

High Prevalence of Antibiotic Resistance and Molecular Characterization of Integrons among *Shigella* Isolates in Eastern China

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A total of 747 *Shigella* isolates were collected from hospitals in Jiangsu Province of China. Susceptibilities to antimicrobials and integrons were tested. A total of 78.3% of *S. flexneri* isolates and a total of 74.3% *S. sonnei* isolates were resistant to at least three antibiotics. Of the *Shigella* isolates, 74.7% had integron I and 82.6% had integron II. The conjunction of the high prevalence of integrons in *Shigella* and high resistance to antimicrobials will lead to rapid dissemination of resistant genes in this region.

Bacterial diarrhea caused by *Shigella* remains a considerable public health problem in the world, especially in developing countries, where the disease may cause as many as 167 million episodes of diarrhea and over a million deaths annually (1). The surveillance of shigellosis studies from multicenters showed that shigellosis was more ubiquitous than previously thought (2). In China, it was estimated that over 800 million diarrhea episodes occurred annually (3), and *Shigella* is one of the principal etiologic organisms for diarrhea (4).

Shigella species are classified by four serogroups, including *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Of them, *S. flexneri* is the predominant species in most of developing countries (2, 5). Antibiotic therapy can limit the duration of shigellosis and shedding of the organism. However, resistance to the most often administered antibiotics has been increasing (5–7). The evidence from Hebei Province of China, Kuwait, and the United States showed that China has a far higher prevalence of resistance for all the bacteria studied (8).

Integrons play an important role in the dissemination and recombination of antimicrobial drug resistance genes (9). They are common in *Salmonella enterica* and make an important contribution to the extent of antimicrobial resistance in this species (10, 11). Recent studies also showed that integrons contributed to the spread of antimicrobial drug resistance genes among shigellosis cases (12). However, there are few data available to describe the prevalence of integrons of *Shigella* spp. circulating in China.

In this study, we collected a total of 747 clinical *Shigella* isolates from the hospitals located in Jiangsu Province of China between January 2008 and November 2010. Stool specimens from patients with either diarrhea or dysentery were collected before the patients received antibiotics therapy and were then screened for *Shigella* spp. by conventional biochemical methods in local hospitals. The ages of patients ranged from 1 to 88 years. We defined diarrhea as the passage of ≥ 3 watery stools in a 24-h period. All isolates were identified using Rapid ID32E strips (bioMérieux Corp., Singapore) on an automatic biochemistry analyzer (Hitachi 917; Boehringer Mannheim, Japan) again. O and H antigens were characterized by slide agglutination with hyperimmune sera (Ningbo Tianrong Bio-pharmaceutical Company Limited), and the serotype was assigned to the Kauffmann-White scheme. Of these *Shigella* isolates, 716 were identified as *Shigella*; 491 (68.6%) were *S. flexneri*, 214 (29.9%) *S. sonnei*, 4 (0.5%) *S. dysenteriae*, and 7 (1.0%) *S. boydii*.

To learn the susceptibility of these *Shigella* isolates to anti-

TABLE 1 Sensitivity of two major *Shigella* isolates to 9 antibiotics^a

Antibiotic	<i>S. flexneri</i> (%)			<i>S. sonnei</i> (%)			Total (%)		
	S	I	R	S	I	R	S	I	R
Ampicillin	25.3	1.9	72.8	25.2	4.0	70.8	25.3	2.5	72.2
Co-amoxiclav	42.1	24.3	33.6	62.9	13.4	23.8	48.2	21.1	30.7
Cephalothin	52.9	17.0	30.1	43.1	27.2	29.7	50.0	20.0	20.0
Cefotaxime	63.7	13.1	23.2	68.3	14.4	17.3	65.1	13.5	21.5
Gentamicin	73.2	5.6	21.2	55.4	5.9	38.6	68.0	5.7	26.3
Nalidixic acid	18.9	3.9	77.2	27.7	3.0	69.3	21.5	3.7	74.9
Norfloxacin	52.5	13.1	34.4	77.2	5.4	17.3	59.8	10.8	29.4
Tetracycline	23.2	2.7	74.1	22.3	3.0	74.8	23.0	2.8	74.3
Co-trimoxazole	30.5	2.7	66.8	21.3	5.0	73.8	27.8	3.4	68.9

^a S, susceptible; I, intermediate; R, resistant.

microbials, the disc diffusion (Kirby-Bauer) method was employed according to Clinical and Laboratory Standards Institute standards (13) using a commercially prepared and dehydrated panel. Ampicillin, co-amoxiclav, cephalothin, cefotaxime, gentamicin, nalidixic acid, norfloxacin, tetracycline, and co-trimoxazole (sulfamethoxazole and trimethoprim [SMZ-TMP]) were involved. Interpretation of inhibition zones was performed according to the CLSI criteria, and *Escherichia coli* ATCC 25922 and ATCC 35218 were used as reference strains for quality control. A total of 491 *S. flexneri* isolates showed high levels of resistance to ampicillin (72.8%), nalidixic acid (77.2%), tetracycline (74.1%), and co-trimoxazole (66.8%). Among a total of 241 *S. sonnei* isolates, high levels of resistance to ampicillin (70.8%), nalidixic acid (69.3%), tetracycline (74.8%), and co-trimoxazole (73.8%) were found (Table 1). Among *S. flexneri* isolates, *S. flexneri* 2a (39.0%), *S. flexneri* 2b (19.3%), *S. flexneri* 1a (17.4%), and *S. flexneri* 4c (12.6%) were the major serotypes. These four serotypes accounted for 87.3% of the *S. flexneri* isolates. Among those serotypes, the resistance of *S. flexneri* 1a to antimicrobials was highest, reaching

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TABLE 2 Proportions of *S. flexneri* isolates with resistance to antimicrobials among various *S. flexneri* serotypes

<i>S. flexneri</i> serotype	% isolates with resistance to:									<i>n</i> ^a (% ^b)
	Ampicillin	Co-amoxiclav	Cephalothin	Cefotaxime	Gentamicin	Nalidixic acid	Norfloxacin	Tetracycline	SMZ-TMP ^d	
1a	88.0	41.0	28.9	28.9	31.3	89.2	51.8	85.5	79.5	83 (17.4)
1b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.0	60.0	5 (1.0)
2a	69.9	35.5	25.8	20.4	17.7	75.3	45.7	71.0	63.4	186 (39.0)
2b	64.1	31.9	29.3	21.7	68.5	68.5	20.7	58.7	52.2	92 (19.3)
3b	100.0	100.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	1 (0.2)
3c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1 (0.2)
4a	100.0	85.7	57.1	42.9	85.7	85.7	42.9	100.0	71.4	7 (1.5)
4b	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	3 (0.6)
4c	81.7	31.7	35.0	23.3	83.3	83.3	13.3	85.0	76.7	60 (12.6)
6	0.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	5 (1.0)
X	89.3	17.9	57.1	46.4	92.9	92.9	21.4	92.9	82.1	28 (5.9)
Y	83.3	0.5	16.7	16.7	83.3	83.3	33.3	83.3	83.3	6 (1.3)
Total ^c	73.8	34.2	29.6	23.7	45.7	78.2	34.8	75.1	67.7	477 (100)

^a *n*, numbers of various serotype *S. flexneri* isolates.

^b Percentages were calculated as the number of various serotype *S. flexneri* isolates/total number of *S. flexneri* isolates × 100.

^c The total percentage and number of *S. flexneri* isolates which were detected with the indicated susceptibility to antibiotics.

^d SMZ-TMP is co-trimoxazole.

88.0% resistance to ampicillin, 89.2% to nalidixic acid, 85.5% to tetracycline, and 79.5% to SMZ-TMP. The high resistance status of serotype 1a isolates was closely followed by that of *S. flexneri* 4c isolates, which showed levels of antimicrobial resistance of 81.7% to ampicillin, 83.3% to both nalidixic acid and gentamicin, 85.0% to tetracycline, and 76.7% to SMZ-TMP (Table 2). Among *S. flexneri* isolates, 78.3% were resistant to at least three antimicrobials. Among *S. sonnei* isolates, 74.3% were resistant to at least three antimicrobials.

Furthermore, we randomly selected 241 isolates to test the status of the two classes of integrons in *Shigella* according to previous methods (14, 15). A total of 74.7% of the isolates had integron I, and 82.6% had integron II. The overall length of integron I was 1,600 bp. The length of integron II of 194 isolates was 2,000 bp; only 1 isolate showed an integron II length of 1,000 bp. The prevalence of integrons I and II in *Shigella* isolates in Jiangsu Province was higher than that in previous studies (14). The resistance to nine antimicrobials in *Shigella* isolates with integron I or/and integron II was far more common than in those without them ($P < 0.01$). Among *Shigella* isolates, *S. flexneri* was the major agent that caused bacterial diarrhea in most Asian countries (1, 16). Our study also showed that *S. flexneri* remains the predominant serotype among *Shigella* strains in Jiangsu Province. However, in previous studies conducted in Henan Province, which is in the central region of China, the *S. flexneri* X variant was the predominant serotype (17). This showed that the major serotypes circulating in various regions of China are different.

The declining susceptibility of bacteria to antimicrobials has become one of the most serious world public health concerns (18–21). In this study, we found a high prevalence of *Shigella* isolates with resistance to many antimicrobials. In China, patients with diarrhea have commonly been given treatment with antibiotics by physicians without the result of bacterial culture; in many cases, patients with diarrhea took antibiotics before they saw a doctor, regardless of whether the diarrhea was caused by bacteria or virus. Therefore, antibiotics abuse is very serious.

Previous studies showed that integrons in *Shigella* isolates were related to resistance to gentamicin, kanamycin, ampicillin, sul-

floxazole, and tetracycline (14). Moreover, the residence of integrons in bacteria is often in the form of conjugative R plasmids which are related to bacterial resistance to ampicillin, cefotaxime, and cephalexin (22). The impact of integrons on resistance of *Shigella* to antibiotics may not be limited only to drug resistance gene transportation. In this study, we found that integrons were associated with resistance of *Shigella* to nine antimicrobials. This suggested that the impact of integrons on antimicrobial resistance may be more complex than we know (23, 24).

The conjunction between the high prevalence of integrons in *Shigella* and high resistance to antimicrobials in this region of China may lead to rapid dissemination of genes conferring resistance in the future. Therefore, further strengthening of surveillance of antimicrobial resistance in China is urgently needed.

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