Serovars and antimicrobial resistance of non-typhoidal *Salmonella* from human patients in Shanghai, China, 2006–2010

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SUMMARY

We conducted a retrospective study on non-typhoidal *Salmonella* isolates from patients with diarrhoea in Shanghai, China, 2006–2010. A total of 1484 isolates of 70 *Salmonella* serovars were recovered from about 18 000 stool specimens. Serovars Enteritidis and Typhimurium were the most prevalent with isolation rates of 27·6% and 25·5%, respectively. The majority (1151, 77·6%) of the isolates were resistant to at least one antimicrobial, and 598 (40·3%) to more than three antimicrobials. Approximately half (50·9%) of the isolates were resistant to nalidixic acid and other resistance rates were sulfisoxazole (47·9%), streptomycin (37·6%), ampicillin (31·3%) and tetracycline (30·5%). Co-resistance to fluoroquinolones and the third- and fourth-generation cephalosporins was also identified.

Key words: Antimicrobial resistance, *Salmonella*, serovars.

INTRODUCTION

Non-typhoidal *Salmonella* infection is a serious threat to global public health, causing an estimated 93·8 million illnesses and 155 000 deaths worldwide every year [1, 2]. Most (95%) *Salmonella* infections in humans are attributed to ingestion of contaminated foods [3]. While usually self-limiting in most individuals, salmonellosis may require antimicrobial drug treatment in infants, the elderly, and immunocompromised

individuals. However, antimicrobial resistance has become increasingly common among *Salmonella*. Because antimicrobial resistance varies widely between and within countries, surveillance is essential to provide data on the magnitude and trends in resistance and to monitor the effect of interventions [4]. The emergence and spread of antimicrobial-resistant *Salmonella*, particularly multidrug-resistant (MDR) strains, has been reported in many countries [5–9] and is increasingly of major concern as these strains reduce the therapeutic options in cases of invasive infections and have serious public health implications. Unfortunately, the spectrum of antimicrobial resistance in *Salmonella* is also increasing and extends to resistance to fluoroquinolones (ciprofloxacin) and third- and fourth-generation

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cephalosporins (ceftriaxone). As these drugs are the primary choice for the treatment of invasive salmonellosis in humans, the appearance of resistance to such antimicrobials is of particular concern.

Despite an estimated high number of salmonellosis cases in many parts of the world, there is a lack of data on the infection and antimicrobial-resistant *Salmonella* in China. The Shanghai Center for Disease Control and Prevention (SCDC), participating in the World Health Organization Global Foodborne Infections Network (WHO-GFN) since 2005, has performed isolation, identification and characterization of foodborne pathogens recovered from patients. The present report provides data on serovars, and antimicrobial resistance of *Salmonella* isolated from patients with diarrhoea in Shanghai from 2006 to 2010.

MATERIALS AND METHODS

Specimen collection

This study was conducted at 24 sentinel hospitals and eight regional SCDC diagnostic laboratories located in five districts of Shanghai (Children's Hospital of Shanghai, and Children's Hospital of Fudan University were added to this project in 2010). Physicians were asked to collect stool samples from patients who presented with ⩾3 loose stools within 1 day and had other symptoms such as fever, vomiting, or abdominal pain [10]. Demographic and clinical information for each case, including age, gender, symptoms, date of illness onset, and date of specimen collection, was collected and electronically transmitted to SCDC.

Bacteriological examination

The clinical laboratories used a standardized procedure to isolate *Salmonella* from stools [11]. Stool specimens were enriched in tetrathionate Brilliant-Green broth or selenite broth for 6–8 h at 37 °C, followed by subcultivation on Hektoen Enteric agar, *Salmonella*-*Shigella* agar, or CHROMagar *Salmonella* agar (Becton Dickinson, USA). The plates were incubated at 37 °C for 18–24 h and presumptive colonies were further tested using triple-sugar-iron agar, motility indole urea agar, L-lysine decarboxylase, and L-galactosidase (*o*-nitrophenyl-L-D-galactopyranoside; ONPG). Isolates were confirmed as *Salmonella* using API 20E test strips (bioMérieux, France). O and H antigens were characterized by slide agglutination with commercial antiserum (S&A Reagents Laboratory, Thailand), and serovars were assigned according to the Kauffmann–White scheme [12].

Antimicrobial susceptibility testing

Isolates were tested for susceptibility to 16 antimicrobials using the Kirby–Bauer disk diffusion method [13]. The antimicrobials were 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), ceftiofur (30μg), amoxicillin/clavulanate acid (30 μg), trimethoprim/sulfamethoxazole (1·25/23·75 μg), ofloxacin (5 μg), cefepime (5 μg), trimethoprim (5 μg), sulfisoxazole (300 μg), and streptomycin (10 μg). *Escherichia coli* ATCC 25 922 and ATCC 35218 were used as quality control organisms. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14].

Statistical analysis

A χ^2 or Fisher's exact test was used for data analysis using SAS v. 9.2 (SAS Institute Inc., USA). A *P* value of <0·05 was considered statistically significant.

RESULTS

Between January 2006 and December 2010, a total of 18 000 stool specimens were cultured from patients with diarrohea in Shanghai, resulting in 1484 (8·2%) *Salmonella* isolates. The number of isolates per annum ranged from 166 to 679 over the sample period (Table 1). Seventy serovars were identified with 10 serovars accounting for nearly 80% (1177) of the isolates, chief of which were Enteritidis (410, 27·6%), Typhimurium (379, 25·5%), Senftenberg (90, 6·1%), Thompson (52, 3·5%), and Agona (45, 3·0%). The other 60 serovars were distributed among the remaining 307 isolates (Table 1).

The age range of patients spanned from 2 days to 91 years. Of 1204 isolates from 2006 to 2010 (except those from Shanghai Children's Hospital and Children's Hospital of Fudan University), 76·4% were from adults aged 18–60 years. However, most isolates (79·6%) from Shanghai Children's Hospital and Children's Hospital of Fudan University in 2010 were recovered from children aged <4 years (Table 2). The male/female ratio of positive isolation was 1·1:1.

Table 1. *Top 10 serovars of* Salmonella *isolates from humans (2006–2010) in Shanghai, China*

	No. (%) of isolates					
Serotype	2006 ($n = 196$)	2007 ($n = 166$)	2008 ($n = 207$)	2009 ($n = 236$)	2010 ($n = 679$)	Total ($n = 1484$)
Enteritidis	44	40	73	62	191	410 (27·6)
Typhimurium	35	30	50	68	196	379 (25·5)
Senftenberg	34	5	14	17	20	90 (6·1)
Thompson	2	22	3	11	14	52 (3·5)
Agona	6	7	4	3	25	45 (3·0)
London	1	0	23	2	18	44 (3·0)
Aberdeen	10	13	3	7	10	43 (2·9)
Infantis	6	15	1	9	10	41 (2·8)
Derby	9	3	2	8	17	39 (2·6)
Meleagridis	1	2	2	4	25	34 (2·3)
Others	48	29	32	45	153	307 (20·7)

Table 2. *Distribution of human* Salmonella *isolates by gender and age (2006–2010), Shanghai, China*

	No. (%)							
	Gender			Age (years)				
Year	Males	Females	Unknown	⩽4	5–17	18–60	>60	Unknown
2006	85 (43·4)	111 (56·6)	0	3	12	158	23	0
2007	85 (51·2)	81 (48·8)	0	1	5	140	20	0
2008	112 (54·1)	87 (42)	8 (3·9)	0	9	164	26	8
2009	122 (51·7)	105 (44·5)	9 (3·8)	7	10	179	31	9
2010*	199 (49·9)	187 (46·9)	13 (3·3)	33	12	279	66	9
2010†	146 (52·1)	98 (35)	36 (12·9)	223	18	0	0	39
Total	749 (50·5)	669 (45·1)	66 (4·4)	267	66	920	166	65

* *Salmonella* data from Shanghai excluding those from Children's Hospital of Shanghai, and Children's Hospital of Fudan University in 2010.
† *Salmonella* data from Children's Hospital of Shanghai, and Children's Hospital of Fudan University in 2010.

Most isolates (77·6%) were resistant to one or more antimicrobials and 50·9% were resistant to nalidixic acid, followed by sulfisoxazole (47·9%), streptomycin (37·6%), ampicillin (31·3%), tetracycline (30·5%) (Table 3). Resistance was also observed, but to a lesser-extent, to ceftiofur (6·4%), amoxicillin/clavulanate acid (4·7%), ceftazidime (3·8%), cefotaxime (3·6%), ciprofloxacin (2·8%), cefepime (2·1%), and ofloxacin (1·4%). Of the top three serovars (Enteritidis, Typhimurium, Senftenberg), Typhimurium exhibited the highest percentages of resistance to each of these drugs (2·4–6·6%) (Fig. 1), whereas Senftenberg displayed resistance only to ceftazidime (3%).

The emergence or increase in resistance to certain antimicrobials during the study period is noteworthy (Table 3). All isolates from 2006 were susceptible to ceftiofur, cefepime, ofloxacin, and ciprofloxacin but by 2009, resistance rates of 17% to ceftiofur, 1·7% to cefepime, 5·1% to ofloxacin, and 6·8% to ciprofloxacin were found. Significant increase in resistance to other antimicrobials was also recorded for isolates from 2006 to 2009: tetracycline (17·3–32·1%, $P < 0·01$), ampicillin (12·3–33%, $P < 0·01$), amoxicillin/clavulanate acid (0·5–6·8%, $P < 0·01$), nalidixic acid (37·2–54·7% $P < 0·01$), trimethoprim (8·2–20·3%, $P < 0·01$), sulfamethoxazole/trimethoprim (8·7–18·2%, $P < 0·01$), gentamicin (5·1–17%, $P < 0·01$), and streptomycin (16·8–45·8%, $P < 0·01$). Interestingly, most antimicrobial resistance rates (except ciprofloxacin and ofloxacin) of strains isolated from Shanghai Children's Hospital and Children's Hospital of Fudan University were higher than those from other hospitals in 2010 (Table 3), indicating difference in antimicrobial treatment between infections from children and adults.

Table 3. *Antimicrobial resistance of* Salmonella *isolates from humans in Shanghai, China, 2006–2010*

Antimicrobial	% of resistance						
	2006 (n = 196)	2007 (n = 166)	2008 (n = 207)	2009 (n = 236)	2010* (n = 399)	2010† (n = 280)	Total (n = 1484)
Tetracycline	17·3	21·1	20·3	30	32·1	51·4	30·5
Ampicillin	12·3	24·1	25·1	33	31·8	56·1	31·3
Amoxicillin/clavulanate acid	0·5	4·8	1	6·8	4·5	8·9	4·7
Ceftiofur	0	0	1·9	17	3	13·9	6·4
Ceftazidime	2·6	0	1	3·8	2	11·4	3·8
Cefepime	0	0	0	1·7	0·8	8·6	2·1
Cefotaxime	0·5	0	1·4	3·8	1·5	12·1	3·6
Nalidixic acid	37·2	39·2	56·5	54·7	49·1	62·5	50·9
Ofloxacin	0	1·2	1	5·1	0·8	0·7	1·4
Ciprofloxacin	0	0·6	1·9	6·8	3·3	2·9	2·8
Trimethoprim	8·2	10·8	9·7	20·3	22·3	36·4	19·7
Sulfamethoxazole/trimethoprim	8·7	10·2	8·7	18·2	23·3	37·5	19·7
Sulfisoxazole	59·2	40·4	41·5	46·2	42·6	58·2	47·9
Chloramphenicol	10·8	12·7	5·3	16·1	15	29·1	15·7
Gentamicin	5·1	4·8	7·7	17	17	29·6	15·2
Streptomycin	16·8	28·9	25·6	45·8	42·9	51·8	37·6

* Antimicrobial resistance data of *Salmonella* from Shanghai excluding those from Children's Hospital of Shanghai, and Children's Hospital of Fudan University in 2010.
† Antimicrobial resistance data of *Salmonella* from Children's Hospital of Shanghai, and Children's Hospital of Fudan University in 2010.

For example, ciprofloxacin is generally not used for young children.

Importantly, a large proportion of the resistant isolates (40·3%) were resistant to ⩾ 3 antimicrobial agents and these were predominantly the serovars Enteritidis (34·4%) and Typhimurium (33·6%), with the most common resistance phenotypes being nalidixic acid, sulfisoxazole and streptomycin. Thirty-eight isolates were resistant to >10 antimicrobials tested and four isolates to 15 antimicrobials, including sulfonamides, fluoroquinolones, tetracyclines, aminoglycosides and β-lactamases. Eight isolates (three Enteritidis and five Typhimurium) were co-resistant to fluoroquinolones and third- and fourth-generation cephalosporins in addition to 5–10 other antimicrobials.

DISCUSSION

This study provided comprehensive data on serovars and antimicrobial susceptibility of non-typhoidal *Salmonella* from diarrhoea patients in Shanghai from 2006 to 2010. Enteritidis and Typhimurium were the most common serovars as reported in other parts of the world [15, 16], although our other top ranking serovars differed from some countries. For example,

Newport, Javiana, I4, [5], 12:i:-, and Heidelberg were among the top 10 serovars reported from the USA and Canada. On the other hand, Senftenberg, Thompson, Agona, London, Aberdeen, Derby and Meleagridis were among the top 10 serovars in this series with Senftenberg the third most frequently isolated serovar in Shanghai. Interestingly, this serovar has mostly been described as an uncommon human pathogen in the past but was one of the 10 most prevalent serovars from animal farms in England and Wales [17], and a predominant serovar from marine environments and seafood, especially in temperate and tropical zones [18].

The worldwide emergence of resistance to antimicrobials in bacterial pathogens is an important public health problem [5, 8, 19] and an increased number of *Salmonella* isolates have become multidrug resistant in many developing and developed countries [20]. Resistance to several clinically important drugs including quinolones/fluoroquinolones and the third- and fourth-generation cephalosporins is particularly troublesome. Studies have shown that *Salmonella* resistant to nalidixic acid also displayed decreased susceptibility to ciprofloxacin, and that infections with ciprofloxacin-resistant *Salmonella* were associated with increased mortality [21]. Nalidixic acid resistance
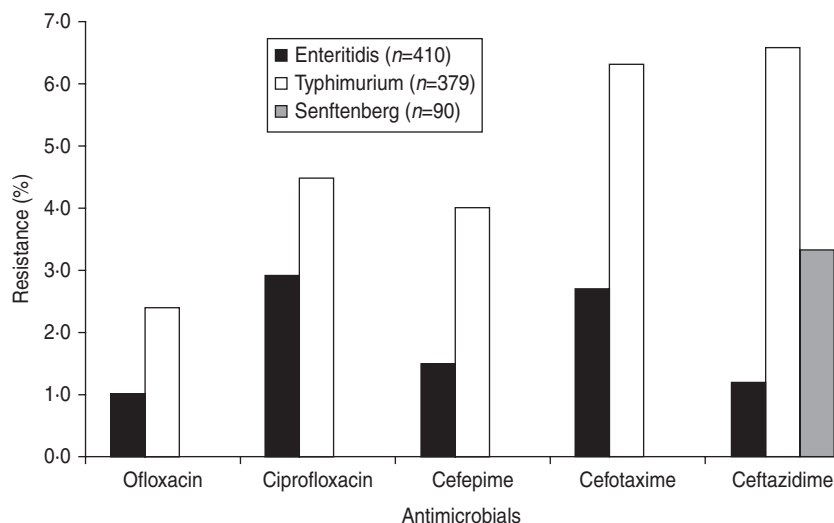
Fig. 1. Resistance to fluoroquinolones, and to third- and fourth-generation cephalosporins in *Salmonella* serovars Enteritidis, Typhimurium and Senftenberg from humans in Shanghai, China.

in non-typhoid *Salmonella* was reported in many countries in the mid-1990s [22–24] and accounted for less than 18% of isolates in Europe in 2000 [9]. More recently nalidixic acid resistance rates of *Salmonella* in India and Taiwan (Typhimurium) were reported to be 20% and 21·6%, respectively [25, 26], but this contrasts with 2% for all *Salmonella* and 0·2% which were ciprofloxacin resistant in the USA in 2010, and 3·8% of *S.* Typhimurium in Korea reported in 2004; none of which were ciprofloxacin resistant [27, 28]. Our finding that nalidixic acid resistance was the most common resistance phenotype (50·9%) in clinical isolates of *Salmonella* in Shanghai, and that 2·8% were also resistant to ciprofloxacin is therefore note-worthy although similar resistance rates for these drugs have been reported in other regions of China, indicating a national trend [29, 30]. The emergence of ciprofloxacin resistance in *Salmonella* is of public health significance as it indicates possible over-prescription in humans and misuse in animals. This may lead to potential failure in the treatment of patients infected with fluoroquinolone-resistant strains and therefore physicians in Shanghai and China in general need to consider determining the antibiogram of all isolates to ensure effective treatment of patients with *Salmonella* infection.

Resistance to sulphonamides has been described in the literature to varying degrees in different countries. In the USA, about 28% S. Typhimurium were resistant to sulfisoxazole [27], whereas 97% and 43% of *S.* Typhimurium were resistant to the drug in Japan and Denmark, respectively [26, 31]. A relatively high level of resistance to sulfisoxazole (47·9%) was also observed in this study.

Cephalosporins are among the most diverse classes of antimicrobials, and are grouped into 'generations' by their antimicrobial properties. Each generation has a broader spectrum of activity than a previous one. Resistance to these drugs has been reported in several countries, albeit at a relatively low rate [26, 27]. Unfortunately, the present study revealed the emergence of co-resistance to fluoroquinolones (ciprofloxacin and ofloxacin), and third-generation (ceftazidime and cefotaxime) and fourth-generation (cefepime) cephalosporins in Enteritidis and Typhimurium isolates. These drugs are among the most widely used worldwide, and are considered critically important in human medicine according to WHO criteria [32] as they are reserved for use in the hospital setting to treat patients with serious and life-threatening infections. The emergence and increased resistance rates to these drugs is therefore particularly alarming. Our findings support the need of continuing active surveillance of antimicrobial resistance in *Salmonella* and other bacterial pathogens as it is essential to the safe and effective use of antimicrobial drugs for humans thereby protecting public health.

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