

1 **Title:** High Prevalence of Antimicrobial Resistance and Molecular Characterization
2 of Integron among *Shigella* Isolates in Eastern China

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23 **Abstract**24 747 *Shigella* isolates were collected from hospitals in Jiangsu province of China.25 Susceptibility to antimicrobials and integron were tested, respectively. 78.3% of *S.*26 *flexneri* and 74.3% *S. sonnei* was resistant to at least three antibiotics. 74.7% *Shigella*

27 isolates have integron I and 82.6% of them have integron II. The conjunction between

28 the high prevalence of integron in *Shigella* and high resistance to antimicrobials will

29 lead to rapid dissemination of resistance gene in this region.

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45 **Text**

46 Bacterial diarrhea caused by *Shigella* remains a considerable public health problem in
47 the world, especially in developing countries where the disease may cause as many as
48 167 million episodes of diarrhea and over a million deaths annually (1). The
49 surveillance of shigellosis studies from multicenter showed that shigellosis was more
50 ubiquitous than previously thought (2). In China, it was estimated that over 800
51 million diarrhea episodes occurred annually(3) , and *Shigella* is one of the principles
52 etiologic organisms for diarrhea(4).

53 *Shigella* species are classified by four serogroups, which includes *S. dysenteriae*,
54 *S.flexneri*, *S.boydii*, and *S. sonnei*. Of them, *S.flexneri* is the predominant species in
55 most of developing countries (2, 5). Antibiotic therapy can limit the duration of
56 shigellosis and shedding of the organism. However, resistance has been increasing to
57 the most often administered antibiotics (5,6,7). The evidence from Hebei Province of
58 China, Kuwait, and the United States showed that China has a far higher prevalence
59 of resistance for all the bacteria studied (8).

60 Integrons play an important role in the dissemination and recombination of
61 antimicrobial drug resistance genes (9). They are common in *Salmonella enterica* and
62 make an important contribution to the extent of antimicrobial resistance in this species
63 (10, 11). Recent studies also showed that integrons contributed to antimicrobial drug
64 resistance genes spread among *Shigellosis* (12). However, there is little data available
65 to describe the prevalence of integron of *Shigella spp.* circulating in China.

66 In this study, we collected a total of 747 clinical *Shigella* isolates from the hospitals

67 located in Jiangsu province of China between January 2008 and November 2010.
68 Stool specimens from patients with either diarrhea or dysentery were collected before
69 they received antibiotics therapy, and then screened for *Shigella* spp. by conventional
70 biochemical methods in local hospitals. The age of patients ranged from 1 to 88 years
71 old. We defined diarrhea as the passage of ≥ 3 watery stools in a 24-hour period. All
72 isolates were identified using Rapid ID32E strips (Biomérieux, Corporate, Singapore)
73 on automatic biochemistry analyzer (Hitachi 917, Boehringer Mannheim, Japan)
74 again. O and H antigens were characterized by slide agglutination with hyperimmune
75 sera (Ningbo Tianrong Bio-pharmaceutical Company Limited) and the serotype was
76 assigned to the Kauffmann-White scheme. Of these *Shigella* isolates, 716 were
77 identified as *Shigella*. 491(68.6%) were *S. flexneri*, 214(29.9%) *S. sonnei*, 4(0.5%) *S.*
78 *dysenteriae*, and 7(1.0%) *S. boydii*.

79 To know the susceptibility of these *Shigella* isolates to antimicrobials, the disc
80 diffusion (Kirby Bauer) method was employed according to Clinical and Laboratory
81 Standards Institute standards(13) using a commercially prepared and dehydrated panel.
82 Ampicillin, co-amoxiclav, cephalothin, cefotaxime, gentamicin, nalidixic acid,
83 norfloxacin, tetracycline, and co-trimoxazole(SMZ-TMP) were involved.
84 Interpretation of inhibition zones was performed according to the CLSI criteria, and
85 *Escherichia coli* ATCC 25922 and ATCC 35218 was used as reference strain for
86 quality control. Among a total of 491 *S. flexneri*, they showed high levels of resistance
87 to ampicillin (72.8%), nalidixic acid (77.2%), tetracycline (74.1%), and
88 co-trimoxazole (66.8%). Among a total of 241 *S. sonnei*, high levels of resistance to

89 ampicillin (70.8%), nalidixic acid (69.3%), tetracycline (74.8%), and co-trimoxazole
90 (73.8%) were showed (table 1). Among *S. flexneri*, *S. flexneri* 2a(39.0%), *S. flexneri*
91 2b(19.3%), *S. flexneri* 1a(17.4%) and *S. flexneri* 4c(12.6%) were the major serotypes.
92 These four serotypes account for 87.3% of *S. flexneri*. Among them, the resistance of
93 *S. flexneri* 1a to antimicrobials was highest, and reached 88.0% to ampicillin, 89.2%
94 to nalidixic acid, 85.5% to tetracycline, and 79.5% to SMZ-TMP. This was closely
95 followed by *S. flexneri* 4c isolates. The antimicrobial resistance of them was 81.7% to
96 ampicillin, 83.3% to both nalidixic acid and gentamicin, 85.0 % to tetracycline, and
97 76.7% to SMZ-TMP (Table 2). Among *S. flexneri*, 78.3% were resistant to at least
98 three antimicrobials. Among *S. sonnei*, 74.3% was resistant to at least three
99 antimicrobials.

100 Furthermore, we randomly selected 241 isolates to test the status of the two classes of
101 integrons in *Shigella* according to the previous methods (14, 15). 74.7% of them had
102 integron I, and 82.6% had integron II. The length of all integron I was 1600bp. The
103 length of integron II of 194 were 2000bp, only one was 1000bp. The prevalence of
104 integron I and II in *Shigella* isolates in Jiangsu province was higher than that in
105 previous studies (16).The resistance to nine antimicrobials in *Shigella* isolates with
106 integron I or/and integron II were far more common than those without them($P<0.01$).

107 Among *Shigella*, *S. flexneri* was the major agents that caused bacterial diarrhea in
108 most Asian countries (1, 19). Our study also showed that *S. flexneri* remains the
109 predominant serotype among *Shigella* in Jiangsu province. However, in previous
110 studies conducted in Henan province which is in the central region of China, the *S.*

111 *flexneri* variant X was the predominated serotype (20). This showed that the major
112 serotype circulating in various regions of China are different.

113 The declining susceptibility of antimicrobials to bacteria has become one of the most
114 serious world public health concerns (21, 22, 23, 24). In this study, we found high
115 prevalence of *Shigella* with resistance to many antimicrobials. In China, it is common
116 that the patients with diarrhea were given antibiotics treatment by physician without
117 the result of bacteria culture, even many patients took antibiotics before they saw a
118 doctor after they had diarrhea whatever diarrhea was caused by bacteria or virus.
119 Therefore, the antibiotics abuse is very serious.

120 Previous studies showed that integrons in *Shigella* were related to the resistance to
121 gentamicin and kanamycin, ampicillin, sulfisoxazole, tetracycline (16). Moreover, the
122 residence of integron in bacteria is often conjugative R plasmids which were related
123 to bacteria against ampicillin, cefotaxim and cephalixin(25). The impact of integron
124 on resistance of *Shigella* to antibiotics may be not only limited to the drug resistance
125 gene transportation. In this study, we found that integron were associated with nine
126 antimicrobial resistance of *Shigella*. This suggested that the impact of integron on
127 antimicrobial resistance may be more complex than as we know (17,18).

128 The conjunction between the high prevalence of integron in *Shigella* and high
129 resistance to antimicrobials in this region may lead to rapid dissemination of
130 resistance gene in future. Therefore, it is urgently needed to further strength
131 surveillance of antimicrobial resistance in China.

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Table 1 The sensitivity of two major *Shigella* isolates to 9 antibiotics

Antibiotics	<i>S. flexneri</i> (%)			<i>S. Sonei</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

S, susceptible; I, intermediate; R, resistance

Table 2 The proportion of *S.flexneri* isolates with resistance to antimicrobial among various *S.flexneri* serotypes

Serotype	Ampicillin	Co-amoxiclav	Cephalothin	Cefotaxime	Gentamicin	Nalidixic			SMZ-		N ^a (% ^b)
						acid	Norfloxacin	Tetracycline	TMP ^d		
<i>S.flexneri</i> 1a	88.0	41.0	28.9	28.9	31.3	89.2	51.8	85.5	79.5	83(17.4)	
<i>S.flexneri</i> 1b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.0	60.0	5(1.0)	
<i>S.flexneri</i> 2a	69.9	35.5	25.8	20.4	17.7	75.3	45.7	71.0	63.4	186(39.0)	
<i>S.flexneri</i> 2b	64.1	31.9	29.3	21.7	68.5	68.5	20.7	58.7	52.2	92(19.3)	
<i>S.flexneri</i> 3b	100.0	100.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	1(0.2)	
<i>S.flexneri</i> 3c	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1(0.2)	
<i>S.flexneri</i> 4a	100.0	85.7	57.1	42.9	85.7	85.7	42.9	100.0	71.4	7(1.5)	
<i>S.flexneri</i> 4b	100.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	3(0.6)	
<i>S.flexneri</i> 4c	81.7	31.7	35.0	23.3	83.3	83.3	13.3	85.0	76.7	60(12.6)	
<i>S.flexneri</i> 6	0.0	0.0	0.0	0.0	100.0	100.0	0.0	100.0	100.0	5(1.0)	
<i>S.flexneri</i> X	89.3	17.9	57.1	46.4	92.9	92.9	21.4	92.9	82.1	28(5.9)	
<i>S.flexneri</i> 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 is the number of various serotype *S.flexneri*

b: the number of various serotype *S.flexneri* /the total *S.flexneri*×100%

c: the total number of *S.flexneri* which were detected for the susceptibility to antibiotics

d: SMZ-TMP is Co-trimoxazole