Analysis of the aetiology of diarrhoea in outpatients in 2007, Henan province, China

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SUMMARY

The purpose of this study was to determine the aetiology of acute diarrhoea and improve knowledge of gastrointestinal pathogens in China. Faecal specimens from 1526 outpatients with diarrhoea were collected from 20 hospitals in Henan province and the prevalence of seven bacterial and five viral diarrhoeagenic pathogens were determined. Bacterial pathogens were recovered from 279/1526 (18·3%) stool specimens and viral pathogens were detected in 178/1526 (11·7%) stool specimens. The top five pathogenic species were diarrhoeagenic *E. coli* ($n = 140$, 9·2%), rotavirus ($n = 79$, 5·2%), *Shigella* spp. ($n = 69$, 4·5%), *Salmonella* spp. ($n = 66$, 4·3%) and norovirus ($n = 56$, 3·7%). The prevalence of pathogens showed correlation with age, season and clinical symptoms. Several dominant serotypes were identified in *Shigella* and *Salmonella* isolates, and high prevalence of multiple drug-resistant isolates was observed in both species. This important information will have a significant effect on public health policy development and resource prioritization practices.

Key words: Aetiology, diarrhoea, outpatients.

INTRODUCTION

Diarrhoeal diseases constitute a major public health problem in the community of both developing and developed countries [1]. Different diarrhoeagenic pathogens, including bacteria, viruses, and parasites, have been identified as the aetiological agents of diarrhoea with most of them being highly related to the consumption of contaminated food products [2]. The features of diarrhoeagenic pathogens may vary from place to place depending on dietary habits, local meteorology, geography, and socioeconomic elements [3, 4]. Epidemiological characterization of these pathogens in the community is the initial step towards introduction of correct interventions for controlling the diseases. Several countries have conducted different prospective population-based or cross-sectional studies to estimate the overall number of foodborne illnesses [5]. Recently, powerful active surveillance networks for diarrhoeagenic pathogens have been established and improved, especially in countries with extensive health resources [6–8].

However, until now, only limited information has been available on the prevalence of the diarrhoeal aetiological spectrum and antimicrobial resistance

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patterns of major diarrhoeagenic bacterial pathogens in China as there is no requirement for hospitals to identify pathogens from stool samples of outpatients. Recently, China CDC has initiated a Diarrhoea Surveillance Network in several provinces, including Henan. The purpose of this study is to describe the prevalence of the main aetiological infectious agents of diarrhoea and the antimicrobial susceptibility of *Salmonella* and *Shigella* from outpatients in Henan province, China.

MATERIALS AND METHODS

Stool specimen collection

Twenty hospitals in Zhengzhou city, Shangqiu city and Suiyang county of Henan province were recruited in this study. These hospitals consisted of tertiary ($n=2$), secondary ($n=6$), and primary ($n=6$) hospitals and community health centres ($n=6$). These hospitals represented the most popular hospital categories in Henan province and were also selected as the sentinel hospitals in the Diarrhoea Surveillance Network of China CDC. The population of Henan province was 99·18 million in 2008, which ranked highest in China. There are 1192 hospitals and 2089 community health centres in this province. The population of Zhengzhou city or Shangqiu city is more than five million and the population of Suiyang county is $>500\,000$. Faecal specimens from outpatients with diarrhoea (defined by World Health Organization guidelines as passage of >3 loose or liquid stools in a 24-h period) were collected from January to December in 2007. From June to September, $\leqslant 30$ faecal specimens were collected from each hospital or health centre every month and $\leqslant 10$ specimens were collected from each hospital or health center in the remaining months. Faecal specimens were collected from each outpatient before antimicrobial treatment. Each day, only the first eligible stool specimen from the outpatient who presented with diarrhoea at each recruiting hospital was collected. Faecal swabs (Cary–Blair transport swabs; Copan, Italy) from each outpatient were shipped to the local CDC laboratiry for bacterial pathogen isolation, and ten ~ 30 g faecal specimen from each outpatient were shipped to the provincial CDC laboratories for virus analysis. Questionnaires regarding information of clinical symptoms as well as general data such as age, gender and place of residence were collected.

Pathogen analysis

Each outpatient contributed $\leqslant 1$ specimen, and each specimen was tested for 12 diarrhoeagenic pathogenic species, including *Aeromonas hydrophila*, *Campylobacter* spp., diarrhoeagenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Yersinia enterocolitica*, astrovirus, adenovirus, noroviruses, rotavirus and sapovirus. The presence of pathogenic bacteria was tested for by routine culture assays on appropriate selective media as follows: diarrhoeagenic *E. coli*, MacConkey agar (BD Diagnostics, China); *Salmonella* spp., BBL™ CHROMagar™ *Salmonella* (BD Diagnostics); *Shigella* spp., *Salmonella-Shigella* agar (BD Diagnostics); *Campylobacter* spp., CCDA agar (Oxoid, China); *A. hydrophila* and *Vibro* spp., thiosulfate-citrate-bile-sucrose agar (BD Diagnostics); *Y. enterocolitica*, Yersinia-selective agar (BD Diagnostics). *Campylobacter* plates were incubated microaerophilically at 42 ± 1 °C; *Yersinia* plates were incubated aerobically at 27 ± 1 °C; all other plates were incubated aerobically at 35 ± 1 °C. From each plate, $\leqslant 3$ suspected colonies were selected and confirmed by API strip tests (bioMérieux, France). *Salmonella* and *Shigella* isolates were further serotyped with commercially available antiserum (Statens Serum Institute, Denmark) through slide agglutination according to the manufacturer's instructions. Pathotypes of diarrhoeagenic *E. coli* were identified through a multiplex PCR method, including enteroaggregative *E. coli* (EAEC), enterohaemorrhagic E. coli (EHEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) [9]. This multiplex PCR amplifies the following target genes: *uidA*, *escV*, *bfpB*, stx_1, stx_2, *elt*, *estIa*, *estIb*, *invE*, *astA*, *aggR*, and *pic*. Adenovirus, astrovirus, norovirus, rotavirus and sapovirus in faecal specimens were detected by reverse transcription multiplex PCR methods [10, 11].

Antimicrobial susceptibility testing

Antimicrobial susceptibility of all *Salmonella* and *Shigella* isolates was determined via the disc diffusion method, and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) interpretive standards [12]. Susceptibility to seven antimicrobials was measured for all isolates, including ampicillin (AMP, 10 μg), cefotaxime (CTX, 30 μg), chloramphenicol (CHL, 30 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NAL, 30 μg), tetracycline (TET, 30 μg)

and trimethoprim–sulfamethoxazole (SXT, 1·25/ 23·75 μg).

Statistical analysis

The differences of antimicrobial resistance frequencies were analysed by χ^2 test and Mantel–Haenszel test using Epi Info v. 6 software (www.cdc. gov/epiinfo/).

RESULTS

From January to December 2007, 1526 stool specimens from individual outpatients with diarrhoea were collected and tested for bacterial and viral pathogens from Zhengzhou city ($n=574$), Shangqiu city ($n=498$) and Suiyang county ($n=454$). The outpatients comprised 515 females and 1011 males that ranged in age from <1 month to 98 years (median age 3 years, mean age 16·4 years). Of 1526 outpatients, 682 subjects had watery diarrhoea, 574 subjects had abdominal pain, 389 subjects had fever ($\geqslant 38$ °C) and 182 subjects had blood in their stool (bloody diarrhoea). A total of 1228 subjects exhibited at least one of the above symptoms, 500 outpatients exhibited more than one symptom, and 102 outpatients exhibited more than two symptoms (Table 1).

Pathogens of 12 diarrhoeagenic pathogenic species were detected in 432/1526 (28·3%) stool specimens. Bacterial pathogens of seven species were recovered from 279/1526 (18·3%) stool specimens and viral pathogens of five species were detected in 178/1526 (11·7%) stool specimens; more than two pathogenic species were found in 60/1526 (3·9%) stool specimens. Both bacterial and viral pathogens were found in 25/ 1526 (1·6%) stool specimens. The top five pathogenic species were diarrhoeagenic E. coli ($n=140$, 9·2%, including EAEC, EHEC, EIEC, EPEC and ETEC), rotavirus ($n=79$, 5·2%), Shigella spp. ($n=69$, 4·5%), Salmonella spp. ($n=66$, 4·3%) and norovirus ($n=56$, 3·7%) (Tables 1 and 2). The median age of patients infected with a bacterial enteric pathogen was 8 years, compared to 1 year for those infected with a viral enteric pathogen and 4 years for outpatients with no pathogen detected.

Stool specimens collected in spring and summer were more likely to yield bacterial pathogens (19·6% and 21·6%, respectively), and those collected in autumn were more likely to yield viral pathogens (23·3%). Stool specimens collected in spring also showed higher prevalence for rotavirus ($n=10$, 6·1%). The pathogen prevalence in three regions showed significant difference: 24·8% (113/454) of specimens from Suiyang county had a positive bacterial culture result, compared to 13·9% (69/498) and 16·9% (97/574) of those from Shangqiu city and Zhengzhou city ($P<0·01$); 16·0% (92/574) of specimens from Zhengzhou city had a positive viral test result, compared to 7·0% (35/498) and 11·2% (51/ 454) of those from Shangqiu city and Suiyang county ($P<0·01$).

The prevalence of clinical symptoms showed a correlation with the distribution of pathogen categories. Outpatients with a positive bacterial culture had a higher prevalence of abdominal pain (119/279, 42·7%) and bloody diarrhoea (49/279, 17·6%), compared to 13·5% (24/178) and 7·9% (14/178) of those with a positive viral test result ($P<0·01$). Outpatients infected by Shigella spp. showed a significantly higher prevalence of bloody diarrhoea (28/69, 40·6%) compared to other diarrhoeagenic pathogens ($P<0·01$). However, outpatients with a positive viral result had a higher prevalence of watery diarrhoea (115/178, 64·6%), compared to those infected with a bacterial enteric pathogen (99/279, 35·5%) or no pathogen detected (478/1094, 43·7%) ($P<0·01$).

The prevalence of pathogen categories also showed a correlation with outpatient age. Stool specimens from outpatients aged <3 years showed similar prevalence of bacterial (113/749, 15·1%) and viral (136/749, 18·2%) pathogens, but a higher prevalence of bacterial pathogens was observed in other age groups (Table 3).

Salmonella serotyping and antimicrobial susceptibility

Of 66 Salmonella isolates, 13 distinct serotypes were identified, including two dominant serotypes: S. Typhimurium ($n=23$, 34·8%) and S. Enteritidis ($n=16$, 24·2%). Minor serotypes included Norwich ($n=7$), London ($n=5$), Derby ($n=5$), Stanley ($n=2$) and Bovismorbificans ($n=2$). Only one isolate was recovered for each of the following serotypes: Agona, Meleagridis, Sanktmarx, Schwarzengrund, Senftenberg and Tennessee.

Of 66 Salmonella isolates, 15 (22·7%) were susceptible to all tested antimicrobials. Resistance to nalidixic acid was the most common ($n=48$, 72·7%), followed by tetracycline ($n=34$, 51·5%), ampicillin ($n=29$, 43·9%), trimethoprim-sulfamethoxazole ($n=27$, 40·9%) and chloramphenicol ($n=24$, 36·4%). The antimicrobial-resistant profiles differed among serotypes. Of 23 S. Typhimurium isolates, 17 (73·9%)

Table 1. *Demographic and illness characteristics in a study of the aetiology of diarrhoea in outpatients in Henan province, China*

Characteristics	Subjects with bacterial pathogen identified on culture ($n=279$)	Subjects with viral pathogen detected by RT–PCR ($n=178$)	Subjects with no pathogen identified ($n=1094$)	All enrolled subjects ($n=1526$)
Male subjects, n (%)	179 (64·2)	126 (70·8)	722 (66·0)	1011 (66·3)
Female subjects, n (%)	100 (35·8)	52 (29·2)	372 (34)	515 (33·7)
Age, median, years (range)	8 (0–98)	1 (0–81)	2 (0–89)	3 (0–98)
Age (years), n (%)				
<3	113 (40·5)	136 (76·4)	520 (47·5)	749 (49·1)
3–19	61 (21·9)	11 (6·2)	192 (17·6)	264 (17·3)
20–49	62 (22·2)	18 (10·1)	272 (24·9)	348 (22·8)
≥50	43 (15·4)	13 (7·3)	110 (10·1)	165 (10·8)
Region, n (%)				
Shangqiu city	69 (24·7)	35 (19·7)	396 (36·2)	498 (32·6)
Suiyang county	113 (40·5)	51 (28·7)	305 (27·9)	454 (29·8)
Zhengzhou city	97 (34·8)	92 (51·7)	393 (35·9)	574 (37·6)
Season, n (%)				
Spring	32 (11·5)	11 (6·2)	121 (11·1)	163 (10·7)
Summer	154 (55·2)	25 (14·0)	543 (49·6)	713 (46·7)
Autumn	85 (30·5)	134 (75·3)	371 (33·9)	575 (37·7)
Winter	8 (2·9)	8 (4·5)	59 (5·4)	75 (4·9)
Symptoms, n (%)				
Fever	83 (29·7)	45 (25·3)	267 (24·4)	389 (25·5)
Abdominal pain	119 (42·7)	24 (13·5)	435 (39·8)	574 (37·6)
Watery diarrhoea	99 (35·5)	115 (64·6)	478 (43·7)	682 (44·7)
Bloody diarrhoea	49 (17·6)	14 (7·9)	131 (12·0)	190 (12·5)

were resistant to at least four antimicrobials. Whereas, in 16 S. Enteritidis isolates, only three (18·9%) were resistant to four or more antimicrobials. Of 27 isolates of minor serotypes, 10 (37·0%) were susceptible to all tested antimicrobials and seven (25·9%) isolates were resistant to at least four antimicrobials. In total, 13 ciprofloxacin-resistant *Salmonella* isolates were recovered, including Typhimurium ($n=9$), Norwich ($n=2$), Enteritidis ($n=1$) and Senftenberg ($n=1$). All these 13 ciprofloxacin-resistant isolates were also resistant to four non-quinolone antimicrobials. Two isolates of different serotypes (Enteritidis and Senftenberg) were resistant to cefotaxime and the Senftenberg isolate also showed resistance to ciprofloxacin.

Shigella serotyping and antimicrobial susceptibility

Of 69 *Shigella* isolates, three dominant serotypes were identified, including *S. flexneri* serotype 2a ($n=21$, 30·4%), *S. flexneri* serotype 4c ($n=13$, 18·8%) and *S. flexneri* serotype 1a ($n=12$, 17·4%). Minor serotypes included *S. flexneri* serotype fx ($n=7$, 10·1%), *S. sonnei* ($n=7$, 10·1%) and *S. flexneri* serotype 2b ($n=6$, 8·7%). Only one isolate was recovered for each of the following serotypes: *S. flexneri* serotype f1b, *S. flexneri* serotype f4a and *S. flexneri* serotype fy.

Of 69 *Shigella* isolates, resistance to nalidixic acid was the most common (69, 100%), followed by tetracycline (67, 97·1%), ampicillin (64, 92·8%), chloramphenicol (60, 87%), trimethoprim–sulfamethoxazole (40, 58·0%) and ciprofloxacin ($n=25$, 36·2%). All 69 *Shigella* isolates were resistant to at least two antimicrobials, and 65 (94·2%) isolates were resistant to at least four antimicrobials. Four dominant antimicrobial-resistant profiles were identified: AMP-CHL-NAL-TET ($n=18$), AMP-CHL-NAL-SXT-TET ($n=14$), AMP-CHL-CIP-NAL-SXT-TET ($n=14$) and AMP-CHL-CIP-NAL-TET ($n=10$). Twenty out of 21 *S. flexneri* serotype f2a isolates were resistant to five ($n=8$) or six ($n=12$) antimicrobials. All 25 ciprofloxacin-resistant isolates were also resistant to three or four non-quinolone antimicrobials and belonged to

Table 2. *Pathogens identified in 1526 stool samples by season and age in a study of the aetiology of diarrhoea in outpatients in Henan province, China*

| | No. of positive samples in different seasons | | | | No. of subjects, by age (years) | | | | |
	Spring (n=163)	Summer (n=713)	Autumn (n=575)	Winter (n=75)	<3 (n=749)	3–19 (n=264)	20–49 (n=348)	⩾50 (n=165)	Total (n=1526)
Bacteria	32 (19·6)	154 (21·6)	85 (14·9)	8 (10·7)	113 (15·1)	61 (23·1)	62 (17·8)	43 (26·1)	279 (18·3)
Salmonella spp.	5 (3·1)	36 (5·0)	24 (4·2)	1 (1·3)	24 (3·2)	16 (6·1)	15 (4·3)	11 (6·7)	66 (4·3)
Shigella spp.	6 (3·7)	40 (5·6)	22 (3·8)	1 (1·3)	28 (3·7)	19 (7·2)	10 (2·9)	12 (7·3)	69 (4·5)
E. coli	21 (12·9)	77 (10·8)	26 (4·5)	2 (2·7)	47 (6·3)	29 (11·0)	31 (8·9)	19 (11·5)	126 (8·3)
EPEC	8 (4·9)	28 (3·9)	5 (0·9)	—	18 (2·4)	6 (2·3)	8 (2·3)	9 (5·5)	41 (2·7)
EHEC	—	17 (2·4)	8 (1·4)	1 (1·3)	12 (1·6)	3 (1·1)	7 (2·0)	4 (2·4)	26 (1·7)
ETEC	2 (1·2)	19 (2·7)	4 (0·7)	—	8 (1·1)	5 (1·9)	8 (2·3)	4 (2·4)	25 (1·6)
EIEC	2 (1·2)	3 (0·4)	—	—	—	2 (0·8)	1 (0·3)	2 (1·2)	5 (0·3)
EAEC	12 (7·4)	21 (2·9)	9 (1·6)	1 (1·3)	13 (1·7)	16 (6·1)	11 (3·2)	3 (1·8)	43 (2·8)
Campylobacter spp.	—	2 (0·3)	11 (1·9)	4 (5·3)	16 (2·1)	—	1 (0·3)	—	17 (1·1)
V. parahaemolyticus	—	—	3 (0·5)	—	1 (0·1)	1 (0·4)	1 (0·3)	—	3 (0·2)
Y. enterocolitica	3 (1·8)	5 (0·7)	1 (0·2)	—	2 (0·3)	3 (1·1)	2 (0·6)	2 (1·2)	9 (0·6)
A. hydrophila	—	7 (1·0)	5 (0·9)	—	5 (0·7)	2 (0·8)	4 (1·1)	1 (0·6)	12 (0·8)
Viruses	11 (6·7)	25 (3·5)	134 (23·3)	8 (10·7)	136 (18·2)	11 (4·2)	18 (5·2)	13 (7·9)	178 (11·7)
Rotavirus	10 (6·1)	13 (1·8)	55 (9·6)	1 (1·3)	64 (8·5)	6 (2·3)	6 (1·7)	3 (1·8)	79 (5·2)
Norovirus	—	8 (1·1)	43 (7·5)	5 (6·7)	41 (5·5)	3 (1·1)	8 (2·3)	4 (2·4)	56 (3·7)
Sapovirus	—	3 (0·4)	26 (4·5)	—	20 (2·7)	—	3 (0·9)	6 (3·6)	29 (1·9)
Astrovirus	—	2 (0·3)	23 (4·0)	2 (2·7)	20 (2·7)	2 (0·8)	3 (0·9)	2 (1·2)	27 (1·8)
Adenovirus	1 (0·6)	—	5 (0·9)	—	6 (0·8)	—	—	—	6 (0·4)

EPEC, Enteropathogenic *E. coli*; EHEC, enterohaemorrhagic E. coli; ETEC, enterotoxigenic *E. coli*; EIEC, enteroinvasive *E. coli*; EAEC, enteroaggregative *E. coli*.
–, Not detected.
Values given are *n* (%).

Table 3. *Pathogen identification and outpatient symptoms in a study of the aetiology of diarrhoea in Henan province, China*

	Fever	Abdominal pain	Watery diarrhoea	Bloody diarrhoea
Bacteria (*n* = 279)	83 (29·7)	119 (42·7)	99 (35·5)	49 (17·6)
Salmonella spp. (*n* = 66)	15 (22·7)	29 (43·9)	22 (33·3)	8 (12·1)
Shigella spp. (*n* = 69)	35 (50·7)	28 (40·6)	12 (17·4)	28 (40·6)
E. coli (*n* = 126)	31 (24·6)	54 (42·9)	49 (38·9)	16 (12·7)
EPEC (*n* = 41)	10 (24·4)	14 (34·1)	13 (31·7)	3 (7·3)
EHEC (*n* = 26)	4 (15·4)	11 (42·3)	13 (50·0)	3 (11·5)
ETEC (*n* = 25)	6 (24·0)	12 (48·0)	8 (32·0)	3 (12·0)
EIEC (*n* = 5)	3 (60·0)	3 (60·0)	4 (80·0)	1 (20·0)
EAEC (*n* = 43)	10 (23·3)	21 (48·8)	15 (34·9)	6 (14·0)
Campylobacter spp. (*n* = 17)	4 (23·5)	5 (29·4)	8 (47·1)	1 (5·9)
V. parahaemolyticus (*n* = 3)	—	—	3 (100·0)	—
Y. enterocolitica (*n* = 9)	3 (33·3)	5 (55·6)	2 (22·2)	2 (22·2)
A. hydrophila (*n* = 12)	3 (25·0)	4 (33·3)	8 (66·7)	—
Viruses (*n* = 178)	45 (25·3)	24 (13·5)	115 (64·6)	14 (7·9)
Rotavirus (*n* = 79)	28 (35·4)	9 (11·4)	50 (63·3)	4 (5·1)
Norovirus (*n* = 56)	13 (23·2)	10 (17·9)	35 (62·5)	2 (3·6)
Sapovirus (*n* = 29)	3 (10·3)	2 (6·9)	21 (72·4)	5 (17·2)
Astrovirus (*n* = 27)	3 (11·1)	3 (11·1)	22 (81·5)	3 (11·1)
Adenovirus (*n* = 6)	1 (16·7)	1 (16·7)	3 (50·0)	—

EPEC, Enteropathogenic *E. coli*; EHEC, enterohaemorrhagic *E. coli*; ETEC, enterotoxigenic *E. coli*; EIEC, enteroinvasive *E. coli*; EAEC, enteroaggregative *E. coli*.
–, Not detected.
Values given are *n* (%).

three *S. flexneri* serotypes: *S. flexneri* 2a (*n* = 20), *S. flexneri* 1a (*n* = 4) and *S. flexneri* 4c (*n* = 1). Six cefotaxime-resistant isolates were also resistant to three or four non-β-lactam antimicrobials and belonged to three serotypes: *S. sonnei* (*n* = 3), *S. flexneri* 1a (*n* = 2) and *S. flexneri* 2a (*n* = 1). The cefotaxime-resistant *S. flexneri* 2a isolate also showed resistance to ciprofloxacin and three other non-quinolone antimicrobials.

DISCUSSION

This study provides useful information regarding the aetiologies of diarrhoea and antimicrobial resistance profiles of major diarrhoeagenic bacteria in densely populated developing regions with inadequate medical resources. More than 25% of 1526 stool specimens were found to have known diarrhoeagenic pathogens that have been reported with variable prevalence in different countries [13–16]. Two major diarrhoeagenic pathogens (*Salmonella* and *Shigella*), were characterized by serotyping and antimicrobial susceptibility. Our data showed multiple drug-resistant (MDR) *Salmonella* and *Shigella* isolates were widely disseminated in recruited outpatients. To the

best of our knowledge, this is the first prospective diarrhoeagenic pathogen spectrum study in outpatients in mainland China.

Although stool specimens were collected from the first outpatient who presented with diarrhoea in each recruiting hospital, 66·3% (1011/1526) of subjects were male and 49·1% (749/1526) of subjects were aged <3 years. This demographic distribution profile was significantly different from other studies [14, 15]. This profile might be a unique demographic pattern for outpatients with diarrhoea in China. Since China implemented the one-child policy, parents take illness in young children very seriously, especially male children. There are now more opportunities for parents to bring their children (especially those aged <3 years) suffering from diarrhoea to see a doctor early in the morning if they have concerns. Further, younger children are more likely to be infected by diarrhoeagenic pathogens because of their dietary habit and immature immune system. Moreover, since most diarrhoeagenic pathogen infections are self-limiting, adults with diarrhoea may not seek medical help or visit a doctor in small clinics or obtain medicines over the counter. As a result, the data of this study might

be biased because more diarrhoea cases of children aged <3 years were included.

In this study, 28·3% of 1526 stool specimens were found to have known diarrhoeagenic pathogens and the top five pathogenic species were diarrhoeagenic *E. coli* ($n=140$, 9·2%), rotavirus ($n=79$, 5·2%), *Shigella* spp. ($n=69$, 4·5%), *Salmonella* spp. ($n=66$, 4·3%) and norovirus ($n=56$, 3·7%). The total diarrhoeagenic pathogen-positive ratio and ranking pattern were different from other studies on the aetiology of diarrhoea; in particular, *Campylobacter* and viral pathogens showed significantly lower prevalence in outpatients with diarrhoea than other studies [15, 17]. The differences in the spectrum of diarrhoeal agents were critically dependent on the employed technology. Since a variety of bacteria species existed in the stool specimens, bacteria similar to the target pathogens may interfere with the recovery of these pathogens. Appropriate selective enrichment step adoption may help to change the ratio of diarrhoeagenic bacterial pathogens in the media and improve the recovery of target pathogens. Other selective methods, such as immunomagnetic beads and better viral RNA extraction methods may also help to improve pathogen detection. In future studies, new techniques should be applied to improve the positive rate of the target pathogens. Another reason might be differences in healthcare-seeking behaviours of the subjects. In this study, close to 50% of the subjects were aged <3 years and two thirds were male, which was different from other studies [4, 15, 18]. Moreover, our study population may differ from the population of other studies because of geographical and dietary habit variations in prevalence of pathogens.

Our data showed outpatients aged >50 years continued to have the highest incidence of bacterial infections. Studies have shown infected elderly subjects are at highest risk for hospitalization and death from most of these infections [19]. MDR *Salmonella* and *Shigella* were especially widely disseminated in these regions which made prompt diagnosis and treatment, as well as careful attention to food safety, particularly important in this age group.

As expected, rotavirus was the most common pathogen ($n=79$, 5·2%), especially for children in autumn. The appreciable detection rates of astroviruses and sapovirus, as reported in Australia, the USA, and France [20–22], suggests that these viruses are also important causes of diarrhoea, especially in children and older subjects (Table 2).

Our data showed that *Shigella* and *S.* Typhimurium isolates had a strong capacity to accumulate antimicrobial resistance mechanisms. More than 90% of *S. flexneri* isolates and more than 70% of *S.* Typhimurium isolates in this study showed MDR (resistant to ⩾3 antimicrobial categories) profiles and were highly resistant to older generation antimicrobials, such as ampicillin, chloramphenicol, nalidixic acid and tetracycline. This rapid accumulation of resistance mechanisms in *Shigella* and *S.* Typhimurium has also been described by other studies which reported the acquisition of resistance mechanisms was through mobile genetic elements [23, 24]. The precise causes of the observed capacity of resistance mechanism accumulation and high prevalence of MDR *Shigella* and *S.* Typhimurium remain unknown. In contrast to *Salmonella* and *E. coli* which are widely distributed, humans and a few primates are the only reservoirs of *Shigella*, and its circulation in humans is through person-to-person contact or consumption of contaminated food, which is facilitated by the low infective dose of *Shigella* (as few as 10 viable organisms). The prevalence of antimicrobial resistance in *Shigella* should be highly related to the volume and categories of antimicrobial consumption in the local community. Inappropriate prescription and easy access to antimicrobials by outpatients could facilitate the selection and spreading of MDR *Shigella* isolates in the local community. Studies have estimated that half of outpatient antimicrobials were prescribed for inappropriate indications, such as viral illness [25]. In China, inappropriate prescriptions are more common, especially through intravenous infusion. The over-the-counter sale of antimicrobials without prescription might be another reason for the resistance dissemination. Since most epidemic *Shigella* isolates in China are MDR, as shown in our data, the abuse of antimicrobials in the community might be the main reason for the wide dissemination of MDR *Salmonella* and *Shigella*.

Fluoroquinolones and third-generation cephalosporins were two popular empirical options to treat severe gastrointestinal infections caused by pathogenic bacteria [26, 27]. However, more than 70% of *Salmonella* and 100% of *Shigella* isolates in this study exhibited resistance to nalidixic acid and decreased susceptibility to ciprofloxacin. These isolates had a high probability of becoming ciprofloxacin resistant, thereby causing treatment failure, as shown in *Salmonella* infection [28]. Besides these, more than 30% of *S.* Typhimurium and *Shigella* spp. isolates

showed resistance to ciprofloxacin. Widespread quinolone resistance in *Shigella* and *S.* Typhimurium has also been documented as a unique characteristic in other studies of China and some Asian countries with easy access of antimicrobials [23, 29]. However, in countries with strict antimicrobial controls, such as the USA, less than 1% of *Shigella* or *S.* Typhimurium isolates were resistant to nalidixic acid and few ciprofloxacin-resistant isolates were identified nationwide [30, 31]. These data further emphasize the necessity of strict antimicrobial application control in developing nations. Besides quinolone resistance, several cefotaxime-resistant *Salmonella* and *Shigella* isolates were also identified; even when only two isolates show co-resistance to both ciprofloxacin and cefotaxime the surveillance agencies should remain alert for the spreading of these isolates. The wide distribution of MDR *Salmonella* and *Shigella* isolates clearly raises the perennial issue of strict control of antimicrobial prescriptions and improved sanitation rather than new antibiotics, as a long-term solution to the plethora of salmonellosis and shigellosis.

Although this aetiological study involved 1526 subjects, it does have limitations. First, we did not include a case-control group as in other studies [15, 32]. Without the control group, we could not rule out the carriers, as indicated in other studies, and obtain the true infection rate and aetiological agents of infection. Compared to other studies, a lower ratio of outpatients with diarrhoeal pathogens was found, this might be caused by the techniques we used and the proficiency of staff. The aetiology of high ratio of unexplained diarrhoea warrants further investigation. Future investigations of the aetiology of diarrhoea should include appropriate control populations to assess the frequencies with which pathogens are found in asymptomatic subjects, and they should also incorporate techniques to identify other pathogens not sought in the present study. However, taken together, these data we present demonstrate the need for – and opportunities in – expanded diarrhoeal aetiological investigations in China.

In summary, aetiological data on diarrhoeal diseases and susceptibility patterns of diarrhoeal pathogens are important information for clinical manage-ment and control strategic planning. These data on the aetiology of diarrhoea and antimicrobial resistance patterns in China will have significant effect on public health policies and resource prioritization practices.

DECLARATION OF INTEREST

None.

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