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Distribution and antimicrobial resistance of enteric pathogens in Chinese paediatric diarrhoea: a multicentre retrospective study, 2008–2013

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SUMMARY

The enteric pathogens causing diarrhoea impair children's health severely. This study retrospectively analysed 1577 pathogens isolated from inpatients and outpatients in six hospitals located in Northern (Inner Mongolia), Northeastern (Hebei), Eastern (Shanghai and Jiangsu), Southern (Hainan) and Central (Hubei) China between 2008 and 2013. Of the 1577 enteric pathogens, *Salmonella* presented with the highest frequency (36·0%), followed by diarrhoeagenic *Escherichia coli* (23·7%), *Staphylococcus aureus* (15·0%), *Shigella* (13·1%), and *Aeromonas* (4·6%). The predominant pathogens varied in different regions of China, with *Salmonella* most prevalent in Shanghai and Hainan, diarrhoeagenic *E. coli* most prevalent in Inner Mongolia, Jiangsu and Hubei, and *Shigella* most prevalent in Hebei. Enteric pathogens were more frequently isolated in males (56·9%) than in females (43·1%). The highest proportion of all enteric pathogens was found in infants (67·6%) with a peak in summer and autumn (68·5%). Antimicrobial susceptibility assay demonstrated that *Shigella* was more resistant to ampicillin, ceftriaxone and sulfamethoxazole than *Salmonella*. Of the top two serotypes in *Salmonella*, Typhimurium was more resistant to ciprofloxacin, sulfamethoxazole and chloramphenicol than Enteritidis ($P < 0·001$). Meanwhile, the resistance rates of *Shigella flexneri* against ampicillin/sulbactam, ciprofloxacin, and chloramphenicol were significantly higher than those of *Shigella sonnei* ($P < 0·001$). Multidrug resistance was apparent in 58·2% of *Shigella* and 45·9% of *Salmonella*, and this phenomenon was more pronounced in *S. flexneri*.

Key words: Antibiotic resistance, bacteriology, diarrhoea, *Salmonella*, *Shigella*.

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INTRODUCTION

Diarrhoeal disease is the second leading cause of morbidity and mortality in children aged <5 years, which represents one of the global public health problems, especially in developing countries. According to a current survey by the World Health Organization, there were nearly 1·7 billion cases of diarrhoea and about 760 000 children die from it every year [1]. The aetiological agents for diarrhoea involve various microorganisms of viruses, bacteria, fungi and parasites. Although the Global Enteric Multicenter Study (GEMS) discovered that rotavirus, *Cryptosporidium*, enterotoxigenic *Escherichia coli* and *Shigella* were the four primary pathogens causing paediatric diarrhoeal disease in sub-Saharan Africa and South Asia [2], the prevalence of enteric pathogens varies in different geographical areas. In developing countries such as Cambodia, Kenya, and Burkina Faso, where the rates of diarrhoea are high because of low income and undernutrition, the most frequently isolated pathogens were enteroaggregative *E. coli* [3–5], but *Shigella* was the most prevalent pathogen in Tehran [6]. In developed countries, diarrhoea is often considered as a minor illness that causes high morbidity but low mortality. A longitudinal study of infectious intestinal disease in the UK (IID2 study) showed *Campylobacter* to be the primary common bacterial cause [7]. Moreover, as investigated by the 2003 global SENTRY Antimicrobial Surveillance Programme, the most frequently collected pathogens in Europe and Latin America were *Salmonella* (56%) and *Shigella* (21%) [8].

In China, about 80 million patients suffer from diarrhoea annually with an incidence of 62·39/10 million, and 52·1% of these patients were aged <5 years [9]. As a result of unbalanced laboratory-testing capabilities, the reported prevalence of pathogens varies by geographical area, and only limited studies of bacterial diarrhoea in children have been reported. Moreover, the nationwide epidemiology of enteric pathogens remains unclear. Thus, a systematic and accurate monitoring of pathogens is required to understand the aetiology of Chinese paediatric diarrhoea.

In this study, we retrospectively analysed the enteric pathogens isolated from stool samples collected in six hospitals located in different regions of China from 2008 to 2013. The epidemiology and antimicrobial resistance of enteric pathogens were systematically investigated, which could provide the basis for a clinical epidemiological study, rational use of antimicrobial agents, and vaccine development.

METHODS

Study design

The surveillance was conducted between January 2008 and December 2013 consecutively across six hospitals from different regions of China, located in Northern (Inner Mongolia), Northeastern (Hebei), Eastern (Shanghai and Jiangsu), Southern (Hainan) and Central (Hubei) China. All these hospitals were third-grade class-A hospitals that undertook healthcare for the majority of children in their region, and therefore were chosen as representative of the prevalent trend of enteric pathogens in children. The data presented pertain to 1577 pathogens (630 strains from Shanghai, 357 from Inner Mongolia, 262 from Hainan, 120 from Jiangsu, 113 from Hubei, 95 from Hebei). All strains were isolated from 62·4% outpatients and 37·6% inpatients.

Ethical approval was not required for this study.

Stool specimens were collected by physicians from children (age 0–14 years) diagnosed with diarrhoea. Diarrhoea was defined as the passage of loose, watery, mucus or bloody stools at least three times in a 24-h period, accompanied by at least one of the following clinical symptoms: nausea, vomiting, abdominal pain, or fever >38 °C. Demographic data of each case including gender, age, and date of specimen collection were obtained from the respective medical record libraries of the hospitals. Patients isolated with the same pathogens for a second time or with incomplete data were excluded from this study.

Detection of bacteria

All stool specimens in each hospital were processed in the microbiology laboratory according to the 'National Guide to Clinical Laboratory Procedures' [10]. Stool samples were cultured directly onto xylose-lysine deoxycholate agar, MacConkey agar and eosin Methylene Blue agar and incubated at 37 °C for 18–24 h to isolate *Salmonella, Shigella* and diarrheagenic *E. coli* (DEC). *Salmonella* samples were first enriched in selenite Brilliant Green broth at 37 °C overnight followed by subcultures in the above media. Sorbitol-MacConkey agar was added to identify *E. coli* O157:H7. To isolate *Campylobacter jejuni*, a modified Campy-BAP medium was used at 42 °C for 48 h in microaerophilic atmosphere in a candle jar. *Vibrio cholerae* was cultured in selective enrichment medium (alkaline peptone water) for 6 h and then subcultured onto thiosulfate-citrate-bile salts-sucrose (TCBS) agar or a double washing plate at

37 °C for 18–24 h. For *Vibrio parahaemolyticus*, stool samples were cultured in alkaline peptone water for 6–8 h and then subcultured onto eosin Methylene Blue, *Salmonella*-*Shigella* agar and TCBS agar at 37 °C overnight. Other enteric pathogens (*Staphylococcus aureus* and *Aeromonas*) were cultured on sheep blood agar. After culturing, suspected bacterial colonies from all media were chosen for further identification by biochemical tests on the basis of standard bacteriological methods [10] and all isolates were finally confirmed using a VITEK 2 compact automated microbiology analyser (bioMérieux, France).

Finally, *Salmonella, Shigella*, and DEC were further serotyped by slide agglutination assay with commercially available antisera (*Salmonella, Shigella*, and DEC sera from Tianrun Biopharmaceuticals, China) according to the manufacturer's instructions. Slide agglutination assay was performed by mixing fresh and single bacterial colonies in serum and sterile saline solution, respectively. A distinct and immediate agglutination in serum was defined as positive. *Salmonella* was serotyped based on O and H antigens. *Shigella* could be typed into four serotypes: *flexneri, sonnei, dysenteriae, and boydii*. For DEC, five categories including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and enterohaemorrhagic *E. coli* (EHEC) were also serotyped based on O, K and H antigens according to the Health Industry Standard of the People's Republic of China (WS271–2007). These serotyping results of DEC were verified by *E. coli* antisera (SSI Diagnostica, Statens Serum Institute, Denmark).

Antimicrobial susceptibility testing

The Kirby–Bauer method [10] was used to determine the antimicrobial susceptibility of *Salmonella* and *Shigella*. The six antimicrobial agents were ampicillin (AMP) 10 μg, ampicillin/sulbactam (SAM) 10 μg, ceftriaxone (CRO) 30 μg, ciprofloxacin (CIP) 5 μg, sulfamethoxazole (SXT) 1·25/23·75 μg, and chloramphenicol (CHL) 30 μg. Resistance criteria for all antimicrobial agents were based on Clinical Laboratory Standards Institute guidelines [11]. *E. coli* ATCC 25922 was used as the control strain for the susceptibility test.

Analysis of data

Whonet 5.6 software [12] was used for susceptibility statistics and analysis. χ^2 test or Fisher's exact test of the SPSS v. 16.0 (SPSS Inc., USA) program was used to test the significance of the data. Data were considered statistically significantly different at $P < 0{\cdot}05$.

RESULTS

Distribution of enteric pathogens

Among the 1577 enteric pathogens, *Salmonella* was found to be associated with paediatric diarrhoeal disease at the highest frequency (36·0%), followed by DEC (23·7%), *S. aureus* (15·0%), *Shigella* (13·1%), *Aeromonas* (4·6%), and *V. parahaemolyticus* (2·3%). However, distribution of these pathogens in different regions varied (Fig. 1). In Shanghai and Hainan, the most frequently isolated pathogen was *Salmonella*, with a frequency of 70·0% and 25·2%, respectively, while DEC was the major pathogen detected in Inner Mongolia, Jiangsu and Hubei (57·1%, 55·8%, and 33·6%, respectively). In Hebei, *Shigella* was the most commonly isolated bacterium (32·6%). Furthermore, the top four detected pathogens annually between 2008 and 2013 were *Salmonella*, DEC, *S. aureus* and *Shigella* (Fig. 2). During the 5 years from 2008 to 2013, the presence of *Salmonella* gradually increased from 29·3% to 50·2%, but *Shigella* decreased from 33·3% to 2·6%.

Distribution of gender and age

Enteric pathogens were mainly distributed in males with a male:female ratio of 1·32:1 (897:680) ($P < 0{\cdot}001$). The gender ratios of *Salmonella*, DEC, *S. aureus* and *Shigella* were 1·36:1, 1·18:1, 1·52:1 and 1·35:1, respectively. Patients aged between 0 and 14 years were divided into four groups including infants (0–1 year, 36·0%), toddlers (1–2 years, 31·6%), preschoolers (3–5 years, 17·3%) and children (6–14 years, 15·1%). Higher proportions of enteric pathogens were isolated in the infant and toddler (0–2 years) groups. However, in Hainan, enteric pathogens were isolated at a significantly higher rate in the 6–14 years age group, which did not occur in the other regions. *Salmonella*, DEC, *S. aureus, Shigella* and *Aeromonas* causing diarrhoea were the most frequently detected pathogens in the 0–2 years age group (infants and toddlers).

Variation by season

Of the 1577 strains, summer and autumn were found to be the two seasons during which pathogens causing

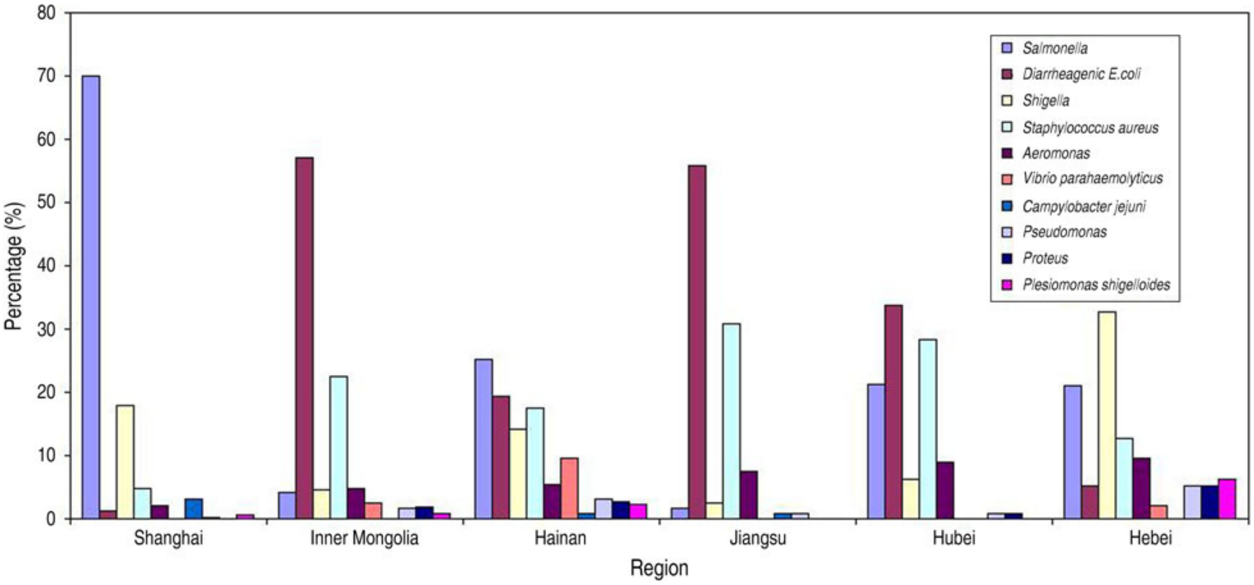

Fig. 1. Proportion of enteric pathogens isolated from different regions in China. During 2008–2013 a total of 1577 strains were isolated from three tier 3 (tertiary) hospitals (Shanghai Children's Hospital, Inner Mongolia Maternal and Children's Hospital, and Hubei Provincial Maternal and Children's Hospital); two tier 2 (municipal) hospitals (Shijiazhuang Municipal Obstetric and Maternal Hospital and Lianyungang Municipal Maternal and Children's Hospital). The total number of bacteria isolated in Hainan province presented includes all hospitals in Hainan province (data collected from the Hainan provincial surveillance system). The percentage in this figure shows the relative portion of each individual enteric pathogen isolated.

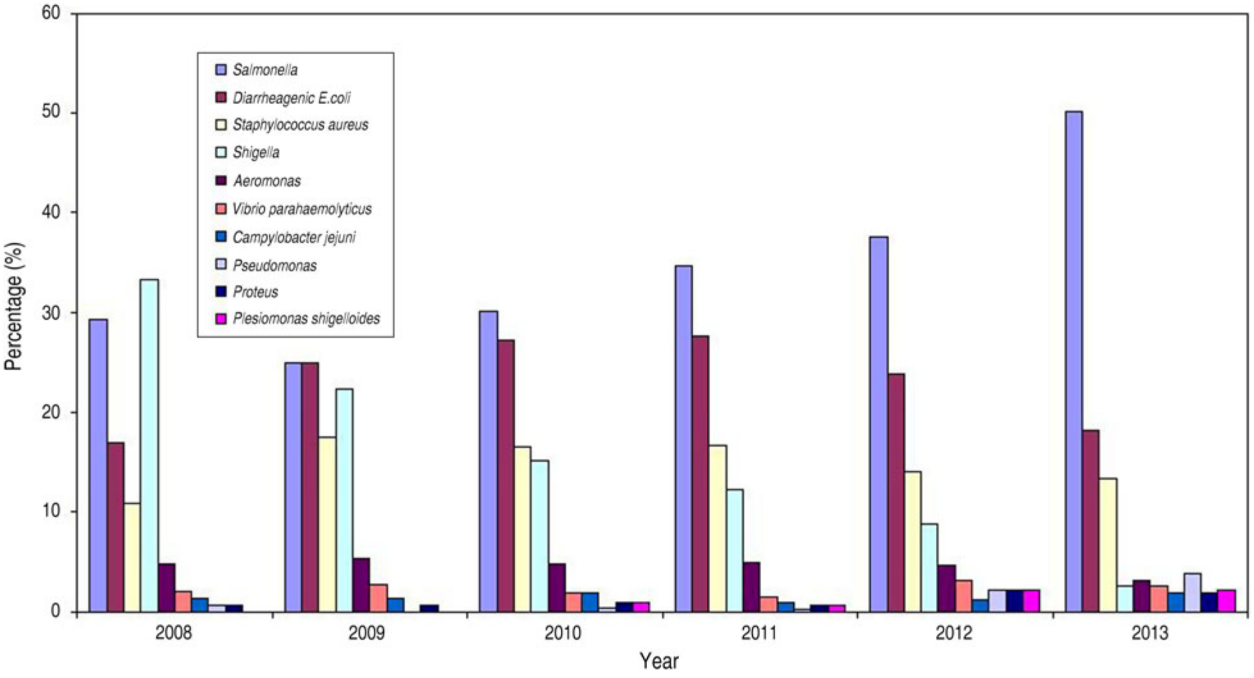

Fig. 2. Enteric pathogens isolated between 2008 and 2013 in China. An interesting trend has been shown for *Salmonella* and *Shigella: Salmonella* increased from 29% in 2008 (and 27% in 2009) up to about 50% in 2013. However, *Shigella* decreased from 33% in 2008 down to 3%.

Table 1. *Common serotypes of* Salmonella *and* Shigella

Pathogen/serotype	*N*	(%)
Salmonella (*N* = 568)		
Typhimurium	217	38·2
Enteritidis	185	32·6
Derby	28	4·9
Stanley	21	3·7
Agona	17	3·0
Choleraesuis	12	2·1
Newport	11	1·9
Thompson	9	1·6
Typhimurium var. Copenhagen	5	0·9
Panama	4	0·7
Montevideo	4	0·7
London	4	0·7
Singapore	3	0·5
Irumu	2	0·4
Turkey	1	0·2
Mbandaka	1	0·2
Virchow	1	0·2
Typhoid	6	1·0
Paratyphoid	1	0·2
Non-typable	36	6·3
Shigella (*N* = 207)		
flexneri	99	47·8
sonnei	89	43
dysenteriae	6	2·9
boydii	7	3·4
Non-typable	6	2·9

diarrhoea appeared significantly more frequently (68·5%, $P < 0{\cdot}001$). Bacterial infection in Hainan between December and January was more common than in the other regions ($P < 0{\cdot}05$). No obvious seasonal trend was found in Jiangsu. Seasonal patterns were seen for diarrhoea associated with *Salmonella, Shigella*, DEC, and *S. aureus. Shigella* was prevalent during September–November, whereas *Salmonella*, DEC and *S. aureus* were prevalent between June and August.

Serotypes of *Salmonella, Shigella* and DEC

As shown in Table 1, 525/568 *Salmonella* isolates (92·4%) were non-typhoidal. The remaining strains were typhoid (6), paratyphoid (1) and non-typable (36). The top five serotypes were Typhimurium (38·2%), Enteritidis (32·6%), Derby (4·9%), Stanley (3·7%) and Agona (3·0%). Other strains belonged to the remaining 12 serotypes. Meanwhile, four serotypes of *Shigella* were detected, including *flexneri* (47·8%), *sonnei* (43·0%), *dysenteriae* (2·9%) and *boydii* (3·4%), except for those that were non-typable (2·9%). Of 373 DEC isolates, 149 (39·9%), 137 (36·7%), 65 (17·4%) and 22 (5·9%) were EPEC, ETEC, EAEC and EIEC, respectively. EHEC was not detected during this period.

Antimicrobial susceptibility

The antimicrobial resistance of *Salmonella* and *Shigella* is summarized in Table 2. Both bacteria showed a higher rate of resistance to ampicillin (>50%) and less resistance to chloramphenicol and ciprofloxacin (<30%). Of the 568 isolated *Salmonella* strains, 59·2% were resistant to ampicillin, 27·6% to sulfamethoxazole, 26·4% to chloramphenicol, 18·7% to ampicillin/sulbactam, 17·1% to ciprofloxacin and 11·4% to ceftriaxone. Of the isolated *Shigella* strains, a high prevalence of resistance was observed for ampicillin (95·2%), followed by sulfamethoxazole (76·1%), ceftriaxone (59·6%), ampicillin/sulbactam (38·3%), chloramphenicol (22·9%), and ciprofloxacin (21·8%). Furthermore, a proportion of Typhimurium strains showed resistance to ciprofloxacin, sulfamethoxazole and chloramphenicol that was significantly higher than that in Enteritidis (28·6% *vs.* 11·4%, 40·6% *vs.* 15·1%, and 44·7% *vs.* 10·3%, respectively) ($P < 0{\cdot}001$). Compared to *S. sonnei, S. flexneri* displayed a much higher resistance rate to ampicillin/sulbactam, ciprofloxacin, and chloramphenicol (58·6% *vs.* 15·7%, 38·4% *vs.* 3·4%, and 42·4% *vs.* 1·1%, respectively) ($P < 0{\cdot}001$).

The emergence of multidrug resistance (MDR) was observed in isolated strains (Table 3). MDR, the phenomenon whereby microorganisms are resistant to ⩾3 antibiotics, was detected in 58·2% (106/182) of *Shigella* strains, which was significantly higher than that of *Salmonella* (45·9%, 161/351) ($P < 0{\cdot}05$). *S. flexneri* showed multiple drug-combination spectrums, of which the ampicillin-chloramphenicolampicillin/sulbactam-sulfamethoxazole-ceftriaxone-ciprofloxacin (ACSamSuCC type) was responsible for most of the MDR strains (18·6%). The rate of MDR in serotype Typhimurium was observed to be higher than the other serotypes of *Salmonella* (60·3% *vs.* 28·6%, $P < 0{\cdot}001$), with the ampicillin-chloramphenicol-sulfamethoxazole-ciprofloxacin (ACSuC) type being responsible for most of the MDR strains (36·2%, 34/94).

DISCUSSION

Enteric pathogens are common agents causing infectious diarrhoea, with *Salmonella, Shigella*, DEC and *Campylobacter* being the most frequent [13, 14]. The

Table 2. *Single antimicrobial resistance of* Salmonella *and* Shigella *(n, %)*

Pathogens	AMP	SAM	CRO	CIP	SXT	CHL
Salmonella						
Typhimurium (*n* = 217)	149 (68·7)	42 (19·4)	25 (11·5)	62 (28·6)	88 (40·6)	97 (44·7)
Enteritidis (*n* = 185)	116 (62·7)	40 (21·6)	24 (13·0)	21 (11·4)	28 (15·1)	19 (10·3)
Salmonella sp. (*n* = 166)	71 (42·8)	24 (14·5)	16 (9·6)	14 (8·4)	41 (24·7)	34 (20·5)
Shigella						
flexneri (*n* = 99)	95 (96·0)	58 (58·6)	62 (62·6)	38 (38·4)	68 (68·7)	42 (42·4)
sonnei (*n* = 89)	84 (94·4)	14 (15·7)	50 (56·2)	3 (3·4)	75 (84·3)	1 (1·1)

AMP, ampicillin; SAM, ampicillin/sulbactam; CRO, ceftriaxone; CIP, ciprofloxacin; SXT, sulfamethoxazole; CHL, chloramphenicol.

Table 3. *Comparison of multidrug resistance of* Salmonella *and* Shigella

Antibiogram	MDR (%)	Non-MDR (%)	χ^2	*P* value
Salmonella				
Typhimurium	94 (60·3)	62 (39·7)	27·239	<0·001
Enteritidis	34 (28·6)	85 (71·4)		
Shigella				
flexneri	70 (71·4)	28 (28·6)	15·182	<0·001
sonnei	36 (42·9)	48 (57·1)		

MDR, Multidrug resistance.

retrospective and multicentre data collected from six different regions throughout the China, demonstrated that *Salmonella, Shigella*, DEC and *S. aureus* were the top four pathogens causing diarrhoea, with the distribution of enteric pathogens showing a clear geographical difference. *Salmonella* was reported as the most frequently isolated pathogen in Southern and Eastern China, DEC was the most prevalent in Northern and Central regions, and *Shigella* was the most commonly isolated bacterium in Northeastern China. Unequal distribution of risk factors such as social and educational conditions, the environment and personal hygienic habits across different regions of China may have been responsible for this geographical difference. Additionally, the pattern indicated that *Shigella* was the most prominent in some regions before 2008. Afterwards, *Shigella* prevalence delined while that of *Salmonella* increased during 2008–2009. After 2010, *Salmonella* became predominant throughout the country. A variety of serovars among *Salmonella* strains were discovered. Enteritidis and Typhimurium are still the most common serovars worldwide [15, 16], although other serotypes made up the proportion. More often, the tendency of diversity in serotypes changed annually, which reflected that it may not result in epidemic diarrhoea but remained in continuous transmission. Moreover, *S. flexneri and S. sonnei* were the major serotypes of *Shigella*, similar to that seen in some Asian countries [17, 18].

The distribution of enteric pathogens differed according to gender, age, season and region. Patients tended to be mainly male rather than female, possibly because boys are more active, which might create greater opportunities for them to become infected with enteric pathogens. Moreover, infants aged <2 years had the greatest prevalence of enteric pathogens. This was because of the gradual reduction in protective antibodies from the mother. Further, infants do not have mature nervous, endocrine, circulatory systems or liver and kidney function and their immune system is not fully developed. Hence compared to preschoolers and children, they are more susceptible to various pathogens.

With the exception of Hainan province, infection reached a peak in summer and autumn, comparable to the regularity of diarrhoea. In these seasons, the climate is hot which facilitates microbial breeding and food is more vulnerable to bacterial contamination. By contrast, we found that enteric pathogens were frequently isolated in winter (December–January) in Hainan, which was almost as high as in summer (June–August). This results from the tropical climate in Hainan province, which is similar to other countries at the same latitude [19, 20]. The reason why bacteria were easily detected in winter in Hainan is that Hainan is located in a subtropical zone and the air temperature remains high all year. However, further study is required to confirm these findings.

Recently, bacterial resistance, which threatens the prevention and treatment of an ever-increasing range of infections, has become an increasingly serious threat to global public health that requires action across all government sectors and society. Patients with infections caused by drug-resistant bacteria are generally at increased risk of worse clinical outcomes and death, and consume more healthcare resources than patients infected with the same bacteria that are not resistant. The inappropriate use of antimicrobial agents in clinics, especially third-generation cephalosporins and quinolones, has led to severe problems of antimicrobial resistance. Our surveillance data showed that 11·4% and 17·1% of *Salmonella* were resistant to ceftriaxone and ciprofloxacin, respectively, which is higher than that reported in developed countries [8, 21] and other parts of Asia [22, 23]. Resistance to ciprofloxacin was evident in 28·6% of Typhimurium strains, which is higher than that of Enteritidis, whereas these two pathogens showed no significant difference in resistance to ceftriaxone. At the same time, about 60% of *Shigella* strains showed resistance to ceftriaxone, and 21·8% showed resistance to ciprofloxacin, both of which are much higher than the rates seen in some countries in Asia, Europe and Latin America [8, 24, 25]. The resistant rate to ciprofloxacin in *S. flexneri* was higher than to *S. sonnei*, but not to ceftriaxone. Quinolones are commonly used for adults and affect articular and cartilage development in children, and they should be used with caution for children. In this study, ciprofloxacin-resistant strains may have resulted from the existing potential drug tolerance or transmission of resistance from adults who were infected with enteric pathogens. In China, quinolones are still used in food-producing animals, which may lead to the evolution of resistant strains. At present, cephalosporin resistance in *Salmonella* and *Shigella* is thought to arise mainly from the production of plasmid-mediated β-lactamase [26, 27], whereas resistance to quinolones results from changes in fluoroquinolone-targeted genes [28, 29]. In addition, resistance patterns to several tested antimicrobials of *Salmonella* and *Shigella* should be further investigated.

MDR makes it difficult to establish an effective treatment for some severe infections caused by *Salmonella* and *Shigella*. MDR is common in enteric pathogen isolates, especially in *Shigella* [24]. In Asian countries [18] and Latin American countries [30], the rates of MDR in *Shigella* were 78% and 29·5%, respectively. In our research, the rate of MDR in *Shigella* was 58·2%, mainly in *S. flexneri*, which was similar to the above results. In addition, for *Salmonella*, the antibiogram of R-type ACSSuT which was defined as resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline was the most common [31]. However, in the present study, the group of ACSuC was also found to contribute partly to MDR, corresponding to the global tendency. Therefore, antimicrobial susceptibility against streptomycin and tetracycline still needs to be explored and their underlying mechanisms further discussed.

In summary, diarrhoea is still a common disease in childhood. Strengthening the monitoring of resistance of intestinal bacteria and summarizing their regularity can provide epidemiological data for further exploration of resistant phenotypes of the pathogens and for elucidation of the relevant mechanisms. The rational use of antimicrobial agents in clinics can reduce the phenomenon of MDR, which is of great significance for better prevention and control of diarrhoea in the future.

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DECLARATION OF INTEREST

None.

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