Original Article

Analysis of Bacterial Pathogens Causing Acute Diarrhea on the Basis of Sentinel Surveillance in Shanghai, China, 2006–2011

Yinghua Zhang1, Yanping Zhao2, Keying Ding1, Xiaoguang Wang1, Xiuhua Chen1, Yun Liu1, and Yue Chen3*

1Department of Microbiology, and 2Department of Epidemiology, Center for Disease Control and Prevention, Minhang District, Shanghai, China; and 3Department of Epidemiology and Community Medicine, University of Ottawa, Canada

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SUMMARY: Acute diarrhea is the most common infectious disease worldwide and its causes vary from one region to another. We aimed to analyze the spectrum and epidemiological characteristics of pathogens from 22,386 outpatients with acute diarrhea on the basis of surveillance data from Shanghai, China, during 2006–2011. The following 8 pathogens were isolated and identified using standard methods: Salmonella, Shigella, Vibrio cholerae, V. parahaemolyticus, enteropathogenic Escherichia coli, enterotoxigenic E. coli, enteroinvasive E. coli, and enterohemorrhagic E. coli. In total, 2,234 strains of pathogens were obtained and the overall isolation rate of these 8 pathogens gradually decreased from 17.1% in 2006 to 7.4% in 2011. V. parahaemolyticus was the most frequently identified pathogen, followed by Shigella and Salmonella. The isolation rate of V. parahaemolyticus notably varied by season, whereas Salmonella and Shigella infections showed little seasonal variation. Age-related variation was also observed. V. parahaemolyticus infection occurred more often in patients aged 20–40 years. S. enterica serovar Enteritidis and S. flexneri were the most common serotypes of Salmonella and Shigella, respectively. The descending trend observed in the isolation rate of pathogens from the current surveillance suggests an urgent requirement or improvement.

INTRODUCTION

Acute diarrhea is an important public health problem, and it remains a leading cause of morbidity and mortality worldwide (1,2). In China, the incidence of acute diarrhea was approximately 55.9/100,000, and approximately 700,000 diarrhea cases were reported annually; it ranked among the top 3 of 38 categories of notifiable infectious diseases (3).

Acute diarrhea can be caused by a wide range of enteric pathogens, varying with geographical area (4–6). A better understanding of the etiology of diarrhea is important for epidemiological surveillance, treatment and prevention. The present study aimed to analyze the spectrum and epidemiological characteristics of enteric pathogens on the basis of a continuous surveillance for outpatients with acute diarrhea in Shanghai, China, from 2006 to 2011. Minhang District, located in southeast Shanghai, has a total of 10 community hospitals and 2 secondary hospitals. Three hospitals were selected every year for routine surveillance of acute diarrhea. Two days per week were randomly selected, and every 10th patient with acute diarrhea who was admitted to intestinal clinics in Minhang District was enrolled in the study, a total of 22,386 outpatients were included during the study period. The number of acute diarrhea cases included in this study substantially varied from year to year. This is largely due to different patient volumes of hospitals that were selected for the surveillance for a particular year. Each patient provided information on age, sex, date of diagnosis and medical care.

MATERIALS AND METHODS

Case definition: Diarrhea was defined as 3 or more loose or watery stools per day or 1 or 2 loose stools in 24 h, accompanied by at least 1 of the following symptoms: nausea, vomiting, abdominal cramps, or fever of 38°C or higher. Acute diarrhea was defined as diarrhea that lasted for less than 14 days before the time of diagnosis. Persistent diarrhea was defined as diarrhea that lasted for more than 14 days at presentation. Patients with persistent diarrhea were excluded from the present study.

Isolation and identification of pathogens: Stool specimens were collected using wide-mouthed sterile plastic containers containing Cary–Blair transport medium. The specimens were transported within 4 h of collection to the Microbiology Laboratory in Minhang District Center for Disease Control and Prevention and processed on the same day. A portion of the specimen was stored at −20°C for repeat detection. Eight enteric pathogens including Salmonella spp., Shigella spp., Vibrio spp., and diarrheagenic Eschericia coli (DEC), including enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), and enterohemorrhagic E. coli (EHEC), were isolated using standard microbiological methods. The isolation

*Corresponding author: Mailing address: Department of Epidemiology and Community Medicine, University of Ottawa, Canada, K1H8M5. Tel: +1-613-562-5800 ext. 8287, Fax: +1-613-562-5466, Email address: Yue.Chen@uottawa.ca
and identification of enteric pathogens were performed using the protocol outlined in the Diagnostic Criteria for Infectious Diarrhea, as detailed by Qu et al. (7). Isolated colonies of suspected enteric pathogens obtained from agar plates were confirmed and identified using the API 20E biochemical system (BioMerieux, Etoile, France). Serotyping was performed by slide agglutination tests using commercially available antisera (anti-
Salmonella antiserum from S&A Reagents Lab Ltd., Bangkok, Thailand; anti-Shigella and anti-V. cholerae antiserum from Denka Seiken, Tokyo, Japan; anti-DEC antiserum from Ningbo Tianrun Bio-Pharmaceutical Co., Ltd., Zhejiang, China). The serotypes of 4 DEC were verified using anti-DEC antisera (Statens Serum Institute SSI Diagnostica, Copenhagen, Denmark).

**Statistical analysis:** Comparative statistics, including the $\chi^2$ test and $\chi^2$ test for trend, were calculated using SPSS20.0 software (SPSS, Inc. and IBM Company, Chicago, IL, USA). A $P$-value ($P < 0.05$) was considered statistically significant.

### RESULTS

**Isolation rates of enteric pathogens:** In total, 2,243 isolates were collected from 22,386 patients with acute diarrheal. The overall isolation rate of enteric pathogens was 17.1% ($439/2,564$) in 2006, 15.7% ($465/2,961$) in 2007, 9.5% ($272/2,849$) in 2008, 8.9% ($264/2,981$) in 2009, 7.2% ($429/5,956$) in 2010, and 7.4% ($374/5,075$) in 2011, with a notable downward trend over time ($\chi^2$ trend = 296.970, $P < 0.01$). Similar downward trends were also observed for *V. parahaemolyticus*, *Salmonella*, and *Shigella*. *V. parahaemolyticus* was identified with the highest frequency among these enteric pathogens, followed by *Salmonella* and *Shigella*, with isolation rates of 8.3%, 0.9%, and 0.8%, respectively (Table 1).

**Seasonal variation:** The enteric pathogens were isolated throughout the year. The isolation rate of *V. parahaemolyticus* showed a distinct seasonal variation with a higher rate in the summer and autumn months. In contrast, remarkable seasonal variation was not observed for *Salmonella* and *Shigella* (Fig. 1).

**Variation in patient age:** The patients were classified into 7 groups on the basis of age. The total isolation rate of enteric pathogens varied with age, with the highest rate (14.5%) being observed in the age group of 20–29 years ($P < 0.01$). The isolation rate of each enteric pathogen significantly differed between different age groups. A large proportion of infections caused by *V. parahaemolyticus* as well as *Salmonella* ($P < 0.05$) was observed in the adult age groups of 20–29 and 30–39 years, whereas patients aged $\leq 10$ years were most susceptible to *Shigella* infection ($P < 0.01$) (Table 2).

**Serotypes of Shigella, Salmonella, and V. parahaemolyticus:** The isolates of *Salmonella*, *Shigella*, and *V. parahaemolyticus* were serotyped (Table 3). Of the 212 isolates of *Shigella*, *S. flexneri* was the most common serogroup (62.3%, 132/212), followed by *S. sonnei* (37.7%, 80/212). *S. dysenteriae* and *S. boydii* were not detected. In total 10 serotypes of *S. flexneri* were detected; the predominant serotypes were 4c (27.8%, 59/132) and 2a (10.4%, 22/132), while any other serotypes were less than 10%.

### Table 1. Number (%) of 8 enteric pathogens isolated from patients with acute diarrhea, 2006 to 2011

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th><em>V. parahaemolyticus</em></th>
<th><em>Shigella</em></th>
<th><em>Salmonella</em></th>
<th>Other 1)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>2,564</td>
<td>372 (14.5)</td>
<td>27 (1.1)</td>
<td>40 (1.6)</td>
<td>0 (0.0)</td>
<td>439 (17.1)</td>
</tr>
<tr>
<td>2007</td>
<td>2,961</td>
<td>339 (11.4)</td>
<td>90 (3.0)</td>
<td>34 (1.1)</td>
<td>2 (0.1)</td>
<td>465 (15.7)</td>
</tr>
<tr>
<td>2008</td>
<td>2,849</td>
<td>208 (7.3)</td>
<td>40 (1.4)</td>
<td>24 (0.8)</td>
<td>0 (0.0)</td>
<td>272 (9.5)</td>
</tr>
<tr>
<td>2009</td>
<td>2,981</td>
<td>213 (7.1)</td>
<td>24 (0.8)</td>
<td>27 (0.9)</td>
<td>0 (0.0)</td>
<td>264 (8.9)</td>
</tr>
<tr>
<td>2010</td>
<td>5,956</td>
<td>402 (6.7)</td>
<td>12 (0.2)</td>
<td>15 (0.3)</td>
<td>0 (0.0)</td>
<td>429 (7.2)</td>
</tr>
<tr>
<td>2011</td>
<td>5,075</td>
<td>315 (6.2)</td>
<td>19 (0.4)</td>
<td>39 (0.8)</td>
<td>1 (0.0)</td>
<td>374 (7.4)</td>
</tr>
<tr>
<td>Total</td>
<td>22,386</td>
<td>1,849 (8.3)</td>
<td>212 (0.9)</td>
<td>179 (0.8)</td>
<td>3 (0.0)</td>
<td>2,243 (10.0)</td>
</tr>
</tbody>
</table>

$\chi^2$ for trend = 296.970, $P < 0.01$.

1): Including *Vibrio cholerae* (1 isolate, O139), enterotoxigenic *E. coli* (2 isolates, O25K19).
Other isolates belonged to 11 different serotypes. 

Derby (13.4\%, 20/179), Typhimurium (20.1\%, 36/179), S. enterica serovar Enteritidis (22.9\%, 41/179), S. enterica serovar Typhimurium (20.1\%, 36/179), S. enterica serovar Derby (13.4\%, 24/179), S. enterica serovar Senftenberg (12.8\%, 23/179), S. enterica serovar Agona (10.6\%, 19/179), and S. enterica serovar Lomita (8.4\%, 15/179). Other isolates belonged to 11 different serotypes. In case of V. parahaemolyticus, only the 315 isolates obtained in 2011 were serotyped. Of these, O3:K6 was the predominant serotype (66.9\%), followed by O4:K8 (19.3\%). Forty-three isolates (28 of O3:KUT, 15 of O1:KUT) were K untypable (KUT) in the current serotyping scheme.

### DISCUSSION

The current large-scale surveillance covering the peak infection seasons over 6 years and a total of 22,386 patients was employed for determining the causative enteric pathogens and epidemiological characteristics of acute diarrhea in Shanghai for the period from 2006 to 2011. Enteric bacterial pathogens were isolated using traditional diagnostic techniques from 10\% of patients with acute diarrhea, which falls within the previously reported range of 4.8\% to 55.1\% (6.8–10). It should be noted that variations pertaining to geographical area, the types of enteric pathogens, and diagnostic methods were observed in these studies.

The isolation rate of enteric pathogens showed a gradual decrease from 17.1\% in 2006 to 7.4\% in 2011. The reasons for this decline, albeit not well understood, may have important public health implications. Data from the present study suggest that these enteric bacterial pathogens likely play less important roles over time because of changes in the pathogen spectrum; therefore, there is an urgent requirement for improvement in the surveillance of acute diarrhea. Since the year 2012, 2 additional bacteria (Campylobacter jejuni and Yersinia enterocolitica) and 4 viruses (Rotavirus, Norwalk virus, Astrovirus, and Adenovirus) have been included routine surveillance. Frequent intervention measures pertaining to these pathogens have been implemented on the basis of surveillance information from previous years. The data from the present study indicate that current surveillance and interventions likely reduce the risk of infections from common enteric pathogens.

The predominant enteric pathogens identified in the present study include V. parahaemolyticus, Shigella and Salmonella. These results are consistent with a those of previous report from Beijing, China (7), with the exception that V. parahaemolyticus was the predominant bacterial pathogen isolated in Shanghai, whereas Shigella was the predominant bacterial pathogen isolated in Beijing. The O3:K6 serotype was predominant among V. parahaemolyticus isolates, which is consistent with certain national and international reports (7,11,12). In accordance with previous reports (12,13), V. parahaemolyticus was the predominant bacterial pathogen isolated in the Shanghai region, possibly because of favorable environmental factors for the pathogen V. parahaemolyticus is widely distributed in temperate, oceanic, and coastal environments. Because of its halophilic characteristics, it is often isolated from seawater, sediments, and a variety of seafood. This bacte-
um is a leading cause of acute diarrhea associated with seafood consumption worldwide (11,13–15). Shanghai is a coastal city located in the eastern part of China. The consumption of contaminated seafood, which is the leading causes of acute diarrhea in adults, is largely responsible for the higher isolation rate of *V. parahaemolyticus* in this region. However, further investigation is required for identifying the type of seafood responsible for *V. parahaemolyticus* infection. The data also revealed a considerable decrease in the isolation rate of *V. parahaemolyticus* during the past 6 years. The sample size varied between the study years and was the largest in the year 2010; the number of *V. parahaemolyticus* isolates was also the highest in the same year. We speculate that a series of control measures implemented by the government from 2006 to 2011 may have reduced the risk of infection and facilitated improvement in hygiene conditions throughout the supply chain, from the distribution of seafood to its consumption.

*Shigella* is another major causative pathogen for diarrhea. In the present surveillance, *Shigella* was isolated from 212 (0.9%) patients; this value is lower than the previous isolation rates (1% to 5%) from certain countries (16,17). Of the 4 species of *Shigella*, namely *S. dysenteriae*, *S. flexneri*, *S. sonnei*, and *S. boydii*.

*S. flexneri* was found to be the most common epidemi-}

The overall isolation rate of *Salmonella* in the present study (0.8%) was lower than that reported previously from China (7,19) as well as developed countries such as the United States and Italy, where a much higher incidence of diarrhea was caused by *Salmonella* (20). More than 2,000 serotypes of *Salmonella* have been discovered globally; however, only a limited number of serotypes are known to cause human disease (21). *S. enterica* serovar Enteritidis was identified as the chief serotype in the present surveillance; this is consistent with a previous report from Beijing, China (7).

In conclusion, we demonstrated that enteric pathogens accounted for approximately 10% of patients with acute diarrhea in Minghang, Shanghai, during period 2006–2011. *V. parahaemolyticus*, *Shigella* spp., and *Salmonella* spp. were the major bacterial pathogens responsible for acute diarrhea in the region. Seasonal and age-related variations were observed in bacterial isolation rates. *S. enterica* serovar Enteritidis and *S. flexneri* were the most common serotypes of *Salmonella* and *Shigella*, respectively. The 8 enteric pathogens included in the present surveillance could not be isolated from approximately 90% of the total samples. Improving the surveillance of acute diarrhea requires additional efforts pertaining to diagnostic capabilities and other important pathogens, which need to be identified.

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Conflicts of interest None to declare.

REFERENCES


