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Clinical Response and Outcome of Infection with *Salmonella enterica* Serotype Typhi with Decreased Susceptibility to Fluoroquinolones: a United States FoodNet Multicenter Retrospective Cohort Study▽

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Patients with typhoid fever due to *Salmonella enterica* serotype Typhi strains for which fluoroquinolones MICs are elevated yet that are classified as susceptible by the current interpretive criteria of the Clinical and Laboratory Standards Institute may not respond adequately to fluoroquinolone therapy. Patients from seven U.S. states with invasive *Salmonella* serotype Typhi infection between 1999 and 2002 were enrolled in a multicenter retrospective cohort study. Patients infected with *Salmonella* serotype Typhi isolates with ciprofloxacin MICs of 0.12 to 1 μg/ml (decreased ciprofloxacin susceptibility but not resistant to ciprofloxacin [DCS]) were compared with patients infected with isolates with ciprofloxacin MICs <0.12 μg/ml for fever clearance time and treatment failure. Of 71 patients, 30 (43%) were female and 24 (34%) were infected with *Salmonella* serotype Typhi with DCS; the median age was 14 years (range, 1 to 51 years). Twenty-one (88%) of 24 isolates with DCS were resistant to nalidixic acid. The median antimicrobial-related fever clearance times in the DCS and non-DCS groups were 92 h (range, 21 to 373 h) and 72 h (range, 19 to 264 h) ($P = 0.010$), respectively, and the fluoroquinolone-related fever clearance times in the DCS and non-DCS groups were 90 h (range, 9 to 373 h) and 64 h (range, 34 to 204 h) ($P = 0.153$), respectively. Four (17%) of 24 patients in the DCS group and 2 (4%) of 46 patients in the non-DCS group (relative risk, 2.5; 95% confidence interval, 1.2 to 5.1) experienced treatment failure. Associations persisted after adjustment for potential confounders. We demonstrate that patients infected with *Salmonella* serotype Typhi isolates with DCS show evidence of a longer time to fever clearance and more frequent treatment failure. Nalidixic acid screening does not detect all isolates with DCS.

Typhoid fever is an acute, generalized infection of the reticuloendothelial system caused by *Salmonella enterica* subsp. *enterica* serotype Typhi and is estimated to cause more than 21 million illnesses and 216,000 deaths worldwide annually (10). Timely treatment with appropriate antimicrobial agents is important in reducing the mortality of invasive infection (12). Resistance to traditional first-line antimicrobial agents, such as ampicillin, chloramphenicol, and trimethoprim-sulfonamide combinations, has emerged worldwide among *Salmonella* serotype Typhi strains (4, 6, 19, 21, 24). Consequently, fluoroquinolones (e.g., ciprofloxacin), which have been available since the 1980s, have become a mainstay of therapy for invasive salmonellosis (2).

The Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) sets standards for antimicrobial susceptibility testing methods and interpretive criteria for the United States. CLSI recommendations are also commonly used in many other countries. The current MIC breakpoints for fluoroquinolones, including ciprofloxacin, for members of the family *Enterobacteriaceae* (including *Salmonella enterica*) are ≥4 μg/ml for resistance and ≤1 μg/ml for susceptibility (18). However, accumulating data indicate that patients infected with *Salmonella*

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serotype Typhi strains with ciprofloxacin MICs of 0.12 to 1 μg/ml (decreased ciprofloxacin susceptibility but no resistance to ciprofloxacin [DCS]) are less likely to respond adequately to fluoroquinolone therapy than patients infected with *Salmonella* serotype Typhi strains with ciprofloxacin MICs of <0.12 μg/ml (9). Furthermore, the proportion of *Salmonella* serotype Typhi strains and strains of other serotypes of *Salmonella* with DCS has risen markedly in recent years worldwide (2). In response to this concern, the CLSI advises physicians and laboratories that fluoroquinolone-susceptible strains of *Salmonella* that are determined to be resistant to the quinolone antimicrobial nalidixic acid, which serves as a marker of DCS, may be associated with clinical failure or a delayed response to treatment in fluoroquinolone-treated patients with extraintestinal infections (18). However, some authorities have proposed that the clinical consequences of typhoid fever due to *Salmonella* serotype Typhi with DCS may be sufficiently adverse to warrant reevaluation of the CLSI interpretive criteria for fluoroquinolones for *Salmonella* strains to reflect more accurately the clinical response to therapy (1).

Most typhoid fever illnesses reported in the United States are acquired abroad (2, 17). Therefore, patient isolates reflect the epidemiology of *Salmonella* serotype Typhi antimicrobial resistance in areas around the world where typhoid fever is endemic. During a 12-month period in 1996 and 1997, 364 patients with typhoid fever were reported to the U.S. Centers for Disease Control and Prevention (CDC). Among 282 patients for whom epidemiologic information was available, 229 (81%) of the illnesses were associated with foreign travel, and the isolates from 20 (9%) of these cases were resistant to nalidixic acid (2). It follows, then, that the management of patients infected with *Salmonella* serotype Typhi with DCS is of considerable importance to clinicians managing typhoid fever in the United States as well as internationally. While the clinical importance of *Salmonella* serotype Typhi with DCS has been examined in Asia (27), no study has been done in the United States. In order to enroll sufficient numbers of patients in the United States, a multicenter study design was needed.

We therefore conducted a multicenter retrospective cohort study to evaluate the impact on the clinical outcome of infection with *Salmonella* serotype Typhi with DCS in the United States in order to inform the clinical management of typhoid fever and to provide data for the reevaluation of the CLSI interpretive criteria for fluoroquinolones.

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MATERIALS AND METHODS

Cohort study. The Foodborne Diseases Active Surveillance Network (FoodNet) (3) of the CDC conducts population-based surveillance for culture-confirmed *Salmonella* serotype Typhi infections at all clinical laboratories within the FoodNet surveillance catchment area. Patients with invasive *Salmonella* serotype Typhi infections (in which *Salmonella* serotype Typhi is isolated from the bloodstream or the bone marrow), as ascertained by FoodNet from 1999 to 2002, who were hospitalized in the FoodNet catchment area, whose medical records could be accessed, and for whom *Salmonella* serotype Typhi isolates were available were included in the cohort study. Seven FoodNet sites participated in the study and included those in California, Connecticut, Georgia, Maryland, Minnesota, Oregon, and Tennessee. These sites had a population of 32 million persons (11% of the U.S. population) in 2002. Within the cohort of hospitalized patients with invasive *Salmonella* serotype Typhi infections, we compared the clinical outcomes for patients infected with *Salmonella* serotype Typhi with ciprofloxacin MICs of 0.12 to 1 μg/ml (DCS) to those for patients infected with *Salmonella* serotype Typhi with ciprofloxacin MICs of <0.12 μg/ml.

FoodNet personnel retrospectively identified hospitalized patients with *Salmonella* serotype Typhi bloodstream or bone marrow infections caused by isolates that had been tested by the National Antimicrobial Resistance Monitoring System (NARMS) laboratory. Eligibility for inclusion in the cohort study was determined. The medical records of the study patients were located at each admitting hospital and were used to complete a structured extraction questionnaire based upon the patient's first admission with *Salmonella* serotype Typhi infection of the blood or bone marrow. The chart extraction questionnaire requested demographic characteristics and epidemiologic data and was used to collect extensive data on the course of the clinical illness. Specific data on the clinical illness included the illness onset date, the hospitalization date, the antimicrobial therapy received before and during hospitalization, the clinical response to therapy (e.g., fever clearance time; fever was defined as a temperature of ≥99.5°F or ≥37.5°C), and whether the patient had a clinical or a microbiologic relapse.

Laboratory procedures. Clinical laboratories forward *Salmonella* serotype Typhi isolates to their state's public health laboratory as a part of routine public health surveillance. State public health laboratories routinely forward all *Salmonella* serotype Typhi isolates to the NARMS laboratory at CDC for testing for susceptibility to ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, sulfamethoxazole, trimethoprim-sulfamethoxaole, and 11 other antimicrobial agents by broth microdilution (Sensititre; Trek Diagnostics, Cleveland, OH).

A selection of *Salmonella* serotype Typhi isolates with DCS and other *Salmonella* serotype Typhi isolates received at the CDC from 1999 to 2002 were designated for quinolone resistance-determining region (QRDR) sequencing. After the confirmation of ciprofloxacin and nalidixic acid susceptibility by Etest (AB Biodisk, Piscataway, NJ), crude DNA from the same subculture used for the Etest was prepared by suspending five colonies in 100 μl of water. A 255-bp region covering the QRDR of *gyrA* (Met52 to Leu137) was amplified with primers gyrA1 (5′-CATGAACGTATTGGGCAATG) and gyrA2 (5′-AGATCG GCCATCAGTTCGTG). The QRDRs of *gyrB*, *parC*, and *parE* were amplified by using previously described primers (11). The PCR mixtures contained 1 μl of the crude DNA suspension, 0.4 mM of each primer, and AmpliTaq Gold PCR master mix (Applied Biosystems, Foster City, CA) in a final volume of 50 μl. The primers were synthesized at the Biotechnology Core Facility, CDC. PCR was carried out in an MJ Research thermal cycler (Waltham, MA) programmed with an initial 5-min denaturing step at 95°C, followed by 30 s at 95°C, 1 min at 55°C, and 30 s at 72°C for 35 cycles. The amplicons were sequenced with the primers described above by using ABI (PE Biosystems, Foster City, CA) BigDye (version 3.1) dye chemistry and ABI 3730XL automated DNA sequencers. Analysis was performed with the BioEdit software program (available at www.mbio.ncsu.edu /BioEdit/bioedit.html) (14). The QRDR DNA sequences of *gyrA*, *gyrB*, *parC*, and *parE* were compared to those of *Salmonella* serotype Typhimurium LT2 (GenBank accession numbers AE008801, AE008878, AE008846, and AE008846, respectively).

The isolates were screened for plasmid-mediated quinolone resistance by multiplex PCR amplification of *qnrA*, *qnrB*, and *qnrS*. Colonies were suspended in 50 μl of water in a microcentrifuge tube and boiled to prepare DNA templates for PCR. The primers used to amplify *qnrA* to give a 516-bp product were 5′-ATTTCTCACGCCAGGATTTG and 5′-GATCGGCAAAGGTTAGGTCA. The primers used to amplify *qnrB* to give a 469-bp product were 5′-GATCGT GAAAGCCAGAAAGG and 5′-ACGATGCCTGGTAGTTGTCC. The primers used to amplify *qnrS* to give a 417-bp product were 5′-ACGACATTCGTC AACTGCAA and 5′-TAAATTGGCACCCTGTAGGC. All six primers were added to the template and the PCR Supermix high fidelity mixture (Invitrogen, Carlsbad, CA). The PCR conditions were 94°C for 45 s, 53°C for 45 s, and 72°C for 60 s cycled 32 times. Clinical isolates that had previously been confirmed to have the *qnr* gene by DNA sequencing were used as positive controls for *qnrA*, *qnrB*, and *qnrS*. Reaction mixtures without a DNA template served as negative controls (13).

Statistical analyses. Questionnaires from which all identifiers had been removed were forwarded to the CDC for entry into the study database and were audited for quality control purposes. Study outcome measures were defined before study implementation. The fluoroquinolone- and antimicrobial-related times to fever clearance were defined as the times from the administration of the first dose of fluoroquinolone and the first dose of antimicrobial, respectively, among patients who received their first dose of fluoroquinolone in the hospital and prior to fever clearance or hospital discharge. Defervescence was defined as a temperature of ≤99.5°F or ≤37.5°C for ≥24 h. Clinical relapse was defined as a relapse of fever more than 48 h after the last dose of antimicrobial but within

TABLE 1. Characteristics of patients enrolled in the retrospective cohort study of hospitalized patients with invasive *Salmonella* serotype Typhi infection, FoodNet sites, 1999 to 2002[a]

Characteristic	All patients	Patients infected with *Salmonella* serotype Typhi isolates with DCS	Patients infected with *Salmonella* serotype Typhi isolates without DCS	*P* value
No. of female patients/total no. (%)	30/70 (43)	9/24 (38)	21/46 (46)	NS[b]
Median (range) age (yr)	14 (1–51)	19 (2–45)	14 (1–51)	NS
No. of patients with foreign travel/total no. (%)	53/71 (75)	21/24 (88)	32/47 (68)	NS
No. of patients who traveled to south Asia/total no. (%)	30/71 (42)	16/24 (67)	14/47 (30)	0.005
No. of patients who were U.S. residents/total no. (%)	24/45 (53)	9/17 (53)	15/28 (54)	NS

[a] The cohort consisted of 71 patients.
[b] NS, not significant.

4 weeks after hospital discharge either as reported by the patient or as clinically documented with a temperature of ≥99.5°F or ≥37.5°C. Microbiologically confirmed relapse was defined as a blood or bone marrow culture positive for *Salmonella* serotype Typhi more than 48 h after the last dose of antimicrobial but within 6 months after hospital discharge. Retreatment was defined as receipt of an antimicrobial anticipated to be effective for the treatment of typhoid fever within 4 weeks of first hospital discharge for suspected persistent or recurrent typhoid fever. Patients with treatment failure were defined as those who received an antimicrobial anticipated to be effective for the treatment of typhoid fever and who remained in hospital for ≥7 days without fever clearance. Patients with fluoroquinolone treatment failure were defined as those who received a fluoroquinolone and who remained in hospital for ≥7 days without fever clearance.

Associations between DCS and clinical outcomes were evaluated by several methods. Outcomes that depended on defervescence times were analyzed by adjusting for censoring and by using survival analytic methods, including both rank-based nonparametric methods, such as log rank tests, and proportional hazard regression. Other outcomes were analyzed by standard chi-square tests and rank-based methods. Comparisons were made without adjustment and after adjustment for potential confounders, including stratification for the most common multiple-drug-resistant phenotype. Statistical analyses were done with SAS software (version 9.1; SAS Institute Inc., Cary, NC).

Research ethics. This study was approved by the institutional review boards of the participating FoodNet sites. Since identifying information was removed prior to data collation at the CDC, the study was determined to be exempt from review by the CDC Institutional Review Board under 45 *Code of Federal Regulations* 46.101(b).

RESULTS

Characteristics and antimicrobial management of study patients. Of 119 patients with culture-confirmed invasive *Salmonella* serotype Typhi infections, as ascertained by the participating FoodNet sites, between 1999 through 2002, 75 (63%) were hospitalized; and of these, the medical records for 73 (97%) were available to the study team. In addition, 14 hospitalized patients with available medical records who resided outside the FoodNet catchment area in Maryland were included. Of the total of 87 hospitalized patients with available medical records, 71 (82%) also had *Salmonella* serotype Typhi isolates available for antimicrobial susceptibility testing and were enrolled in the cohort study. Of the 71 patients enrolled, 30 (43%) were female, 12 (17%) were from California, 16 (23%) were from Connecticut, 8 (11%) were from Georgia, 18 (25%) were from Maryland, 10 (14%) were from Minnesota, 6 (8%) were from Oregon, and 1 (1%) was from Tennessee. None of the patients died. Of the 71 patients enrolled, medical chart review indicated that 53 (75%) had reported foreign travel in the 30 days prior to illness onset and that for 30 (42%) travel to south Asia was specifically recorded. Compared with all other study participants, those who traveled to south Asia during the 30 days before illness onset were more likely to have been infected with *Salmonella* serotype Typhi with DCS (relative risk [RR], 2.2; $P = 0.005$). Other characteristics of the enrolled patients are summarized in Table 1. Patients infected with *Salmonella* serotype Typhi with DCS had more days of antimicrobial use and more days of fluoroquinolone use than those infected with *Salmonella* serotype Typhi without DCS. These and other aspects of the antimicrobial management of the study patients are summarized in Table 2.

Laboratory evaluation of *Salmonella* serotype Typhi isolates. Of the *Salmonella* serotype Typhi isolates from the 71 patients enrolled in the study, all but 1 of the isolates were recovered within 2 days of hospitalization and 24 (34%) isolates had DCS. Of 24 *Salmonella* serotype Typhi isolates with DCS, 21 (87%) were resistant to nalidixic acid and 5 (7%) were multidrug resistant; each of these multidrug-resistant isolates was resistant to at least ampicillin, chloramphenicol, sulfamethoxazole, and trimethoprim-sulfamethoxazole (resistance type ACSuTm). Resistance type ACSuTm isolates were 3.5 times more likely (95% confidence interval [CI], 2.4 to 5.1) to also have DCS than isolates that did not have this multidrug-resistant phenotype. One (4%) isolate with DCS also met a CLSI screening criterion for extended-spectrum β-lactamase (ESBL) production. All except 1 of the 47 *Salmonella* serotype Typhi isolates without DCS were fully susceptible to all antimicrobials tested; 1 (2%) isolate was resistant to nalidixic acid.

The QRDRs of the *gyrA*, *gyrB*, *parC*, and *parE* genes for 20 (83%) of 24 *Salmonella* serotype Typhi isolates with DCS were sequenced. Sixteen (80%) had one *gyrA* point mutation (none of the isolates had more than one *gyrA* point mutation); 15 of those 16 isolates had a mutation at codon 83 (14 had a serine-to-tyrosine substitution and 1 had a serine-to-phenylalanine substitution), and 1 of the 16 isolates had a mutation at codon 87 (aspartic acid-to-asparginine substitution). Six (13%) of 47 *Salmonella* serotype Typhi isolates without DCS were sequenced; 1 (17%) isolate had a *gyrA* mutation (aspartic acid-to-tyrosine substitution at codon 87). None of the isolates had a *gyrB*, *parC*, or *parE* mutation; and no *qnr* genes were detected by PCR.

Clinical outcomes for patients with *Salmonella* serotype Typhi infections. The antimicrobial-related fever clearance times could be determined for 67 (94%) patients. The fluoroquinolone-related fever clearance times could be determined for 22 (38%) of 58 patients who received their first dose of a fluoroquinolone in the hospital and prior to defervescence

TABLE 2. Antimicrobial management of patients enrolled in the retrospective cohort study of hospitalized patients with invasive *Salmonella* serotype Typhi infection, FoodNet sites, 1999 to 2002[a]

Characteristic	All patients	Patients infected with *Salmonella* serotype Typhi isolates with DCS	Patients infected with *Salmonella* serotype Typhi isolates without DCS	*P* value
Antimicrobial use prior to hospitalization (no. of patients/total no. [%])				
Took any antimicrobial prior to hospitalization	31/67 (46)	12/24 (50)	19/43 (44)	NS[b]
Took fluoroquinolone prior to hospitalization	9/67 (13)	4/24 (17)	5/43 (12)	NS
Antimicrobial use during hospitalization (no. of patients/total no. [%])				
Took any antimicrobial during hospitalization	71/71 (100)	24/24 (100)	47/47 (100)	NS
Took fluoroquinolone during hospitalization	32/71 (45)	14/24 (58)	18/47 (39)	NS
First took fluoroquinolone during hospitalization	22/58 (38)	11/20 (55)	11/38 (29)	NS
Aggregate antimicrobial use (prior to and during hospitalization)				
Median (range) no. of days of antimicrobial use prior to hospital discharge	6 (2–31)	8 (2–31)	6 (2–16)	0.017
Median (range) no. of days of fluoroquinolone use prior to hospital discharge	0 (0–31)	5 (0–31)	0 (0–11)	0.023
Median (range) no. of days of fluoroquinolone use prior to hospital discharge among patients treated with fluoroquinolones	5 (0–31)	8 (0–31)	5 (0–11)	0.011
Use of any fluoroquinolone (no. of patients/total no. [%])	35/71 (49)	15/24 (63)	20/47 (43)	NS
Exclusive fluoroquinolone use (no. of patients/total no. [%])	4/68 (6)	1/24 (4)	3/44 (7)	NS
Use of any cephalosporin (no. of patients/total no. [%])	61/71 (86)	21/24 (88)	40/47 (85)	NS
Median (range) no. of antimicrobial classes used[c]	2 (1–4)	2 (1–4)	2 (1–4)	NS

[a] The cohort consisted of 71 patients.
[b] NS, not significant.
[c] Number of classes of antimicrobial agents used to treat typhoid fever, including penicillins, cephalosporins, sulfa drugs, fluoroquinolones, aminoglycosides, macrolides, and carbepenems.

(Table 2). One (5%) of these patients infected with a *Salmonella* serotype Typhi strain without DCS was excluded from the analysis of the time to the loss of fever because the patient was afebrile at time zero. The median antimicrobial-related times to fever clearance were 92 h (range, 21 to 373 h) for 23 patients infected with *Salmonella* serotype Typhi with DCS and 72 h (range, 19 to 264 h) for 44 patients infected with *Salmonella* serotype Typhi isolates that were fully susceptible to ciprofloxacin (Fig. 1), and this difference was statistically significant ($P = 0.010$). The median fluoroquinolone-related times to fever clearance were 90 h (range, 9 to 373 h) for 11 patients infected with *Salmonella* serotype Typhi with DCS and 64 h (range, 34 to 204 h) for 10 patients infected with *Salmonella* serotype Typhi isolates that were fully susceptible to ciprofloxacin (Fig. 2), and this difference was not statistically significant ($P = 0.153$). Four (17%) of 24 patients infected with *Salmonella* serotype Typhi with DCS and 2 (4%) of 46 patients infected with *Salmonella* serotype Typhi without DCS (RR, 2.5; 95% CI, 1.2 to 5.1) experienced treatment failures, and this association persisted after the ACSuTm resistance type was

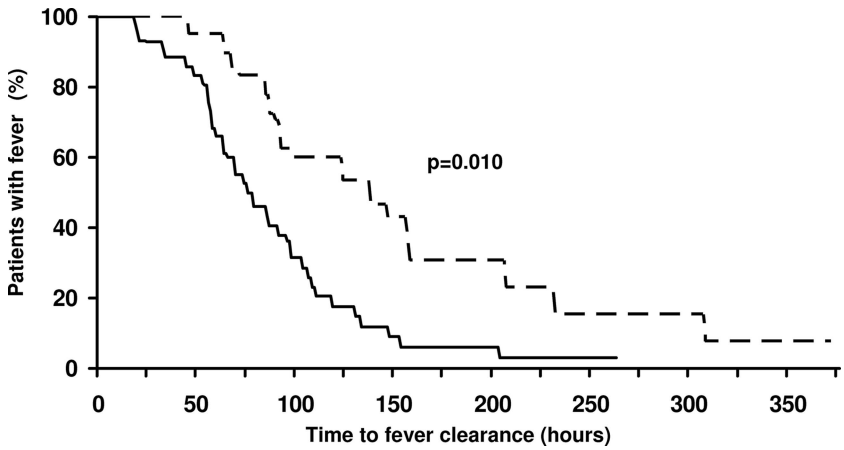

FIG. 1. Time to fever clearance from time of administration of the first hospital dose of antimicrobial among hospitalized typhoid fever patients, FoodNet sites, 1999 to 2002. Solid line, patients infected with isolates without DCS; dashed line, patients infected with isolates with DCS. Fever was defined as a temperature of ≥99.5°F or ≥37.5°C. The estimated median fever clearance times were 157 h (95% CI, 93 to 232 h) for the group of patients infected with isolates with DCS and 86 h (95% CI, 64 to 107 h) for the group of patients infected with isolates without DCS.

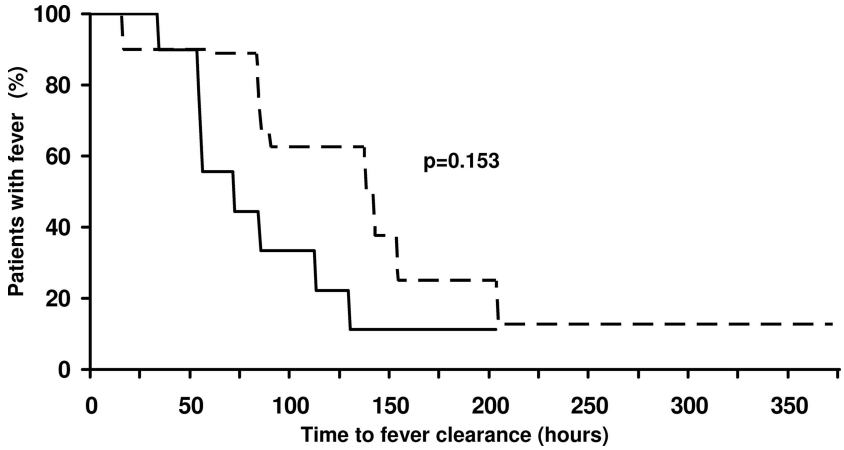

FIG. 2. Time to fever clearance from time of administration of the first hospital dose of a fluoroquinolone antimicrobial among hospitalized typhoid fever patients, FoodNet sites, 1999 to 2002. Solid line, patients infected with isolates without DCS; dashed line, patients infected with isolates with DCS. Fever was defined as a temperature of ≥99.5°F or ≥37.5°C. The estimated median fever clearance times were 142 h (95% CI, 85 to 204 h) for the group of patients infected with isolates with DCS and 72 h (95% CI, 55 to 130 h) for the group of patients infected with isolates without DCS.

controlled for (RR, 2.2; 95% CI, 1.0 to 4.7). Fluoroquinolone treatment failure occurred in 2 (18%) of 11 patients infected with isolates with DCS and in 1 (10%) of 10 patients infected with isolates without DCS, although this difference was not statistically significant either before (RR, 2.1; 95% CI, 0.7 to 6.1) or after (RR, 1.9; 95% CI, 0.6 to 6.5) the ACSuTm resistance type was controlled for. Clinical relapses could be ascertained in only 3 (12%) of 25 patients: 2 (20%) of 10 patients infected with isolates with DCS and 1 (7%) of 15 patients infected with isolates without DCS. One microbiologically confirmed relapse occurred in the group of patients infected with isolates with DCS. There were no deaths.

DISCUSSION

We show that in the United States hospitalized patients with typhoid fever due to *Salmonella* serotype Typhi with DCS (ciprofloxacin MICs, 0.12 to 1 μg/ml) experience longer times to fever clearance and more frequent treatment failures than patients with typhoid fever due to *Salmonella* serotype Typhi without DCS (ciprofloxacin MICs, <0.12 μg/ml). In addition, these patients receive longer inpatient courses of antimicrobial therapy. Although our study was limited by the relatively small number of patients with typhoid fever in the United States and by the use of a retrospective study design, these findings are consistent with the findings of prospective studies done in settings where typhoid fever is endemic (27). Since the proportion of *Salmonella* serotype Typhi isolates with DCS reported in the United States grew from 19% in 1999 to 38% in 2003 (2, 17) and since *Salmonella* serotype Typhi isolates with DCS occur at even higher proportions in other countries (20, 22), these findings are of considerable clinical and public health importance (28).

Although our study was not large enough to investigate an effect of DCS on mortality, treatment of typhoid fever patients with an appropriate antimicrobial agent is known to significantly reduce the typhoid fever case fatality rate (26). It is unknown whether the impaired clinical response seen among typhoid fever patients with DCS in this study would be associated with an increased rate of typhoid fever complications and death in a larger study. However, it is likely that the effect of DCS on the clinical outcome would be magnified in a setting in which the premorbid health of typhoid fever patients is poor; in which access to health care services is limited; and in which alternative antimicrobial agents, such as extended-spectrum cephalosporins, are unavailable (9). The majority of global cases of typhoid fever occur under such conditions of poverty (10), and it is notable that most patients enrolled in our study acquired their infection outside of the United States and predominantly in south Asia. Furthermore, this study could not capture cases of typhoid fever diagnosed and treated among traveling American citizens and residents prior to their return home. This limitation could have led to a reduced rate of ascertainment of the adverse clinical outcomes of typhoid fever.

We found that the presence of multidrug resistance, particularly resistance type ACSuTm, correlated with DCS among *Salmonella* serotype Typhi isolates in this study. Since the patients enrolled in this study received a number of antimicrobial agents besides fluoroquinolones, we sought to confirm that the trend toward a poorer clinical response seen among patients infected with *Salmonella* serotype Typhi isolates with DCS was not being driven by the failure of *Salmonella* serotype Typhi isolates with resistance type ACSuTm to respond to the traditional first-line antimicrobials used for the treatment of typhoid fever, such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Since the association between DCS with a prolonged fever clearance time and treatment failure persisted after the multidrug-resistant phenotype was controlled for, it is likely that DCS was responsible for the trend toward a poorer clinical response observed in this study. In this study, ceftriaxone therapy was commonly used in addition to fluoroquinolone therapy, so it is also possible that ESBL production could drive a prolonged fever clearance time and treatment failure (5). However, only one *Salmonella* serotype Typhi isolate had a ceftriaxone MIC ≥2 μg/ml, a CLSI

screening criterion for ESBL production (7), and there was no association between this phenotype and DCS. Since possible ESBL production was found in only one patient, there was no association between DCS and ESBL production; and since ceftriaxone was used equally for patients infected with *Salmonella* serotype Typhi with DCS and patients infected with *Salmonella* serotype Typhi isolates without DCS (Table 2), the decreased susceptibility of *Salmonella* serotype Typhi to extended-spectrum cephalosporins was not responsible for the trend toward poorer outcomes observed among patients infected with isolates with DCS in this study. Furthermore, these findings support the continued use of extended-spectrum cephalosporins as alternatives to fluoroquinolones in the presence of DCS.

Screening of the *Salmonella* serotype Typhi isolates recovered from patients with invasive infection for nalidixic acid resistance has been advocated to identify those at increased risk of fluoroquinolone treatment failure due to infection with isolates with DCS (18). However, our findings and those of others (8) indicate that this approach does not identify all *Salmonella* serotype Typhi isolates with DCS. It is possible that discordant nalidixic acid and fluoroquinolone susceptibility testing results may be driven by mechanisms of resistance other than chromosomal point mutations in the genes that code for the enzymes DNA gyrase (*gyrA*) and topoisomerase IV (*parC*). We screened a subset of isolates for loci recently identified to be associated with plasmid-mediated quinolone resistance (*qnrA*, *qnrB*, *qnrS*), and all isolates were negative. It is possible that altered cell membrane permeability (15), efflux pumps (29), or other mechanisms may contribute to the discordant nalidixic acid and fluoroquinolone susceptibility testing results, but we were unable to study these mechanisms. Whatever the mechanism, the discordance of nalidixic acid and fluoroquinolone susceptibility testing results adds weight to the argument that the fluoroquinolone MICs for invasive *Salmonella* serotype Typhi isolates should be measured in order to identify all isolates with DCS (25). Since the capacity to measure MICs is not available in many laboratories in settings where typhoid fever is endemic, research on the adjustment of fluoroquinolone disk diffusion interpretive criteria is also warranted.

This study had a number of limitations. The relatively small number of typhoid fever illnesses that occur in the United States (2) means that even in a multicenter study design conducted over 4 years, the statistical power to detect outcomes of interest was modest. While we were able to demonstrate significantly longer times to the loss of fever and more frequent treatment failures for all antimicrobials in the group infected with isolates with DCS, only statistically nonsignificant trends could be detected for the subset of patients for whom fluoroquinolone use was clearly captured in the medical record. Furthermore, the study design was observational, and few patients were treated exclusively with fluoroquinolones, limiting our ability to attribute differences in clinical outcomes to fluoroquinolone treatment failure. We addressed this limitation by ascertaining the antimicrobials used in addition to fluoroquinolones and the type of resistance other than DCS and controlling for it when it was thought to potentially have a confounding effect. The retrospective observational design also meant that we had no control over whether or when patients received their first dose of a fluoroquinolone. Of the 71 patients enrolled in the study, only 32 were treated with fluoroquinolones. For the survival analysis, which consisted of the antimicrobial- and fluoroquinolone-related times to fever clearance, we had to restrict the analysis to patients who received their first dose of antimicrobial or their first dose of fluoroquinolone in the hospital and prior to defervescence, further restricting the number of cases eligible for the survival analysis. In addition, some patients were discharged from the hospital prior to defervescence, resulting in censored data. The retrospective nature of the study required clinical information to be extracted from medical records. However, because the records of the medications received and the regular periodic measurement of temperature were likely to have been reliably collected, we think that the main outcome measure of the study was robust. A larger prospective study conducted in an area where typhoid fever is endemic is needed to understand how DCS may affect the rates of complication and death on a global level.

We demonstrated that patients with typhoid fever due to *Salmonella* serotype Typhi isolates with DCS experienced longer times to fever clearance and more frequent treatment failures than patients with typhoid fever due to *Salmonella* serotype Typhi isolates without DCS. We confirm that screening for nalidixic acid resistance does not always detect *Salmonella* serotype Typhi isolates with DCS. These findings are consistent with those of studies conducted in settings where typhoid fever is endemic and suggest that the fluoroquinolone breakpoints for *Salmonella* serotype Typhi should be reevaluated (16, 23, 27). We suggest that fluoroquinolone MIC measurement rather than screening of invasive *Salmonella* serotype Typhi isolates for nalidixic acid resistance should be considered. Larger prospective studies in areas where typhoid fever is endemic are urgently needed to determine whether DCS is also associated with increased rates of typhoid fever complications and death.

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