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A 10-year surveillance of antimicrobial susceptibility patterns in *Shigella sonnei* isolates circulating in Jiangsu Province, China

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ABSTRACT

Objectives: The rapid emergence of drug-resistant *Shigella sonnei* is a serious public health problem. This study aimed to characterise the antimicrobial resistance patterns, molecular subtypes, and integron types and resistance gene cassettes in *S. sonnei* from Jiangsu Province, China.
Methods: In total, 340 *S. sonnei* were collected in 2002–2011 throughout Jiangsu Province. Antimicrobial susceptibility testing, pulsed-field gel electrophoresis (PFGE), PCR amplification of integrons, restriction fragment length polymorphism (RFLP) and DNA sequencing of cassette regions were performed.
Results: Resistance rates to ampicillin (67.7%), nalidixic acid (75.2%), tetracycline (73.7%) and trimethoprim/sulfamethoxazole (68.7%) remained high. Strains from Centre and South Jiangsu showed higher resistance and multiresistance rates compared with the North. PFGE analysis indicated that large-scale clonal transmission among different cities occurred several times during 10 years. Among all strains, 55.9% (190/340) harboured class 1 integrons, 80.3% (273/340) harboured class 2 integrons and 49.4% (168/340) harboured an atypical class 1 integron. Resistance rates to nine antimicrobials in the class 1 integron-positive group were significantly higher than in the negative group ($P < 0.05$). Seven different gene cassettes were detected in class 1 integrons. The most prevalent type was *aacA4–cmlA1* (114/286). Class 2 integrons carried the gene cassette array *dfrA1–sat1–aadA1*, and the atypical class 1 integron carried bla_{OXA-30}*–aadA1*.
Conclusions: The increasing antimicrobial resistance and significant clonal transmission of *S. sonnei* circulating in Jiangsu were closely related to the high prevalence of integrons and gene cassettes. Long-term cross-regional monitoring of antimicrobial resistance is urgently required for *S. sonnei*.

1. Introduction

Shigella is a major gastrointestinal pathogen accounting for 5–10% of diarrhoeal diseases or bacillary dysentery throughout the world [1]. In China, up to 1.7 million episodes of shigellosis occurred each year, with up to 0.20 million patients hospitalised [2]. Among the four species of *Shigella* (*Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*), *S. sonnei* has been the predominant species in developed countries and is often the second most prevalent species in developing countries for many years [3–5]. Recently, *S. sonnei* has become increasingly important due to the growing rate of this species in many developing countries [6,7].

Antimicrobial treatment is usually recommended for shigellosis as it can reduce the duration of symptoms and the severity of illness by shortening the period of pathogen excretion [8]. However, owing to long-term overuse of antimicrobials, drug-resistant and multidrug-resistant (MDR) *Shigella* isolates prevail worldwide [9,10]. Evidence from China [11], Bangladesh [5], Belgium [12], Gabon [13] and the USA [14] indicated that China has a much higher prevalence of resistance to most commonly used antimicrobials.

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The close correlation between antimicrobial resistance and the presence of integrons containing gene cassettes has been frequently reported in studies from various countries and regions [10,15,16]. However, limited information is available on the following three aspects: the regional level of the antimicrobial resistance profile; the genotype level of the dominating endemic strains; and long-term surveillance and comparison of integrons and gene cassettes.

Therefore, this study was designed to investigate the antimicrobial resistance patterns, epidemic clones and integrons characteristics of strains collected during a 10-year period (2002–2011) in Jiangsu Province, China.

2. Materials and methods

2.1. Sample collection

A total of 340 *S. sonnei* were collected between 2002 and 2011 from outpatients and inpatients in hospitals throughout Jiangsu Province. Jiangsu Province, a populous and prosperous province in China, was divided into three regions comprised of 13 provincial cities, the South (Nanjing, Suzhou, Wuxi, Changzhou and Zhenjiang), the Centre (Yangzhou, Taizhou and Nantong) and the North (Xuzhou, Suqian, Huai'an, Yancheng and Lianyungang). As shown in Table 1, South Jiangsu exhibited the highest isolation rate of *S. sonnei* (186/340; 54.7%), followed by the Centre (84/340; 24.7%) and the North (70/340; 20.6%).

All strains were identified by API system (bioMérieux, Marcy-l'Étoile, France) and were confirmed as *S. sonnei* by slide agglutination with *Shigella* antisera (Lanzhou Institute of Biological Products, Lanzhou, China).

2.2. Antimicrobial susceptibility testing

Susceptibility to nine commonly used antimicrobials was determined by the disk diffusion method. The antimicrobial agents were as follows: ampicillin (10 μg); amoxicillin/clavulanic acid (AMC) (30 μg); cefalotin (30 μg); cefotaxime (30 μg); gentamicin (120 μg); nalidixic acid (30 μg); norfloxacin (10 μg); tetracycline (30 μg); and trimethoprim/sulfamethoxazole (SXT) (25 μg). Results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [17]. *Escherichia coli* ATCC 25922 was used as a quality control strain.

In view of the small sample size ($n = 21$ strains) and different antimicrobials used in antimicrobial susceptibility testing in 2002–2005, this study only compared the resistance rates of *S. sonnei* collected in 2006–2011.

Table 1
Source of *Shigella sonnei* strains ($n = 340$), by year and by city, 2002–2011.

Characteristic	No. of strains	%
Year		
2002	6	1.8
2003	2	0.6
2004	6	1.8
2005	7	2.1
2006	19	5.6
2007	48	14.1
2008	89	26.2
2009	98	28.8
2010	33	9.7
2011	32	9.4
City		
Changzhou	75	22.1
Huai'an	15	4.4
Lianyungang	21	6.2
Nanjing	36	10.6
Nantong	63	18.5
Suqian	0	0.0
Suzhou	22	6.5
Taizhou	4	1.2
Wuxi	28	8.2
Xuzhou	27	7.9
Yancheng	7	2.1
Yangzhou	17	5.0
Zhenjiang	25	7.4

2.3. Pulsed-field gel electrophoresis (PFGE)

To determine DNA fingerprinting profiles, the 340 *S. sonnei* strains were analysed by PFGE according to the US Centers for Disease Control and Prevention (CDC) PulseNet protocol [18] and were digested with the restriction enzyme *Xba*I (Takara, Dalian, China). Electrophoresis was performed with a CHEF-DR II system (Bio-Rad, Hercules, CA) under the following conditions: switching time from 2.2 s to 54.2 s at 6 V for 17 h with an angle of 120°. *Salmonella enterica* serovar Braenderup H9812 was used as a molecular weight standard. PFGE banding patterns were compared using BioNumerics software v.4.0 (Applied Maths, Sint-Martens-Latem, Belgium) based on the Dice coefficient. Dendrograms were constructed by the unweighted-pair group method with arithmetic mean (UPGMA), with a position tolerance of 1% and optimisation of 0.5%. Strains sharing ≥90% similarity in DNA fingerprinting profiles were defined as a cluster [19].

2.4. Identification of integrons and gene cassettes

Class 1, 2 and 3 integrons were detected by PCR using degenerate primers hep35 and hep36 targeted to conserved regions of integrase genes *intI1*, *intI2* and *intI3* [20]. PCR products were analysed by restriction fragment length polymorphism (RFLP) to classify integrons [21]. Atypical class 1 integrons were also screened in *S. sonnei* using primers int1f and is16 [20].

The variable region of class 1 and 2 integrons was amplified using the primers 5′CS1/3′CS1 and 5′CS2/3′CS2, respectively, as described previously [22]. PCR products of variable regions were digested by the restriction enzyme *Hinf*I (Takara). Same-sized amplicons with the same RFLP pattern were considered as one type. At least one representative of each type of amplicon was sequenced to identify the gene cassettes. Sequence analysis and alignment was performed using BLAST programs available at the National Center for Biotechnology Information (NCBI) website.

2.5. Statistical analysis

Statistical analysis was performed with Stata v.7.0 software (StataCorp LP, College Station, TX). Pearson's χ^2 test was used to analyse the antimicrobial resistance of isolates from different regions. Fisher's exact test was applied to compare antimicrobial resistance between integron-positive and integron-negative groups. A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Resistance trends from 2006 to 2011

A wide of spectrum of antimicrobial resistance was observed in 319 *S. sonnei* strains isolated from 2006 to 2011 (Table 2). The resistance rates to ampicillin (67.7%), nalidixic acid (75.2%), tetracycline (73.7%) and SXT (68.7%) remained at a high level during 6 years, whereas resistance to cefotaxime and norfloxacin was relatively lower (17.9% and 21.0%, respectively). Of the nine antimicrobials tested, a sharp resistance change was observed for gentamicin, with a three-fold increase from 26.3% in 2006 to 78.1%

Table 2
Antimicrobial resistance of *Shigella sonnei*, 2006–2011.

Antimicrobial agent	Resistant [% (*n*)]						
	2006 (*N* = 19)	2007 (*N* = 48)	2008 (*N* = 89)	2009 (*N* = 98)	2010 (*N* = 33)	2011 (*N* = 32)	Overall (*N* = 319)
Ampicillin	52.6 (10)	81.3 (39)	48.3 (43)	77.6 (76)	60.6 (20)	87.5 (28)	67.7 (216)
AMC	52.6 (10)	97.9 (47)	39.3 (35)	30.6 (30)	0.0 (0)	18.8 (6)	40.1 (128)
Cefalotin	52.6 (10)	33.3 (16)	15.7 (14)	45.9 (45)	27.3 (9)	25.0 (8)	32.0 (102)
Cefotaxime	5.3 (1)	14.6 (7)	12.4 (11)	30.6 (30)	21.2 (7)	3.1 (1)	17.9 (57)
Gentamicin	26.3 (5)	8.3 (4)	39.3 (35)	34.7 (34)	33.3 (11)	78.1 (25)	35.7 (114)
Nalidixic acid	78.9 (15)	95.8 (46)	61.8 (55)	76.5 (75)	63.6 (21)	87.5 (28)	75.2 (240)
Norfloxacin	26.3 (5)	25.0 (12)	4.5 (4)	46.9 (46)	0.0 (0)	0.0 (0)	21.0 (67)
Tetracycline	73.7 (14)	93.8 (45)	58.4 (52)	73.5 (72)	72.7 (24)	87.5 (28)	73.7 (235)
SXT	68.4 (13)	72.9 (35)	64.0 (57)	65.3 (64)	81.8 (27)	71.9 (23)	68.7 (219)

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole.

in 2011. A decrease in resistance to AMC (from 52.6% to 18.8%) was observed.

3.2. Regional distribution of resistance rates

Of the 319 *S. sonnei* strains isolated from 2006 to 2011, 176 were collected from South Jiangsu, 78 from the Centre and 65 from the North (Table 3). The proportions of resistant strains from these three regions showed a significant difference for eight of the nine antimicrobials tested, the only exception being tetracycline. Resistance to ampicillin, cefalotin, cefotaxime and norfloxacin was higher in Centre Jiangsu, whilst resistance to gentamicin, nalidixic acid and SXT was higher in the South. The North isolates showed low rates of antimicrobial resistance.

3.3. Time and regional distribution of multidrug-resistant (MDR) Shigella sonnei

Multidrug resistance (resistance to three or more classes of antimicrobial agents) was detected in 74.3% (237/319) of the strains, and the MDR rates remained at a high level from 2006 to 2011 (78.9%, 93.8%, 61.8%, 75.5%, 60.6% and 87.5%, respectively).

This study analysed the MDR rates of different regions in Jiangsu Province (Fig. 1). MDR strains were most frequent among South Jiangsu (77.3%), followed by the Centre (76.9%) and the North (63.1%). MDR rates in 13 cities are displayed in Fig. 1: Taizhou (100.0%), Xuzhou (91.7%) and Suzhou (90.5%) showing the highest MDR rates.

3.4. Pulsed-field gel electrophoresis profile

Among 32 *S. sonnei* isolated in 2011, three PFGE clusters were identified. A total of 31 isolates were grouped into cluster SA1 (24 isolates), SA2 (2 isolates) and SA4 (5 isolates). These clusters spread in five, one and two cities, respectively (Supplementary Fig. S1A).

Among 33 *S. sonnei* isolated in 2010, five PFGE clusters were observed. All 33 isolates were grouped into cluster SB1 (15 isolates), SB2 (3 isolates), SB3 (2 isolates), SB4 (6 isolates) and SB5 (7 isolates). Two (40.0%) of the five clusters spread in two cities, one (20.0%) in four cities, one (20.0%) in five cities and, notably, there was one PFGE cluster spread in eight different cities (Supplementary Fig. S1B).

During 10 years, similar clonal transmission among different cities was revealed several times in Jiangsu Province. The dendrograms of PFGE patterns of all strains are displayed in Supplementary material.

Table 3
Antimicrobial resistance of *Shigella sonnei* from South, Centre and North Jiangsu, 2006–2011.

Antimicrobial agent	Resistant [% (*n*)]			*P*-value
	South (*N* = 176)	Centre (*N* = 78)	North (*N* = 65)	
Ampicillin	69.9 (123)	76.9 (60)	50.8 (33)	0.003
AMC	38.1 (67)	33.3 (26)	53.8 (35)	0.032
Cefalotin	24.4 (43)	51.3 (40)	29.2 (19)	<0.001
Cefotaxime	13.6 (24)	30.8 (24)	13.8 (9)	0.003
Gentamicin	40.9 (72)	38.5 (30)	18.5 (12)	0.005
Nalidixic acid	79.0 (139)	78.2 (61)	61.5 (40)	0.016
Norfloxacin	9.1 (16)	48.7 (38)	20.0 (13)	<0.001
Tetracycline	76.7 (135)	75.6 (59)	63.1 (41)	0.093
STX	76.1 (134)	65.4 (51)	52.3 (34)	0.001

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole.

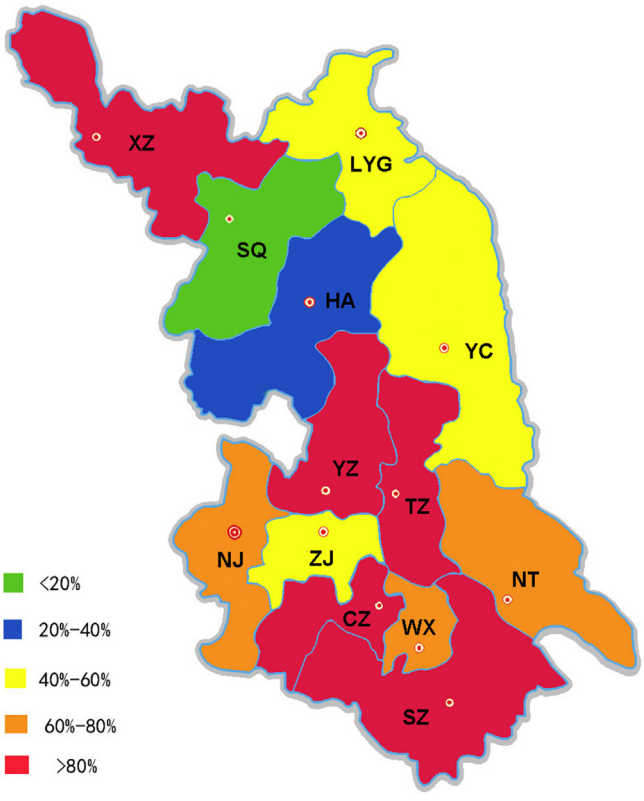

Fig. 1. Multidrug resistance rates in different cities of Jiangsu Province. CZ, Changzhou; HA, Huai'an; LYG, Lianyungang; NJ, Nanjing; NT, Nantong; SQ, Suqian; SZ, Suzhou; TZ, Taizhou; WX, Wuxi; XZ, Xuzhou; YC, Yancheng; YZ, Yangzhou; ZJ, Zhenjiang.

Table 4
Rates of integron-positive strains from 2002–2011.

Year	Class 1 integron	Class 2 integron	Atypical class 1 integron
2002	0.0 (0/6)	100.0 (6/6)	100.0 (6/6)
2003	50.0 (1/2)	50.0 (1/2)	100.0 (2/2)
2004	50.0 (3/6)	100.0 (6/6)	100.0 (6/6)
2005	100.0 (7/7)	100.0 (7/7)	100.0 (7/7)
2006	57.9 (11/19)	94.7 (18/19)	84.2 (16/19)
2007	77.1 (37/48)	93.8 (45/48)	87.5 (42/48)
2008	33.7 (30/89)	75.3 (67/89)	23.6 (21/89)
2009	66.3 (65/98)	74.5 (73/98)	56.1 (55/98)
2010	48.5 (16/33)	72.7 (24/33)	18.2 (6/33)
2011	62.5 (20/32)	81.3 (26/32)	21.9 (7/32)
Total	55.9 (190/340)	80.3 (273/340)	49.4 (168/340)

3.5. Integrons detected in Shigella sonnei

The detection rate of integrons in *S. sonnei* was generally high (Table 4). Among all strains, 55.9% (190/340) harboured class 1 integrons, 80.3% (273/340) harboured class 2 integrons and 49.4% (168/340) harboured an atypical class 1 integron. None of the strains harboured a class 3 integron.

To determine the role of class 1 integron in drug resistance, strains were divided into two groups (class 1 integron-positive and class 1 integron-negative). Resistance rates to the nine antimicrobials in the class 1 integron-positive group were significantly higher than the class 1 integron-negative group ($P < 0.05$) (Table 5).

3.6. Resistance gene cassettes of integrons

The variable regions of class 1, class 2 and atypical class 1 integrons were amplified and analysed in all integrase-positive strains. Five amplicons (0.15, 0.75, 1.5, 1.6 and 2.2 kb) were identified in variable regions of class 1 integron. Sequencing of the variable region revealed that the 0.75-kb amplicon carried one gene cassette (*dfrA5*); the 1.5-, 1.6- and 2.2-kb amplicons carried two gene cassettes (*dfrA1–aadA1*, *dfrA17–aadA5* and *aacA4–cmlA1*, respectively); the 0.15-kb amplicon carried 5′CS and 3′CS (Table 6). In total, seven different gene cassettes were detected in class 1 integrons. The most prevalent type was *aacA4–cmlA1* (114/286).

Only one amplicon of 2.0 kb in size was identified in class 2 integrons, which carried *dfrA1–sat1–aadA1*. The gene cassette array bla_{OXA-30}*–aadA1* was detected in a 2.4-kb amplicon of an atypical class 1 integron.

4. Discussion

In this study, only 40 (11.8%) of 340 *S. sonnei* isolates were obtained from 2002 to 2006, whereas 300 isolates (88.2%) were obtained in the following 5 years in Jiangsu Province, which was a rapidly developing area in China. The noticeable increase of *S. sonnei* has also been observed in other fast-developing countries [23], and in some areas such as Beijing and Vietnam [6,24]. *S. sonnei* has even replaced *S. flexneri* to become the predominant subgroup. The pattern shift might due to improvements in the economic situation, environmental conditions and hygiene habits in these regions. Another finding was that only two *S. sonnei* isolates were collected in 2003 during the severe acute respiratory syndrome (SARS) epidemic. Since the main transmission mode of *Shigella* is person-to-person contact or consumption of contaminated food [25], fewer people getting out and eating out in order to avoid SARS infections may have resulted in the obvious decrease of *S. sonnei* isolates.

Antimicrobial resistance of *Shigella* has become one of the most serious global public health concerns [14]. Over the past few decades, *Shigella* have become resistant to most of the widely used antimicrobials [26,27]. In this study, the resistance patterns of *S. sonnei* were analysed from three different angles: changing resistance categorised by year; changing resistance categorised by geographical region; and MDR analysis.

Table 5
Correlation between antibiogram profile and class 1 integrons in *Shigella sonnei*, 2006–2011.

Antimicrobial agent	Class 1 integron-positive ($N = 179$)			Class 1 integron-negative group ($N = 140$)			*P*-value
	%R (*n*)	%I (*n*)	%S (*n*)	%R (*n*)	%I (*n*)	%S (*n*)	
Ampicillin	86.6 (155)	0.6 (1)	12.8 (23)	43.6 (61)	2.9 (4)	53.6 (75)	<0.001
AMC	50.3 (90)	15.1 (27)	34.6 (62)	27.1 (38)	6.4 (9)	66.4 (93)	<0.001
Cefalotin	39.7 (71)	22.9 (41)	37.4 (67)	22.1 (31)	25.7 (36)	52.1 (73)	0.003
Cefotaxime	23.5 (42)	14.0 (25)	62.6 (112)	10.7 (15)	9.3 (13)	80.0 (112)	0.002
Gentamicin	40.8 (73)	6.7 (12)	52.5 (94)	29.3 (41)	2.9 (4)	67.9 (95)	0.015
Nalidixic acid	88.8 (159)	2.2 (4)	8.9 (16)	57.9 (81)	9.3 (13)	32.9 (46)	<0.001
Norfloxacin	31.3 (56)	5.0 (9)	63.7 (114)	7.9 (11)	5.0 (7)	87.1 (122)	<0.001
Tetracycline	87.7 (157)	0.6 (1)	11.7 (21)	55.7 (78)	2.9 (4)	41.4 (58)	<0.001
SXT	78.8 (141)	2.8 (5)	18.4 (33)	55.7 (78)	10.0 (14)	34.3 (48)	<0.001

%R/I/S, percent resistant/intermediate/susceptible; AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole.

Table 6
Gene cassette arrays in class 1 integrons from *Shigella sonnei*, 2002–2011.

Approximate length (kb)	No. of isolates	Gene cassette array
0.15	22	5′CS and 3′CS
1.5	5	*dfrA1–aadA1*
1.6	4	*dfrA17–aadA5*
2.2	42	*aacA4–cmlA1*
0.15 + 1.5	1	5′CS and 3′CS, *dfrA1–aadA1*
0.15 + 2.2	52	5′CS and 3′CS, *aacA4–cmlA1*
0.75 + 2.2	1	*dfrA5,aacA4–cmlA1*
1.5 + 2.2	13	*dfrA1–aadA1*, *aacA4–cmlA1*
1.6 + 2.2	1	*dfrA17–aadA5*, *aacA4–cmlA1*
0.15 + 0.75 + 2.2	1	5′CS and 3′CS, *dfrA5*, *aacA4–cmlA1*
0.15 + 1.5 + 2.2	4	5′CS and 3′CS, *dfrA1–aadA1*, *aacA4–cmlA1*

In this study during the period 2006–2011, more than one-half of *S. sonnei* isolates were resistant to ampicillin (67.7%), nalidixic acid (75.2%), tetracycline (73.7%) and SXT (68.7%). Resistance to these antimicrobials is commonly reported and the frequency of resistance differs by country. In Belgium, an 18-year research showed that resistance rates to these four antimicrobials were 19.2%, 3.8%, 59.3% and 85.9%, respectively [12]. In the USA during 2000–2010 [28], resistance rates to the common drugs nalidixic acid (1%), tetracycline (18%) and SXT (33%) were dramatically lower than those observed in the current study, whilst ampicillin had a similar resistance rate of 74%. In addition, in Eastern China high levels of resistance to ampicillin (70.8%), nalidixic acid (69.3%), tetracycline (74.8%) and SXT (73.8%) were reported [9]. The widespread nature of nalidixic acid and fluoroquinolone resistance has been documented in many countries [29,30]. In the current study, resistance to nalidixic acid was found to be very high, with a peak at 95.8% in 2007, whilst the third-generation fluoroquinolone norfloxacin showed a lower resistance rate of 21.0% on average. Cefotaxime also had a lower frequency of resistance compared with cefalotin. Thus, the third-generation fluoroquinolones and cephalosporins are recommended as first-line drugs to treat infections caused by *S. sonnei*.

This study further analysed the resistance of *S. sonnei* in different regions of Jiangsu Province. Resistance rates of isolates from the South, Centre and North differed significantly for eight of nine antimicrobials tested. North Jiangsu exhibited low resistance rates compared with the Centre and the South. The great geographical variation found in resistance patterns may be ascribed to the various prescribing practises of doctors in different regions. This calls for strong monitoring of the local resistance patterns in order to provide effective empirical treatment regimens.

Together with the increased single-drug resistance, the frequency of MDR isolates during 6 years remained at a high level, slowly rising from 78.9% in 2006 to 87.5% in 2011. The same trend had already been revealed a few years ago in a long-term surveillance in Belgium, where the MDR rate of *S. sonnei* increased from 55.9% in 1990 to 80.2% in 2007, reaching a peak of 89.0% during 2004–2005 [12]. Geographical distribution of multidrug resistance indicated that South Jiangsu represented the predominant MDR region (77.3%), followed by the Centre (76.9%) and the North (63.1%).

The declining susceptibility to commonly used antimicrobials and the emergence of MDR *S. sonnei* could be highly related to inappropriate prescription and easy access to antimicrobials among outpatients. It is therefore important to strictly control antimicrobial application and to monitor local resistance in order to facilitate the rational use of antimicrobial agents.

This high frequency of MDR *S. sonnei* strains has led to tremendous interest in molecular typing and determination of resistance mechanisms. Bacterial molecular typing is frequently applied for outbreak surveillance and phylogenetic investigation. Strain-specific fingerprints generated are used to facilitate the identification of disease transmission routes and sources [7–11]. To date, various genotyping methods including biotyping, multilocus variable-number tandem-repeat analysis (MLVA) and PFGE have been used for the epidemiological investigation of *S. sonnei* [31,32]. Biotyping is a relatively earlier phenotypic method with insufficient discrimination.

Although MLVA has a higher discriminatory power for distinguishing epidemiological relationships, it is not a universal method and is not 100% reproducible, and it has no well-established protocol for surveillance networks [5]. On the other hand, PFGE, as the acceptable gold-standard technique for molecular typing, has the standardised PulseNet PFGE protocol for interlaboratory comparison of results [18] and has been successfully used for subtyping sporadic or epidemic isolates of *Shigella* [11,24]. PFGE is an electrophoresis-based method that can directly or indirectly reflect pathogen variation, in other words, the changing in DNA sequence.

In this study, PFGE was used to characterise the homology of all 340 *S. sonnei* isolates. The results revealed that many PFGE clusters contained strains from multiple geographical regions; some clusters even contained strains from six to seven cities throughout the province. Large-scale transmission of different clones was found in different years. In 2009, cluster SC6 containing 33 isolates spread between Changzhou and Nantong; in 2008, cluster SD5 containing 35 isolates was prevalent in Wuxi, Zhenjiang, Suzhou, Lianyungang, Nanjing and Xuzhou; and in 2007, cluster SE1 containing 35 isolates was present in Xuzhou, Lianyungang, Changzhou, Taizhou, Suzhou, Yancheng and Huai'an, a total of 7 cities. Similarly, the clonal spread of *S. sonnei* has also been described by other studies in Malaysia during 1997–2009 [32] and on a global scale [33]. These observations emphasise the necessity of monitoring the molecular subtypes of *S. sonnei* from endemic regions and controlling outbreaks of *S. sonnei*.

The most commonly applied method of integron detection is to use the specific primer of various integrons for single PCR and to examine them separately, which is time consuming and complicated. This study used degenerate primers combined with restriction enzyme digestion to detect class 1, 2 and 3 integrons concurrently. A total of 55.9% of *S. sonnei* isolates carried class 1 integrons in Jiangsu Province, perceptibly higher than that in Bangladesh (2.5%) [5], South Korea (14.9%) [16] and Zhejiang Province of China (12.9%) [21]. This difference may due to geographical variation or the increased prevalence of class 1 integrons in recent years. Class 2 integrons were detected in 80.3% of *S. sonnei* isolates, which was in concordance with previous reports (100.0% in Gabon [13], 100.0% in South Korea [16], 81.0% in Uzbekistan [34] and 80.6% in Zhejiang Province [21]). Class 3 integrons were not detected in any isolates. Furthermore, an atypical class 1 integron, ever present in Africa, Europe and America [35], was also detected with a positive rate of 49.4%. Overall, the current study searched for four classes of integron (classes 1, 2 and 3 and atypical class 1 integrons) in all isolates obtained. The results showed that class 2 integrons remained the preponderant integron among *S. sonnei* strains but it was not as dominant as reported previously. It is well known that classes 1 and 2 and atypical class 1 integrons play an important role in increased antimicrobial resistance. This was supported by the fact that the resistance rates of the class 1 integron-positive group were all statistically higher than the class 1 integron-negative group for all nine antimicrobials, thus integrons were associated with resistance of *S. sonnei* to multiple antimicrobials at the phenotype level.

Further analysis of the variable region sequencing showed the close correlation between drug resistance and the presence of integrons at the genotype level. Four of five amplicons carried one or two gene cassettes; only one amplicon (0.15 kb) carried the skeletal structure of 5′CS and 3′CS. The class 1 integron containing 0.15-kb and 2.2-kb amplicons was the most common type in this study, and *aacA4–cmlA1* was the most prevalent gene cassette, conferring resistance to aminoglycoside and chloramphenicol, respectively. The gene cassette array *dfrA1–sat1–aadA* in class 2 integrons conferred resistance to trimethoprim, streptothricin and streptomycin [16,36]. The gene cassette $bla_{\text{OXA-30}}$–*aadA1*, conferring resistance to β-lactams and aminoglycosides, was detected in an atypical class 1 integron. The decrease of AMC resistance is probably due to the decrease of the number of strains harbouring an atypical class 1 integron, which contains $bla_{\text{OXA-30}}$ encoding an enzyme conferring resistance to AMC. So far, a similar

atypical class 1 integron has been reported in China, India and France [21,37–39].

In conclusion, this study systematically analysed the subtype shift, antimicrobial resistance patterns, genetic homology and molecular characteristics of integrons in 340 *S. sonnei* collected from 2002 to 2011 in Jiangsu Province, China. The multicentre, large-sample research, globally unusual, is of great significance for the clinical therapy, prevention and control of *S. sonnei* infection in Jiangsu Province, China.

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Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at **http://dx.doi.org/10.1016/j.jgar.2017.03.009**.

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