1</i>	AMP, FIS, SXT, STR	60	Unknown
DH/38786	2	0.125	<i>qnrS1</i>	AMP, FIS, SXT, STR, TET	80	Unknown
DH/38301	4	0.125	<i>qnrS1</i>	AMP, FIS, SXT, STR, TET	80	Unknown
DH/38988	4	0.25	<i>qnrB6 + aac(6′)-Ib-cr</i>	FIS, STR, SXT, TET	60	IncN + ColE

<sup>a</sup> AMP, ampicillin; CIP, ciprofloxacin; FIS, sulfisoxazole; NAL, nalidixic acid; SXT, trimethoprim-sulfamethoxazole; STR, streptomycin; TET, tetracycline. Additional drugs tested: amikacin, amoxicillin-clavulanic acid, chloramphenicol, ceftriaxone, cefoxitin, gentamicin, kanamycin, and ceftiofur.

<sup>b</sup> —, not applicable.

India and in Asian travel-associated *Shigella* cases in the United States (4, 11, 15). This report supports the hypothesis that ciprofloxacin-resistant *Shigella* infections are imported into the United States from Asian countries. Physicians treating patients with diarrheal illness should always ask about recent travel because it may affect treatment decisions (8). The emergence of *Shigella* infections with decreased susceptibility to ciprofloxacin is a public health concern, especially since PMQR determinants may spread among other members of the *Enterobacteriaceae*. Continued surveillance for resistance to clinically important antimicrobials among *Shigella* isolates, as well as collection of exposure and travel history data and studies on the spread of resistance determinants, are necessary to guide public health interventions.

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