Laboratory monitoring of bacterial gastroenteric pathogens *Salmonella* and *Shigella* in Shanghai, China 2006–2012

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SUMMARY

In 2006 we initiated an enhanced laboratory-based surveillance of *Salmonella* and *Shigella* infections in Shanghai, China. A total of 4483 *Salmonella* and 2226 *Shigella* isolates were recovered from stool specimens by 2012. In 80 identified *Salmonella* serovars, Enteritidis (34·5%) and Typhimurium (26·2%) were the most common. *Shigella* (*S.*) *sonnei* accounted for 63·9% of human *Shigella* infections over the same time period, and replaced *S. flexneri* to become the primary cause of shigellosis since 2010. Overall, a high level of antimicrobial resistance was observed in *Salmonella* and *Shigella*, particularly to nalidixic acid, ampicillin, and tetracycline. Ciprofloxacin resistance was common in *Salmonella* Typhimurium (21·0%) and *S. flexneri* (37·6%). The cephalosporin resistance in both pathogens also increased over the years, ranging from 3·4% to 7·0% in *Salmonella*, and from 10·4% to 28·6% in *Shigella*. Resistance to multiple antimicrobials was also identified in a large number of the isolates. This study provides insight into the distribution of *Salmonella* and *Shigella* in diarrhoeal diseases.

Key words: Antimicrobial susceptibility, gastroenteritis, *Salmonella*, *Shigella*.

INTRODUCTION

Infections of the gastrointestinal tract with an increasingly recognized array of bacterial, parasitic, and viral pathogens have represented a major public health burden throughout the world [1]. The symptoms of gastrointestinal infections range from a mild self-limiting attack to severe complications, such as kidney failure and meningitis [2]. Gastrointestinal infection remains a common illness in developed countries, but a major cause of morbidity and mortality in developing nations, particularly for young children, the elderly, and those with immune suppression [3–5]. Epidemiological studies of gastrointestinal infections have determined several transmission modes associated with the disease, including consumption of contaminated food and water, and direct contact through the person-to-person or person-to-animals route [1, 6].

Salmonella and *Shigella* are two of the most common bacterial gastroenteric pathogens. It is estimated that *Shigella* annually causes 80·0–165·0 million cases and over 600 000 deaths worldwide, with about 100 times higher incidence reported in developing

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countries than in developed countries [7]. Children aged <5 years comprise the majority of infections and fatal outcomes [4, 7]. *Salmonella* is slightly less prevalent, causing 61·8–131·6 million cases with 155 000 deaths each year [8]. Despite recent public health efforts to reduce salmonellosis, the number of infections with certain *Salmonella* serotypes, such as Enteritidis, has increased in many countries [9]. Antimicrobial therapy is performed for severe salmonellosis and shigellosis to reduce the duration of symptoms and limit transmission by shortening excretion period. However, both *Salmonella* and *Shigella* are capable of obtaining resistance against clinically important antibiotics, such as fluoroquinolones, by point mutation or horizontal gene transfer [10, 11]. In addition, antimicrobial resistance of *Salmonella* and *Shigella* is highly associated with the type of serovar or species [10, 12], and presents various patterns in different nations [10], or even in different areas within the same country [13, 14]. These factors present challenges to antimicrobial treatment, and should be taken in to consideration before applying antimicrobials empirically.

Gastrointestinal infections can be controlled successfully when there are adequate inputs of public health measures, and surveillance of laboratory-confirmed infections has proven to be an essential tool for monitoring disease incidence, detecting outbreaks, characterizing enteric infections, directing and evaluating the implementation of effective control strategies [1]. National surveillance programmes have been established for the epidemiological monitoring of major foodborne gastroenteric pathogens in North America, Europe, and Australia [1]. China initiated its National Infectious Disease Internet Reporting System in 2005, which collects information on bacterial infections commonly transmitted via food, and the reporting symptoms include cholera, dysentery, and other infectious diarrhoea [12]. With the assistance of the World Health Organization Global Foodborne Infections Network (WHO GFN), Shanghai Center for Disease Control and Prevention (CDC) started enhanced laboratory-based monitoring for bacterial gastroenteric pathogens in 2006. *Salmonella* and *Shigella* were the first two pathogens in the programme, and others such as *Campylobacter*, Shiga toxin-producing *Escherichia coli*, and *Vibrio* were added thereafter. This report describes the prevalence and characteristics of *Salmonella* and *Shigella* isolated from human clinical samples in Shanghai from 2006 to 2012.

MATERIAL AND METHODS

Specimen collection

This study was conducted between 2006 and 2012 in 26 sentinel hospitals and eight regional Shanghai CDC diagnostic laboratories in five districts of Shanghai, China. Of the 26 participating hospitals, 13 were added to the programme since 2010. Physicians were asked to collect stool samples from all patients (aged 2–90 years) who sought hospital care with diarrhoea and other gastroenteritis symptoms such as fever, vomiting or abdominal pain. The samples were then inoculated into Carry–Blair medium and transferred to the diagnostic laboratories within 4 h.

Bacterial isolation and identification

The specimens were tested for *Salmonella* and *Shigella* using the following protocol. For *Salmonella* isolation, stool specimens were enriched in tetrathionate Brilliant-Green broth (BD, USA) at 37 °C for 8 h, and then inoculated onto *Salmonella-Shigella* agar (Oxoid, UK), and CHROMagar *Salmonella* agar (CHROMagar, France), respectively, for incubation at 37 °C up to 24 h. Single colonies were transferred for further testing onto triple sugar iron (TSI) slant, motility indole-urea agar, L-lysine decarboxylase, and L-galactosidase, and confirmed as *Salmonella* using API 20E test strips (bioMérieux, France). For *Shigella* isolation, the samples were streaked onto xylose lysine deoxycholate (XLD) agar (BD) and *Salmonella-Shigella* agar for incubation at 37 °C for 18–24 h. Similarly, colourless and transparent colonies were screened first using TSI slant and motility indole-urea agar, and the presumptive positive *Shigella* isolates were confirmed with API 20E strips (bioMérieux).

Bacterial serotyping

The O and H antigens of *Salmonella* isolates were characterized using slide agglutination with commercial antiserum (SSI, Denmark), and a serotype was assigned according to the Kauffmann–White scheme. All *Shigella* isolates were serotyped using polyvalent antisera (i.e. A, A1, B, C, C1, C1–3, D) and monovalent antisera (i.e. *S. dysenteriae* 1–12, *S. flexneri* I–VI, *S. boydii* 1–18, *S. sonnei* I–II, and group factor 4, 6, 7) (Denka Seiken, Japan). *Shigella* serotypes were

Table 1. *Serotype distribution of* Salmonella *isolated from human gastrointestinal infections in Shanghai, 2006–2012*

Leading serovar								
Rank	2006 (n=253)	2007 (n=226)	2008 (n=358)	2009 (n=300)	2010 (n=686)	2011 (n=1394)	2012 (n=1266)	Total (n=4483)
1	Enteritidis 22·5%	Enteritidis 21·7%	Enteritidis 21·8%	Enteritidis 30·7%	Typhimurium 28·9%	Enteritidis 42·8%	Enteritidis 38·5%	Enteritidis 34·5%
2	Typhimurium 20·9%	Typhimurium 20·8%	Typhimurium 15·9%	Typhimurium 28·3%	Enteritidis 27·8%	Typhimurium 24·5%	Typhimurium 31·0%	Typhimurium 26·2%
3	Senftenberg 14·6%	Thompson 14·2%	London 8·7%	Senftenberg 6%	Meleagridis 3·6%	Thompson 3·5%	Thompson 3·9%	Senftenberg 5·2%
4	Aberdeen 4·3%	Aberdeen 8·0%	Senftenberg 8·4%	Thompson 4%	Senftenberg 3·1%	Derby 3·4%	Derby 3·0%	Thompson 3·2%
5	Derby 4·0%	Senftenberg 4·9%	Derby 2·2%	Derby 3·7%	London 2·6%	Infantis 2·4%	Agona 2·6%	Derby 2·6%
Subtotal	66·4%	69·5%	57·0%	72·7%	66·0%	76·7%	79%	71·7%

interpreted according to the Manual of Clinical Microbiology criteria [15].

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Salmonella* and *Shigella* were evaluated using the Kirby–Bauer disk diffusion method for 16 antimicrobial agents, including ampicillin (10 μg), amoxicillin/clavulanate acid (30 μg), cefepime (5 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftiofur (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), nalidixic acid (30 μg), ofloxacin (5 μg), streptomycin (10 μg), sulfisoxazole (300 μg), tetracycline (30 μg), trimethoprim (5 μg), and trimethoprim/sulfamethoxazole (1·25/23·75 μg). *Escherichia coli* ATCC 25922 and ATCC 35218 were used as quality control organisms, and isolates were classified as resistant, intermediate, or susceptible according to Clinical Laboratory Standards guidelines [16–18].

RESULTS

Species and serotype distribution

Through the years 2006–2012, a total of 4483 *Salmonella* and 2226 *Shigella* isolates were recovered from human stool samples. The number of isolates per annum ranged from 226 to 1266 for *Salmonella*, and 186–534 for *Shigella*. The difference resulted from the addition of 13 hospitals to the programme in 2010. Eighty *Salmonella* serovars were identified with five serovars accounting for 71·7% of the total isolates, including Enteritidis, Typhimurium, Senftenberg, Thompson, and Derby (Table 1). Several other serovars emerged as significant contributors to salmonellosis in several individual years; for example, *Salmonella* Aberdeen in 2007, and *Salmonella* London in 2008.

Within the same period, 63·9% of the 2226 *Shigella* isolates belonged to *S. sonnei*, and 36·0% to *S. flexneri*. *S. sonnei* caused less than half of shigellosis cases in 2006 and 2007, but gradually became the major species over *S. flexneri*, especially in 2010 and 2011. Most *S. flexneri* infections in Shanghai were caused by serotypes 2a and 4c (Table 2), of which serotype 4c accounted for more than a quarter of shigellosis cases before 2007, but was overtaken by serotypes 2a and 1a in 2008.

Table 2. *Species and serotype distribution of* Shigella *isolated from human gastrointestinal infection in Shanghai, 2006–2012*

Rank	Leading species or serotype							
	2006 (n=314)	2007 (n=249)	2008 (n=379)	2009 (n=186)	2010 (n=307)	2011 (n=534)	2012 (n=257)	Total (n=2226)
1	S. sonnei 49%	S. sonnei 41·3%	S. sonnei 64·6%	S. sonnei 29·6%	S. sonnei 80·5%	S. sonnei 84·8%	S. sonnei 65·0%	S. sonnei 63·9%
2	S. flexneri 4c 28%	S. flexneri 4c 25·7%	S. flexneri 2a 20·1%	S. flexneri 2a 25·3%	S. flexneri 2a 6·8%	S. flexneri 2a 6·4%	S. flexneri 2a 20·2%	S. flexneri 2a 11·8%
3	S. flexneri 1a 9·2%	S. flexneri 2a 10·4%	S. flexneri 1a 6·1%	S. flexneri 1a 24·7%	S. flexneri 1a 6·5%	S. flexneri 1a 2·4%	S. flexneri 1a 3·9%	S. flexneri 4c 9·5%
4	S. flexneri 2a 3·5%	S. flexneri 1a 8·8%	S. flexneri 4c 3·7%	S. flexneri 4c 9·7%	S. flexneri 4c 3·3%	S. flexneri 4c 2·2%	S. flexneri 2c 3·9%	S. flexneri 1a 7·4%
5	S. flexneri 2b 3·2%	S. flexneri 2b 4·4%	S. flexneri x 1·6%	S. flexneri y 2·2%	S. flexneri 2b 1·6%	S. flexneri 2b 1·5%	S. flexneri 4c 1·9%	S. flexneri 2b 1·5%
Subtotal	93·0%	90·8%	96·0%	91·4%	98·7%	97·3%	94·9%	94·5%

Antimicrobial susceptibility

Regardless of serovar, resistance to sulfamethoxazole was most common in *Salmonella*, followed by nalidixic acid, streptomycin, ampicillin, and tetracycline (Table 3). Resistance was also observed, but to a less extent to ciprofloxacin and cephalosporins including cefotaxime, ceftazidime, cefepime, and ceftiofur. Different antimicrobial resistance profiles were apparent between Enteritidis and Typhimurium (Table 3). Notably, Typhimurium exhibited higher resistance to ciprofloxacin than Enteritidis (21% *vs.* 5·4%). A significant finding was the high resistance rate of nalidixic acid in China. Additionally, 11·3% of *Salmonella* isolates were resistant to more than nine antimicrobials tested, and 10·9% presented the resistance pattern of ACSStT (ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline) (data not shown).

A total of 1724 *Shigella* isolates collected from 2008 to 2012 displayed extremely high resistance (>70·0%) against ampicillin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, trimethoprim, and trimethoprim/sulfamethoxazole (Table 3). Consequently, as many as 79·6% of *Shigella* isolates had resistance patterns consisting of more than eight antimicrobials tested (data not shown). *Shigella* isolates had more reduced susceptibility to extended-spectrum cephalosporins than *Salmonella*, with resistance rates ranging from 10·4% to 28·6% (Table 3). A similar discrepancy in antimicrobial resistance profiles was also detected between *S. sonnei* and *S. flexneri*. For instance, ciprofloxacin resistance was present in almost one third of *S. flexneri* isolates, but only in 1·4% of *S. sonnei*.

Trend of antimicrobial resistance

The dynamic change of resistance to selected antimicrobials over the years was summarized among leading *Salmonella* serovars and *S. sonnei* (Fig. 1). Resistance to nalidixic acid in both serovars was most common throughout the study, followed by ampicillin and tetracycline, while resistance to cephalosporins was much lower. Ciprofloxacin resistance was only observed in 2% of Typhimurium cases in 2006, and reached a peak of 39·8% in 2011. Notably, a large increase in resistance in Enteritidis isolates was identified against cefotaxime, ciprofloxacin, and tetracycline, and in Typhimurium isolates against cefotaxime in 2009.

Table 3. *Antimicrobial susceptibility of* Salmonella *and* Shigella *isolated from human gastrointestinal infections in Shanghai, 2006–2012*

Antimicrobials	Salmonella			Shigella*		
	S. Enteritidis (n = 1550)	S. Typhimurium (n = 1174)	All Salmonella (n = 4483)	S. sonnei (n = 1248)	S. flexneri (n = 473)	All Shigella (n = 1724)
Amoxicllin	4·4	12·6	7·1	1	64·7	18·4
Ampicillin	59·7	55·7	41·6	84·2	95·5	87·2
Cefepime	4·8	5·1	4·0	13·5	15·4	14·0
Cefotaxime	7·4	9·7	7·0	27·9	30·7	28·6
Ceftazidime	3·7	7·0	5·0	9·6	12·5	10·4
Ceftiofur	2·7	4·7	3·4	n.t.	n.t.	n.t.
Chloramphenicol	4·1	41·1	17·8	1·6	92·4	26·5
Ciprofloxacin	5·4	21·0	10·7	1·4	37·6	11·4
Gentamicin	11·7	31·6	15·0	73·8	10·4	56·3
Nalidixic acid	96·0	66·0	57·9	97·7	97·0	97·5
Ofloxacin	0·6	1·7	1·4	0·6	32·4	9·3
Streptomycin	48·0	65·8	47·6	97·9	97·9	97·7
Sulfonamides	60·9	68·3	59·2	77·5	61·1	72·9
Tetracycline	26·9	55·1	29·3	73·5	95·5	79·4
Trimethoprim	9·3	40·1	21·0	98·4	97·7	98·1
Trimethoprim + sulfamethoxazole	9·5	41·3	21·1	85·1	63·2	78·9

n.t., Not tested.
* Shigella isolated from 2008 to 2012 were tested for antimicrobial susceptibility.
Values given are percentages.

Of the 1248 *S. sonnei* isolates, resistance to ampicillin and tetracycline remained high (>70%) until 2011, then fell sharply by 1/2 to 2/3 in 2012. Extended-spectrum cephalosporin resistance in *S. sonnei* varied over time, but at a higher level compared to that of *Salmonella*. In particular, a marked increase was observed for cefotaxime in 2010, when half of the *S. sonnei* isolates showed resistance. Ciprofloxacin resistance was relatively low (i.e. 0–5·5%) in *S. sonnei*.

DISCUSSION

The distribution of *Salmonella* serovars and *Shigella* species in human infections may change geographically and temporally, and affect implementation of effective prevention and control strategies. Enteritidis and Typhimurium were the two most common serovars associated with human salmonellosis as reported in many countries [8, 19]. Enteritidis was the leading serovar in Shanghai over 7 years, while studies in other provinces in China reported Typhimurium as the primary cause with prevalence from 19·2% to 45·2% [13, 20, 21]. Our data also showed that Senftenberg was the third most frequently isolated serovar in Shanghai. However, it was rarely reported in

human pathogens in the past, but has been found to persist in feed, and feed materials in feed factories as well as poultry, poultry farms and the processing environment [22–24]. Retrospective analysis did not detect any genetically related isolates in any food samples during the same period, indicating that contact with diverse animals may play an important role in those infections.

Since 2010, *S. sonnei* exceeded *S. flexneri* in causing more human illness in Shanghai, similar to what was reported in Beijing and Chengdu, China [10, 25]. These cities have undergone considerable socioeconomic development since the 1990s; therefore, it is not unexpected that the disease trend has changed from a pattern typical of developing countries to the one frequently seen in industrialized nations [26, 27]. Although *S. flexneri* serotype 2a was still the most prevalent serotype in human disease, *S. flexneri* 4c as an emerging variant was persistently circulating in China and causing the majority of *S. flexneri* infections until 2007 [28]. Given that human immunity to *S. flexneri* is serotype specific [29], such emergence and significant shifts of serotypes between years requires updating of vaccine development. A recently used live serotype 2a vaccine in China cannot provide
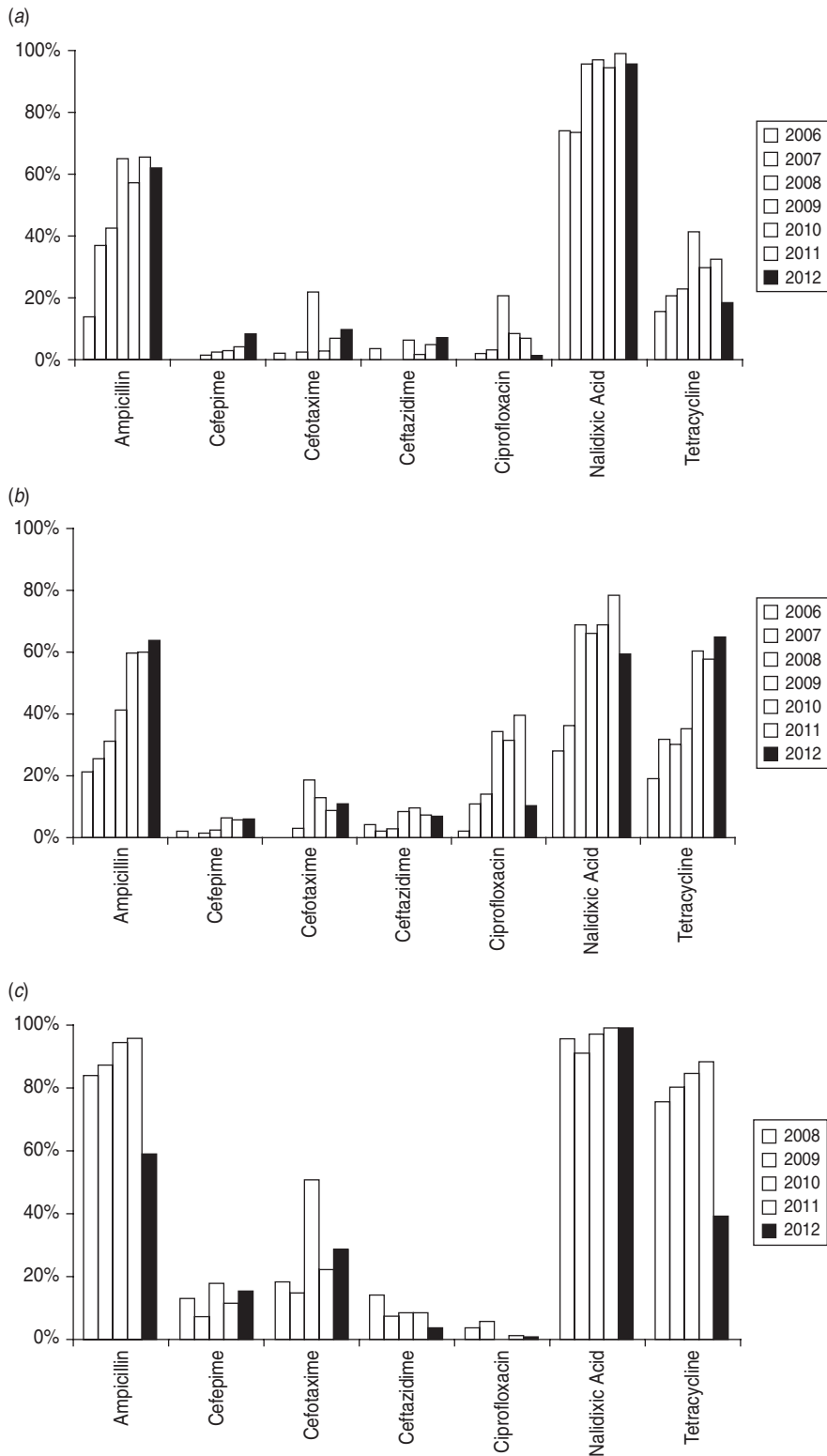
Fig. 1. Antimicrobial resistance of (*a*) *Salmonella* Enteritidis, (*b*) *Salmonella* Typhimurium, and (*c*) *Shigella sonnei* from human gastrointestinal infections. The *Salmonella* strains tested were from 2006 to 2012 and the *Shigella* strains were from 2008 to 2012 only.

cross-protection against 4c [28]. Current vaccine research emphasizes the use of mixed vaccines to confer protection against multiple *Shigella* serotypes [29].

Antimicrobial resistance in bacterial pathogens remains a worldwide public health problem, particularly concerning the emergence and increasing levels of resistance to several clinically important drugs [30]. Fluoroquinolones and extended-spectrum cephalosporins are two common empirical choices for the treatment of severe gastroenteritis, and infection with ciprofloxacin-resistant *Salmonella* tends to be more associated with increased mortality [31]. The widespread nalidixic acid resistance in *Salmonella* and *Shigella* isolates and the high proportion of ciprofloxacin resistance in *Salmonella* Typhimurium and *S. flexneri* were particularly troublesome. The high resistance to these drugs as reported in other provinces of China indicated a potential national trend [10, 14, 28]. In China, over-the-counter purchase and over-reliance on antibiotics for disease therapy, infection prevention, and animal growth promotion are common phenomena in healthcare settings and veterinary practice. The overuse and misuse of antimicrobial agents in hospitals in China may be a critical factor, where the average antibiotic prescription rate was 48·4%, much higher than that reported in USA (15·3%) and northern Europe (<1%) [32]. Stricter antimicrobial control may explain why nalidixic acid resistance in *Salmonella* and *Shigella* was rarely reported in the USA [33]. Additionally, a large number of isolates in this study was resistant to multiple drugs; the survival of fluoroquinolone-resistant bacteria would therefore be greatly enhanced if it was co-resistant to other commonly prescribed antibiotics. Unregulated antibiotic use on the farm for growth promotion and infection prevention may also assist in selection of antimicrobial-resistant bacteria, which can spread to humans through the food production chain [30]. Physicians should be aware of the current epidemiological status of fluoroquinolone-resistant *Salmonella* and *Shigella*, and test antibiograms of all isolates to ensure effective treatment.

Cephalosporins are mainly reserved for the treatment of salmonellosis in children, and severe and life-threatening cases in adults. Unfortunately, the present study revealed the emergence of third- (ceftazidime and cefotaxime) and fourth-generation cephalosporins (cefepime) in *Salmonella* and *Shigella* in Shanghai. Interestingly, both pathogens developed resistance faster to commonly prescribed cefotaxime than cefepime and ceftazidime over the same period, indicating a

selective pressure due to the high frequency of cefotaxime use. Cefotaxime-resistant *S. sonnei* was only isolated in sporadic cases in China in the past [25], Similar to fluoroquinolone, a few cephalosporins resistant *Salmonella* and *Shigella* isolates were detected in developed countries with better antimicrobial practice [33].

In conclusion, this 7-year laboratory-based surveillance in Shanghai successfully monitored the shift of prevalence of major *Salmonella* and *Shigella* subgroups in causing gastrointestinal infections, the increasing trend of antimicrobial resistance, and the rise of *S. sonnei* with cephalosporin resistance. These findings underscore the need for greater efforts to preserve important antibiotic classes such as fluoroquinolones and cephalosporins. Action from the authorities is urgently required to guide appropriate antimicrobial practice in the clinical setting, in order to lower the selection pressure for resistance in *Salmonella, Shigella*, and other bacterial pathogens, and reverse the increasing trend, if possible.

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DECLARATION OF INTEREST

None.

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