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Antimicrobial Susceptibility of *Shigella sonnei* Isolates in Japan and Molecular Analysis of *S. sonnei* Isolates with Reduced Susceptibility to Fluoroquinolones

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We performed susceptibility testing with *Shigella sonnei* isolates from imported and domestic cases of infection in Japan during 2001 and 2002. Some *S. sonnei* isolates were resistant to nalidixic acid, tetracycline, and trimethoprim-sulfamethoxazole. Most of the nalidixic acid-resistant strains showed reduced susceptibility to fluoroquinolones but did not show fluoroquinolone resistance.

Shigella species remain an important cause of gastrointestinal illness manifested by watery diarrhea, which may progress to mucoid bloody diarrhea. The annual number of *Shigella* episodes throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries, with 1.1 million deaths (9). Each year, many people who travel from an industrialized contry to a developing country in tropical areas encounter diarrhea caused by a variety of enteric pathogens which are acquired by ingestion of contaminated food and water. In Japan, there are about 600 bacteriologically confirmed cases of shigellosis each year. *Shigella sonnei* has become the primary cause of shigellosis in Japan (12). Most of the shigellosis in Japan is travel related, with infections occurring in developing countries in southeast Asia. We performed antimicrobial susceptibility tests with *S. sonnei* isolates from imported and domestic isolates in Japan. Some of the *S. sonnei* isolates recovered showed resistance to several kinds of antimicrobial agents. Furthermore, we analyzed the molecular basis of the acquired resistance to nalidixic acid (NA).

The bacterial strains used in this study were collected from regional public health institutes in Japan, and all isolates were obtained from a stool culture of patients and identified by biochemical and serological testing on the basis of standard criteria (3). A total of 58 clinical isolates of *S. sonnei* were used, of which 19 were isolated from domestic cases and 39 were isolated from imported cases. Each strain was selected from independent incidences. The domestic isolates were recovered from patients who did not have a recent history of travel to a foreign country. The imported isolates were recovered from patients who had a record of recent international travel.

MICs for the *S. sonnei* isolates were determined by using either the Etest (Aska Diagnostics, Tokyo, Japan) or broth microdilution method. The MICs of chloramphenicol (CP), ampicillin (AP), streptomycin (SM), kanamycin (KM), gentamicin (GM), tetracycline (TC), fosfomycin (FOM), NA, norfloxacin (NFLX), ciprofloxacin (CPFX), ofloxacin (OFLX), levofloxacin (LVFX), sparfloxacin (SPFX), cefoperazone (CPZ), ceftriaxone (CTRX), cefotaxime (CTX), and imipenem (IPM) were determined by Etest, and the MIC of trimethoprim-sulfamethoxazole (TS) (1:19) was determined by the broth microdilution method. The susceptibility testing by Etest was carried out according to the manufacturer's instructions, and the susceptibility testing by the broth microdilution method followed the NCCLS method (11). The primers used for the PCR amplification of the *gyrA* genes and the sequencing of the quinolone resistance-determining region (QRDR) of the *gyrA* genes were previously described (7).

We report here the results of susceptibility and molecular analyses of clinical isolates of *S. sonnei* recovered from imported and domestic cases during the period 2001 to 2002 in Japan. Fifty-eight strains of *S. sonnei* isolated in Japan were tested for susceptibility to 18 antimicrobial agents. All of the isolates tested in this study were susceptible to kanamycin, gentamicin, norfloxacin, fosfomycin, ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, cefoperazone, ceftriaxone, cefotaxime, and imipenem (Table 1).

Resistance to chloramphenicol and ampicillin was infrequent (range, 0 to 8%) in both domestic and imported isolates. Resistance to trimethoprim-sulfamethoxazole and tetracycline was highly frequent (69 and 89%, respectively) in imported isolates. In contrast, the frequencies of resistance to trimethoprim-sulfamethoxazole and tetracycline ranged from about 32 to 37% in domestic isolates. We found that 26% of isolates in both imported and domestic cases were NA-resistant strains, and most of them showed reduced susceptibility to several fluoroquinolones (Tables 1 and 2). The major patterns of resistance were resistance to TC and TS (36%) and TC, TS, and NA (24%) in imported cases and resistance to NA (21%) and TC and TS (21%) in domestic cases (data not shown).

The MICs of fluoroquinolones were compared between NA-resistant and NA-susceptible strains (Table 2). The MICs were higher in the NA-resistant than NA-susceptible strains. Most of the NA-resistant *S. sonnei* strains showed reduced susceptibility to the fluoroquinolones tested but did not show resistance. The current NCCLS breakpoint for resistance to ciprofloxacin is $\geq$4 μg/ml. Strains for which the MIC of ciprofloxacin was $\geq$0.125 μg/ml and $\leq$2 μg/ml were considered

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TABLE 1. Susceptibilities of clinical isolates of *S. sonnei*

Drug(s)	MIC (μg/ml)[a]								Breakpoint (μg/ml) for resistance
	Domestic isolates (19 strains)				Imported isolates (39 isolates)				
	Range	50%	90%	Resistant (%)[b]	Range	50%	90%	Resistant (%)[b]	
Chloramphenicol	4–8	8	8	0	4–>256	8	16	5	≥32
Ampicillin	2–>256	2	4	5	1–>256	2	16	8	≥32
Streptomycin	8–512	64	256	—	8–512	256	512	—	NA[c]
Kanamycin	2–4	4	4	0	2–8	4	4	0	≥64
Gentamicin	0.5–1	0.5	1	0	0.5–1	0.5	1	0	≥16
Trimethoprim-sulfamethoxazole (1:19)	4–>1024	8	>1,024	37	2–>1,024	>1,024	>1,024	82	≥4/76
Tetracycline	1–>256	2	>256	32	2–>256	>256	>256	69	≥16
Fosfomycin	2–16	4	8	0	2–32	8	16	0	≥256
Nalidixic acid	1–>256	2	>256	26	1–>256	2	>256	26	≥32
Norfloxacin	0.064–1	0.064	1	0	0.008–1	0.064	1	0	≥16
Ciprofloxacin	0.008–0.25	0.008	0.25	0	0.008–0.25	0.008	0.125	0	≥4
Ofloxacin	0.064–1	0.064	1	0	0.032–1	0.064	1	0	≥8
Levofloxacin	0.016–0.25	0.032	0.25	0	0.016–0.25	0.032	0.25	0	≥8
Sparfloxacin	0.008–0.25	0.016	0.25	—	0.008–0.25	0.016	0.25	—	NA[c]
Cefoperazon	0.032–32	0.125	0.25	0	0.032–4	0.125	0.5	0	≥64
Ceftriaxone	0.032–0.064	0.032	0.064	0	0.016–0.125	0.032	0.064	0	≥64
Cefotaxime	0.016–0.125	0.032	0.064	0	0.016–0.125	0.064	0.125	0	≥64
Imipenem	0.125–0.25	0.25	0.25	0	0.125–0.5	0.25	0.25	0	≥16

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] The percentages of isolates resistant to the antimicrobial agents are based on the breakpoints of the NCCLS. —, no breakpoint given.
[c] NA, interpretive breakpoint for *Enterobacteriaceae* is not available in the NCCLS breakpoints.

to have reduced susceptibility to ciprofloxacin among the *S. enterica* serovars Typhi and Paratyphi A (1, 8, 13), because typhoid fever and paratyphoid fever caused by these strains did not respond to fluoroquinolone therapy when the strains were judged "susceptible" based on the NCCLS breakpoint in susceptibility tests. Similarly, we considered *S. sonnei* strains for which the MIC of ciprofloxacin was ≥0.125 μg/ml and ≤2 μg/ml to have reduced susceptibility to ciprofloxacin. Several studies have shown that resistance to nalidixic acid and reduced susceptibility to fluoroquinolones have increased among *Shigella* spp. (5, 6). Despite the low level of resistance to fluoroquinolones, the efficacy of fluoroquinolone treatment may be reduced in humans infected with *S. sonnei* strains that are regarded as having reduced susceptibility to fluoroquinolone, as seen in infections by the isolates of *S. enterica* serovars Typhi and Paratyphi A with reduced susceptibility to fluoroquinolone. The breakpoint for reduced susceptibility is important when assessing the efficacy of fluoroquinolones. The breakpoint for reduced susceptibility was different for each fluoroquinolone antibiotic. In this study, the breakpoints for reduced susceptibility were 0.125, 0.25, 0.25, 0.125, and 0.064 μg/ml for ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, and sparfloxacin, respectively (Table 2).

In all of the NA-resistant strains, a QRDR in the *gyrA* gene

TABLE 2. Comparison of MICs of fluoroquinolones between nalidixic acid-resistant and susceptible *S. sonnnei* isolates

Phenotype for NA resistance or susceptibility	No. of strains for which fluoroquinolone MIC (μg/ml) is:								Breakpoint for reduced susceptibility to fluoroquinolones tested
	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	
Ciprofloxacin									
NA-R	1			3	5	6			0.125
NA-S	37	4		2					
Norfloxacin									
NA-R			1			1	6	7	0.25
NA-S	3		3	35	2				
Ofloxacin									
NA-R			1			3	3	8	0.25
NA-S			3	37	3				
Levofloxacin									
NA-R		1		1	3	10			0.125
NA-S		12	27	4					
Sparfloxacin									
NA-R	1			4	1	9			0.064
NA-S	14	24	5						

[a] NA-R; NA-resistant strain, NA-S; NA-susceptible strain.

TABLE 3. MICs of NA-resistant *S. sonnnei* strains

Parameter (no. of strains)	MIC (μg/ml)					
	Nalidixic acid	Ciprofloxacin	Norfloxacin	Ofloxacin	Levofloxacin	Sparfloxacin
gyrA mutation in codon 87 (5)						
Range	64–>256	0.008–0.125	0.032–0.5	0.032–0.5	0.016–0.125	0.008
MIC_{50}	64	0.004	0.25	0.25	0.125	0.064
MIC_{90}	>256	0.125	0.5	0.5	0.125	0.064
gyrA mutation in codon 83 (10)[a]						
Range	>256	0.125–0.25	0.5–1	0.5–1	0.25	0.125–0.25
MIC_{50}	>256	0.25	1	1	0.25	0.25
MIC_{90}	>256	0.25	1	1	0.25	0.25

[a] Domestic and imported strains.

coding for a region associated with NA resistance was sequenced. All NA-resistant strains had a single point mutation in either codon 83 or 87 of GyrA. All five NA-resistant isolates from domestic cases had the same mutations in codon 83 of GyrA, which led to the replacement of Ser (TCC)-83 with Leu (TGG)-83. Out of 10 NA-resistant isolates from imported cases, five strains had a mutation in codon 83 and the others had a mutation in codon 87 of GyrA. The five NA-resistant isolates from imported cases had the same mutation in codon 83 (Ser to Leu). One strain with a mutation in codon 87 of GyrA had a novel alteration at the codon, which led to replacement of Asp (GAC) with Ala (GCC). This alteration has not been reported in *Shigella gyrA* mutations previously. The others had a mutation which led to Asp (GAC)-87 being replaced with Tyr (TAC)-87, which has been reported in other papers (4).

The MICs at which 90% and 50% of the isolates tested are inhibited (MIC_{90} and MIC_{50}, respectively) of several fluoroquinolones for isolates were compared among the strains with mutations in codons 83 and 87 of GyrA (Table 3). The MIC_{90} and MIC_{50} seemed to be slightly lower for the strains with a single mutation in codon 87 than for those with a mutation in codon 83 (Table 3). We found only one strain which was resistant to NA but highly susceptible to ciprofloxacin and other fluoroquinolones (Table 2). A genetic analysis revealed this strain to have a mutation in codon 87. Furthermore, we analyzed the *parC* genes of NA-resistant strains but did not find any mutations responsible for the fluoroquinolone resistance. A mutational analysis of the *S. sonnei* isolates suggested that the reduced susceptibility to fluoroquinolones was due to mutation of the *gyrA* gene.

Fluoroquinolones are the first choice for the treatment of shigellosis caused by multidrug-resistant strains. Recently, the emergence of *Shigella dysenteriae* that is resistant to fluoroquinolones such as ciprofloxacin, norfloxacin, and ofloxacin has been reported from India and Bangradesh (2, 10). The emergence of ciprofloxacin-resistant *Shigella* strains is a major problem in the treatment of shigellosis. In this study, we did not find any fluoroquinolone-resistant *S. sonnei* strains in Japan. The surveillance of the antimicrobial resistance of *S. sonnei* isolates should be continued, particularly to monitor the emergence of strains which are fully resistant to fluoroquinolones.

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