ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2007, p. 195–197 Vol. 51, No. 1
0066-4804/07/$08.00+0 doi:10.1128/AAC.00222-06

Increase in Nalidixic Acid Resistance among Non-Typhi *Salmonella enterica* Isolates in the United States from 1996 to 2003▽

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Received 20 February 2006/Returned for modification 5 April 2006/Accepted 24 October 2006

Fluoroquinolones commonly are used to treat adult *Salmonella* infections. Fluoroquinolone treatment has failed for persons infected with nalidixic acid-resistant *Salmonella*. From 1996 to 2003, state public health laboratories forwarded 12,252 non-Typhi *Salmonella enterica* isolates to the Centers for Disease Control and Prevention for antimicrobial susceptibility testing; 203 (1.6%) of the isolates were nalidixic acid resistant, and 14 (7%) of those were ciprofloxacin resistant. Resistance to nalidixic acid significantly increased from 0.4% in 1996 to 2.3% in 2003. All ciprofloxacin-resistant isolates had at least one point mutation in the quinolone resistance determining region (QRDR) of *gyrA* and did not harbor *qnr* or have point mutations in the QRDR of *gyrB*, *parC*, or *parE*. Continued surveillance of antimicrobial resistance among non-Typhi *S. enterica* isolates is needed to mitigate the increasing prevalence of nalidixic acid resistance.

Each year, an estimated 1.4 million persons are infected with non-Typhi *Salmonella enterica* in the United States, resulting in 15,000 hospitalizations and 400 deaths (20). Although most *Salmonella* infections result in mild-to-moderate self-limiting gastroenteritis, severe infections, such as bacteremia and meningitis, may occur. Antimicrobial agents are not essential for the treatment of most *Salmonella* infections but may be lifesaving for patients with severe infections. The prevalence of resistance among *Salmonella* to several antimicrobial agents, including ampicillin and trimethoprim-sulfamethoxazole, has increased in recent decades (3). Fluoroquinolones (e.g., ciprofloxacin) are the most commonly used antimicrobial agents in the United States for the treatment of *Salmonella* infections in adults.

Fluoroquinolones are broad-spectrum antimicrobial agents that target DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV genes (*parE* and *parC*) in non-Typhi *S. enterica* (17). Isolates with nalidixic acid resistance commonly have decreased susceptibility to ciprofloxacin (16). Most isolates with nalidixic acid resistance have a single point mutation in the quinolone resistance determining region (QRDR) of *gyrA*; isolates with ciprofloxacin resistance have two QRDR point mutations (16). Recently, plasmid-mediated *qnr* resistance has been found among nalidixic acid-resistant non-Typhi *S. enterica* isolates (7). Public health surveillance for resistance to nalidixic acid is useful in monitoring emerging fluoroquinolone resistance. Furthermore, fluoroquinolone treatment has failed for patients infected with nalidixic acid-resistant *Salmonella* (5, 9, 11, 17). We therefore reviewed national surveillance data for evidence of emerging fluoroquinolone resistance. We describe an increase in nalidixic acid resistance among human non-Typhi *S. enterica* isolates in the United States between 1996 and 2003.

MATERIALS AND METHODS

State public health department laboratories routinely receive *Salmonella* isolates from clinical diagnostic laboratories for serotyping as part of public health surveillance. Once received, isolates are confirmed as *Salmonella* and are serotyped according to the Kaufmann-White scheme (2). State public health laboratories participating in the National Antimicrobial Resistance Monitoring System (NARMS) systematically selected every 10th non-Typhi *S. enterica* isolate from 1996 to 2002 and every 20th isolate in 2003 to send to the CDC for antimicrobial susceptibility testing. In 1996, 14 states (California [Alameda, Los Angeles, and San Francisco counties], Colorado, Connecticut, Florida, Georgia, Kansas, Maryland, Massachusetts, Minnesota, New Jersey, New York [Bronx, Brooklyn, New York, Queens, and Richmond counties], Oregon, Washington, and West Virginia) participated in NARMS surveillance, representing 30% of the U.S. population. In 1999, Tennessee was added and New York began statewide participation, resulting in 40% of the U.S. population participating in NARMS. In 2002, Arizona, Hawaii, Louisiana, Maine, Michigan, Montana, Nebraska, New Mexico, South Dakota, Texas, and Wisconsin were added, resulting in 65% of the U.S. population (26 states) participating in NARMS. In 2003, all 50 states participated in NARMS.

At the CDC, isolates were tested for antimicrobial susceptibilities to amikacin, amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole via a semiautomated broth microdilution system (Sensititre; Trek Diagnostics, Westlake, OH). Partial-range MICs were determined. CLSI (formerly NCCLS) interpretive criteria were used when available (nalidixic acid resistance is defined as a MIC of ≥32 μg/ml); because there are no CLSI interpretive criteria for ceftiofur, we defined ceftiofur resistance as a MIC of ≥8 μg/ml (12). We defined decreased susceptibility to ciprofloxacin as a MIC of ≥0.12 μg/ml.

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▽ Published ahead of print on 6 November 2006.

All ciprofloxacin-resistant isolates (MIC ≥ 32 μg/ml) were screened for *qnr*, and their QRDR of *gyrA*, *gyrB*, *parC*, and *parE* were sequenced as previously described (7).

Data were analyzed using SAS version 9.1 and SPSS version 13.0. Additional isolates of the same serotype submitted from the same patient within a 1-year time period were excluded from analyses. A Wilcoxon rank-sum test was performed to compare the median ages of persons with nalidixic acid-resistant and -susceptible infections. A multivariate logistic regression analysis was performed to assess the change in nalidixic acid resistance among non-Typhi *S. enterica* isolates tested by NARMS in 2003 compared with data for 1996, adding a variable site into the model to take into account site variation.

RESULTS

Between 1996 and 2003, 12,252 non-Typhi *S. enterica* isolates were received and tested at the CDC; of those, 203 (1.6%) were resistant to nalidixic acid. The percentage of isolates resistant to nalidixic acid was 0.4% (5/1,324) in 1996, 0.9% (12/1,301) in 1997, 1.4% (20/1,460) in 1998, 1.1% (16/1,498) in 1999, 2.5% (34/1,377) in 2000, 2.6% (37/1,419) in 2001, 1.8% (36/2,008) in 2002, and 2.3% (43/1,865) in 2003. Using the logistic regression model, isolates were seven times more likely to be resistant to nalidixic acid in 2003 than in 1996 (95% confidence interval, 2.6, 17.7).

Of 203 nalidixic acid-resistant *S. enterica* isolates serotyped, 63 (31%) were serotype Enteritidis, 22 (11%) serotype Typhimurium, 20 (10%) serotype Virchow, 18 (9%) serotype Paratyphi A, 11 (5%) serotype Senftenberg, and 7 (3%) serotype Berta. Among serotypes with at least 5 nalidixic acid-resistant isolates, the serotypes with the highest proportion of isolates that were nalidixic acid resistant were Paratyphi A (49%; 18/37) and Virchow (43%; 20/46).

Nalidixic acid-resistant isolates were more likely than nalidixic acid-susceptible isolates to be resistant to ampicillin (23% versus 16%), gentamicin (9% versus 2%), kanamycin (13% versus 4%), tetracycline (28% versus 19%), and trimethoprim-sulfamethoxazole (15% versus 2%); all P values were <0.01. The most common multidrug resistance phenotype among nalidixic acid-resistant isolates was resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (R-type ACSSuT). Fifteen (7%) of the 203 nalidixic acid-resistant isolates were at least R-type ACSSuT; five R-type ACSSuT isolates were additionally resistant to amoxicillin-clavulanic acid, cephalothin, cefoxitin, and ceftiofur, with decreased susceptibility (MIC of ≥2 μg/ml) to ceftriaxone. Among the 203 nalidixic acid-resistant isolates, 185 (91%) had decreased susceptibility to ciprofloxacin; 14 (7%) isolates were resistant to ciprofloxacin. Of the 14 isolates that were ciprofloxacin resistant, 9 (64%) were serotype Senftenberg, 3 (21%) serotype Schwarzengrund, 1 (7%) serotype Indiana, and 1 (7%) serotype Typhimurium.

Thirty-three (66%) states submitted at least one nalidixic acid-resistant *Salmonella* isolate. Age information was available for 185 (91%) persons infected with nalidixic acid-resistant *Salmonella* and 10,419 (86%) persons infected with nalidixic acid-susceptible *Salmonella*. Persons infected with nalidixic acid-resistant *Salmonella* were older (median age, 31 years) than persons infected with susceptible *Salmonella* (median age, 21 years) ($P < 0.01$). There was no difference in gender distribution between persons infected with nalidixic acid-resistant and -susceptible *Salmonella*.

Specimen information was available for 97% of the isolates. Nalidixic acid-resistant isolates were more likely to have been isolated from blood than nalidixic acid-susceptible isolates (13% versus 5%; $P < 0.01$). The most common serotypes of nalidixic acid-resistant *S. enterica* isolated from blood were Paratyphi A (15 isolates) and Enteritidis (4 isolates). Among the 658 serotyped isolates from blood, 15 (58%) of 26 nalidixic acid-resistant isolates were serotype Paratyphi A, compared with 14 (2%) of 632 nalidixic acid-susceptible isolates ($P < 0.01$); in contrast, 4 (15%) nalidixic acid-resistant isolates were serotype Enteritidis, compared with 145 (23%) of nalidixic acid-susceptible isolates.

All 14 ciprofloxacin-resistant isolates had >1 point mutation in QRDR of *gyrA*; all 14 isolates had a point mutation at codon 83 (9 isolates with S83Y and 5 with S83F). Thirteen isolates also had a point mutation at codon 87 (12 isolates with D87G and 1 with D87N). No isolates harbored *qnr* or had point mutations in the QRDR of *gyrB*, *parC*, or *parE*.

DISCUSSION

Nalidixic acid resistance is increasing among non-Typhi *S. enterica* isolates in the United States. Over the past 7 years, there has been a sevenfold significant increase in the proportion of non-Typhi *S. enterica* isolates that are resistant to nalidixic acid. Most (91%) of these isolates also showed decreased susceptibility to ciprofloxacin; 7% were ciprofloxacin resistant. All of the ciprofloxacin-resistant isolates in this study had at least one point mutation in the QRDR of *gyrA*; none of the ciprofloxacin-resistant isolates harbored *qnr* or had point mutations in the QRDR of *gyrB*, *parC*, or *parE*. Nalidixic acid resistance is noteworthy because fluoroquinolones are the most commonly used antimicrobial agent for the treatment of invasive *Salmonella* infections in adults in the United States, and fluoroquinolone treatment has failed for patients with nalidixic acid-resistant *Salmonella* infections (5, 9, 11, 17). Furthermore, we found that nalidixic acid-resistant *Salmonella* isolates were more commonly isolated from blood than susceptible isolates, suggesting that nalidixic acid-resistant *Salmonella* may be associated with more severe infections (19). If nalidixic acid-resistant *Salmonella* is associated with more severe disease, further research is needed to determine whether increased severity is due to serotype, resistance genotype, or other reasons.

Several studies have suggested that the use of fluoroquinolones in veterinary medicine contributes to the emergence and dissemination of nalidixic acid resistance in *Salmonella* among food animals, which may be transmitted to humans (1, 11). The high prevalence of nalidixic acid resistance among *Salmonella* serotype Enteritidis isolates is noteworthy, because sporadic serotype Enteritidis infections of humans have been associated with international travel and, among domestically acquired infections, eating chicken and turkey (10). The first approved uses of fluoroquinolones in food animals in the United States were in chickens and turkeys in 1995. Use in cattle was approved in 1999. The use of fluoroquinolones in chickens and turkeys has resulted in the emergence and dissemination of fluoroquinolone-resistant *Campylobacter*, which is transmitted to humans through contaminated poultry (8). Persons infected with fluoroquinolone-resistant *Campylobacter* have a longer duration of diarrhea than persons infected with susceptible

strains (13). These and other data contributed to the decision by the FDA to discontinue the use of fluoroquinolones in chickens and turkeys (6). As of September 2005, it is illegal to use fluoroquinolones in chickens and turkeys in the United States (18). Use of fluoroquinolones and other antimicrobial agents for humans also may contribute to transmission of nalidixic acid-resistant *Salmonella* to humans, particularly among persons who travel to countries where quinolone resistance is more common among *Salmonella* strains (15).

The increasing prevalence of nalidixic acid resistance among *Salmonella* isolates should be considered in light of a modest concurrent decline in the incidence of *Salmonella* infections in the United States. From 1996 to 2003, the incidence of laboratory-confirmed *Salmonella* infections declined 17% in the Food-borne Diseases Active Surveillance Network (4). Despite the decline in the incidence of *Salmonella* infections in the United States, the prevalence of nalidixic acid resistance among *Salmonella* isolates has increased. Recent analysis, combining Food-borne Diseases Active Surveillance Network and NARMS data, indicates an increase in the incidence of nalidixic acid-resistant *Salmonella* infections, suggesting an increased human health burden of nalidixic acid-resistant *Salmonella* in the United States (14). Continued surveillance of antimicrobial resistance among non-Typhi *S. enterica* and continued education of veterinary and human medicine practitioners on appropriate use of antimicrobial agents are needed to mitigate the increasing prevalence of nalidixic acid resistance among *Salmonella*.

ACKNOWLEDGMENT

This work was funded by an interagency agreement between the U.S. Food and Drug Administration and the CDC.

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