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Complete List of Authors:	Gong, Xiaohuan; Shanghai Municipal Center for Disease Control and Prevention, Division of Infectious Disease Control and Prevention Wu, Huan-yu; Shanghai Municipal Center for Disease Control and Prevention, Division of Infectious Disease Control and Prevention Li, Jian; Shanghai Municipal Center for Disease Control and Prevention, Division of Infectious Disease Control and Prevention Xiao, Wen-jia; Shanghai Municipal Center for Disease Control and Prevention, Division of Infectious Disease Control and Prevention Zhang, Xi; Shanghai Municipal Center for Disease Control and Prevention, Microbiology Laboratory Chen, Min; Shanghai Municipal Center for Disease Control and Prevention, Microbiology Laboratory Teng, Zheng; Shanghai Municipal Center for Disease Control and Prevention, Microbiology Laboratory Pan, Hao; Shanghai Municipal Center for Disease Control and Prevention, Division of Infectious Disease Control and Prevention Yuan, Zheng-an ; Shanghai Municipal Center for Disease Control and Prevention
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Epidemiology, etiology and seasonality of infectious diarrhea in adult outpatients through active surveillance in Shanghai, China, 2012-2016

Xiao-huan Gong[†], Huan-yu Wu[†], Jian Li, Wen-jia Xiao, Xi Zhang, Min Chen, Zheng Teng, Hao Pan[*], Zheng-an Yuan[*]

[†] Equal contributor：Xiao-huan Gong, Huan-yu Wu

[*] Corresponding author:

Zheng-an Yuan

Email: yuanzhengan@scdc.sh.cn; Phone:+86 21 62758710

Hao Pan

Email: panhao@scdc.sh.cn; Phone:+86 21 62758710

Division of Infectious Disease Control and Prevention, Shanghai Municipal Center for Disease Control and Prevention, No. 1380, West Zhongshan Road, Shanghai 200336, China.

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Email addresses:

Xiao-huan Gong: gloriag1988@163.com

Huan-yu Wu: wuhuanyu@scdc.sh.cn

Jian Li: lijian@scdc.sh.cn

Wen-jia Xiao: xiaowenjia@scdc.sh.cn

Xi Zhang: zhangxi@scdc.sh.cn

Min Chen: chenmin@scdc.sh.cn

Zheng Teng: tengzheng@scdc.sh.cn

Hao Pan: panhao@scdc.sh.cn

Zheng-an Yuan: yuanzhengan@scdc.sh.cn

ABSTRACT

Objectives

This study aimed to identify the epidemiology, clinical characteristics, etiology and seasonality of sporadic infectious diarrhea in adults in Shanghai.

Setting

This study was based on a citywide, active continuous hospital-based diarrhea surveillance network established by Shanghai CDC. There were 22 sentinel hospitals in all 16 districts (9 primary-level hospitals, 6 secondary-level hospitals and 7 tertiary-level hospitals), which were selected using PPS sampling method.

Participants

From 1 May 2012 through 31 May 2016, 95284 patients were enrolled in surveillance system, of whom 90713 were included in this study. Among 8797 patients whose' stool samples were collected and detected, 4392 patients were male.

Results

The positive rate was 47.96%. Bacterial and viral infections accounted for 27.19% and 69.07% separately. Norovirus was the most common pathogen (43.10%), followed by rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. Patients between 30-44 and 45-59 years were more vulnerable to infectious diarrhea and viral diarrhea. Those aged 30-44 years were the most vulnerable to *V. parahaemolyticus* (aOR versus 60+ years: 2.04 [1.47-2.78]) and norovirus (aOR versus 60+ years: 1.32

[1.12-1.56]). Bacterial (except *V. parahaemolyticus*) diarrhea was characterized by fever, abdominal pain and loose stool; whilst viral diarrhea was characterized by nausea, vomiting and watery stool. A seasonal distribution of infectious diarrhea was observed with larger peaks in winter and smaller peaks in summer. Winter peaks were mainly due to norovirus and rotavirus, and summer peaks were due to bacterial infections. An emerging spring peak of norovirus around March was observed in the past 3 years.

Conclusion

Viral infections were predominant, and norovirus played a leading role. A seasonal distribution was observed and an emerging spring peak of norovirus was noted. Our findings highlight the necessity for conducting an active, comprehensive surveillance in adults, to monitor changing dynamics in the epidemiology and etiology of infectious diarrhea.

Key Words

Diarrhea, Surveillance, Epidemiology, Etiology, Sporadic, Bacteria, Virus, China

Strengths and limitations of this study

- ✧ This is the first study in Shanghai identifying the etiology and epidemiology of adult infectious diarrhea in sporadic outpatients from a continuous active diarrhea surveillance enhanced with comprehensive laboratory testing for common enteric bacteria and virus.
- ✧ Etiology of adult infectious diarrhea in Shanghai, including bacteria and virus, was detailed in this study.
- ✧ Seasonality of adult infectious diarrhea and relevant contribution of different enteric pathogens in seasonal trend were demonstrated in detail.
- ✧ Since information and detection results were collected from 22 hospitals and 16 laboratories, there was a chance of bias caused by the different levels and conditions of hospitals and laboratories. Also admission rate bias and recall bias was difficult to avoid.
- ✧ Only mild diarrhea patients were included in surveillance, severe diarrhea patients or asymptomatic patients were not studies in our research.
- ✧ As for seasonality, only descriptive data of every month or statistical tests of seasons were demonstrated. No statistical methods were used to analyze the successive time series.

BACKGROUND

Diarrhea is generally characterized by the frequent passage of loose or liquid stools. It is usually a symptom of gastrointestinal infections caused by bacterial, viral or parasitic pathogens, which spread through contaminated food or drinking-water or from person-to-person[1]. According to WHO, rotavirus and *Diarrheagenic Escherichia coli* (DEC) are the two most common etiological agents of diarrhea in developing countries[1]. However, norovirus was found the most prevalent pathogen of infectious diarrhea in adults in China CDC's research[2], and *Vibrio parahaemolyticus* (*V. parahaemolyticus*) was the most common enteric pathogen in acute bacterial gastroenteritis[3]. The etiology of infectious diarrhea differs among regions depending on economic development, local climate and geography [4, 5].

Nearly 1.7 billion cases and 1.3 million deaths due to diarrhea occur worldwide every year.[1, 6] Diarrhea causes substantial medical and healthcare costs and thus has a high economic impact on society[7]. Diarrhea remains one of the major causes of disease burden worldwide, despite significant progress in sanitation status and public health awareness. Mortality due to diarrhea fell 20% in recent 10 years, while it is still leading common cause of life loss (ranking fifth) globally[6]. To react to this worldwide health issue, we established the Shanghai Diarrhea Comprehensive Surveillance System since 2012, which is an active continuous surveillance system and which this research is based on.

Most of current studies of diarrhea have focused on children under 5 years old[8-12].. Consequently, limited data about the epidemiology and etiology of infectious diarrhea

in adults is available[13-15]. Although diarrhea accounts for only 2% deaths of adults[16], they may play a role in enteric infection transmission to other susceptible populations such as immunocompromised patients. Furthermore, there is rare research on the etiology of infectious diarrhea in adults in China[2, 3, 17, 18], especially based on a continuous active surveillance with comprehensive laboratory detection of enteric bacteria and viruses. Better understanding to the epidemiology, etiology and seasonality of infectious diarrhea in adults would be valuable for planning and adopting targeted preventive measures and antimicrobial therapy.

The objective of this study was to identify the epidemiology, clinical characteristics, etiology and pathogen seasonality of infectious diarrhea in adult sporadic outpatients through an active continuous hospital-based diarrhea surveillance in Shanghai, and to explore to develop targeted policy of disease prevention and control in the future.

METHODS

Shanghai Diarrhea Comprehensive Surveillance System

The Shanghai Diarrhea Comprehensive Surveillance System conducts active, population-based surveillance on diarrhea outpatients. It consists of adult surveillance and children surveillance. The adult surveillance was established with 6 sentinel hospitals in May 2012, and incorporated 16 additional sentinel hospitals in August 2013. Municipal CDC, district CDCs and sentinel hospitals cooperate to maintain the surveillance, and share information and detection results through a dedicated online system. The 22 sentinel hospitals (9 primary-level hospitals, 6 secondary-level

hospitals and 7 tertiary-level hospitals) were selected using Probability Proportionate to Size (PPS) sampling method among all hospitals which had enteric disease clinics in all 16 districts of Shanghai. Different sampling intervals were allocated to different sentinel hospitals considering the hospitals' location, classification and annual number of diarrhea patients comprehensively, for use of collecting fecal specimens.

Surveillance subjects were defined as patients who visited the enteric disease clinics of sentinel hospitals, with 3 or more loose or liquid stools per day, or more frequently than normal for the individual (World Health Organization's definition of diarrhea)[19]. All surveillance subjects were interviewed by doctors using a standardized questionnaire in hospitals. Demographic, epidemiological and medical information of all surveillance subjects was obtained and recorded into the dedicated online system. Epidemiologically-linked outbreak cases were excluded via inquiry.

Laboratory Tests

Fecal specimens were collected from surveillance subjects in accordance with sampling intervals by trained medical staff, as a part of standard medical care. If the sampling interval of a sentinel hospital is X, then fecal specimens are collected from the Xth, 2Xth, 3Xth,…nXth surveillance subjects in this sentinel hospital. Approximately 8~10g (mL) of stool was collected and then dispensed into two containers: (1) a tube with Cary-Blair (C-B) culture medium for bacteria testing and (2) a sterile plastic cup for virus testing. Nucleic Acid was extracted from fecal specimens (20% w/v or v/v suspensions in PBS) using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).

All specimens were detected for 8 bacterial pathogens [*Vibrio cholera (V. cholera)*, *Shigella* spp., *Salmonella* spp., *V. parahaemolyticus*, *Campylobacter jejuni* (*C. jejuni*), *Yersinia enterocolitica (Y. enterocolitica)*, *Campylobacter coli* (*C. coli*), DEC (including EPEC, ETEC, EHEC, EAggEC, EIEC)], and 5 viral pathogens (norovirus, rotavirus, astrovirus, sapovirus, and adenovirus). Bacteria were isolated using different mediums at proper temperatures after preparation. The mediums included ChromID *Vibrio* and TCBS for *V. cholera* and *V. parahaemolyticus*, MAC for DEC, XLD for *Shigella* spp. and *Salmonella* spp., etc.. Bacteria were identified using biochemical tests. An automatic biochemical identification system was used for DEC. Serum agglutination tests were employed to subtype *Shigella* spp., *Salmonella* spp., *V. cholera* and DEC. Astrovirus, norovirus, sapovirus and rotavirus were detected using real-time reverse transcription- polymerase chain reaction assays (rRT-PCR) and adenovirus was detected using rPCR. All molecular assays were performed using the appropriate respective commercial kits (Shanghai Zhijiang Biotechonology Co., Ltd.) according to the manufacturer's instructions.

Samples were scored as positive if at least one of enteric pathogens was isolated or identified. A bacterial infection means enteric bacteria was isolated and no viruses were identified. A viral infection means enteric virus was identified and no bacteria were isolated. Samples were scored as simplex infection if one of the 13 enteric pathogens was isolated or identified; as a mixed infection if at least two of these pathogens were isolated or identified; as a bacterial-viral mixed infection if at least one bacteria was isolated and one virus was identified.

Statistical Analysis

Data were analyzed using Statistical Analysis Software (SAS) version 9.3. Numbers and percentages were computed for categorical variables. Cochren-Mantel-Haenszel test was used for comparison of categorical variables. Binary logistic model and general logit model were used for binary dependent variables and multi-category disordered dependent variables respectively, to calculated adjusted odds ratio (aOR) and to explore the association between etiology and characteristics of infectious diarrhea after adjusting for confounders. Variables of age group, suburb, gender, season, and epidemiological histories were put into model and selected by stepwise methods. Two-tailed p values < 0.05 was considered statistically significant.

This study focused on the adult diarrhea patients with age $\geqslant$18 years. Age group was defined as 18-29,30-44, 45-59, and 60+ years, according to the Global Burden of Disease 2000 and surveillance diarrhea patients 'age distribution[20]. Season was defined by the climatic characteristics of Shanghai, spring means March to May, and summer means June to August, and autumn means September to November, and winter means December to February.

Ethics

The study protocol was approved by the Institutional Ethics Review Committee of the Shanghai Municipal Center for Disease Control and Prevention.

RESULTS

From 1 May 2012 through 31 May 2016, a total of 95284 patients were enrolled in

Shanghai diarrhea comprehensive surveillance system, of whom 4571 (4.80%) were not included in this study for the following reasons: 401 (0.42%) patients did not report clinical signs of diarrhea, 379 (0.40%) patients visited the enteric disease clinics within 14 days and thus were considered as the same episodes, 11 (0.01%) patients sought clinical care > 60 days after onset of diarrhea, 212 (0.22%) patients were not infectious diarrhea with other explicit diagnosis, and 3568 (3.74%) patients were younger than 18 years. Among 90713 adult diarrhea patients, 8797 (9.70%) patients' stool samples were collected and detected. These 8797 patients were included for further analysis.

1. Prevalence of Enteric Bacteria and Viruses

A total of 4657 pathogens were identified or isolated from 4219 (positive rate 47.96%) stool samples of the 8797 samples. There are 1147 bacterial infections (27.19%), 2914 viral infections (69.07%) and 158 bacterial-viral mixed infections (infected with at least 1 bacteria and 1 virus, 3.74%). Excluding mixed-infection samples, *V. parahaemolyticus* infections, DEC infections and *Salmonella* spp. infections were the most frequently bacterial infections, respectively with positive rate 4.50%, 3.43% and 2.90%. Excluding mixed-infection samples, norovirus infections and rotavirus infections were the most frequently viral infections, with positive rates 19.82% and 8.12%, respectively. Positive rates of other enteric viral infections were as follows: sapovirus, 1.93%; astrovirus, 1.56%; and adenovirus, 0.35%. Positive rates of enteric bacterial infections were as follows: *C.jejuni*, 1.13%; *Shigella* spp., 0.22%; *C. coli*, 0.08%; *Y. enterocolitica*, 0.01%; and *Staphylococcus aureus*, 0.01%. In addition, there

were 343 (3.90%) mixed infections.

Isolated DEC consisted of 216 ETEC, 131 EPEC, 84 EAggEC, 2 EIEC and 1 EHEC. Identified noroviruses consisted of 281 GI and 1726 GII. Identified rotaviruses consisted of 766 rotavirus group A, 6 rotavirus group B and 15 rotavirus group C.

2. Demographic and Epidemiological Characteristics

The median age was 46 (IQR 30-60) years. Of 8797 patients, 22.94% aged 18-29 years, 24.57% aged 30-44 years, 25.79% aged 45-59 years, and 26.70% aged equal to or older than 60 years. A significantly difference in distribution of age groups could be found among comparison of positive and negative diarrhea patients (p=0.0150), comparison of bacterial and viral and bacterial-viral infections (p=0.0074), and comparison of different enteric pathogens infections (p<0.0001) (Table 1). There were 4392 (49.93%) male patients, with a higher male proportion in positive diarrhea patients (p=0.0472), DEC infections (aOR=1.29, 95%CI=1.02-1.64) and norovirus infections (aOR=1.22, 95%CI=1.08-1.36) (Table 1 and Table 2).

Table1 Demographic and epidemiological characteristics of diarrhea outpatient adults by different infections

	Positive (n=4219)	Negative (n=4578)	*P*	Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)	*P*	*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)	Other infections§ (n=808)	*P*
Gender, N (%)														
Male	2153 (51.03)	2239 (48.91)	**0.0472**	577 (50.31)	1497 (51.37)	79 (50.00)	0.8005	184 (46.46)	164 (54.30)	128 (50.20)	946 (54.24)	326 (45.66)	405 (50.12)	**0.0011**
Age, N (%)														
18-29 years	941 (22.32)	1074 (23.52)	**0.0150**	292 (25.48)	611 (20.97)	38 (24.20)	**0.0074**	109 (27.53)	74 (24.50)	43 (16.93)	384 (22.03)	118 (16.53)	213 (26.39)	**<0.0001**
30-44 years	1084 (25.71)	1074 (23.52)		298 (26.00)	748 (25.68)	38 (24.20)		119 (30.05)	72 (23.84)	57 (22.44)	473 (27.14)	158 (22.13)	205 (25.40)	
45-59 years	1112 (26.38)	1153 (25.25)		294 (25.65)	768 (26.36)	50 (31.85)		105 (26.52)	78 (25.83)	76 (29.92)	426 (24.44)	231 (32.35)	196 (24.29)	
60+ years	1079 (25.59)	1266 (27.72)		262 (22.86)	786 (26.98)	31 (19.75)		63 (15.91)	78 (25.83)	78 (30.71)	460 (26.39)	207 (28.99)	193 (23.92)	
Living region, , N (%)														
Suburb	2401 (56.91	2975 (64.98)	**<0.0001**	665 (57.98)	1645 (56.45)	91 (57.59)	0.6661	257 (64.90)	170 (56.29)	149 (58.43)	1019 (58.43)	403 (56.44)	403 (49.88)	**<0.0001**
Epidemiological history , N (%)														
Had a medical history of enteric disease in the past 6 months	17 (0.40)	47 (1.03)	**0.0006**	5 (0.44)	12 (0.41)	0 (0.00)	0.7132	1 (0.25)	1 (0.33)	2 (0.78)	8 (0.46)	2 (0.28)	3 (0.37)	0.9001
Had consumed suspicious food within 5 days before onset	1914 (45.37)	1865 (40.74)	**<0.0001**	490 (42.72)	1350 (46.33)	74 (46.84)	0.1073	179 (45.20)	111 (36.75)	117 (45.88)	847 (48.57)	282 (39.50)	378 (46.78)	**<0.0001**
Had went out within 7 days before onset	78 (1.85)	46 (1.00)	**0.0010**	29 (2.53)	48 (1.65)	1 (0.63)	0.0881	7 (1.77)	8 (2.65)	5 (1.96)	34 (1.95)	6 (0.84)	18 (2.23)	0.3226
Had kept or had contact with pets.	814 (19.29)	604 (13.19)	**<0.0001**	224 (19.53)	556 (19.08)	34 (21.52)	0.7304	55 (13.89)	65 (21.52)	41 (16.08)	323 (18.52)	123 (17.23)	207 (25.62)	**<0.0001**

§ Simplex infections;

Bold face: $P<0.05$

Table2 Adjusted odds ratio of demographic and epidemiological characteristics comparing positive detection with negative detection in diarrhea outpatients*

	Positive (n=4219)		Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)		*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)
Male vs female	1.09 (1.00-1.19)		1.07 (0.94-1.22)	1.1 (0.99-1.22)	1.04 (0.75-1.43)		0.89 (0.72-1.09)	**1.29 (1.02-1.64)**	1.11 (0.86-1.44)	**1.22 (1.08-1.36)**	0.88 (0.75-1.05)
Age (years)											
18-29	1.10 (0.97-1.25)		**1.32 (1.09-1.59)**	0.99 (0.85-1.14)	1.52 (0.93-2.44)		**1.92 (1.41-2.7)**	1.11 (0.79-1.54)	**0.64 (0.44-0.94)**	1.03 (0.88-1.22)	**0.75 (0.58-0.97)**
30-44	**1.28 (1.14-1.45)**		**1.28 (1.06-1.56)**	**1.28 (1.11-1.47)**	1.54 (0.94-2.50)		**2.04 (1.47-2.78)**	1.02 (0.73-1.43)	0.83 (0.58-1.19)	**1.32 (1.12-1.56)**	1.08 (0.84-1.35)
45-59	**1.19 (1.06-1.35)**		1.2 0 (1.00-1.47)	**1.16 (1.01-1.33)**	**1.85 (1.18-2.94)**		**1.72 (1.25-2.38)**	1.06 (0.77-1.47)	1.06 (0.76-1.47)	1.09 (0.93-1.28)	**1.33 (1.08-1.67)**
60+¶	1.00		1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
Suburb	**0.80 (0.72-0.88)**		**0.80 (0.68-0.92)**	**0.80 (0.71-0.89)**	0.83 (0.58-1.19)		0.96 (0.76-1.23)	**0.75 (0.57-0.98)**	0.82 (0.62-1.09)	**0.85 (0.75-0.97)**	**0.79 (0.66-0.95)**
Had a medical history of enteric disease in the past 6 months	**0.41 (0.23-0.73)**		0.42 (0.17-1.08)	**0.43 (0.22-0.85)**	0		0.24 (0.03-1.76)	0.34 (0.05-2.46)	0.71 (0.17-2.94)	0.47 (0.21-1.01)	0.32 (0.07-1.39)
Had consumed suspicious food within 5 days before onset	**1.18 (1.08-1.29)**		1.06 (0.93-1.22)	**1.24 (1.12-1.38)**	1.26 (0.82-1.75)		1.22 (0.99-1.51)	0.84 (0.66-1.08)	0.24 (0.96-1.61)	**1.31 (1.17-1.48)**	0.99 (0.83-1.18)
Had kept or had contact with pets	**1.33 (1.17-1.5)**		**1.57 (1.30-1.90)**	**1.21 (1.04-1.40)**	**1.62 (1.05-2.48)**		1.17 (0.85-1.63)	**1.79 (1.29-2.47)**	1.20 (0.83-1.75)	**1.26 (1.06-1.48)**	1.00 (0.78-1.27)

* Data are adjusted odds ratio (95%CI) in binary logistic model or general logit model

§ Simplex infections

¶ Reference group in logistic regression model

Bold face: $P<0.05$

Adjusted odds ratios of age were shown in Table 2. Patients between 30-44 and 45-59 years were more vulnerable to infectious diarrhea and viral diarrhea. Those aged 30-44 years were the most vulnerable to *V. parahaemolyticus* (aOR versus 60+ years group: 2.04 [1.47-2.78]) and norovirus (aOR versus 60+ years group: 1.32 [1.12-1.56]). In addition, patients in 18-29 years group had a significantly lower odds of experiencing infectious diarrhea (aOR=0.85, 95% CI=0.76-0.97), viral infections (aOR=0.78, 95% CI=0.67-0.90), norovirus infections (aOR=0.78, 95% CI=0.66-0.92) and rotavirus infections (aOR=0.70, 95% CI=0.54-0.92) compared with 30-44 years group. Patients in 18-29 years group had a significantly lower odds of experiencing viral infections (aOR=0.85, 95% CI=0.74-0.98), *Salmonella* spp. infections (aOR=0.61, 95% CI=0.41-0.89) and rotavirus infections (aOR=0.56, 95% CI=0.44-0.72) compared with 45-59 years group. Patients in 30-44 years group had a significantly higher odds experiencing norovirus infections (aOR=1.22, 95% CI=1.03-1.43) compared with 40-45 years group.

Among diarrhea patients, 5376 (85.67%) visited the hospitals in suburb. Less positive patients lived in suburb area compared with negative patients ($p<0.0001$, Table 1). Comparing different enteric pathogen infections, the proportions of patients in suburb were significantly different ($p<0.0001$). More diarrhea patients infected with *V. parahaemolyticus* (64.90%) lived in suburb area. Patients living in suburb area were less likely to get infected with enteric pathogens (aOR=0.75-0.85) except *V. parahaemolyticus* infections and *Salmonella* spp. infections (Table 2).

64 (0.73%) patients had a medical history of enteric disease in the past 6 months.

Within 5 days before onset, 3779 (42.96%) patients had a history of consuming suspicious food. 124 (1.41%) patients had a history of go out within 7 days before onset. And 1418 (16.12%) patients kept or had contact with pets. When compared with negative patients, a higher proportion of positive patients has a history of consuming suspicious food within 5 days before onset ($p<0.0001$), had a history of going out within 7 days before onset ($p=0.0010$), and kept or had contact with pets ($p<0.0001$), while a lower proportion had a medical history of enteric disease in the past 6 months ($p=0.0006$) (Table 1). Epidemiological history, including consuming suspicious food and keeping or contacting with pets, was significantly associated with higher odds of infectious diarrhea, viral infections and norovirus infections. A medical history of enteric disease was significantly associated with lower odds of infectious diarrhea (Table 2).

3. Clinical Symptoms

Of positive diarrhea patients, 13.11% reported fever, 41.91% reported nausea, 28.21% reported vomiting, and 49.09% reported abdominal pain (Table 3). Watery stool and loose stool were common, respectively accounting 76.27% and 20.93%. Compared with negative diarrhea patients, positive patients reported more fever ($p=0.0009$), nausea ($p<0.0001$), vomiting ($p<0.0001$) and watery stool ($p<0.0001$), while fewer abdominal pain (<0.0001).

The distributions of clinical symptoms by different infections were significantly different (Table 3). Diarrhea patients infected with bacteria reported more fever (19.09%, $p<0.0001$), abdominal pain (64.60%, $p<0.0001$) and loose stool (23.28%,

p<0.0001). Diarrhea patients infected with virus reported more nausea (43.34%%, p=0.0175), vomiting (30.13%, p=0.0001) and watery stool (78.35%, p<0.0001). Diarrhea patients infected with *V. parahaemolyticus* featured more nausea (56.27%), vomiting (41.41%), abdominal pain (71.9%) and watery stool (81.57%). Patients infected with DEC featured fewer nausea (28.81%) and vomiting (13.58%). Patients infected with *Salmonella* spp. featured more fever (28.24%). Patients infected with norovirus featured fewer fever (9.69%) and abdominal pain (44.55%).

Table3 Clinical symptoms in diarrhea outpatients by different infections

	Positive (n=4219)	**Negative (n=4578)**	***P***	**Bacterial infections (n=1147)**	**Viral infections (n=2914)**	**Bacterial-viral Mixed infections (n=158)**	***P***	***V. parahaemolyticus*** **§ (n=396)**	**DEC§ (n=302)**	***Salmonella* spp.§ (n=255)**	**Norovirus§ (n=1744)**	**Rotavirus§ (n=714)**	**Other infections§ (n=808)**	***P***
Fever, N (%)	553 (13.11)	495 (10.81)	**0.0009**	219 (19.09)	312 (10.71)	22 (13.92)	**<0.0001**	46 (11.62)	43 (14.24)	72 (28.24)	169 (9.69)	96 (13.45)	127 (15.72)	**<0.0001**
Nausea, N (%)	1768 (41.91)	1561 (34.10)	**<0.0001**	442 (38.54)	1263 (43.34)	63 (39.87)	**0.0175**	224 (56.27)	87 (28.81)	71 (27.84)	790 (45.30)	309 (43.28)	287 (35.52)	**<0.0001**
Vomiting, N (%)	1190 (28.21)	916 (20.01)	**<0.0001**	269 (23.45)	878 (30.13)	43 (27.22)	**0.0001**	164 (41.41)	41 (13.58)	37 (14.51)	595 (34.12)	195 (27.31)	158 (19.55)	**<0.0001**
Abdominal pain, N (%)	2071 (49.09)	2446 (53.43)	**<0.0001**	741 (64.60)	1257 (43.14)	73 (46.20)	**<0.0001**	285 (71.97)	170 (56.29)	151 (59.22)	777 (44.55)	321 (44.96)	367 (45.42)	**<0.0001**
Fecal property, N (%)														
Watery	3218 (76.27)	3150 (68.81)	**<0.0001**	814 (70.97)	2283 (78.35)	121 (76.58)	**<0.0001**	323 (81.57)	202 (66.89)	179 (70.20)	1344 (77.06)	583 (81.65)	587 (72.65)	**<0.0001**
Loose	883 (20.93)	1202 (26.26)		267 (23.28)	583 (20.01)	33 (20.89)		54 (13.64)	85 (28.15)	61 (23.92)	372 (21.33)	121 (16.95)	190 (23.51)	
Mucous	72 (1.71)	143 (3.12)		38 (3.31)	31 (1.06)	3 (1.90)		8 (2.02)	11 (3.64)	11 (4.31)	18 (1.03)	6 (0.84)	18 (2.23)	
Else	46 (1.09)	83 (1.81)		28 (2.44)	17 (0.58)	1 (0.63)		11 (2.78)	4 (1.32)	4 (1.57)	10 (0.57)	4 (0.56)	13 (1.61)	

§ Simplex infections;

Bold face: $P<0.05$

4. Pathogen Spectrum and Seasonality

In term of descriptive data, the enteric pathogens spectrum of infectious diarrhea patients displayed a yearly seasonal trend (Figure 1). In general, viruses were predominant during November to March of every seasonal cycle, accounting for more than 80% in every month. Bacteria were predominant during June to August of almost every seasonal cycle, accounting for more than 60% in every month. September and October were the transition period from bacteria to viruses, and April and May were the transition period from viruses to bacteria. Norovirus and rotavirus both showed yearly seasonal trends. Rotavirus peaked in winter months, especially in December and January. Norovirus displayed a less distinct and broader seasonality. Norovirus clustered around autumn and winter, while a smaller peak appeared in March of 2014 and 2015. In the seasonal cycle from 2015-2016, norovirus peaked in March 2016. *V. parahaemolyticus*, DEC and *Salmonella* spp. all showed yearly seasonal trends. These three enteric bacteria peaked in August, and *Salmonella* spp. showed a smaller peak (Figure 2).

Figure 1 insert here.

Figure 2 insert here.

In term of statistical analysis, there were significantly different season distribution in comparison of positive and negative diarrhea patients ($p<0.0001$), comparison of bacterial and viral and bacterial-viral infections ($p<0.0001$), and comparison of different enteric pathogens infections ($p<0.0001$). More bacterial infections appeared in summer (54.58%) and more viral infections appeared in winter (44.51%). The

proportion of winter was lower among norovirus infections (34.86%) compared with among rotavirus infections (67.37%).

Patients in summer were 1.55-4.39 times more likely to have simplex bacterial diarrhea and 0.16-0.20 times less likely to have simplex viral diarrhea compared with in spring. Patients in autumn were 2.02-3.38 times more likely to have *V. parahaemolyticus* infections and DEC infections, and 0.69-0.77 times less likely to have simplex viral diarrhea compared with in spring. Patients in winter were 1.60-5.61 times more likely to have simplex viral infections, and 0.14-0.56 times less likely to have simplex bacterial diarrhea compared with in spring (Table 4).

Table4 Seasonality of diarrhea outpatients by different infections*

	Negative (n=4578)	Positive (n=4219)		Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)		*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)
Season [(No.(%)]		***P*<0.0001**		***P* <0.0001**				***P* <0.0001**				
Spring	867 (18.94)	877 (20.79)		149 (12.99)	695 (23.85)	33 (20.89)		34 (8.59)	21 (6.95)	41 (16.08)	462 (26.49)	101 (14.15)
Summer	1746 (38.14)	927 (21.97)		626 (54.58)	260 (8.92)	41 (25.95)		252 (63.64)	178 (58.94)	123 (48.24)	180 (10.32)	32 (4.48)
Autumn	1238 (27.04)	1031 (24.44)		322 (28.07)	662 (22.72)	47 (29.75)		106 (26.77)	96 (31.79)	72 (28.24)	494 (28.33)	100 (14.01)
Winter	727 (15.88)	1384 (32.80)		50 (4.36)	1297 (44.51)	37 (23.42)		4 (1.01)	7 (2.32)	19 (7.45)	608 (34.86)	481 (67.37)
Season [aOR (95%CI)]												
Spring^		1.00		1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
summer		**0.54 (0.48-0.61)**		**2.16 (1.77-2.64)**	**0.18 (0.16-0.23)**	0.62 (0.39-1.00)		**3.65 (2.53-5.29)**	**4.39 (2.77-6.96)**	**1.55 (1.07-2.23)**	**0.20 (0.17-0.24)**	**0.16 (0.10-0.24)**
autumn		**0.85 (0.75-0.97)**		1.59 (0.28-1.97)	**0.69 (0.60-0.79)**	1.04 (0.66-1.65)		**2.2 (1.48-3.28)**	**3.38 (2.09-5.47)**	1.26 (0.85-1.87)	**0.77 (0.66-0.9)**	**0.69 (0.52-0.93)**
winter		**1.91 (1.67-2.18)**		**0.40 (0.29-0.56)**	**2.26 (1.98-2.59)**	1.36 (0.84-2.20)		**0.14 (0.05-0.40)**	**0.39 (0.16-0.92)**	**0.56 (0.32-0.97)**	**1.60 (1.37-1.88)**	**5.61 (4.42-7.11)**

* Data are adjusted odds ratio (95%CI) in binary logistic model or general logit model

§ Simplex infections;

¶ Reference group in logistic regression model

Bold face: $P<0.05$

DISCUSSION

This study is the first study in Shanghai identifying the etiology and epidemiology of adult infectious diarrhea in sporadic outpatients from a continuous active diarrhea surveillance enhanced with comprehensive laboratory testing for common enteric bacteria and virus. It also adds to the limited number of studies investigating adult cases of infectious diarrhea in China. The Shanghai Diarrhea Comprehensive Surveillance System used Probability Proportionate to Size (PPS) sampling method and was conducted among 22 sentinel hospitals in all 16 districts of Shanghai continuously since May 2012, data from which are more representative and more feasible to be extrapolated to the city's population by avoiding the influence of clusters and season-specific cases.

Etiology of adult infectious diarrhea in Shanghai was detailed in this study. At least one enteric pathogen was found in 47.96% adult diarrhea patients' stools. Viral infections are predominant and bacteria were isolated from many cases. These findings were consistent with those Wang 's research in Beijing[2]. The comparison between our study with another research in Africa, which found bacterial more frequent than virus in diarrhea patients, showed that the etiology of infectious diarrhea has obvious divergence among regions depending on economic development and geography [4, 5].

We found that norovirus was the most common enteric pathogen, accounting for over 40% of all cases, followed by rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. The proportion of norovirus was higher than the sum proportion of rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. This result confirmed norovirus's leading role in adult infectious diarrhea in China, and was similar to the research finding in sporadic gastroenteritis in both developing and developed countries [21-24]. And it is observed that norovirus infections were more than twice as that of rotavirus in adult patients in Shanghai. Rotavirus ranked second to norovirus. This results was consistent with studies in Russia [24] and Shanghai, China[25], while inconsistent with study in France[26]. Yet according to WHO, rotavirus are most common etiological

agents of diarrhea in developing countries, which may due to rotavirus's important role in children. As leading cause of severe diarrhea in children, the pathogenic role and disease burden of rotavirus in adults had been underestimated. Rotavirus needs more attention in routine clinical diagnosis and vaccination program.

According to this study, *V. parahaemolyticus*, DEC and *Salmonella* spp. were common bacteria in adult infectious diarrhea. The prevalence of these three bacterial infections was similar between 2.90~4.50%, much lower than viral infections. In previous studies，*V. parahaemolyticus*, DEC and *Salmonella* spp. were also among the most prevalent pathogen in adult infectious diarrhea in different regions of China and worldwide[1, 2, 17, 18, 27]. Although diarrhea due to *V. parahaemolyticus* decreased since 1998[28, 29], *V. parahaemolyticus* was still the leading cause of adult bacterial infections in this study. However, *Shigella* spp. was also among frequent bacteria in several studies before 2013[18, 28, 29]. This study showed that positive rate of *Shigella* spp. infections was only 0.22% during 2012-2016 in Shanghai, which may due to the downward trend of *Shigella* spp. infections over time [29].

This study showed that there was association between adult infectious diarrhea and patient age. In general, patients between 30-59 years were more likely to have infectious diarrhea and viral diarrhea than age groups of 18-29 and 60+ years. This was partly consistent with a study in France which found incidence of acute diarrhea in youth group was higher than elderly group [26]. Elderly people (≥60years) were the least likely to get infected with *V. parahaemolyticus*, whereas people aged 30-44 years were the most likely among adult age groups. The similar findings were observed in a study in Shanghai[29]. This may be related to more seafood consumption in young adults, which is an important risk factor in *V. parahaemolyticus* infections[30] .In contrast to other studies which found elderly people more vulnerable to norovirus[22, 31], our study discovered that the highest proportion in norovirus infections was 30-44 years old. And considering the results of general logit model adjusting for other factors, 30-44 years patients were the most

vulnerable to norovirus. Patients aged 18-29 years had the lowest odds experiencing rotavirus diarrhea.

People living in rural area were more susceptible to DEC, norovirus and rotavirus, which may because city environment provided more chance for pathogen to transmit.

In regarding to clinical symptoms in general, bacterial diarrhea was characterized by fever, abdominal pain and loose stool, while viral diarrhea was characterized by nausea, vomiting and watery stool. However the symptom of *V. parahaemolyticus* infections showed more like viral infections. In addition, abdominal pain was common in *V. parahaemolyticus* infections. These findings of *V. parahaemolyticus* were in accordance with a research in Shanghai during 1998-2013[28]. The symptoms of DEC and *Salmonella* spp. were similar except fever. The proportion of fever was highest in *Salmonella* spp. (28.24%) while lowest in norovirus (9.69%). The proportion of fever in norovirus infections was much lower in comparison with some studies[26, 31], while the proportion in *Salmonella* spp. infections was close to another research [28]. The proportion of abdominal pain was highest in *V. parahaemolyticus* (71.97%) while much lower in norovirus (44.55%) and rotavirus (44.96%).

This study also demonstrated the seasonality of adult infectious diarrhea and relevant contribution of different enteric pathogens in seasonal trend. A seasonal distribution of adult infectious diarrhea was observed with a large peak in winter and a small peak in summer. Winter peak was mainly due to norovirus and rotavirus, which was in line with previous study[32, 33]. Summer peak was smaller, due to low proportion of bacterial infections. What should be noted was that there was a peak around March due to norovirus in 2014-2016, ever higher than the summer peak in 2015-2016 season cycle. This emerging spring peak was possibly because of the increased activity of a novel norovirus GII.17 [34]. Rotavirus showed a distinct peak in December and January (significantly winter VS summer aOR=35.67), which was consistent with researches in Shanghai and Iran [25, 35], while different from a study in London (peak from January through May) [36] and a study in Russia (peak from December through May) [24]. However, norovirus

displayed a broader seasonality peaking around autumn and winter (significantly winter VS summer OR=8.00) in this study and a study in Netherland [9]. Bacterial infections, included *V. parahaemolyticus*, DEC and *Salmonella* spp., showed a yearly seasonality peaking in summer (often in August), with significantly summer VS winter OR 25.00, 11.11 and 2.78 respectively. This was similar in Enserink's study [9], whereas autumn peak of bacterial infections was observed in some studies [25, 37]. The seasonality of infectious diarrhea may due to the climate, biological characteristics of pathogens and people's diet habit of Shanghai.

There are several limitations that need to be acknowledged. First, information and detection results were collected from 22 hospitals and 16 laboratories. Though detection methods & materials were unified and regular trainings were hold, there was still a chance of bias caused by the different levels and conditions of hospitals and laboratories. Admission rate bias should also be taken into consideration as patients may have a preference when visiting hospitals of different levels or in different regions. Second, the recall bias of epidemiological information was difficult to avoid. And the data of exposure history was important for infectious diarrhea. Third, only mild diarrhea patients were included in surveillance, severe diarrhea patients or asymptomatic patients were not studies in our research. Fourth, as for seasonality, only descriptive data of every month or statistical tests of seasons were demonstrated. No statistical methods were used to analyze the successive time series, which was because of the limit seasonal cycles of existing data. In the future, after accumulating enough data during several years, time series analysis could be taken to explore the inherent natural order and to forecasting prospective trend.

CONCLUSIONS

In conclusion, this study provides a detailed picture about the epidemiology, etiology and seasonal pathogen spectrum of adult infectious diarrhea in Shanghai. Viral infections are predominant, and norovirus is the most common enteric pathogen detected in our surveillance. Other common pathogens include rotavirus, *V.*

parahaemolyticus, DEC and *Salmonella* spp.. Patients between 30-59 years were more vulnerable to infectious diarrhea and viral diarrhea. A seasonal distribution was observed with larger peaks in winter and smaller peaks in summer. Winter peak was mainly due to norovirus and rotavirus, and summer peak was due to bacterial infections. An emerging spring peak of norovirus around March was observed in recent 3 years. Our findings highlight the necessity for conducting an active, comprehensive surveillance for both bacterial and viral enteric pathogens in adults, to monitor the changing dynamics in the epidemiology and etiology of infectious diarrhea. These findings promote to understand adult infectious diarrhea thoroughly and to develop targeted prevention strategies.

LIST OF ABBREVIATIONS

aOR: Adjusted odds ratio; *C. coli*: *Campylobacter coli*; *C. jejuni*: *Campylobacter jejuni*; DEC*: Diarrheagenic Escherichia coli*; EAggEC: Enteroaggregative escherichia coli; EHEC: Enterohemorrhagic escherichia coli; EIEC: Enteroinvasive escherichia coli; EPEC: Enteropathogenic escherichia coli; ETEC: Enterotoxigenic escherichia coli; PPS: Probability Proportionate to Size; rRT-PCR: real-time Reverse Transcription -Polymerase Chain Reaction; SAS: Statistical Analysis Software; *V. cholera: Vibrio cholera; V. parahaemolyticus*: *Vibrio parahaemolyticus;* WHO: World Health Organization; *Y. enterocolitica: Yersinia enterocolitica;*

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Xiao-huan Gong performed the statistical analysis and drafted the manuscript. Hao Pan and Huan-yu Wu placed the surveillance system into effect. Jian Li designed the study of the surveillance system. Wen-jia Xiao participated in the management of the system. Xi Zhang, Min Chen and Zheng Teng carried out the

management and quality control of the laboratory tests. Zheng-an Yuan conceived of the study. All authors read and approved of the final manuscript.

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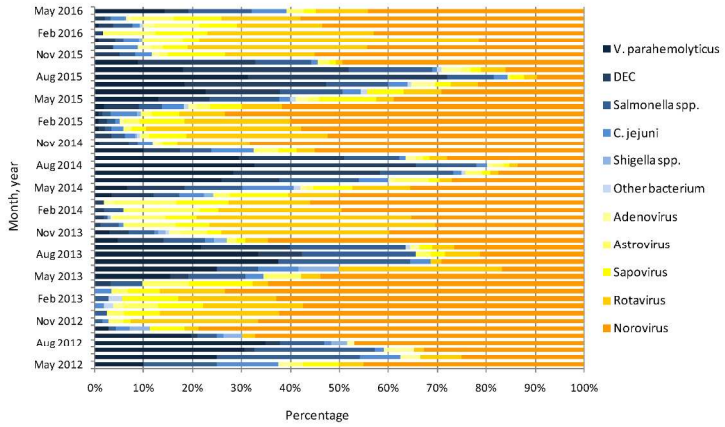

Figure 1 Pathogen spectrum of major enteric pathogens in adults with infectious diarrhea by month in Shanghai, May 2012-May 2016

Figure 1 Pathogen spectrum of major enteric pathogens in adults with infectious diarrhea by month in Shanghai, May 2012-May 2016

297x210mm (300 x 300 DPI)

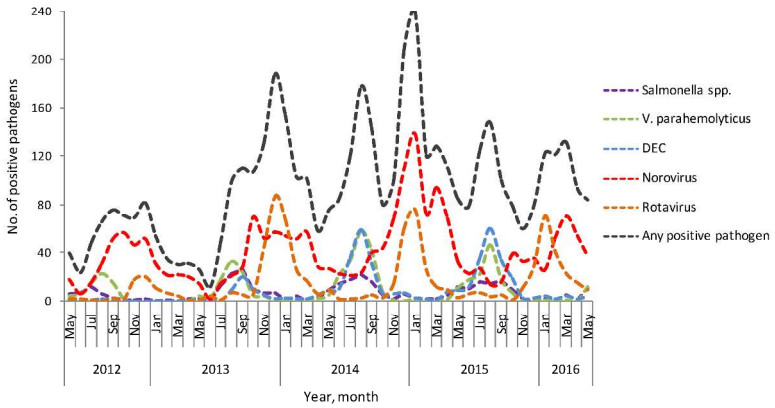

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

297x210mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7-8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10
Bias	9	Describe any efforts to address potential sources of bias	7-10
Study size	10	Explain how the study size was arrived at	7-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	7-8/10
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10-11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-21
		(*b*) Report category boundaries when continuous variables were categorized	10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	22-26
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	22-26
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	27

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiology, etiology and seasonality of infectious diarrhea in adult outpatients through active surveillance in Shanghai, China, 2012-2016: a cross-sectional study

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Keywords:	INFECTIOUS DISEASES, BACTERIOLOGY, VIROLOGY

Epidemiology, etiology and seasonality of infectious diarrhea in adult outpatients through active surveillance in Shanghai, China, 2012-2016: a cross-sectional study

Xiao-huan Gong[†], Huan-yu Wu[†], Jian Li, Wen-jia Xiao, Xi Zhang, Min Chen, Zheng Teng, Hao Pan[*], Zheng-an Yuan[*]

[†] Equal contributor：Xiao-huan Gong, Huan-yu Wu

[*] Corresponding author:

Zheng-an Yuan

Email: yuanzhengan@scdc.sh.cn; Phone:+86 21 62758710

Hao Pan

Email: panhao@scdc.sh.cn; Phone:+86 21 62758710

Division of Infectious Disease Control and Prevention, Shanghai Municipal Center for Disease Control and Prevention, No. 1380, West Zhongshan Road, Shanghai 200336, China.

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Email addresses:

Xiao-huan Gong: gloriag1988@163.com

Huan-yu Wu: wuhuanyu@scdc.sh.cn

Jian Li: lijian@scdc.sh.cn

Wen-jia Xiao: xiaowenjia@scdc.sh.cn

Xi Zhang: zhangxi@scdc.sh.cn

Min Chen: chenmin@scdc.sh.cn

Zheng Teng: tengzheng@scdc.sh.cn

Hao Pan: panhao@scdc.sh.cn

Zheng-an Yuan: yuanzhengan@scdc.sh.cn

ABSTRACT

Objectives

This study aimed to identify the epidemiology, clinical characteristics, etiology and seasonality of sporadic infectious diarrhea in adults in Shanghai.

Setting

This study was based on a citywide, active continuous hospital-based diarrhea surveillance network established by Shanghai CDC. There were 22 sentinel hospitals in all 16 districts (9 primary-level hospitals, 6 secondary-level hospitals and 7 tertiary-level hospitals), which were selected using Probability Proportionate to Size (PPS) sampling method.

Participants

From 1 May 2012 through 31 May 2016, 90713 patients were included in this study. Among 8797 patients whose' stool samples were collected and detected, 4392 patients were male.

Results

The positive rate was 47.96%. Bacterial and viral infections accounted for 27.19% and 69.07% separately. Norovirus was the most common pathogen (43.10%), followed by rotavirus, *V. parahaemolyticus*, *Diarrheagenic Escherichia coli* (DEC) and *Salmonella* spp.. Patients between 30-44 and 45-59 years were more likely to infectious diarrhea and viral diarrhea. Those aged 30-44 years were the most likely to

V. parahaemolyticus (aOR versus 60+ years: 2.04 [1.47-2.78]) and norovirus (aOR versus 60+ years: 1.32 [1.12-1.56]). Bacterial (except *V. parahaemolyticus*) diarrhea was characterized by fever, abdominal pain and loose stool; whilst viral diarrhea was characterized by nausea, vomiting and watery stool. A seasonal distribution of infectious diarrhea was observed with larger peaks in winter and smaller peaks in summer. Winter peaks were mainly due to norovirus and rotavirus, and summer peaks were due to bacterial infections. An emerging spring peak of norovirus around March was observed in the past 3 years.

Conclusion

Viral infections were predominant, and norovirus played a leading role. A seasonal distribution was observed and an emerging spring peak of norovirus was noted. Our findings highlight the necessity for conducting an active, comprehensive surveillance in adults, to monitor changing dynamics in the epidemiology and etiology of infectious diarrhea.

Key Words

Diarrhea, Surveillance, Epidemiology, Etiology, Sporadic, Bacteria, Virus, China

Strengths and limitations of this study

- ✧ This is the first study in Shanghai identifying the etiology and epidemiology of adult infectious diarrhea in sporadic outpatients from a continuous active diarrhea

surveillance enhanced with comprehensive laboratory testing for common enteric bacteria and virus.

- ✧ Etiology of adult infectious diarrhea in Shanghai, including bacteria and virus, was detailed in this study.
- ✧ Seasonality of adult infectious diarrhea and relevant contribution of different enteric pathogens in seasonal trend were demonstrated in detail.
- ✧ Since information and detection results were collected from 22 hospitals and 16 laboratories, there was a chance of bias caused by the different levels and conditions of hospitals and laboratories. Also admission rate bias and recall bias was difficult to avoid.
- ✧ As for seasonality, only descriptive data of every month or statistical tests of seasons were demonstrated. No statistical methods were used to analyze the successive time series.

BACKGROUND

Diarrhea is generally characterized by the frequent passage of loose or liquid stools. It is usually a symptom of gastrointestinal infections caused by bacterial, viral or parasitic pathogens, which spread through contaminated food or drinking-water or from person-to-person[1]. According to WHO, rotavirus and *Diarrheagenic Escherichia coli* (DEC) are the two most common etiological agents of diarrhea in developing countries[1]. However, norovirus was found the most prevalent pathogen

of infectious diarrhea in adults in China CDC's research[2], and *Vibrio parahaemolyticus* (*V. parahaemolyticus*) was the most common enteric pathogen in acute bacterial gastroenteritis[3]. The etiology of infectious diarrhea differs among regions depending on economic development, local climate and geography [4, 5].

Nearly 1.7 billion cases and 1.3 million deaths due to diarrhea occur worldwide every year.[1, 6] Diarrhea causes substantial medical and healthcare costs and thus has a high economic impact on society[7]. Diarrhea remains one of the major causes of disease burden worldwide, despite significant progress in sanitation status and public health awareness. Mortality due to diarrhea fell 20% in recent 10 years, while it is still leading common cause of life loss (ranking fifth) globally[6]. To react to this worldwide health issue, we established the Shanghai Diarrhea Comprehensive Surveillance System since 2012, which is an active continuous surveillance system and which this research is based on.

Most of current studies of diarrhea have focused on children under 5 years old[8-12].. Consequently, limited data about the epidemiology and etiology of infectious diarrhea in adults is available[13-15]. Although diarrhea accounts for only 2% deaths of adults[16], they may play a role in enteric infection transmission to other susceptible populations such as immunocompromised patients. Furthermore, there is rare research on the etiology of infectious diarrhea in adults in China[2, 3, 17, 18], especially based on a continuous active surveillance with comprehensive laboratory detection of enteric bacteria and viruses. Better understanding to the epidemiology, etiology and seasonality of infectious diarrhea in adults would be valuable for planning and

adopting targeted preventive measures and antimicrobial therapy.

The objective of this study was to identify the epidemiology, clinical characteristics, etiology and pathogen seasonality of infectious diarrhea in adult sporadic outpatients through an active continuous hospital-based diarrhea surveillance in Shanghai, and to explore to develop targeted policy of disease prevention and control in the future.

METHODS

Shanghai Diarrhea Comprehensive Surveillance System

The Shanghai Diarrhea Comprehensive Surveillance System conducts active, population-based surveillance on diarrhea outpatients. It consists of adult surveillance and children surveillance. The adult surveillance was established with 6 sentinel hospitals in May 2012, and incorporated 16 additional sentinel hospitals in August 2013. Municipal CDC, district CDCs and sentinel hospitals cooperate to maintain the surveillance, and share information and detection results through a dedicated online system. The 22 sentinel hospitals (9 primary-level hospitals, 6 secondary-level hospitals and 7 tertiary-level hospitals) were selected using Probability Proportionate to Size (PPS) sampling method among all hospitals which had enteric disease clinics in all 16 districts of Shanghai. Different sampling intervals were allocated to different sentinel hospitals considering the hospitals' location(district distribution), classification(hospital level distribution) and annual number of diarrhea patients (workload and operability)comprehensively, for use of collecting fecal specimens, ranging from 3:1 to 20:1.

Surveillance subjects were defined as patients who visited the enteric disease clinics of sentinel hospitals, with 3 or more loose or liquid stools per day, or more frequently than normal for the individual (World Health Organization's definition of diarrhea)[19]. Demographic, epidemiological and medical information of all surveillance subjects was obtained using a standardized questionnaire, and recorded into the dedicated online system. Epidemiologically-linked outbreak cases were excluded via inquiry.

Patient and Public Involvement

Patients involved were informed about the development and procedure of the surveillance, and interviewed by doctors in sentinel hospitals.

Laboratory Tests

Fecal specimens were collected from surveillance subjects in accordance with sampling intervals by trained medical staff, as a part of standard medical care. If the sampling interval of a sentinel hospital is X, then fecal specimens are collected from the Xth, 2Xth, 3Xth,…nXth surveillance subjects in this sentinel hospital. Approximately 8~10g (mL) of stool was collected and then dispensed into two containers: (1) a tube with Cary-Blair (C-B) culture medium for bacteria testing and (2) a sterile plastic cup for virus testing. Nucleic Acid was extracted from fecal specimens (20% w/v or v/v suspensions in PBS) using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).

All specimens were detected for 8 bacterial pathogens [*Vibrio cholera (V. cholera)*,

Shigella spp., *Salmonella* spp., *V. parahaemolyticus*, *Campylobacter jejuni* (*C. jejuni*), *Yersinia enterocolitica (Y. enterocolitica)*, *Campylobacter coli* (*C. coli*), DEC (including EPEC, ETEC, EHEC, EAggEC, EIEC)], and 5 viral pathogens (norovirus, rotavirus, astrovirus, sapovirus, and enteric adenovirus). Bacteria were isolated using different mediums at proper temperatures after preparation. The mediums included ChromID *Vibrio* and TCBS for *V. cholera* and *V. parahaemolyticus*, MAC for DEC, XLD for *Shigella* spp. and *Salmonella* spp., etc.. Bacteria were identified using biochemical tests. An automatic biochemical identification system was used for DEC. Serum agglutination tests were employed to subtype *Shigella* spp., *Salmonella* spp., *V. cholera* and DEC. Astrovirus, norovirus, sapovirus and rotavirus were detected using real-time reverse transcription- polymerase chain reaction assays (rRT-PCR) and enteric adenovirus was detected using rPCR. All molecular assays were performed using the appropriate respective commercial kits (Shanghai Zhijiang Biotechonology Co., Ltd.) according to the manufacturer's instructions.

Samples were scored as positive if at least one of enteric pathogens was isolated or identified. A bacterial infection means enteric bacteria was isolated and no viruses were identified. A viral infection means enteric virus was identified and no bacteria were isolated. Samples were scored as simplex infection if one of the 13 enteric pathogens was isolated or identified; as a mixed infection if at least two of these pathogens were isolated or identified; as a bacterial-viral mixed infection if at least one bacteria was isolated and one virus was identified.

Statistical Analysis

Data were analyzed using Statistical Analysis Software (SAS) version 9.3. Numbers and percentages were computed for categorical variables. Cochren-Mantel-Haenszel test was used for comparison of categorical variables. Binary logistic model and general logit model were used for binary dependent variables and multi-category disordered dependent variables respectively, to calculated adjusted odds ratio (aOR) and to explore the association between etiology and characteristics of infectious diarrhea after adjusting for confounders. Variables of age group, suburb, gender, season, and epidemiological histories were put into model and selected by stepwise methods. Age group, gender, suburb, season, consumption of suspicious food, medical history of enteric disease, and whether to keep a pet were included in the final model. Two-tailed p values < 0.05 was considered statistically significant.

This study focused on the adult diarrhea patients with age $\geqslant$18 years. Age group was defined as 18-29,30-44, 45-59, and 60+ years, according to the Global Burden of Disease 2000 and surveillance diarrhea patients 'age distribution[20]. Patients who visited hospitals in suburb area were grouped in "suburb". Patients who visited hospitals in rural area were grouped in "rural". Season was defined by the climatic characteristics of Shanghai, spring means March to May, and summer means June to August, and autumn means September to November, and winter means December to February. Suspicious food meant the suspicious food that may cause diarrhea, such as food which was contaminated by diarrhea pathogen.

Ethics

The study protocol was approved by the Institutional Ethics Review Committee of the Shanghai Municipal Center for Disease Control and Prevention.

RESULTS

From 1 May 2012 through 31 May 2016, a total of 95284 patients were enrolled in Shanghai diarrhea comprehensive surveillance system, of whom 4571 (4.80%) were not included in this study for the following reasons: 401 (0.42%) patients did not report clinical signs of diarrhea, 379 (0.40%) patients visited the enteric disease clinics within 14 days and thus were considered as the same episodes, 11 (0.01%) patients sought clinical care > 60 days after onset of diarrhea, 212 (0.22%) patients were not infectious diarrhea with other explicit diagnosis, and 3568 (3.74%) patients were younger than 18 years. Among 90713 adult diarrhea patients, 8797 (9.70%) patients' stool samples were collected and detected. These 8797 patients were included for further analysis.

1. Prevalence of Enteric Bacteria and Viruses

A total of 4657 pathogens were identified or isolated from 4219 (positive rate 47.96%) stool samples of the 8797 samples. There are 1147 bacterial infections (27.19%), 2914 viral infections (69.07%) and 158 bacterial-viral mixed infections (infected with at least 1 bacteria and 1 virus, 3.74%). Excluding mixed-infection samples, *V. parahaemolyticus* infections, DEC infections and *Salmonella* spp. infections were the most frequently bacterial infections, respectively with positive rate 4.50%, 3.43% and

2.90%. Excluding mixed-infection samples, norovirus infections and rotavirus infections were the most frequently viral infections, with positive rates 19.82% and 8.12%, respectively. Positive rates of other enteric viral infections were as follows: sapovirus, 1.93%; astrovirus, 1.56%; and enteric adenovirus, 0.35%. Positive rates of enteric bacterial infections were as follows: *C.jejuni*, 1.13%; *Shigella* spp., 0.22%; *C. coli*, 0.08%; *Y. enterocolitica*, 0.01%; and *Staphylococcus aureus*, 0.01%. In addition, there were 343 (3.90%) mixed infections.

Isolated DEC consisted of 216 ETEC, 131 EPEC, 84 EAggEC, 2 EIEC and 1 EHEC. Identified noroviruses consisted of 281 GI and 1726 GII. Identified rotaviruses consisted of 766 rotavirus group A, 6 rotavirus group B and 15 rotavirus group C.

2. Demographic and Epidemiological Characteristics

The median age was 46 (IQR 30-60) years. Of 8797 patients, 22.94% aged 18-29 years, 24.57% aged 30-44 years, 25.79% aged 45-59 years, and 26.70% aged equal to or older than 60 years. A significantly difference in positive rate within different age groups could be found among comparison of positive and negative diarrhea patients ($p=0.0150$), comparison of bacterial and viral and bacterial-viral infections ($p=0.0074$), and comparison of different enteric pathogens infections ($p<0.0001$) (Table 1). There were 4392 (49.93%) male patients, with a higher male proportion in positive diarrhea patients ($p=0.0472$), DEC infections (aOR=1.29, 95%CI=1.02-1.64) and norovirus infections (aOR=1.22, 95%CI=1.08-1.36) (Table 1 and Table 2).

Table1 Demographic and epidemiological characteristics of diarrhea outpatient adults by different infections

	Positive (n=4219)	Negative (n=4578)	*P*	Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)	*P*	*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)	Other infections§ (n=808)	*P*
Gender, N (%)														
Male	2153 (51.03)	2239 (48.91)	**0.0472**	577 (50.31)	1497 (51.37)	79 (50.00)	0.8005	184 (46.46)	164 (54.30)	128 (50.20)	946 (54.24)	326 (45.66)	405 (50.12)	**0.0011**
Age, Positive rate (%)														
18-29 years	941 (46.70)	1074	**0.0150**	292 (14.49)	611 (30.32)	38 (1.89)	**0.0074**	109 (5.41)	74 (3.67)	43 (2.13)	384 (19.06)	118 (5.86)	213 (10.57)	**<0.0001**
30-44 years	1084 (53.80)	1074		298 (14.79)	748 (37.12)	38 (1.89)		119 (5.91)	72 (3.57)	57 2.83)	473 (23.47)	158 (7.84)	205 (10.17)	
45-59 years	1112 (55.19)	1153		294 (14.59)	768 (38.11)	50 (2.48)		105 (5.21)	78 (3.87)	76 (3.77)	426 (21.14)	231 (11.46)	196 (9.73)	
60+ years	1079 (53.55)	1266		262 (13.00)	786 (39.01)	31 (1.54)		63 (3.13)	78 (3.87)	78 (3.87)	460 (22.83)	207 (10.27)	193 (9.58)	
Living region, , Positive rate (%)														
Suburb	2401 (44.66)	2975	**<0.0001**	665 (12.37)	1645 (30.60)	91 (1.69)	0.6661	257 (4.78)	170 (3.16)	149 (2.77)	1019 (18.95)	403 (7.50)	403 (7.50)	**<0.0001**
Rural	1818(53.14)	1603		482(14.09)	1269(37.09)	67(1.96)		139(4.06)	132(3.86)	255(3.10)	725(21.19)	311(9.09)	405(11.84)	
Epidemiological history , N (%)														
Had a medical history of enteric disease in the past 6 months	17 (0.40)	47 (1.03)	**0.0006**	5 (0.44)	12 (0.41)	0 (0.00)	0.7132	1 (0.25)	1 (0.33)	2 (0.78)	8 (0.46)	2 (0.28)	3 (0.37)	0.9001
Had consumed suspicious food within 5 days before onset	1914 (45.37)	1865 (40.74)	**<0.0001**	490 (42.72)	1350 (46.33)	74 (46.84)	0.1073	179 (45.20)	111 (36.75)	117 (45.88)	847 (48.57)	282 (39.50)	378 (46.78)	**<0.0001**
Had went out within 7 days before onset	78 (1.85)	46 (1.00)	**0.0010**	29 (2.53)	48 (1.65)	1 (0.63)	0.0881	7 (1.77)	8 (2.65)	5 (1.96)	34 (1.95)	6 (0.84)	18 (2.23)	0.3226
Had kept or had contact with pets.	814 (19.29)	604 (13.19)	**<0.0001**	224 (19.53)	556 (19.08)	34 (21.52)	0.7304	55 (13.89)	65 (21.52)	41 (16.08)	323 (18.52)	123 (17.23)	207 (25.62)	**<0.0001**

§ Simplex infections;

Bold face: $P<0.05$

Cochren-Mantel-Haenszel test was used for comparison of categorical variables.

Table2 Adjusted odds ratio of demographic and epidemiological characteristics comparing positive detection with negative detection in diarrhea outpatients*

	Positive (n=4219)		Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)		*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)
Male vs female	1.09 (1.00-1.19)		1.07 (0.94-1.22)	1.1 (0.99-1.22)	1.04 (0.75-1.43)		0.89 (0.72-1.09)	**1.29 (1.02-1.64)**	1.11 (0.86-1.44)	**1.22 (1.08-1.36)**	0.88 (0.75-1.05)
Age (years)											
18-29	1.10 (0.97-1.25)		**1.32 (1.09-1.59)**	0.99 (0.85-1.14)	1.52 (0.93-2.44)		**1.92 (1.41-2.7)**	1.11 (0.79-1.54)	**0.64 (0.44-0.94)**	1.03 (0.88-1.22)	**0.75 (0.58-0.97)**
30-44	**1.28 (1.14-1.45)**		**1.28 (1.06-1.56)**	**1.28 (1.11-1.47)**	1.54 (0.94-2.50)		**2.04 (1.47-2.78)**	1.02 (0.73-1.43)	0.83 (0.58-1.19)	**1.32 (1.12-1.56)**	1.08 (0.84-1.35)
45-59	**1.19 (1.06-1.35)**		1.2 0 (1.00-1.47)	**1.16 (1.01-1.33)**	**1.85 (1.18-2.94)**		**1.72 (1.25-2.38)**	1.06 (0.77-1.47)	1.06 (0.76-1.47)	1.09 (0.93-1.28)	**1.33 (1.08-1.67)**
60+¶	1.00		1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
Suburb	**0.80 (0.72-0.88)**		**0.80 (0.68-0.92)**	**0.80 (0.71-0.89)**	0.83 (0.58-1.19)		0.96 (0.76-1.23)	**0.75 (0.57-0.98)**	0.82 (0.62-1.09)	**0.85 (0.75-0.97)**	**0.79 (0.66-0.95)**
Had a medical history of enteric disease in the past 6 months	**0.41 (0.23-0.73)**		0.42 (0.17-1.08)	**0.43 (0.22-0.85)**	0		0.24 (0.03-1.76)	0.34 (0.05-2.46)	0.71 (0.17-2.94)	0.47 (0.21-1.01)	0.32 (0.07-1.39)
Had consumed suspicious food within 5 days before onset	**1.18 (1.08-1.29)**		1.06 (0.93-1.22)	**1.24 (1.12-1.38)**	1.26 (0.82-1.75)		1.22 (0.99-1.51)	0.84 (0.66-1.08)	0.24 (0.96-1.61)	**1.31 (1.17-1.48)**	0.99 (0.83-1.18)
Had kept or had contact with pets	**1.33 (1.17-1.5)**		**1.57 (1.30-1.90)**	**1.21 (1.04-1.40)**	**1.62 (1.05-2.48)**		1.17 (0.85-1.63)	**1.79 (1.29-2.47)**	1.20 (0.83-1.75)	**1.26 (1.06-1.48)**	1.00 (0.78-1.27)

* Data are adjusted odds ratio (95%CI) in binary logistic model or general logit model

§ Simplex infections

¶ Reference group in logistic regression model

Bold face: $P<0.05$

Adjusted odds ratios of age were shown in Table 2. Patients between 30-44 and 45-59 years were more likely to infectious diarrhea and viral diarrhea. Those aged 30-44 years were the most likely to *V. parahaemolyticus* (aOR versus 60+ years group: 2.04 [1.47-2.78]) and norovirus (aOR versus 60+ years group: 1.32 [1.12-1.56]). In addition, patients in 18-29 years group had a significantly lower odds of experiencing infectious diarrhea (aOR=0.85, 95% CI=0.76-0.97), viral infections (aOR=0.78, 95% CI=0.67-0.90), norovirus infections (aOR=0.78, 95% CI=0.66-0.92) and rotavirus infections (aOR=0.70, 95% CI=0.54-0.92) compared with 30-44 years group. Patients in 18-29 years group had a significantly lower odds of experiencing viral infections (aOR=0.85, 95% CI=0.74-0.98), *Salmonella* spp. infections (aOR=0.61, 95% CI=0.41-0.89) and rotavirus infections (aOR=0.56, 95% CI=0.44-0.72) compared with 45-59 years group. Patients in 30-44 years group had a significantly higher odds experiencing norovirus infections (aOR=1.22, 95% CI=1.03-1.43) compared with 40-45 years group.

Among diarrhea patients, 5376 (85.67%) visited the hospitals in suburb. The positive rates in suburb and rural groups were significantly different($p<0.0001$, Table 1). Comparing different enteric pathogen infections, the positive rates of patients in suburb and rural groups were significantly different ($p<0.0001$). More diarrhea patients infected with *V. parahaemolyticus* (64.90%) lived in suburb area. Patients living in suburb area were less likely to get infected with enteric pathogens (aOR=0.75-0.85) except *V. parahaemolyticus* infections and *Salmonella* spp. infections (Table 2).

64 (0.73%) patients had a medical history of enteric disease in the past 6 months. Within 5 days before onset, 3779 (42.96%) patients had a history of consuming suspicious food. 124 (1.41%) patients had a history of go out within 7 days before onset. And 1418 (16.12%) patients kept or had contact with pets. When compared with negative patients, a higher proportion of positive patients has a history of consuming suspicious food within 5 days before onset ($p<0.0001$), had a history of going out within 7 days before onset ($p=0.0010$), and kept or had contact with pets ($p<0.0001$), while a lower proportion had a medical history of enteric disease in the past 6 months ($p=0.0006$) (Table 1). Epidemiological history, including consuming suspicious food and keeping or contacting with pets, was significantly associated with higher odds of infectious diarrhea, viral infections and norovirus infections. A medical history of enteric disease was significantly associated with lower odds of infectious diarrhea (Table 2).

3. Clinical Symptoms

Of positive diarrhea patients, 13.11% reported fever, 41.91% reported nausea, 28.21% reported vomiting, and 49.09% reported abdominal pain (Table 3). Watery stool and loose stool were common, respectively accounting 76.27% and 20.93%. Compared with negative diarrhea patients, positive patients reported more fever ($p=0.0009$), nausea ($p<0.0001$), vomiting ($p<0.0001$) and watery stool ($p<0.0001$), while fewer abdominal pain (<0.0001).

The distributions of clinical symptoms by different infections were significantly different (Table 3). Diarrhea patients infected with bacteria reported more fever

(19.09%, p<0.0001), abdominal pain (64.60%, p<0.0001) and loose stool (23.28%, p<0.0001). Diarrhea patients infected with virus reported more nausea (43.34%%, p=0.0175), vomiting (30.13%, p=0.0001) and watery stool (78.35%, p<0.0001).

Diarrhea patients infected with *V. parahaemolyticus* featured more nausea (56.27%), vomiting (41.41%), abdominal pain (71.9%) and watery stool (81.57%). Patients infected with DEC featured fewer nausea (28.81%) and vomiting (13.58%). Patients infected with *Salmonella* spp. featured more fever (28.24%). Patients infected with norovirus featured fewer fever (9.69%) and abdominal pain (44.55%).

Table3 Clinical symptoms in diarrhea outpatients by different infections

	Positive (n=4219)	**Negative (n=4578)**	***P***	**Bacterial infections (n=1147)**	**Viral infections (n=2914)**	**Bacterial-viral Mixed infections (n=158)**	***P***	***V. parahaemolyticus*§ (n=396)**	**DEC§ (n=302)**	***Salmonella* spp.§ (n=255)**	**Norovirus§ (n=1744)**	**Rotavirus§ (n=714)**	**Other infections§ (n=808)**	***P***
Fever, N (%)	553 (13.11)	495 (10.81)	**0.0009**	219 (19.09)	312 (10.71)	22 (13.92)	**<0.0001**	46 (11.62)	43 (14.24)	72 (28.24)	169 (9.69)	96 (13.45)	127 (15.72)	**<0.0001**
Nausea, N (%)	1768 (41.91)	1561 (34.10)	**<0.0001**	442 (38.54)	1263 (43.34)	63 (39.87)	**0.0175**	224 (56.27)	87 (28.81)	71 (27.84)	790 (45.30)	309 (43.28)	287 (35.52)	**<0.0001**
Vomiting, N (%)	1190 (28.21)	916 (20.01)	**<0.0001**	269 (23.45)	878 (30.13)	43 (27.22)	**0.0001**	164 (41.41)	41 (13.58)	37 (14.51)	595 (34.12)	195 (27.31)	158 (19.55)	**<0.0001**
Abdominal pain, N (%)	2071 (49.09)	2446 (53.43)	**<0.0001**	741 (64.60)	1257 (43.14)	73 (46.20)	**<0.0001**	285 (71.97)	170 (56.29)	151 (59.22)	777 (44.55)	321 (44.96)	367 (45.42)	**<0.0001**
Fecal property, N (%)														
Watery	3218 (76.27)	3150 (68.81)	**<0.0001**	814 (70.97)	2283 (78.35)	121 (76.58)	**<0.0001**	323 (81.57)	202 (66.89)	179 (70.20)	1344 (77.06)	583 (81.65)	587 (72.65)	**<0.0001**
Loose	883 (20.93)	1202 (26.26)		267 (23.28)	583 (20.01)	33 (20.89)		54 (13.64)	85 (28.15)	61 (23.92)	372 (21.33)	121 (16.95)	190 (23.51)	
Mucous	72 (1.71)	143 (3.12)		38 (3.31)	31 (1.06)	3 (1.90)		8 (2.02)	11 (3.64)	11 (4.31)	18 (1.03)	6 (0.84)	18 (2.23)	
Else	46 (1.09)	83 (1.81)		28 (2.44)	17 (0.58)	1 (0.63)		11 (2.78)	4 (1.32)	4 (1.57)	10 (0.57)	4 (0.56)	13 (1.61)	

§ Simplex infections;

Bold face: $P<0.05$

Cochren-Mantel-Haenszel test was used for comparison of categorical variables

4. Pathogen Spectrum and Seasonality

In term of descriptive data, the enteric pathogens spectrum of infectious diarrhea patients displayed a yearly seasonal trend (Figure 1). In general, viruses were predominant during November to March of every seasonal cycle, accounting for more than 80% in every month. Bacteria were predominant during June to August of almost every seasonal cycle, accounting for more than 60% in every month. September and October were the transition period from bacteria to viruses, and April and May were the transition period from viruses to bacteria. Norovirus and rotavirus both showed yearly seasonal trends. Rotavirus peaked in winter months, especially in December and January. Norovirus displayed a less distinct and broader seasonality. Norovirus clustered around autumn and winter, while a smaller peak appeared in March of 2014 and 2015. In the seasonal cycle from 2015-2016, norovirus peaked in March 2016. *V. parahaemolyticus*, DEC and *Salmonella* spp. all showed yearly seasonal trends. These three enteric bacteria peaked in August, and *Salmonella* spp. showed a smaller peak (Figure 2).

Figure 1 insert here.

Figure 2 insert here.

In term of statistical analysis, there were significantly different season distribution in comparison of positive and negative diarrhea patients ($p<0.0001$), comparison of bacterial and viral and bacterial-viral infections ($p<0.0001$), and comparison of different enteric pathogens infections ($p<0.0001$). More bacterial infections appeared in summer (54.58%) and more viral infections appeared in winter (44.51%). The

proportion of winter was lower among norovirus infections (34.86%) compared with among rotavirus infections (67.37%).

Patients in summer were 1.55-4.39 times more likely to have simplex bacterial diarrhea and 0.16-0.20 times less likely to have simplex viral diarrhea compared with in spring. Patients in autumn were 2.02-3.38 times more likely to have *V. parahaemolyticus* infections and DEC infections, and 0.69-0.77 times less likely to have simplex viral diarrhea compared with in spring. Patients in winter were 1.60-5.61 times more likely to have simplex viral infections, and 0.14-0.56 times less likely to have simplex bacterial diarrhea compared with in spring (Table 4).

Table4 Seasonality of diarrhea outpatients by different infections*

	Negative (n=4578)	Positive (n=4219)		Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)		*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)
Season [(No.(%)]		***P*<0.0001**		***P* <0.0001**				***P* <0.0001**				
Spring	867 (18.94)	877 (20.79)		149 (12.99)	695 (23.85)	33 (20.89)		34 (8.59)	21 (6.95)	41 (16.08)	462 (26.49)	101 (14.15)
Summer	1746 (38.14)	927 (21.97)		626 (54.58)	260 (8.92)	41 (25.95)		252 (63.64)	178 (58.94)	123 (48.24)	180 (10.32)	32 (4.48)
Autumn	1238 (27.04)	1031 (24.44)		322 (28.07)	662 (22.72)	47 (29.75)		106 (26.77)	96 (31.79)	72 (28.24)	494 (28.33)	100 (14.01)
Winter	727 (15.88)	1384 (32.80)		50 (4.36)	1297 (44.51)	37 (23.42)		4 (1.01)	7 (2.32)	19 (7.45)	608 (34.86)	481 (67.37)
Season [aOR (95%CI)]												
Spring^		1.00		1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
summer		**0.54 (0.48-0.61)**		**2.16 (1.77-2.64)**	**0.18 (0.16-0.23)**	0.62 (0.39-1.00)		**3.65 (2.53-5.29)**	**4.39 (2.77-6.96)**	**1.55 (1.07-2.23)**	**0.20 (0.17-0.24)**	**0.16 (0.10-0.24)**
autumn		**0.85 (0.75-0.97)**		1.59 (0.28-1.97)	**0.69 (0.60-0.79)**	1.04 (0.66-1.65)		**2.2 (1.48-3.28)**	**3.38 (2.09-5.47)**	1.26 (0.85-1.87)	**0.77 (0.66-0.9)**	**0.69 (0.52-0.93)**
winter		**1.91 (1.67-2.18)**		**0.40 (0.29-0.56)**	**2.26 (1.98-2.59)**	1.36 (0.84-2.20)		**0.14 (0.05-0.40)**	**0.39 (0.16-0.92)**	**0.56 (0.32-0.97)**	**1.60 (1.37-1.88)**	**5.61 (4.42-7.11)**

* Data are adjusted odds ratio (95%CI) in binary logistic model or general logit model

§ Simplex infections;

¶ Reference group in logistic regression model

Bold face: $P<0.05$

Cochren-Mantel-Haenszel test was used for comparison of categorical variables

DISCUSSION

This study is the first study in Shanghai identifying the etiology and epidemiology of adult infectious diarrhea in sporadic outpatients from a continuous active diarrhea surveillance enhanced with comprehensive laboratory testing for common enteric bacteria and virus. It also adds to the limited number of studies investigating adult cases of infectious diarrhea in China. The Shanghai Diarrhea Comprehensive Surveillance System used Probability Proportionate to Size (PPS) sampling method and was conducted among 22 sentinel hospitals in all 16 districts of Shanghai continuously since May 2012, data from which are more representative and more feasible to be extrapolated to the city's population by avoiding the influence of clusters and season-specific cases.

Etiology of adult infectious diarrhea in Shanghai was detailed in this study. At least one enteric pathogen was found in 47.96% adult diarrhea patients' stools. Viral infections are predominant and bacteria were isolated from many cases. These findings were consistent with those Wang 's research in Beijing[2]. We found that norovirus was the most common enteric pathogen, accounting for over 40% of all cases, followed by rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. The proportion of norovirus was higher than the sum proportion of rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. This result confirmed norovirus's leading role in adult infectious diarrhea in China, and was similar to the research finding in sporadic gastroenteritis in both developing and developed countries [21-24]. And it is observed that norovirus infections were more than twice as that of rotavirus in adult patients in Shanghai. Rotavirus ranked second to norovirus. This results was consistent with studies in Russia [24] and Shanghai, China[25], while inconsistent with study in France[26]. Yet according to WHO, rotavirus are most common etiological agents of diarrhea in developing countries, which may due to rotavirus's important role in children. As leading cause of severe diarrhea in children, the pathogenic role and disease burden of rotavirus in adults had been underestimated. Rotavirus needs more attention in routine clinical diagnosis and vaccination

program.

According to this study, *V. parahaemolyticus*, DEC and *Salmonella* spp. were common bacteria in adult infectious diarrhea. The prevalence of these three bacterial infections was similar between 2.90~4.50%, much lower than viral infections. In previous studies，*V. parahaemolyticus*, DEC and *Salmonella* spp. were also among the most prevalent pathogen in adult infectious diarrhea in different regions of China and worldwide[1, 2, 17, 18, 27]. Although diarrhea due to *V. parahaemolyticus* decreased since 1998[28, 29], *V. parahaemolyticus* was still the leading cause of adult bacterial infections in this study. However, *Shigella* spp. was also among frequent bacteria in several studies before 2013[18, 28, 29]. This study showed that positive rate of *Shigella* spp. infections was only 0.22% during 2012-2016 in Shanghai, which may due to the downward trend of *Shigella* spp. infections over time [29].

This study showed that there was association between adult infectious diarrhea and patient age. In general, patients between 30-59 years were more likely to have infectious diarrhea and viral diarrhea than age groups of 18-29 and 60+ years. This was partly consistent with a study in France which found incidence of acute diarrhea in youth group was higher than elderly group [26]. Elderly people (⩾60years) were the least likely to get infected with *V. parahaemolyticus*, whereas people aged 30-44 years were the most likely among adult age groups. The similar findings were observed in a study in Shanghai[29]. This may be related to more seafood consumption in young adults, which is an important risk factor in *V. parahaemolyticus* infections[30] .In contrast to other studies which found elderly people more likely to norovirus[22, 31], our study discovered that the highest proportion in norovirus infections was 30-44 years old. And considering the results of general logit model adjusting for other factors, 30-44 years patients were the most likely to norovirus. Patients aged 18-29 years had the lowest odds experiencing rotavirus diarrhea.

People living in rural area were more susceptible to DEC, norovirus and rotavirus, which may because city environment provided more chance for pathogen to transmit.

In regarding to clinical symptoms in general, bacterial diarrhea was characterized by fever, abdominal pain and loose stool, while viral diarrhea was characterized by nausea, vomiting and watery stool. However the symptom of *V. parahaemolyticus* infections showed more like viral infections. In addition, abdominal pain was common in *V. parahaemolyticus* infections. These findings of *V. parahaemolyticus* were in accordance with a research in Shanghai during 1998-2013[28]. The symptoms of DEC and *Salmonella* spp. were similar except fever. The proportion of fever was highest in *Salmonella* spp. (28.24%) while lowest in norovirus (9.69%). The proportion of fever in norovirus infections was much lower in comparison with some studies[26, 31], while the proportion in *Salmonella* spp. infections was close to another research [28]. The proportion of abdominal pain was highest in *V. parahaemolyticus* (71.97%) while much lower in norovirus (44.55%) and rotavirus (44.96%).

This study also demonstrated the seasonality of adult infectious diarrhea and relevant contribution of different enteric pathogens in seasonal trend. A seasonal distribution of adult infectious diarrhea was observed with a large peak in winter and a small peak in summer. Winter peak was mainly due to norovirus and rotavirus, which was in line with previous study[32, 33]. Summer peak was smaller, due to low proportion of bacterial infections. What should be noted was that there was a peak around March due to norovirus in 2014-2016, ever higher than the summer peak in 2015-2016 season cycle. This emerging spring peak was possibly because of the increased activity of a novel norovirus GII.17 [34]. Rotavirus showed a distinct peak in December and January (significantly winter VS summer aOR=35.67), which was consistent with researches in Shanghai and Iran [25, 35], while different from a study in London (peak from January through May) [36] and a study in Russia (peak from December through May) [24]. However, norovirus displayed a broader seasonality peaking around autumn and winter (significantly winter VS summer OR=8.00) in this study and a study in Netherland [9]. Bacterial infections, included *V. parahaemolyticus*, DEC and *Salmonella* spp., showed a yearly seasonality peaking in summer (often in August), with

significantly summer VS winter OR 25.00, 11.11 and 2.78 respectively. This was similar in Enserink's study [9], whereas autumn peak of bacterial infections was observed in some studies [25, 37]. The seasonality of infectious diarrhea may due to the climate, biological characteristics of pathogens and people's diet habit of Shanghai.

There are several limitations that need to be acknowledged. First, information and detection results were collected from 22 hospitals and 16 laboratories. Though detection methods & materials were unified and regular trainings were hold, there was still a chance of bias caused by the different levels and conditions of hospitals and laboratories. Admission rate bias should also be taken into consideration as patients may have a preference when visiting hospitals of different levels or in different regions. Second, the recall bias of epidemiological information was difficult to avoid. And the data of exposure history was important for infectious diarrhea. Third, only diarrhea patients who visited the enteric disease clinics were included in surveillance, severe diarrhea patients or asymptomatic patients were possibly not studies in our research. Fourth, as for seasonality, only descriptive data of every month or statistical tests of seasons were demonstrated. No statistical methods were used to analyze the successive time series, which was because of the limit seasonal cycles of existing data. In the future, after accumulating enough data during several years, time series analysis could be taken to explore the inherent natural order and to forecasting prospective trend.

CONCLUSIONS

In conclusion, this study provides a detailed picture about the epidemiology, etiology and seasonal pathogen spectrum of adult infectious diarrhea in Shanghai. Viral infections are predominant, and norovirus is the most common enteric pathogen detected in our surveillance. Other common pathogens include rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. Patients between 30-59 years were more likely to infectious diarrhea and viral diarrhea. A seasonal distribution was observed with larger peaks in winter and smaller peaks in summer. Winter peak was mainly due to norovirus and rotavirus, and summer peak was due to

bacterial infections. An emerging spring peak of norovirus around March was observed in recent 3 years. Our findings highlight the necessity for conducting an active, comprehensive surveillance for both bacterial and viral enteric pathogens in adults, to monitor the changing dynamics in the epidemiology and etiology of infectious diarrhea. These findings promote to understand adult infectious diarrhea thoroughly and to develop targeted prevention strategies.

LIST OF ABBREVIATIONS

aOR: Adjusted odds ratio; *C. coli*: *Campylobacter coli*; *C. jejuni*: *Campylobacter jejuni*; DEC*: Diarrheagenic Escherichia coli*; EAggEC: Enteroaggregative escherichia coli; EHEC: Enterohemorrhagic escherichia coli; EIEC: Enteroinvasive escherichia coli; EPEC: Enteropathogenic escherichia coli; ETEC: Enterotoxigenic escherichia coli; PPS: Probability Proportionate to Size; rRT-PCR: real-time Reverse Transcription -Polymerase Chain Reaction; SAS: Statistical Analysis Software; *V. cholera: Vibrio cholera; V. parahaemolyticus*: *Vibrio parahaemolyticus;* WHO: World Health Organization; *Y. enterocolitica: Yersinia enterocolitica;*

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Xiao-huan Gong performed the statistical analysis and drafted the manuscript. Hao Pan and Huan-yu Wu placed the surveillance system into effect. Jian Li designed the study of the surveillance system. Wen-jia Xiao participated in the management of the system. Xi Zhang, Min Chen and Zheng Teng carried out the management and quality control of the laboratory tests. Zheng-an Yuan conceived of the study. All authors read and approved of the final manuscript.

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DATA SHARING STATEMENT

No additional data are available for public sharing.

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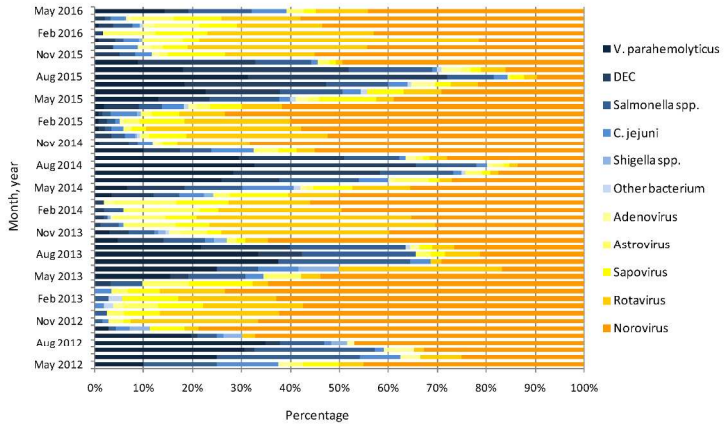
Figure 1 Pathogen spectrum of major enteric pathogens in adults with infectious diarrhea by month in Shanghai, May 2012-May 2016

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016



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297x210mm (300 x 300 DPI)

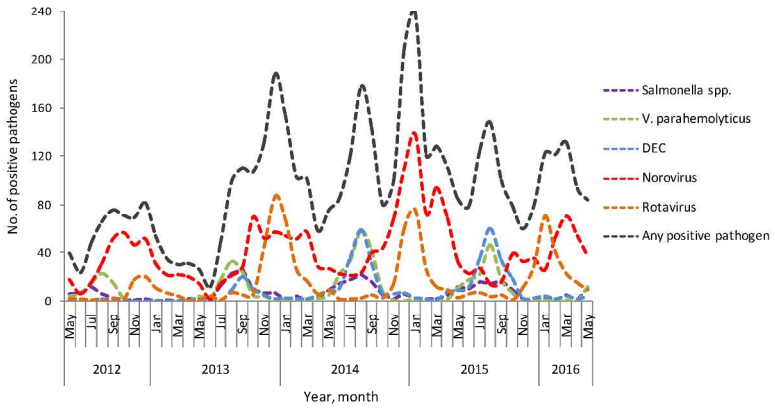

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

297x210mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7-8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10
Bias	9	Describe any efforts to address potential sources of bias	7-10
Study size	10	Explain how the study size was arrived at	7-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	7-8/10
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10-11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-21
		(*b*) Report category boundaries when continuous variables were categorized	10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	22-26
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	22-26
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	27

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Epidemiology, etiology and seasonality of infectious diarrhea in adult outpatients through active surveillance in Shanghai, China, 2012-2016: a cross-sectional study

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Epidemiology, etiology and seasonality of infectious diarrhea in adult outpatients through active surveillance in Shanghai, China, 2012-2016: a cross-sectional study

Xiao-huan Gong[1†], Huan-yu Wu[1†], Jian Li[2], Wen-jia Xiao[1], Xi Zhang[1], Min Chen[1], Zheng Teng[1], Hao Pan[1*], Zheng-an Yuan[1*]

[†] Equal contributor：Xiao-huan Gong, Huan-yu Wu

[*] Corresponding author:

Zheng-an Yuan

Email: yuanzhengan@scdc.sh.cn; Phone:+86 21 62758710

Hao Pan

Email: panhao@scdc.sh.cn; Phone:+86 21 62758710

[1] Division of Infectious Disease Control and Prevention, Divison of Pathogen Detection, Shanghai Municipal Center for Disease Control and Prevention, No. 1380, West Zhongshan Road, Shanghai 200336, China.

[2] Clinical Research Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Key Words:

Diarrhea, Surveillance, Epidemiology, Etiology, Sporadic, Bacteria, Virus, China

Email addresses:

Xiao-huan Gong: gloriag1988@163.com

Huan-yu Wu: wuhuanyu@scdc.sh.cn

Jian Li: lijian@scdc.sh.cn

Wen-jia Xiao: xiaowenjia@scdc.sh.cn

Xi Zhang: zhangxi@scdc.sh.cn

Min Chen: chenmin@scdc.sh.cn

Zheng Teng: tengzheng@scdc.sh.cn

Hao Pan: panhao@scdc.sh.cn

Zheng-an Yuan: yuanzhengan@scdc.sh.cn

ABSTRACT

Objectives

This study aimed to identify the epidemiology, clinical characteristics, etiology and seasonality of sporadic infectious diarrhea in adults in Shanghai.

Setting

This study was based on a citywide, active continuous hospital-based diarrhea surveillance network established by Shanghai CDC. There were 22 sentinel hospitals in all 16 districts (9 primary-level hospitals, 6 secondary-level hospitals and 7 tertiary-level hospitals), which were selected using Probability Proportionate to Size (PPS) sampling method.

Participants

From 1 May 2012 through 31 May 2016, 90713 patients were included in this study. Among 8797 patients whose stool samples were collected and detected, 4392 patients were male.

Results

The positive rate was 47.96%. Bacterial and viral infections accounted for 27.19% and 69.07% separately. Norovirus was the most common pathogen (43.10%), followed by rotavirus, *V. parahaemolyticus*, *Diarrheagenic Escherichia coli* (DEC) and *Salmonella* spp.. Patients between 30-44 and 45-59 years were more likely to have infectious diarrhea and viral diarrhea. Those aged 30-44 years were the most

likely to get infected with *V. parahaemolyticus* (aOR versus 60+ years: 2.04 [1.47-2.78]) and norovirus (aOR versus 60+ years: 1.32 [1.12-1.56]). Bacterial (except *V. parahaemolyticus*) diarrhea was characterized by fever, abdominal pain and loose stool; whilst viral diarrhea was characterized by nausea, vomiting and watery stool. A seasonal distribution of infectious diarrhea was observed with larger peaks in winter and smaller peaks in summer. Winter peaks were mainly due to norovirus and rotavirus, and summer peaks were due to bacterial infections. An emerging spring peak of norovirus around March was observed in the past 3 years.

Conclusion

Viral infections were predominant, and norovirus played a leading role. A seasonal distribution was observed and an emerging spring peak of norovirus was noted. Our findings highlight the necessity for conducting an active, comprehensive surveillance in adults, to monitor changing dynamics in the epidemiology and etiology of infectious diarrhea.

Key Words

Diarrhea, Surveillance, Epidemiology, Etiology, Sporadic, Bacteria, Virus, China

Strengths and limitations of this study

- ✧ This is the first study in Shanghai identifying the etiology and epidemiology of adult infectious diarrhea in sporadic outpatients from a continuous active diarrhea

surveillance enhanced with comprehensive laboratory testing for common enteric bacteria and virus.

- ✧ Seasonality of adult infectious diarrhea and relevant contribution of different enteric pathogens in seasonal trend were demonstrated in detail.
- ✧ Etiology of adult infectious diarrhea in Shanghai, including bacteria and virus, was detailed in this study.
- ✧ Since information and detection results were collected from 22 hospitals and 16 laboratories, there was a chance of bias caused by different levels and conditions of hospitals and laboratories. Also admission rate bias and recall bias were difficult to avoid.
- ✧ As for seasonality, only descriptive data of every month or statistical tests of seasons were demonstrated. No statistical methods were used to analyze the successive time series.

BACKGROUND

Diarrhea is generally characterized by the frequent passage of loose or liquid stools. It is usually a symptom of gastrointestinal infections caused by bacterial, viral or parasitic pathogens, which spread through contaminated food or drinking-water or from person-to-person[1]. According to WHO, rotavirus and *Diarrheagenic Escherichia coli* (DEC) are the two most common etiological agents of diarrhea in developing countries[1]. However, norovirus was found the most prevalent pathogen

of infectious diarrhea in adults in China CDC's research[2], and *Vibrio parahaemolyticus* (*V. parahaemolyticus*) was the most common enteric pathogen in acute bacterial gastroenteritis[3]. The etiology of infectious diarrhea differs among regions depending on economic development, local climate and geography [4, 5].

Nearly 1.7 billion cases and 1.3 million deaths due to diarrhea occur worldwide every year.[1, 6] Diarrhea causes substantial medical and healthcare costs and thus has a high economic impact on society[7]. Diarrhea remains one of the major causes of disease burden worldwide, despite significant progress in sanitation status and public health awareness. Mortality due to diarrhea fell 20% in recent 10 years, while it is still leading common cause of life loss (ranking fifth) globally[6]. To react to this worldwide health issue, Shanghai CDC have established the Shanghai Diarrhea Comprehensive Surveillance System since 2012, which is an active continuous surveillance system this research is based on.

Most of current studies of diarrhea have focused on children under 5 years old[8-12].. Consequently, limited data about the epidemiology and etiology of infectious diarrhea in adults is available[13-15]. Although diarrhea accounts for only 2% deaths of adults[16], they may play a role in enteric infection transmission to other susceptible populations such as immunocompromised patients. Furthermore, there is rare research on the etiology of infectious diarrhea in adults in China[2, 3, 17, 18], especially based on a continuous active surveillance with comprehensive laboratory detection of enteric bacteria and viruses. Better understanding of the epidemiology, etiology and seasonality of infectious diarrhea in adults would be valuable for planning and

adopting targeted preventive measures and antimicrobial therapy.

The objectives of this study were to identify the epidemiology, clinical characteristics, etiology and pathogen seasonality of infectious diarrhea in adult sporadic outpatients through an active continuous hospital-based diarrhea surveillance in Shanghai, and to explore to develop targeted policy of disease prevention and control in the future.

METHODS

Shanghai Diarrhea Comprehensive Surveillance System

The Shanghai Diarrhea Comprehensive Surveillance System conducts active, population-based surveillance on diarrhea outpatients. It consists of adult surveillance and children surveillance. The adult surveillance was established with 6 sentinel hospitals in May 2012, and incorporated 16 additional sentinel hospitals in August 2013. Municipal CDC, district CDCs and sentinel hospitals cooperate to maintain the surveillance, and share information and detection results through a dedicated online system. The 22 sentinel hospitals (9 primary-level hospitals, 6 secondary-level hospitals and 7 tertiary-level hospitals) were selected using Probability Proportionate to Size (PPS) sampling method among all hospitals which had enteric disease clinics in all 16 districts of Shanghai. Different sampling intervals were allocated to different sentinel hospitals considering the hospitals' location(district distribution), classification(hospital level distribution) and annual number of diarrhea patients (workload and operability)comprehensively, for use of collecting fecal specimens, ranging from 3:1 to 20:1.

Surveillance subjects were defined as patients who visited the enteric disease clinics of sentinel hospitals, with 3 or more loose or liquid stools per day, or more frequent than normal for the individual (World Health Organization's definition of diarrhea)[19]. Demographic, epidemiological and medical information of all surveillance subjects was obtained using a standardized questionnaire, and recorded into the dedicated online system. Epidemiologically-linked outbreak cases were excluded via inquiry.

Patient and Public Involvement

Patients involved were informed about the development and procedure of the surveillance, and interviewed by doctors in sentinel hospitals.

Laboratory Tests

Fecal specimens were collected from surveillance subjects in accordance with sampling intervals by trained medical staff, as a part of standard medical care. If the sampling interval of a sentinel hospital is X, then fecal specimens are collected from the Xth, 2Xth, 3Xth,…nXth surveillance subjects in this sentinel hospital. Approximately 8~10g (mL) of stool was collected and then dispensed into two containers: (1) a tube with Cary-Blair (C-B) culture medium for bacteria testing and (2) a sterile plastic cup for virus testing. Nucleic Acid was extracted from fecal specimens (20% w/v or v/v suspensions in PBS) using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany).

All specimens were detected for 8 bacterial pathogens [*Vibrio cholera (V. cholera)*,

Shigella spp., *Salmonella* spp., *V. parahaemolyticus*, *Campylobacter jejuni* (*C. jejuni*), *Yersinia enterocolitica (Y. enterocolitica)*, *Campylobacter coli* (*C. coli*), DEC (including EPEC, ETEC, EHEC, EAggEC, EIEC)], and 5 viral pathogens (norovirus, rotavirus, astrovirus, sapovirus, and enteric adenovirus). Bacteria were isolated using different mediums at proper temperatures after preparation. The mediums included ChromID *Vibrio* and TCBS for *V. cholera* and *V. parahaemolyticus*, MAC for DEC, XLD for *Shigella* spp. and *Salmonella* spp., etc.. Bacteria were identified using biochemical tests. An automatic biochemical identification system was used for DEC. Serum agglutination tests were employed to subtype *Shigella* spp., *Salmonella* spp., *V. cholera* and DEC. Astrovirus, norovirus, sapovirus and rotavirus were detected using real-time reverse transcription- polymerase chain reaction assays (rRT-PCR) and enteric adenovirus was detected using rPCR. All molecular assays were performed using the appropriate respective commercial kits (Shanghai Zhijiang Biotechonology Co., Ltd.) according to the manufacturer's instructions.

Samples were scored as positive if at least one of enteric pathogens was isolated or identified. A bacterial infection means enteric bacteria was isolated and no viruses were identified. A viral infection means enteric virus was identified and no bacteria were isolated. Samples were scored as simplex infection if one of the 13 enteric pathogens was isolated or identified; as a mixed infection if at least two of these pathogens were isolated or identified; as a bacterial-viral mixed infection if at least one bacteria was isolated and one virus was identified.

Statistical Analysis

Data were analyzed using Statistical Analysis Software (SAS) version 9.3. Numbers and percentages were computed for categorical variables. Cochren-Mantel-Haenszel test was used for comparison of categorical variables. Binary logistic model and general logit model were used for binary dependent variables and multi-category disordered dependent variables respectively, to calculated adjusted odds ratio (aOR) and to explore the association between etiology and characteristics of infectious diarrhea after adjusting for confounders. Variables of age group, suburb, gender, season, and epidemiological histories were put into model and selected by stepwise methods. Age group, gender, suburb, season, consumption of suspicious food, medical history of enteric disease, and whether to keep a pet were included in the final model. Two-tailed p values < 0.05 was considered statistically significant.

This study focused on the adult diarrhea patients with age $\geqslant$18 years. Age group was defined as 18-29,30-44, 45-59, and 60+ years, according to the Global Burden of Disease 2000 and surveillance diarrhea patients 'age distribution[20]. Patients who visited hospitals in suburb areas were grouped in "suburb". Patients who visited hospitals in rural areas were grouped in "rural". Season was defined by the climatic characteristics of Shanghai, spring means March to May, and summer means June to August, and autumn means September to November, and winter means December to February. Suspicious food meant the suspicious food that patients self reported and doctors thought that may cause diarrhea, such as food which was contaminated by diarrhea pathogen.

Ethics

The study protocol was approved by the Institutional Ethics Review Committee of the Shanghai Municipal Center for Disease Control and Prevention.

RESULTS

From 1 May 2012 through 31 May 2016, a total of 95284 patients were enrolled in Shanghai diarrhea comprehensive surveillance system, of whom 4571 (4.80%) were not included in this study for the following reasons: 401 (0.42%) patients did not report clinical signs of diarrhea, 379 (0.40%) patients visited the enteric disease clinics within 14 days and thus were considered as the same episodes, 11 (0.01%) patients sought clinical care > 60 days after onset of diarrhea, 212 (0.22%) patients were not infectious diarrhea and have other explicit diagnosis, and 3568 (3.74%) patients were younger than 18 years. Among 90713 adult diarrhea patients, 8797 (9.70%) patients' stool samples were collected and detected. These 8797 patients were included for further analysis.

1. Prevalence of Enteric Bacteria and Viruses

A total of 4657 pathogens were identified or isolated from 4219 (positive rate 47.96%) stool samples of the 8797 samples. There are 1147 bacterial infections (27.19%), 2914 viral infections (69.07%) and 158 bacterial-viral mixed infections (infected with at least 1 bacteria and 1 virus, 3.74%). Excluding mixed-infection samples, *V. parahaemolyticus* infections, DEC infections and *Salmonella* spp. infections were the most frequent bacterial infections, respectively with positive rate 4.50%, 3.43% and

2.90%. Excluding mixed-infection samples, norovirus infections and rotavirus infections were the most frequent viral infections, with positive rates 19.82% and 8.12%, respectively. Positive rates of other enteric viral infections were as follows: sapovirus, 1.93%; astrovirus, 1.56%; and enteric adenovirus, 0.35%. Positive rates of enteric bacterial infections were as follows: *C.jejuni*, 1.13%; *Shigella* spp., 0.22%; *C. coli*, 0.08%; *Y. enterocolitica*, 0.01%; and *Staphylococcus aureus*, 0.01%. In addition, there were 343 (3.90%) mixed infections.

Isolated DEC consisted of 216 ETEC, 131 EPEC, 84 EAggEC, 2 EIEC and 1 EHEC. Identified noroviruses consisted of 281 GI and 1726 GII. Identified rotaviruses consisted of 766 rotavirus group A, 6 rotavirus group B and 15 rotavirus group C.

2. Demographic and Epidemiological Characteristics

The median age was 46 (IQR 30-60) years. Of 8797 patients, 22.94% aged 18-29 years, 24.57% aged 30-44 years, 25.79% aged 45-59 years, and 26.70% aged equal to or older than 60 years. A significant difference in positive rate within different age groups could be found among comparison of positive and negative diarrhea patients (p=0.0150), comparison of bacterial and viral and bacterial-viral infections (p=0.0074), and comparison of different enteric pathogens infections (p<0.0001) (Table 1). There were 4392 (49.93%) male patients, with a higher male proportion in positive diarrhea patients (p=0.0472), DEC infections (aOR=1.29, 95%CI=1.02-1.64) and norovirus infections (aOR=1.22, 95%CI=1.08-1.36) (Table 1 and Table 2).

Table1 Demographic and epidemiological characteristics of diarrhea outpatient adults by different infections

	Positive (n=4219)	Negative (n=4578)	*P*	Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)	*P*	*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)	Other infections§ (n=808)	*P*
Gender, N (%)														
Male	2153 (51.03)	2239 (48.91)	**0.0472**	577 (50.31)	1497 (51.37)	79 (50.00)	0.8005	184 (46.46)	164 (54.30)	128 (50.20)	946 (54.24)	326 (45.66)	405 (50.12)	**0.0011**
Age, Positive rate (%)														
18-29 years	941 (46.70)	1074	**0.0150**	292 (14.49)	611 (30.32)	38 (1.89)	**0.0074**	109 (5.41)	74 (3.67)	43 (2.13)	384 (19.06)	118 (5.86)	213 (10.57)	**<0.0001**
30-44 years	1084 (53.80)	1074		298 (14.79)	748 (37.12)	38 (1.89)		119 (5.91)	72 (3.57)	57 2.83)	473 (23.47)	158 (7.84)	205 (10.17)	
45-59 years	1112 (55.19)	1153		294 (14.59)	768 (38.11)	50 (2.48)		105 (5.21)	78 (3.87)	76 (3.77)	426 (21.14)	231 (11.46)	196 (9.73)	
60+ years	1079 (53.55)	1266		262 (13.00)	786 (39.01)	31 (1.54)		63 (3.13)	78 (3.87)	78 (3.87)	460 (22.83)	207 (10.27)	193 (9.58)	
Living region, , Positive rate (%)														
Suburb	2401 (44.66)	2975	**<0.0001**	665 (12.37)	1645 (30.60)	91 (1.69)	0.6661	257 (4.78)	170 (3.16)	149 (2.77)	1019 (18.95)	403 (7.50)	403 (7.50)	**<0.0001**
Rural	1818(53.14)	1603		482(14.09)	1269(37.09)	67(1.96)		139(4.06)	132(3.86)	255(3.10)	725(21.19)	311(9.09)	405(11.84)	
Epidemiological history , N (%)														
Had a medical history of enteric disease in the past 6 months	17 (0.40)	47 (1.03)	**0.0006**	5 (0.44)	12 (0.41)	0 (0.00)	0.7132	1 (0.25)	1 (0.33)	2 (0.78)	8 (0.46)	2 (0.28)	3 (0.37)	0.9001
Had consumed suspicious food within 5 days before onset	1914 (45.37)	1865 (40.74)	**<0.0001**	490 (42.72)	1350 (46.33)	74 (46.84)	0.1073	179 (45.20)	111 (36.75)	117 (45.88)	847 (48.57)	282 (39.50)	378 (46.78)	**<0.0001**
Had went out within 7 days before onset	78 (1.85)	46 (1.00)	**0.0010**	29 (2.53)	48 (1.65)	1 (0.63)	0.0881	7 (1.77)	8 (2.65)	5 (1.96)	34 (1.95)	6 (0.84)	18 (2.23)	0.3226
Had kept or had contact with pets.	814 (19.29)	604 (13.19)	**<0.0001**	224 (19.53)	556 (19.08)	34 (21.52)	0.7304	55 (13.89)	65 (21.52)	41 (16.08)	323 (18.52)	123 (17.23)	207 (25.62)	**<0.0001**

§ Simplex infections;

Bold face: $P<0.05$

Cochren-Mantel-Haenszel test was used for comparison of categorical variables.

Table2 Adjusted odds ratio of demographic and epidemiological characteristics comparing positive detection with negative detection in diarrhea outpatients*

	Positive (n=4219)		Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)		*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)
Male vs female	1.09 (1.00-1.19)		1.07 (0.94-1.22)	1.1 (0.99-1.22)	1.04 (0.75-1.43)		0.89 (0.72-1.09)	**1.29 (1.02-1.64)**	1.11 (0.86-1.44)	**1.22 (1.08-1.36)**	0.88 (0.75-1.05)
Age (years)											
18-29	1.10 (0.97-1.25)		**1.32 (1.09-1.59)**	0.99 (0.85-1.14)	1.52 (0.93-2.44)		**1.92 (1.41-2.7)**	1.11 (0.79-1.54)	**0.64 (0.44-0.94)**	1.03 (0.88-1.22)	**0.75 (0.58-0.97)**
30-44	**1.28 (1.14-1.45)**		**1.28 (1.06-1.56)**	**1.28 (1.11-1.47)**	1.54 (0.94-2.50)		**2.04 (1.47-2.78)**	1.02 (0.73-1.43)	0.83 (0.58-1.19)	**1.32 (1.12-1.56)**	1.08 (0.84-1.35)
45-59	**1.19 (1.06-1.35)**		1.2 0 (1.00-1.47)	**1.16 (1.01-1.33)**	**1.85 (1.18-2.94)**		**1.72 (1.25-2.38)**	1.06 (0.77-1.47)	1.06 (0.76-1.47)	1.09 (0.93-1.28)	**1.33 (1.08-1.67)**
60+¶	1.00		1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
Suburb	**0.80 (0.72-0.88)**		**0.80 (0.68-0.92)**	**0.80 (0.71-0.89)**	0.83 (0.58-1.19)		0.96 (0.76-1.23)	**0.75 (0.57-0.98)**	0.82 (0.62-1.09)	**0.85 (0.75-0.97)**	**0.79 (0.66-0.95)**
Had a medical history of enteric disease in the past 6 months	**0.41 (0.23-0.73)**		0.42 (0.17-1.08)	**0.43 (0.22-0.85)**	0		0.24 (0.03-1.76)	0.34 (0.05-2.46)	0.71 (0.17-2.94)	0.47 (0.21-1.01)	0.32 (0.07-1.39)
Had consumed suspicious food within 5 days before onset	**1.18 (1.08-1.29)**		1.06 (0.93-1.22)	**1.24 (1.12-1.38)**	1.26 (0.82-1.75)		1.22 (0.99-1.51)	0.84 (0.66-1.08)	0.24 (0.96-1.61)	**1.31 (1.17-1.48)**	0.99 (0.83-1.18)
Had kept or had contact with pets	**1.33 (1.17-1.5)**		**1.57 (1.30-1.90)**	**1.21 (1.04-1.40)**	**1.62 (1.05-2.48)**		1.17 (0.85-1.63)	**1.79 (1.29-2.47)**	1.20 (0.83-1.75)	**1.26 (1.06-1.48)**	1.00 (0.78-1.27)

* Data are adjusted odds ratio (95%CI) in binary logistic model or general logit model

§ Simplex infections

¶ Reference group in logistic regression model

Bold face: $P<0.05$

Adjusted odds ratios of age were shown in Table 2. Patients between 30-44 and 45-59 years were more likely to have infectious diarrhea and viral diarrhea. Those aged 30-44 years were the most likely to get infected with *V. parahaemolyticus* (aOR versus 60+ years group: 2.04 [1.47-2.78]) and norovirus (aOR versus 60+ years group: 1.32 [1.12-1.56]). In addition, patients in 18-29 years group had a significantly lower odds of experiencing infectious diarrhea (aOR=0.85, 95% CI=0.76-0.97), viral infections (aOR=0.78, 95% CI=0.67-0.90), norovirus infections (aOR=0.78, 95% CI=0.66-0.92) and rotavirus infections (aOR=0.70, 95% CI=0.54-0.92) compared with 30-44 years group. Patients in 18-29 years group had a significantly lower odds of experiencing viral infections (aOR=0.85, 95% CI=0.74-0.98), *Salmonella* spp. infections (aOR=0.61, 95% CI=0.41-0.89) and rotavirus infections (aOR=0.56, 95% CI=0.44-0.72) compared with 45-59 years group. Patients in 30-44 years group had a significantly higher odds experiencing norovirus infections (aOR=1.22, 95% CI=1.03-1.43) compared with 40-45 years group.

Among diarrhea patients, 5376 (85.67%) visited the hospitals in suburb. The positive rates in suburb and rural groups were significantly different($p<0.0001$, Table 1). Comparing different enteric pathogen infections, the positive rates of patients in suburb and rural groups were significantly different ($p<0.0001$). More diarrhea patients infected with *V. parahaemolyticus* (64.90%) lived in suburb areas. Patients living in suburb areas were less likely to get infected with enteric pathogens (aOR=0.75-0.85) except *V. parahaemolyticus* infections and *Salmonella* spp. infections (Table 2).

64 (0.73%) patients had a medical history of enteric disease in the past 6 months. Within 5 days before onset, 3779 (42.96%) patients had a history of consuming suspicious food. 124 (1.41%) patients had a history of going out within 7 days before onset. And 1418 (16.12%) patients kept or had contact with pets. When compared with negative patients, a higher proportion of positive patients had a history of consuming suspicious food within 5 days before onset ($p<0.0001$), had a history of going out within 7 days before onset ($p=0.0010$), and kept or had contact with pets ($p<0.0001$), while a lower proportion had a medical history of enteric disease in the past 6 months ($p=0.0006$) (Table 1). Epidemiological history, including consuming suspicious food and keeping or contacting with pets, was significantly associated with higher odds of infectious diarrhea, viral infections and norovirus infections. A medical history of enteric disease was significantly associated with lower odds of infectious diarrhea (Table 2).

3. Clinical Symptoms

Of positive diarrhea patients, 13.11% reported fever, 41.91% reported nausea, 28.21% reported vomiting, and 49.09% reported abdominal pain (Table 3). Watery stool and loose stool were common, respectively accounting for 76.27% and 20.93%. Compared with negative diarrhea patients, positive patients reported more fever ($p=0.0009$), nausea ($p<0.0001$), vomiting ($p<0.0001$) and watery stool ($p<0.0001$), but fewer abdominal pain (<0.0001).

The distributions of clinical symptoms by different infections were significantly different (Table 3). Diarrhea patients infected with bacteria reported more fever

(19.09%, p<0.0001), abdominal pain (64.60%, p<0.0001) and loose stool (23.28%, p<0.0001). Diarrhea patients infected with virus reported more nausea (43.34%%, p=0.0175), vomiting (30.13%, p=0.0001) and watery stool (78.35%, p<0.0001).

Diarrhea patients infected with *V. parahaemolyticus* featured more nausea (56.27%), vomiting (41.41%), abdominal pain (71.9%) and watery stool (81.57%). Patients infected with DEC featured fewer nausea (28.81%) and vomiting (13.58%). Patients infected with *Salmonella* spp. featured more fever (28.24%). Patients infected with norovirus featured fewer fever (9.69%) and abdominal pain (44.55%).

Table3 Clinical symptoms in diarrhea outpatients by different infections

	Positive (n=4219)	Negative (n=4578)	*P*	Bacterial infections (n=1147)	Viral infections (n=2914)	Bacterial-viral Mixed infections (n=158)	*P*	*V. parahaemolyticus*§ (n=396)	DEC§ (n=302)	*Salmonella* spp.§ (n=255)	Norovirus§ (n=1744)	Rotavirus§ (n=714)	Other infections§ (n=808)	*P*
Fever, N (%)	553 (13.11)	495 (10.81)	**0.0009**	219 (19.09)	312 (10.71)	22 (13.92)	**<0.0001**	46 (11.62)	43 (14.24)	72 (28.24)	169 (9.69)	96 (13.45)	127 (15.72)	**<0.0001**
Nausea, N (%)	1768 (41.91)	1561 (34.10)	**<0.0001**	442 (38.54)	1263 (43.34)	63 (39.87)	**0.0175**	224 (56.27)	87 (28.81)	71 (27.84)	790 (45.30)	309 (43.28)	287 (35.52)	**<0.0001**
Vomiting, N (%)	1190 (28.21)	916 (20.01)	**<0.0001**	269 (23.45)	878 (30.13)	43 (27.22)	**0.0001**	164 (41.41)	41 (13.58)	37 (14.51)	595 (34.12)	195 (27.31)	158 (19.55)	**<0.0001**
Abdominal pain, N (%)	2071 (49.09)	2446 (53.43)	**<0.0001**	741 (64.60)	1257 (43.14)	73 (46.20)	**<0.0001**	285 (71.97)	170 (56.29)	151 (59.22)	777 (44.55)	321 (44.96)	367 (45.42)	**<0.0001**
Fecal property, N (%)														
Watery	3218 (76.27)	3150 (68.81)	**<0.0001**	814 (70.97)	2283 (78.35)	121 (76.58)	**<0.0001**	323 (81.57)	202 (66.89)	179 (70.20)	1344 (77.06)	583 (81.65)	587 (72.65)	**<0.0001**
Loose	883 (20.93)	1202 (26.26)		267 (23.28)	583 (20.01)	33 (20.89)		54 (13.64)	85 (28.15)	61 (23.92)	372 (21.33)	121 (16.95)	190 (23.51)	
Mucous	72 (1.71)	143 (3.12)		38 (3.31)	31 (1.06)	3 (1.90)		8 (2.02)	11 (3.64)	11 (4.31)	18 (1.03)	6 (0.84)	18 (2.23)	
Else	46 (1.09)	83 (1.81)		28 (2.44)	17 (0.58)	1 (0.63)		11 (2.78)	4 (1.32)	4 (1.57)	10 (0.57)	4 (0.56)	13 (1.61)	

§ Simplex infections;

Bold face: $P<0.05$

Cochren-Mantel-Haenszel test was used for comparison of categorical variables

4. Pathogen Spectrum and Seasonality

In term of descriptive data, the enteric pathogens spectrum of infectious diarrhea patients displayed a yearly seasonal trend (Figure 1). In general, viruses were predominant during November to March of every seasonal cycle, accounting for more than 80% in every month. Bacteria were predominant during June to August of almost every seasonal cycle, accounting for more than 60% in every month. September and October were the transition period from bacteria to viruses, and April and May were the transition period from viruses to bacteria. Norovirus and rotavirus both showed yearly seasonal trends. Rotavirus peaked in winter months, especially in December and January. Norovirus displayed a less distinct and broader seasonality. Norovirus clustered around autumn and winter, while a smaller peak appeared in March of 2014 and 2015. In the seasonal cycle from 2015-2016, norovirus peaked in March 2016. *V. parahaemolyticus*, DEC and *Salmonella* spp. all showed yearly seasonal trends. These three enteric bacteria peaked in August, and *Salmonella* spp. showed a smaller peak (Figure 2).

Figure 1 insert here.

Figure 2 insert here.

In term of statistical analysis, there were significantly different season distributions in comparison of positive and negative diarrhea patients ($p<0.0001$), comparison of bacterial and viral and bacterial-viral infections ($p<0.0001$), and comparison of different enteric pathogens infections ($p<0.0001$). More bacterial infections appeared in summer (54.58%) and more viral infections appeared in winter (44.51%). The

proportion of winter was lower among norovirus infections (34.86%) compared with among rotavirus infections (67.37%).

Patients in summer were 1.55-4.39 times more likely to have simplex bacterial diarrhea and 0.16-0.20 times less likely to have simplex viral diarrhea compared with in spring. Patients in autumn were 2.02-3.38 times more likely to have *V. parahaemolyticus* infections and DEC infections, and 0.69-0.77 times less likely to have simplex viral diarrhea compared with in spring. Patients in winter were 1.60-5.61 times more likely to have simplex viral infections, and 0.14-0.56 times less likely to have simplex bacterial diarrhea compared with in spring (Table 4).

Table4 Seasonality of diarrhea outpatients by different infections*

	Negative (n=4578)	**Positive (n=4219)**		**Bacterial infections (n=1147)**	**Viral infections (n=2914)**	**Bacterial-viral Mixed infections (n=158)**		***V. parahaemolyticus*** **§ (n=396)**	**DEC§ (n=302)**	***Salmonella* spp.§ (n=255)**	**Norovirus§ (n=1744)**	**Rotavirus§ (n=714)**
Season [(No.(%)]		***P*<0.0001**		***P* <0.0001**				***P* <0.0001**				
Spring	867 (18.94)	877 (20.79)		149 (12.99)	695 (23.85)	33 (20.89)		34 (8.59)	21 (6.95)	41 (16.08)	462 (26.49)	101 (14.15)
Summer	1746 (38.14)	927 (21.97)		626 (54.58)	260 (8.92)	41 (25.95)		252 (63.64)	178 (58.94)	123 (48.24)	180 (10.32)	32 (4.48)
Autumn	1238 (27.04)	1031 (24.44)		322 (28.07)	662 (22.72)	47 (29.75)		106 (26.77)	96 (31.79)	72 (28.24)	494 (28.33)	100 (14.01)
Winter	727 (15.88)	1384 (32.80)		50 (4.36)	1297 (44.51)	37 (23.42)		4 (1.01)	7 (2.32)	19 (7.45)	608 (34.86)	481 (67.37)
Season [aOR (95%CI)]												
Spring^		1.00		1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
summer		**0.54 (0.48-0.61)**		**2.16 (1.77-2.64)**	**0.18 (0.16-0.23)**	0.62 (0.39-1.00)		**3.65 (2.53-5.29)**	**4.39 (2.77-6.96)**	**1.55 (1.07-2.23)**	**0.20 (0.17-0.24)**	**0.16 (0.10-0.24)**
autumn		**0.85 (0.75-0.97)**		1.59 (0.28-1.97)	**0.69 (0.60-0.79)**	1.04 (0.66-1.65)		**2.2 (1.48-3.28)**	**3.38 (2.09-5.47)**	1.26 (0.85-1.87)	**0.77 (0.66-0.9)**	**0.69 (0.52-0.93)**
winter		**1.91 (1.67-2.18)**		**0.40 (0.29-0.56)**	**2.26 (1.98-2.59)**	1.36 (0.84-2.20)		**0.14 (0.05-0.40)**	**0.39 (0.16-0.92)**	**0.56 (0.32-0.97)**	**1.60 (1.37-1.88)**	**5.61 (4.42-7.11)**

* Data are adjusted odds ratio (95%CI) in binary logistic model or general logit model

§ Simplex infections;

¶ Reference group in logistic regression model

Bold face: $P<0.05$

Cochren-Mantel-Haenszel test was used for comparison of categorical variables

DISCUSSION

This study is the first study in Shanghai to identify the etiology and epidemiology of adult infectious diarrhea in sporadic outpatients from a continuous active diarrhea surveillance enhanced with comprehensive laboratory testing for common enteric bacteria and virus. It also adds to the limited number of studies investigating adult cases of infectious diarrhea in China. The Shanghai Diarrhea Comprehensive Surveillance System used Probability Proportionate to Size (PPS) sampling method and was conducted among 22 sentinel hospitals in all 16 districts of Shanghai continuously since May 2012, data from which are more representative and more feasible to be extrapolated to the city's population by avoiding the influence of clusters and season-specific cases.

Etiology of adult infectious diarrhea in Shanghai was detailed in this study. At least one enteric pathogen was found in 47.96% of adult diarrhea patients' stools. Viral infections are predominant and bacteria were isolated from many cases. These findings were consistent with those of Wang 's research in Beijing[2]. We found that norovirus was the most common enteric pathogen, accounting for over 40% of all cases, followed by rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. The proportion of norovirus was higher than the sum proportion of rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. This result confirmed norovirus's leading role in adult infectious diarrhea in China, and was similar to the research finding in sporadic gastroenteritis in both developing and developed countries [21-24]. And it is observed that norovirus infections were more than twice that of rotavirus in

adult patients in Shanghai. Rotavirus ranked second to norovirus. This results were consistent with studies in Russia [24] and Shanghai, China[25], while inconsistent with study in France[26]. Yet according to WHO, rotavirus is most common etiological agents of diarrhea in developing countries, which may be due to rotavirus's important role in children. As the leading cause of severe diarrhea in children, the pathogenic role and disease burden of rotavirus in adults has been underestimated. Rotavirus needs more attention in routine clinical diagnosis and vaccination program.

According to this study, *V. parahaemolyticus*, DEC and *Salmonella* spp. were common bacteria in adult infectious diarrhea. The prevalence of these three bacterial infections was between 2.90~4.50%, much lower than viral infections. In previous studies，*V. parahaemolyticus*, DEC and *Salmonella* spp. were also among the most prevalent pathogen in adult infectious diarrhea in different regions of China and worldwide[1, 2, 17, 18, 27]. Although diarrhea due to *V. parahaemolyticus* has decreased since 1998[28, 29], *V. parahaemolyticus* was still the leading cause of adult bacterial infections in this study. However, *Shigella* spp. was also among frequent bacteria in several studies before 2013[18, 28, 29]. This study showed that positive rate of *Shigella* spp. infections was only 0.22% during 2012-2016 in Shanghai, which may be due to the downward trend of *Shigella* spp. infections over time [29].

This study showed that there was association between adult infectious diarrhea and patient age. In general, patients between 30-59 years were more likely to have infectious diarrhea and viral diarrhea than age groups of 18-29 and 60+ years. This was partly consistent with a study in France which found incidence of acute diarrhea

in youth group was higher than elderly group [26]. Elderly people (⩾60years) were the least likely to get infected with *V. parahaemolyticus*, whereas people aged 30-44 years were the most likely among adult age groups. The similar findings were observed in a study in Shanghai[29]. This may be related to more seafood consumption in young adults, which is an important risk factor in *V. parahaemolyticus* infections[30] .In contrast to other studies which found elderly people more likely to get infected with norovirus[22, 31], our study discovered that the highest proportion in norovirus infections was 30-44 years old. And considering the results of general logit model adjusting for other factors, 30-44 years patients were the most likely to get infected to norovirus. Patients aged 18-29 years had the lowest odds experiencing rotavirus diarrhea.

People living in rural areas were more susceptible to DEC, norovirus and rotavirus, which may be because city environment provided more chances for pathogens to transmit.

In regard to clinical symptoms in general, bacterial diarrhea was characterized by fever, abdominal pain and loose stool, while viral diarrhea was characterized by nausea, vomiting and watery stool. However the symptoms of *V. parahaemolyticus* infections showed more like viral infections. In addition, abdominal pain was common in *V. parahaemolyticus* infections. These findings of *V. parahaemolyticus* were in accordance with a research in Shanghai during 1998-2013[28]. The symptoms of DEC and *Salmonella* spp. were similar except fever. The proportion of fever was the highest in *Salmonella* spp. (28.24%) while lowest in norovirus (9.69%). The

proportion of fever in norovirus infections was much lower in comparison with some studies[26, 31], while the proportion in *Salmonella* spp. infections was close to another research [28]. The proportion of abdominal pain was the highest in *V. parahaemolyticus* (71.97%) while much lower in norovirus (44.55%) and rotavirus (44.96%).

This study also demonstrated the seasonality of adult infectious diarrhea and relevant contribution of different enteric pathogens in seasonal trend. A seasonal distribution of adult infectious diarrhea was observed with a large peak in winter and a small peak in summer. Winter peak was mainly due to norovirus and rotavirus, which was in line with previous study[32, 33]. Summer peak was smaller, due to low proportion of bacterial infections. What should be noted was that there was a peak around March due to norovirus in 2014-2016, even higher than the summer peak in 2015-2016 season cycle. This emerging spring peak was possibly because of the increased activity of a novel norovirus GII.17 [34]. Rotavirus showed a distinct peak in December and January (significantly winter VS summer aOR=35.67), which was consistent with researches in Shanghai and Iran [25, 35], while different from a study in London (peak from January through May) [36] and a study in Russia (peak from December through May) [24]. However, norovirus displayed a broader seasonality peaking around autumn and winter (significantly winter VS summer OR=8.00) in this study and a study in Netherland [9]. Bacterial infections, included *V. parahaemolyticus*, DEC and *Salmonella* spp., showed a yearly seasonality peaking in summer (often in August), with significantly summer VS winter OR 25.00, 11.11 and

2.78 respectively. This was similar in Enserink's study [9], whereas autumn peak of bacterial infections was observed in some studies [25, 37]. The seasonality of infectious diarrhea may be due to the climate, biological characteristics of pathogens and people's diet habit of Shanghai.

There are several limitations that need to be acknowledged. First, information and detection results were collected from 22 hospitals and 16 laboratories. Though detection methods & materials were unified and regular trainings were held, there was still a chance of bias caused by the different levels and conditions of hospitals and laboratories. Admission rate bias should also be taken into consideration as patients may have a preference when visiting hospitals of different levels or in different regions. Second, the recall bias of epidemiological information was difficult to avoid. And the data of exposure history was important for infectious diarrhea. Third, only diarrhea patients who visited the enteric disease clinics were included in surveillance, severe diarrhea patients or asymptomatic patients were possibly not studied in our research. Fourth, as for seasonality, only descriptive data of every month or statistical tests of seasons were demonstrated. No statistical methods were used to analyze the successive time series, because of the limit seasonal cycles of existing data. In the future, after accumulating enough data for several years, time series analysis could be taken to explore the inherent natural order and to forecast prospective trend.

CONCLUSIONS

In conclusion, this study provides a detailed picture about the epidemiology, etiology and seasonal pathogen spectrum of adult infectious diarrhea in Shanghai. Viral

infections are predominant, and norovirus is the most common enteric pathogen detected in our surveillance. Other common pathogens include rotavirus, *V. parahaemolyticus*, DEC and *Salmonella* spp.. Patients between 30-59 years were more likely to have infectious diarrhea and viral diarrhea. A seasonal distribution was observed with larger peaks in winter and smaller peaks in summer. Winter peak was mainly due to norovirus and rotavirus, and summer peak was due to bacterial infections. An emerging spring peak of norovirus around March was observed in recent 3 years. Our findings highlight the necessity for conducting an active, comprehensive surveillance for both bacterial and viral enteric pathogens in adults, to monitor the changing dynamics in the epidemiology and etiology of infectious diarrhea. These findings help us to understand adult infectious diarrhea better and to develop targeted prevention strategies.

LIST OF ABBREVIATIONS

aOR: Adjusted odds ratio; *C. coli*: *Campylobacter coli*; *C. jejuni*: *Campylobacter jejuni*; DEC*: Diarrheagenic Escherichia coli*; EAggEC: Enteroaggregative escherichia coli; EHEC: Enterohemorrhagic escherichia coli; EIEC: Enteroinvasive escherichia coli; EPEC: Enteropathogenic escherichia coli; ETEC: Enterotoxigenic escherichia coli; PPS: Probability Proportionate to Size; rRT-PCR: real-time Reverse Transcription -Polymerase Chain Reaction; SAS: Statistical Analysis Software; *V. cholera: Vibrio cholera; V. parahaemolyticus*: *Vibrio parahaemolyticus;* WHO: World Health Organization; *Y. enterocolitica: Yersinia enterocolitica;*

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Xiao-huan Gong performed the statistical analysis and drafted the manuscript. Hao Pan and Huan-yu Wu put the surveillance system into effect. Jian Li designed the study of the surveillance system. Wen-jia Xiao participated in the management of the system. Xi Zhang, Min Chen and Zheng Teng carried out the management and quality control of the laboratory tests. Zheng-an Yuan conceived of the study. All authors read and approved of the final manuscript.

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Planning.

DATA SHARING STATEMENT

No additional data are available for public sharing.

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Figure 1 Pathogen spectrum of major enteric pathogens in adults with infectious diarrhea by month in Shanghai, May 2012-May 2016

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

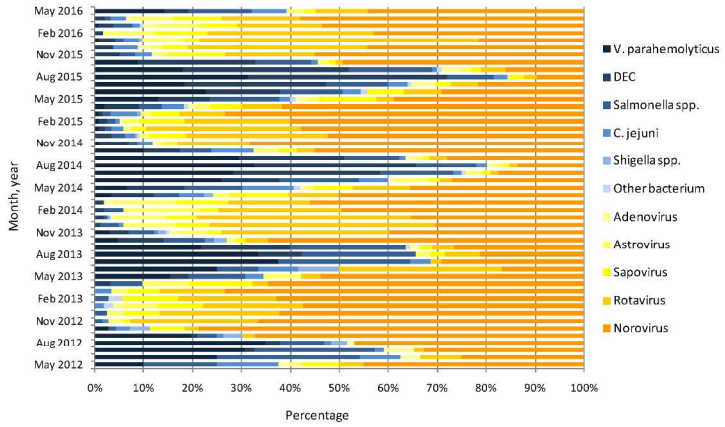

Figure 1 Pathogen spectrum of major enteric pathogens in adults with infectious diarrhea by month in Shanghai, May 2012-May 2016

Figure 1 Pathogen spectrum of major enteric pathogens in adults with infectious diarrhea by month in Shanghai, May 2012-May 2016

297x210mm (300 x 300 DPI)

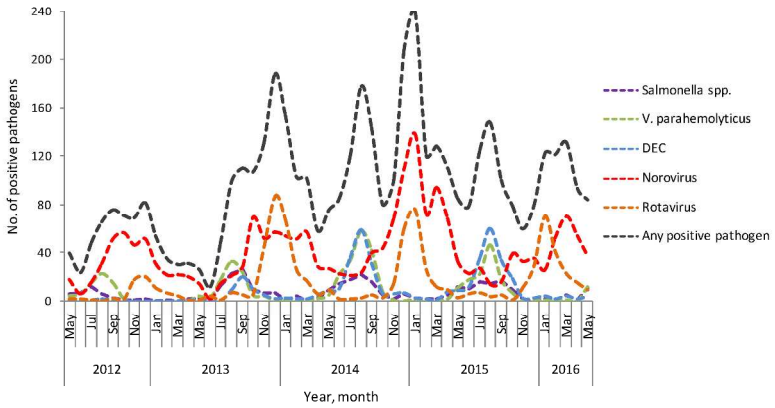

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

Figure2 Seasonality of major enteric pathogens in adult with infectious diarrhea in Shanghai, May 2012-May 2016

297x210mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7-8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7-8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-10
Bias	9	Describe any efforts to address potential sources of bias	7-10
Study size	10	Explain how the study size was arrived at	7-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	7-8/10
		(*e*) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10-11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	10-11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-21
		(*b*) Report category boundaries when continuous variables were categorized	10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	22-26
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	22-26
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	27

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.