Antibiotic Susceptibilities of *Salmonella enterica* Serovar Typhi and *S. enterica* Serovar Paratyphi A Isolated from Patients in Japan

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The antibiotic susceptibilities of 62 strains of *Salmonella enterica* serovar Typhi and 37 strains of *S. enterica* serovar Paratyphi A were investigated with 18 antibiotics. Eighteen *S. enterica* serovar Typhi isolates and five *S. enterica* serovar Paratyphi A isolates were resistant to one or more antimicrobial agents, among which 10 *S. enterica* serovar Typhi isolates were nalidixic acid resistant and also showed decreased ciprofloxacin susceptibility.

Enteric fever remains an important public health problem in many countries of the world. Typhoid fever is a sometimes fatal infection of adults and children that causes bacteremia and inflammatory destruction of the intestine and other organs. Typhoid fever is endemic in developing countries, especially in southeast Asia and Africa. Chloramphenicol has been a choice of treatment for typhoid fever for about 40 years, but alternative drugs for treatment are now required by the emergence of multidrug-resistant (MDR) *Salmonella enterica* serovar Typhi (resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole). Fluoroquinolones have proven to be effective for the treatment of typhoid fever caused by MDR strain, and have become the drugs for the first line of treatment of typhoid fever (2, 6). But some *S. enterica* serovar Typhi strains resistant to fluoroquinolones have already been reported (5, 10, 18). Further, several failures of clinical treatment of typhoid patients with ciprofloxacin and other fluoroquinolones have also been reported (4, 20, 21). Geographically, the emergence and spread of these resistant organisms have been reported from developing countries, particularly from Vietnam (15, 21), the Indian subcontinent (5, 11, 16, 19), and Tajikistan (12, 13). In this study, the susceptibility of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A strains isolated in Japan was investigated by the determination of the MICs of 18 kinds of antimicrobial agents, and the increases of the drug resistances of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A strains and the implications for treatment are discussed.

The following totals were reported in Japan: for *S. enterica* serovar Typhi, 76 isolates were collected in 1997, 62 isolates were collected in 1998, and 87 isolates were collected in 1999, and for *S. enterica* serovar Paratyphi A, 36 isolates were collected in 1997, 49 isolates were collected in 1998, and 28 isolates were collected in 1999. A total of 62 *S. enterica* serovar Typhi and 37 *S. enterica* serovar Paratyphi A isolates that were collected from 1997 to 1999 in Japan were randomly selected from the isolates of each year and were investigated in this study. All the isolates were obtained from either a blood culture or stool culture of individual patients and identified by biochemical and serological tests on the basis of standard criteria. Phage typing was performed with the phage set provided by the World Health Organization (WHO) International Phage Typing Laboratory for Salmonella at Colindale, London, United Kingdom. The standard phage typing technique described by Anderson et al. (1) was employed throughout. Antibiotics were obtained from the manufacturers as laboratory powders of defined potency and were reconstituted in their recommended diluent to yield stock solutions that were kept frozen. Antimicrobial agent powders used in these studies were provided as follows: norfloxacin (Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan), ciprofloxacin (Bayer Pharmaceutical Co., Ltd., Tokyo, Japan), ofloxacin and levofloxacin (Dai-ichi Pharmaceutical Co., Ltd., Tokyo, Japan), tosufloxacin (Toyama Chemical Co., Ltd., Tokyo, Japan), sparfloxacin (Dainippon Pharmaceutical Co., Ltd., Tokyo, Japan), cefoperazone (Toyama Chemical Co., Ltd.), ceftriaxone (Roche Japan, Tokyo, Japan), cefotaxime (Hoechst Marion Roussel, Tokyo, Japan), fosfomycin (Meiji Seika Kaisha, Ltd., Tokyo, Japan), aztreonam (Ezai, Tokyo, Japan), azithromycin (Pfizer Inc., New York, N.Y.), imipenem (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan), and ampicillin, chloramphenicol, sulfamethoxazole, trimethoprim, and nalidixic acid (Wako, Tokyo, Japan). Glucose-6-phosphate was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan), and was added to a final concentration of 50 μg/ml into the medium containing each concentration of fosfomycin. Broth microdilution antimicrobial susceptibility testing was performed in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) methodology (14). Susceptibility testing was performed with cation-adjusted Mueller-Hinton broth. The reagent powders were dissolved in Mueller-Hinton broth and distributed to the wells of microdilution trays. Each tray was inoculated with 5×10^4 CFU per well to yield a final volume of 0.1 ml per well. The tray was incubated at 35°C for 18 h. Appropriate quality control strains, *Escherichia coli* ATCC 25922 and *Staphylococcus*

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TABLE 1. Susceptibilities of clinical isolates of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A to 18 antimicrobial agents

| Drugs | MIC (μg/ml) | | | | | | Incidence of resistant strains (%)[a] | | Breakpoint (μg/ml) for resistance |
| | *S. enterica* serovar Typhi | | | *S. enterica* serovar Paratyphi A | | | *S. enterica* serovar Typhi | *S. enterica* serovar Paratyphi A | |
	Range	50%	90%	Range	50%	90%			
Chloramphenicol	2.0–>256	4	>256	2.0–>256	8	8	19.4	2.7	≥32
Ampicillin	0.25–>256	0.5	>256	2.0–>256	2	2	19.4	2.7	≥32
Streptomycin	8.0–>1,024	16.0	>1,024	0.25–>1,024	32.0	64.0	–	–	–[b]
Trimethoprim-sulfa-methoxazole (1:19)	0.5–>256	1	>256	0.5–>256	4	4	19.4	2.7	≥4/76
Nalidixic acid	2.0–>256	4	>256	8.0–>256	8	16	16.1	5.4	≥32
Fosfomycin	1.0–128	8	16	2.0–64	32.0	64.0	0.0	0.0	≥256
Norfloxacin	0.031–2.0	0.125	1	0.125–4.0	0.25	0.5	0.0	0.0	≥16
Ciprofloxacin	0.008–0.5	0.016	0.25	0.031–2.0	0.063	0.125	0.0	0.0	≥4
Ofloxacin	0.031–1.0	0.063	0.5	0.004–4.0	0.25	0.5	0.0	0.0	≥8
Levofloxacin	0.016–0.5	0.031	0.25	0.125–2.0	0.125	0.125	0.0	0.0	≥8
Tosufloxacin	0.004–0.25	0.031	0.125	0.031–1.0	0.063	0.063	–	–	–
Sparfloxacin	0.008–0.25	0.016	0.25	0.016–1.0	0.031	0.063	–	–	–
Cefoperazone	0.031–8.0	0.25	4	0.25–4.0	0.5	1	0.0	0.0	≥64
Ceftriaxone	0.016–0.063	0.031	0.063	0.016–0.5	0.031	0.063	0.0	0.0	≥64
Cefotaxime	0.031–0.125	0.063	0.063	0.063–0.125	0.063	0.125	0.0	0.0	≥64
Aztreonam	0.031–0.25	0.031	0.25	0.063–0.25	0.125	0.25	0.0	0.0	≥32
Azithromycin	1.0–4.0	2	4	2.0–8.0	4	4	–	–	–
Imipenem	0.25–1.0	0.25	0.5	0.25–4.0	0.5	2	0.0	0.0	≥16

[a] The percentages of resistance to the antimicrobial agents are based on the breakpoints of the NCCLS (14). –, no breakpoints are given.
[b] –, The interpretive breakpoints for *Enterobacteriaceae* were not available in the NCCLS breakpoints.

aureus ATCC 29213, were included in each test. The recorded MICs of all of the antibiotics were the lowest concentrations that completely inhibited visible growth of the test strain. The MICs at which 50% of the isolates tested were inhibited (MIC$_{50}$s) and MIC$_{90}$s were calculated in accordance with current NCCLS methodology.

Table 1 summarizes the drugs used in this study, the MIC range, MIC$_{50}$s and MIC$_{90}$s of the antibiotics tested, and the rate of resistance to each antibiotic tested. Of the drugs tested, fluoroquinolones and extended-spectrum cephalosporins were the most effective against *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A. The MIC$_{90}$s of fluoroquinolones for *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A were extremely low compared to those of traditional drugs (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole). The MIC$_{90}$s of chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, nalidixic acid, and streptomycin for *S. enterica* serovar Typhi were larger than 256 μg/ml, but those for *S. enterica* serovar Paratyphi A were within the susceptible range. The incidences of MDR strains of *S. enterica* serovar Typhi were 15% in 1997, 20% in 1998, and 22.7% in 1999, and those of *S. enterica* serovar Paratyphi A were none in both 1997 and 1998 and 8.3% in 1999. These data show that resistance to antimicrobial agents, and in particular to traditional drugs, is increasing year by year. Traditional agents do not seem to be effective for the treatment of *S. enterica* serovar Typhi infection when considering the MIC$_{90}$s, and therefore, they might be no longer useful as the first line of treatment for *S. enterica* serovar Typhi infection. Most cases of MDR *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A infection in Japan show a history of travel to the Indian subcontinent and southeast Asia. Most patients with E1 phage-typed *S. enterica* serovar Typhi isolates had recently returned from the Indian subcontinent, and most of the phage-type E1 strains were MDR strains (data not shown). We also observed nali-

dixic acid-resistant strains of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A. The incidences of nalidixic acid-resistant strains of *S. enterica* serovar Typhi were 10% in 1997, 5% in 1998, and 31.8% in 1999, and those of *S. enterica* serovar Paratyphi A were none in 1997, 7.7% in 1998, and 8.3% in 1999. Five of a total of 10 nalidixic acid-resistant *S. enterica* serovar Typhi strains were MDR strains.

Using the breakpoints recommended by the NCCLS (MICs of ≤1 and ≥4 μg/ml for ciprofloxacin are considered to indicate susceptibility and resistance, respectively) (14), we did not observe the typical strains resistant to ciprofloxacin for both *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A in this study. However, we found strains that had a decreased susceptibility to ciprofloxacin, for which the MICs ranged from 0.125 to 2.0 μg/ml. Among the strains tested, 10 (16.1%) of 62 *S. enterica* serovar Typhi isolates and 2 (5.4%) of 37 *S. enterica* serovar Paratyphi A isolates exhibited reduced susceptibility to ciprofloxacin. The MIC of ciprofloxacin for these strains was about 10 times larger than that for susceptible strains (Table

TABLE 2. MICs of fluoroquinolones and other drugs for *S. enterica* serovar Typhi strains resistant or susceptible to nalidixic acid

| Drugs | MIC (μg/ml) | | | | | |
| | Resistant strains (*n* = 10) | | | Susceptible strains (*n* = 52) | | |
	Range	50%	90%	Range	50%	90%
Ciprofloxacin	0.125–0.5	0.25	0.5	0.008–0.031	0.016	0.031
Ofloxacin	0.5–1.0	0.5	1.0	0.031–0.25	0.063	0.25
Norfloxacin	0.5–2.0	1.0	2.0	0.031–0.25	0.063	0.25
Levofloxacin	0.25–0.5	0.25	0.5	0.031–0.25	0.063	0.125
Tosufloxacin	0.016–0.25	0.125	0.25	0.004–0.25	0.016	0.016
Sparfloxacin	0.016–0.25	0.25	0.25	0.008–0.25	0.016	0.016
Cefoperazone	0.25–8.0	0.5	4.0	0.031–4.0	0.25	2.0
Ceftriaxone	0.031–0.063	0.063	0.063	0.016–0.063	0.031	0.063
Cefotaxime	0.031–0.063	0.063	0.063	0.031–0.125	0.063	0.063

2). The strains with decreased ciprofloxacin susceptibility were also uniformly resistant to nalidixic acid. The MICs of fluoroquinolones for 10 nalidixic acid-resistant and 52 susceptible *S. enterica* serovar Typhi isolates are shown in Table 2. The MICs of ciprofloxacin, ofloxacin, norfloxacin, and levofloxacin for nalidixic acid-resistant strains ranged from 0.125 to 2.0 μg/ml, and all of the nalidixic acid-resistant strains showed a decreased susceptibility to ciprofloxacin, ofloxacin, norfloxacin, and levofloxacin. However, the MICs of tosufloxacin and sparfloxacin for nalidixic acid-resistant strains ranged from 0.016 to 0.25 μg/ml. These results suggest that some strains with decreased ciprofloxacin susceptibility were still susceptible to tosufloxacin and sparfloxacin. Further, the strains with decreased ciprofloxacin susceptibility were fully susceptible to extended-spectrum cephalosporins, including cefoperazone, ceftriaxone, and cefotaxime, in vitro, which might suggest that these antibiotics could be appropriate therapy for urgent cases of typhoid fever. However, attention should be paid to the emergence of strains resistant to extended-spectrum cephalosporins in the near future. Recently, *S. enterica* serovar Typhi isolates with decreased ciprofloxacin susceptibility (MIC, ≥0.125 μg/ml) have become the subject of worldwide attention (3, 9, 13, 18). Threlfall et al. reported that the incidence of *S. enterica* serovar Typhi isolates with decreased ciprofloxacin susceptibility in the United Kingdom increased from 0.9% in 1991 to 33% in 1999 (20). We also observed that the incidence of *S. enterica* serovar Typhi isolates with decreased ciprofloxacin susceptibility in Japan increased from 10% in 1997 to 31.8% in 1999. Furthermore, about 67% of the isolates from patients with a history of travel to India showed a decreased susceptibility to ciprofloxacin in 1999 (data not shown). We also analyzed the quinolone resistance-determining region of the *gyrA* gene of the *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A isolates with decreased ciprofloxacin susceptibility. All of the *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A isolates with decreased ciprofloxacin susceptibility had a point mutation in the quinolone resistance-determining region of the *gyrA* gene (data not shown), as reported for other *Salmonella* serovars (7, 8, 17).

In conclusion, we demonstrated the presence of *S. enterica* serovar Typhi isolates and *S. enterica* serovar Paratyphi A isolates with decreased fluoroquinolone susceptibility in Japan. It may be necessary to alert clinicians to recognize the existence of the strains showing decreased fluoroquinolone susceptibility. The surveillance for antimicrobial resistance of *S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi A isolates should be continued, particularly to monitor the emergence of strains fully resistant to fluoroquinolones.

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