ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 2142–2144 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.01288-08

Emergence of Plasmid-Mediated Quinolone Resistance among Non-Typhi *Salmonella enterica* Isolates from Humans in the United States[▽]

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Received 25 September 2008/Returned for modification 28 October 2008/Accepted 27 January 2009

Plasmid-mediated quinolone resistance determinants are emerging among gram-negative pathogens. Here we report results of a retrospective study investigating the prevalence of *aac(6′)-Ib-cr*, *qepA*, and *qnr* genes among 19,010 human isolates of non-Typhi *Salmonella enterica* collected in the United States from 1996 to 2006.

Approximately 1.4 million people in the United States are infected with non-Typhi *Salmonella enterica* (NTS) annually, resulting in 15,000 hospitalizations and >400 deaths (14). Severe invasive infections are commonly treated with the fluoroquinolone ciprofloxacin (CIP). Although endogenous topoisomerase mutations are an important source of fluoroquinolone resistance in *Enterobacteriaceae* (6), three recently described plasmid-mediated mechanisms confer decreased susceptibility to ciprofloxacin: QepA efflux, QNR proteins, and AAC(6′)-Ib-cr (12). The last is a mutant aminoglycoside acetyltransferase [AAC(6′)-Ib] which modifies CIP and norfloxacin (13). Here we report the prevalence of plasmid-mediated quinolone resistance mechanisms among human isolates of NTS submitted to the National Antimicrobial Resistance Monitoring System (NARMS) from 1996 to 2006.

(Portions of the data were presented at the ASM Conference on Antimicrobial Resistance in Zoonotic Bacteria and Foodborne Pathogens, 15 to 18 June 2008, Copenhagen, Denmark.)

State and local public health laboratories participating in NARMS submitted every 10th (1996 to 2002) or every 20th (2003 to 2006) NTS isolate that they received to the Centers for Disease Control and Prevention for susceptibility testing. MICs were determined for 14 to 17 antimicrobial agents by broth microdilution (Sensititre; Trek Diagnostics, Westlake, OH). Isolates that exhibited decreased susceptibility to CIP (≥0.25 mg/liter) were included in the study. Data for 2006 are preliminary and may be subject to change.

The *qepA* and *qnr* genes were detected as previously described (5, 10). A SYBR green-based real-time PCR assay using previously described primers was developed to screen isolates for the *aac(6′)-Ib* gene (9). PCR mixtures contained 1× SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA), 0.4 μM (each) primer, 2 μl template DNA, and sterile water to a final volume of 25 μl. Thermal cycling conditions were as follows: 10 min at 95°C, followed by 35 cycles of 95°C for 20 s, 55°C for 30 s, and 72°C for 30 s. Finally, a melt-curve analysis between 70 and 90°C was performed. Amplification, data acquisition, and data analysis were carried out in a RotorGene 6000 (Corbett Research, Mortlake, NSW, Australia). The presence of the *aac(6′)-Ib-cr* variant was confirmed by direct sequencing of PCR products. Chromosomal topoisomerase mutations were detected as previously described (5).

Among the 19,010 NTS isolates submitted to NARMS between 1996 and 2006, 283 (1.5%) displayed decreased susceptibility to CIP. Of these, 273 were available for PCR screening. Eight (2.9%) isolates, including isolates of serotypes Typhimurium ($n = 1$), Newport ($n = 1$), Senftenberg ($n = 4$), Typhimurium var. O:5− ($n = 1$), and Cubana ($n = 1$), harbored the *aac(6′)-Ib* gene. Seven isolates contained wild-type *aac(6′)-Ib*; one contained the two point mutations (Trp102Arg and Asp179Tyr) characteristic of the *aac(6′)-Ib-cr* variant (Table 1). This isolate displayed a CIP MIC of 1.0 mg/liter and contained in addition to *aac(6′)-Ib-cr* a mutation in the *gyrA* gene (Asp87Tyr). The isolate was serotype Typhimurium var. O:5− and was isolated from a 4-year-old child in Massachusetts in 2005 (Table 1). The patient's family reported a history of travel to China. The patient did not receive antibiotic treatment and recovered without complications.

The prevalence of *qnr* genes among NTS isolates submitted to NARMS between 1996 and 2003 has been reported elsewhere (5). Among NTS isolates exhibiting decreased susceptibility to CIP collected in 2004 to 2006, 17 (9.8%) carried *qnr* genes: 11 isolates harbored *qnrS1*, four carried *qnrB5*, one carried *qnrB2*, and one isolate carried *qnrA1* (Table 1). Typhimurium ($n = 5$), Corvallis ($n = 3$), Montevideo ($n = 2$), and Saintpaul ($n = 2$) were the most common serotypes harboring *qnr* genes. None of the isolates in the present study carried the *qepA* gene.

The *aac(6′)-Ib-cr* mechanism was originally reported in 2003 in a clinical isolate of *Escherichia coli* collected in Shanghai, China (16). Since then the *aac(6′)-Ib-cr* variant has appeared in several countries in Asia, North America, and Europe (1, 2, 4, 9, 11, 15). Interestingly, the *aac(6′)-Ib-cr*-positive NTS isolate described here was isolated after travel to Shanghai, China. In China, the gene has been detected in both human

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▽ Published ahead of print on 17 February 2009.

TABLE 1. Characteristics of *Salmonella* isolates harboring *qnr* or the *aac(6′)-Ib-cr* gene

Isolate no.	Yr of collection	*Salmonella enterica* serotype	Submitting state[a]	Resistance phenotype[b]	CIP MIC (mg/liter)	*aac(6′)-Ib*/*qnr* variant
AM20376	2004	Corvallis	MI	STR, SUL, TET	0.5	*qnrS1*
AM19383	2004	Montevideo	NY	SXT	0.5	*qnrS1*
AM21420	2004	Telelkebir	RI	ND[c]	0.5	*qnrB5*
AM25008	2005	Typhimurium var. O:5−	MA	AMP, CHL, KAN, NAL, SUL, SXT, TET	1	*aac(6′)-Ib-cr*
AM25736	2006	Corvallis	IL	STR, SUL, TET	0.25	*qnrS1*
AM25927	2006	Corvallis	WI	ND	0.25	*qnrS1*
AM25798	2006	Enteritidis	CT	AMP, STR	0.5	*qnrS1*
AM30594	2006	Saintpaul	PA	TET	0.5	*qnrS1*
AM26790	2006	Saintpaul	OR	SXT, TET	0.5	*qnrS1*
AM27315	2006	Typhimurium	MI	STR, SUL, TET	0.5	*qnrS1*
AM27549	2006	Typhimurium	PA	STR, SUL, TET	0.5	*qnrS1*
AM29365	2006	Typhimurium	NY	ND	0.5	*qnrS1*
AM29869	2006	Typhimurium	WA	ND	0.5	*qnrS1*
AM27031	2006	Kiambu	CA	NAL	0.5	*qnrB5*
AM26209	2006	Aqua	TN	ND	0.25	*qnrB5*
AM27125	2006	Typhimurium	NY	AMP, CHL, STR, SUL, TET	0.5	*qnrB5*
AM28764	2006	Cubana	NY	GEN, KAN, NAL, STR, SUL, SXT	0.5	*qnrB2*
AM28704	2006	Montevideo	IL	KAN, SUL, SXT, TET	0.25	*qnrA1*

[a] CA, California; CT, Connecticut; FL, Florida; IL, Illinois; MA, Massachusetts; MI, Michigan; MN, Minnesota; NY, New York; OR, Oregon; PA, Pennsylvania; RI, Rhode Island; TN, Tennessee; WA, Washington; WI, Wisconsin.

[b] AMP, ampicillin; CHL, chloramphenicol; GEN, gentamicin; NAL, nalidixic acid; KAN, kanamycin; STR, streptomycin; SUL, sulfamethoxazole or sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline.

[c] ND, none detected.

and veterinary *Enterobacteriaceae* isolates, with high frequency (>50%) among clinical *E. coli* isolates (7, 8, 13).

The low prevalence (0.4%) of *aac(6′)-Ib-cr* among human isolates of NTS contrasts with a previous U.S. report in which 44 of 313 (14%) isolates of *Klebsiella pneumoniae*, *E. coli*, and *Enterobacter* spp. carried the gene (9). The difference in *aac(6′)-Ib-cr* prevalence among *Enterobacteriaceae* might be due to several factors. First, the gene might have been introduced recently into *Salmonella* populations; the fact that the first *aac(6′)-Ib-cr*-positive NARMS isolate of *Salmonella* appeared in 2005 supports the recent emergence of the mechanism as opposed to late discovery of an established mechanism. Second, the different prevalences might be due to instability of the genetic element hosting the gene or a higher fitness cost associated with gene carriage in *Salmonella* compared with other *Enterobacteriaceae.* Finally, selective pressures facilitating selection and maintenance of *aac(6′)-Ib-cr* (e.g., use of aminoglycosides and fluoroquinolones) may differ among various *Enterobacteriaceae* reservoirs. Further studies of *Salmonella* from humans and animals are necessary to determine sources of *aac(6′)-Ib-cr* and its first appearance in *Salmonella* populations.

A previous study reported 10 (0.08%) *qnr*-positive NTS NARMS isolates from 1996 to 2003 (5). Here we report 17 (0.3%) *qnr*-positive isolates among 6,057 isolates submitted in 2004 to 2006. The fact that 14 of these were collected in 2006 and originated from 10 different states is of concern, as it indicates that *qnr* genes may be increasing among NTS isolates in the United States. The fact that *qnr* genes were found in four serotypes (Berta, Mbandaka, Bovismorbificans, and Anatum) in 1996 to 2003 and in nine additional serotypes (Typhimurium, Corvallis, Saintpaul, Montevideo, Telelkebir, Kiambu, Enteritidis, Aqua, and Cubana) in 2004 to 2006 further supports the dissemination of the genes.

This is the first description of *qnrA* among NARMS human isolates of NTS. This gene has previously been reported among isolates of serotype Enteritidis in China (3). In the United States, *qnrA* has primarily been found among isolates of *Klebsiella pneumoniae* and *Enterobacter* spp. (12).

In conclusion, we show that the *aac(6′)-Ib-cr* gene is present among NTS isolates in the United States collected from humans, although the mechanism appears to be rare. Furthermore, we report a notable increase in *qnr* genes among NTS isolates submitted to NARMS in 2006. Vigilant surveillance and prospective studies are necessary to determine changes in prevalence and the basis for continued appearance. Judicious use of antimicrobial agents in human and veterinary medicine will be crucial for limiting further spread of plasmid-mediated quinolone resistance among *Enterobacteriaceae.*

We thank the NARMS participating public health laboratories for submitting the isolates, John McQuiston and Matt Mikoleit for confirming *Salmonella* serotypes, and the Massachusetts Department of Public Health for providing patient interview information.

This work was supported by an interagency agreement between CDC and the Food and Drug Administration Center for Veterinary Medicine.

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