Plasmid-mediated Quinolone Resistance among Non-Typhi Salmonella enterica Isolates, USA

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We determined the prevalence of plasmid-mediated quinolone resistance mechanisms among non-Typhi Salmonella spp. isolated from humans, food animals, and retail meat in the United States in 2007. Six isolates collected from humans harbored aac(6′)-Ib-cr or a qnr gene. Most prevalent was qnrS1. No animal or retail meat isolates harbored a plasmid-mediated mechanism.

Severe Salmonella enterica infections are commonly treated with fluoroquinolones (e.g., ciprofloxacin) (1). In the United States, the antimicrobial drug susceptibility of Salmonella spp. isolated from humans, food animals, and retail meats is systematically monitored by the National Antimicrobial Resistance Monitoring System (NARMS). This program is a collaborative effort of the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) and the US Department of Agriculture (USDA). Antimicrobial susceptibility to fluoroquinolones among Salmonella spp. has been monitored since the program’s inception in 1996.

Although fluoroquinolone resistance in Enterobacteriaceae is predominantly due to topoisomerase mutations, 3 plasmid-mediated mechanisms have been described that confer decreased susceptibility to ciprofloxacin: quinolone resistance proteins (Qnr), Aac(6′)-Ib-cr, and QepA efflux (2). The Qnr proteins protect the DNA-gyrase from quinolones, Aac(6′)-Ib-cr modifies quinolones with a piperazinyl group, and QepA is involved in active efflux (2). Because patients have experienced treatment failure when infected with Salmonella isolates that displayed decreased susceptibility to fluoroquinolones, plasmid-mediated mechanisms are clinically relevant (3).

A survey of 12,253 NARMS non-Typhi Salmonella (NTS) isolates collected from humans from 1996 through 2003 identified 10 (0.08%) qnr-positive isolates (4). A second survey of NARMS NTS collected from humans during 2004–2006 showed an increase in the proportion of isolates harboring plasmid-mediated quinolone resistance mechanisms. Among 6,057 isolates, 17 qnr-positive isolates and 1 aac(6′)-Ib-cr-positive isolate were detected, representing 0.3% of the NTS collected during that time (5).

The increase in plasmid-mediated quinolone resistance among NTS isolated from humans in the United States prompted further studies to determine continued presence among NTS of human origin and possible reservoirs of these mechanisms. In this study, we investigated plasmid-mediated quinolone resistance mechanisms among NARMS NTS isolated from humans, food animals, and retail meat in the United States in 2007.

The Study

In 2007, 54 NARMS-participating public health laboratories from all 50 states forwarded every 20th human isolate of NTS to CDC. Similarly, NTS isolated from retail meat (chicken breasts, ground turkey, ground beef, and pork chops) were submitted by 10 states that participated in CDC’s Foodborne Diseases Active Surveillance Network (FoodNet) for analysis at FDA-CVM. NTS from food animals were obtained from carcass rinsates (chicken), carcass swab specimens (turkey, cattle, and swine), and ground products (chicken, turkey, and beef). Animal samples were collected by the Food Safety Inspection Service of the USDA from federally inspected slaughter and processing plants throughout the United States and sent to USDA facilities in Athens, Georgia, for further analysis.

At each agency, MICs were determined by broth microdilution (Sensititer; Trek Diagnostics, Westlake, OH, USA). Human, animal, and retail meat isolates of NTS that displayed decreased susceptibility to ciprofloxacin (MIC ≥0.25 mg/L) were included in our study. For each isolate, genomic DNA was prepared by lysing the bacteria at 95°C and collecting the supernatant after centrifugation. PCRs with previously described primers were used to screen isolates for qepA, aac(6′)-Ib-cr, and qnr genes (qnrA, B, C, D, S) (6–10). Positive controls were included for qepA (Escherichia coli TOP10 pAT851), qnrA (S. enterica serotype Montevideo AM28704), qnrB (S. enterica serotype Berta AM04589), qnrS (S. enterica serotype Montevideo AM28704), and qnrA (S. enterica serotype Berta AM04589).
Bovismorbificans AM12888) and aac(6')-Ib-cr (E. coli 36564). For isolates with positive results in the screening, amplicons were confirmed by direct sequencing by using a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Among 2,165 isolates of NTS collected from humans in 2007, 51 (2.4%) displayed decreased susceptibility to ciprofloxacin. Among 320 NTS obtained from retail meat, 5 (1.6%) showed decreased susceptibility to ciprofloxacin, and among the 1,915 isolates obtained from animal sources, 5 (0.3%) showed such susceptibility. Six (11.8%) of the 51 human isolates carried a plasmid-mediated mechanism that affected quinolones; 5 isolates harbored a qnr gene, and 1 isolate contained the aac(6')-Ib-cr gene (Table). None of the isolates harbored the qepA gene. Sequencing of the 5 qnr-positive isolates showed 3 qnrS and 2 qnrB variants among 4 serotypes (Beaudesert, Corvallis, Enteritidis, and Typhimurium) (Table). The aac(6')-Ib-cr gene was found in an isolate of serotype Thompson, and sequencing confirmed the 2 point mutations (Trp102Arg and Asp179Tyr) characteristic of the ciprofloxacin-modifying variant. The MIC of ciprofloxacin among the qnr-positive isolates ranged from 0.25 mg/L to 0.5 mg/L. All isolates from humans were susceptible to nalidixic acid (MIC range 8–16 mg/L). None of the isolates collected from animal and retail meat by the USDA and FDA in 2007 harbored these mechanisms. However, animals and food have been detected among NARMS human NTS and was first detected in an isolate of serotype Bovismorbificans collected in 2000 (4). The gene was later reported in 11 isolates (serotypes Corvallis, Enteritidis, Montevideo, Saintpaul, and Typhimurium) collected by NARMS during 2004–2006 (5).

That qnr genes could only be detected among Salmonella isolates obtained from humans warrants further exploration. One factor that could influence the number of Qnr-producing Salmonella isolates among humans in the United States is the extent of travel-associated infections. Two patients in this study had a history of international travel before illness onset. Another factor that could lead to the development of Qnr-producing Salmonella isolates is the in vivo transfer of resistance from other qnr-bearing Enterobacteriaceae.

Our study does not suggest that food animals and meat in the United States are major sources of Salmonella isolates that harbor plasmid-mediated quinolone resistance mechanisms. However, animals and food have been described as reservoirs for these mechanisms elsewhere. A high prevalence of Enterobacteriaceae with qnr and aac(6')-Ib-cr have been reported among companion and food animals in the People’s Republic of China and qnr-positive Salmonella isolates have been found in poultry in Europe (11,12). Thus, other food and meat sources, not investigated in the current study, may serve as reservoirs for these mechanisms.

Fluoroquinolone resistance among isolates of NTS has important public health implications because ciprofloxacin is commonly used to treat invasive infections of Salmonella spp. in adults. Although plasmid-mediated quinolone resistance mechanisms do not, by themselves, confer clinical resistance to ciprofloxacin, they may promote the selection of mutations that do (13). In addition, studies have shown

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**Table. Characteristics of non-Typhi Salmonella enterica isolates harboring qnr or the aac(6')-Ib-cr gene, collected through NARMS, 2007**

| Isolate no. | S. enterica serovar | Submitting site | Resistance phenotype | Ciprofloxacin MIC, mg/L | aac6'/Ib/qnr variant |
|-------------|---------------------|-----------------|----------------------|-------------------------|---------------------|
| AM31035     | Thompson            | NY              | AMP, SUL             | 0.5                     | qnrB19             |
| AM30827     | Typhimurium         | CA              | STR, SUL, TET        | 0.5                     | qnrS1              |
| AM31228     | Corvallis           | VA              | ND                   | 0.5                     | qnrS1              |
| AM33914     | Typhimurium         | NC              | ND                   | 0.5                     | qnrS1              |
| AM31434     | Enteritidis         | LX              | SUL, SXT, TET        | 0.25                    | qnrS1              |
| AM33097     | Beaudesert          | CA              | ND                   | 0.25                    | qnrS1              |

*NARMS, National Antimicrobial Resistance Monitoring System; NY, New York; AMP, ampicillin; SUL, sulfamethoxazole or sulfisoxazole; CA, California; STR, streptomycin; TET, tetracycline; VA, Virginia; ND, none detected; NC, North Carolina; LX, Los Angeles; SXT, trimethoprim/sulfamethoxazole.
that patients infected with isolates that display low-level fluoroquinolone resistance may respond poorly to treatment, prompting a reconsideration of MIC breakpoints in clinical medicine (3,14). To avoid further dissemination of plasmid-mediated quinolone resistance among Salmonella and other Enterobacteriaceae isolates in the United States, prudent use of antimicrobial agents in both human and veterinary medicine will be crucial. Continued surveillance for resistant bacteria among human, animal, and food sources remains critical.

Acknowledgments

We thank the NARMS participating public health laboratories, the Retail Foods Survey Working Group, and the Food Safety Inspection Service laboratories for submitting the isolates. We also thank the California and Virginia Divisions of Public Health for providing patient interviews; Kathryn Lupoli for serotype confirmations; and the National Veterinary Services Laboratories, Ames, Iowa, for serotyping the animal isolates.

This work was supported by an interagency agreement between CDC, USDA, and FDA-CVM.

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