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Antimicrobial resistance of *Shigella* spp. from humans in Shanghai, China, 2004–2011

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ABSTRACT

A retrospective study conducted on patients with diarrhea in Shanghai, China from 2004–2011, indicated that of 77,600 samples collected, 1,635 (2.1%) tested positive for *Shigella*. Species isolated included *S. sonnei* (1,066, 65.1%), *S. flexneri* (569, 34.7%), and *S. boydii* (3, 0.2%). Most of the *Shigella* isolates were found to be resistant to streptomycin (98.7%), trimethoprim (98.0%), ampicillin (92.1%), and nalidixic acid (91.7%). Additionally, many isolates were resistant to tetracycline (86.9%), trimethoprim + sulfamethoxazole (80.1%), sulfisoxazole (76.8%) and gentamicin (55.5%). Approximately 80% of the isolates were resistant to at least eight antimicrobial agents, 14% to at least ten antimicrobials tested and 10 isolates to fourteen antimicrobials, including sulfonamides, fluoroquinolones, tetracyclines, aminoglycosides and β-lactamases. Importantly, co-resistance to fluoroquinolones and the third- and fourth-generation cephalosporins was also identified. The high levels of resistance to antimicrobial agents commonly used in clinical medicine presents a great challenge to treating patients with shigellosis.

1. Introduction

Shigellosis is one of the most common diarrheal diseases in humans worldwide. Approximately 125 million cases of *Shigella* infections occur annually in Asia, of which 14,000 are fatal (Bardhan et al., 2010). In China, shigellosis is one of the top four notifiable infectious diseases, with 1.7 million episodes of bacillary dysentery, and 200,000 patients admitted to hospitals each year (Qiu et al., 2011; Wang et al., 2006). The distribution of *Shigella* spp. depends on the country: *S. flexneri* is predominant in developing countries, whilst *S. sonnei* is most reported in developed countries (Kotloff et al., 1999; Niyogi, 2005; Wang et al., 2006; Ye et al., 2010). However, *S. sonnei* has become dominant in some Asian countries in recent years (Bangtrakulnonth et al., 2008; Filliol-Toutain et al., 2011; Salmanzadeh-Ahrabi et al., 2007). Better understanding of *Shigella* in different geographic regions may assist in the recognition and tracing of pathogens and in the implementation of proper treatment and control strategies (Kotloff et al., 1999).

Shigellosis is often treated with antibiotics to reduce excretion and to shorten the duration of illness (Salam & Bennish, 1991). However, the progressive increase in antimicrobial resistance, especially multi-drug resistance among *Shigella* due to the antimicrobial overuse, is becoming a critical public health problem (Salmanzadeh-Ahrabi et al., 2007). Because the prevalence of antimicrobial-resistant *Shigella* varies widely between and within countries, surveillance is essential for providing information on the magnitude and trends in antimicrobial resistance and for monitoring the effect of interventions. A number of international studies have shown that multidrug-resistant (MDR) *Shigella* infections are widespread (Alici et al., 2006; Opintan & Newman, 2007; Shiferaw et al., 2012; Xia et al., 2011) and can be acquired during travel. Delaying treatment, pending culture and sensitivity results, can prolong illness and contagiousness. Treatment with an antibiotic can be an important public health control measure, especially in an outbreak setting (Hoffman & Shillam, 1990).

Shanghai Center for Disease Control and Prevention (SCDC) has participated in the World Health Organization Global Foodborne Infections Network since 2005. The SCDC has regularly performed isolation, identification and characterization of foodborne pathogens recovered patients. In the present study, we described the distribution of *Shigella* isolates and its antimicrobial resistance patterns from a passive surveillance study conducted from 2004 to 2011 in Shanghai.

2. Materials and methods

2.1. Specimen collection

Clinical specimens were collected from outpatients in hospitals located in 10 districts of Shanghai from January 2004 to December 2011, except in 2009 when two hospitals were not able to provide the isolates. During sampling, stool specimens were collected from diarrhea patients with clinically suspected dysentery and submitted

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Table 1
Number and percentage of the different *Shigella* species and serotypes isolated in Shanghai from 2004 to 2011.

Species	No. (%) of *Shigella* species and *S. flexneri* serotypes								
	2004	2005	2006	2007	2008	2009	2010	2011	Total
S. flexneri	28 (100)	71 (83.5)	21 (48.4)	66 (56.9)	116 (32.3)	131 (70.4)	60 (19.5)	76 (14.6)	569 (34.7)
1a	0	4	0	13	19	46	20	13	115 (20.2)
1b	0	0	0	0	0	1	0	0	1 (0.2)
1c	0	3	3	0	2	3	0	0	11 (1.9)
1	0	0	0	0	0	2	0	1	3 (0.5)
2a	0	2	3	16	70	47	21	32	191 (33.6)
2b	1	7	1	5	0	2	5	8	29 (5.1)
2c	1	12	0	0	5	2	0	3	23 (4.0)
3a	0	1	0	1	1	2	2	0	7 (1.2)
4a	0	1	0	1	0	0	0	0	2 (0.4)
4c	26	32	12	26	13	18	10	12	149 (26.2)
4	0	1	0	0	0	2	0	1	4 (0.7)
6	0	0	0	1	0	0	1	2	4 (0.7)
X	0	8	0	3	4	2	0	2	19 (3.3)
Y	0	0	2	0	2	4	1	2	11 (1.9)
S. sonnei	0 (0)	14 (16.5)	22 (51.1)	50 (43.1)	237 (67.1)	55 (29.6)	247 (80.5)	441 (84.8)	1066 (65.1)
S. boydii	0	0	0	0	0	0	0	3	3 (0.2)
Total	28	85	43	116	353	186	307	520	1638

to the Microbiology Laboratory of SCDC. Demographic and clinical information for each case, including age, gender, symptoms, date of illness onset, and date of specimen collection, were collected and electronically transmitted to SCDC.

2.2. Bacteriological examination

Samples were cultured for *Shigella* by streaking directly onto *Salmonella-Shigella* (SS) agar (Tian Tan Biologic Technology Company, China) and incubated overnight at 37 °C. Typical *Shigella* colonies were streaked onto Kligler iron agar. *Shigella* isolates were identified according to their biochemical characteristics described previously (Nataro et al., 2011).

2.3. Serotyping

Serological identification was performed using slide agglutination with polyvalent somatic (O) antigen grouping sera, followed by testing with monovalent antisera (Denka Seiken, Japan) for specific serotype determination. The slide agglutination method was based on the manufacturer's instructions (Japan Institute of Health Diagnostic Serum Shigella, Japan).

2.4. Antimicrobial susceptibility testing

The *Shigella* isolates were tested for antimicrobial susceptibility to 15 antimicrobials using the Kirby-Bauer disk diffusion method (CLSI, 2009). The antimicrobials included ampicillin (10 μg), cefotaxime (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), gentamicin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), amoxicillin/clavulanate acid (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), ofloxacin (5 μg), cefepime (5 μg), trimethoprim (5 μg), sulfisoxazole (300 μg), streptomycin (10 μg). *Escherichia coli* ATCC 25922 and ATCC 35218 were used as quality control organisms. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010).

2.5. Statistical analysis

chi-Square or Fisher's exact test was used for data analysis using SAS9.2 (SAS Institute, Cary, NC). A *P*-value of < 0.05 was considered statistically significant.

3. Results

Of 77,600 collected stool samples, 1,635 samples (2.1%) were positive for *Shigella*. Among the *Shigella* isolates identified to species level, 1,066 (65.1%) were *S. sonnei*, 569 (34.7%) were *S. flexneri*, and 3 (0.2%) were *S. boydii* (Table 1). No *S. dysenteriae* was identified. Except for 2008, from 2004 to 2009, *S. flexneri* was the most predominant species found in diarrhea patients in Shanghai. Interestingly, *S. sonnei* became the most common species after 2009. The three *S. boydii* isolates were all recovered in 2011. Fourteen serotypes were identified among the *S. flexneri* isolates, and *S. flexneri* 2a was the most common serotype (191/569, 33.6%), followed by serotypes 4c [IV:(7),8] (149/569, 26.2%) and 1a (115/569, 20.2%).

Information on ages of 682 of the 824 patients was included in the surveillance project from 2010 to 2011. Patient ages ranged from 2 months to 90 years. Approximately 21% (n = 143) of the *Shigella* isolates were from patients between 0 and 4 years of age, and 25.5% (n = 174) were from those between 20 and 29 years of age (Table 2). Data on patient gender were available for 686 of the 824 patients from 2010 to 2011. The distribution of *S. flexneri* and *S. sonnei* by gender was different. Approximately 59% (n = 60) of the patients infected with *S. flexneri* were male, whereas more female patients (55.8%, n = 326) were infected with *S. sonnei*.

High levels of antimicrobial resistance among the *Shigella* isolates included streptomycin (98.7%), trimethoprim (98.0%), ampicillin

Table 2
Distribution of *Shigella* isolates by gender and age (2010-2011), Shanghai, China.

	2010		2011		Total n = 824
	SF (n = 60)	SS (n = 247)	SF (n = 76)	SS (n = 441)	
Age (years)					
≤4	12	52	15	64	143
5–9	0	21	4	44	69
10–19	2	9	10	43	64
20–29	3	25	7	139	174
30–39	1	18	12	51	82
40–49	3	8	6	25	42
50–59	3	12	8	33	56
≥60	1	5	12	34	52
Unknown	35	97	2	8	142
Gender					
Males	15	62	45	196	318
Females	11	88	31	238	368
unknown	34	97	0	7	138

SF, *S. flexneri*; SS, *S. sonnei*.