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The prevalence of diarrhea-causing bacterial strains in Tehran, Iran

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ABSTRACT

Diarrheal disease is one of the main causes of death in developing countries. This study was performed to the prevalence of diarrhea-causing bacterial strains admitted to the Military hospitals and Imam Khomeini Medical Center Hospital. This cross-sectional study was conducted in a period of 9 months from June 2014 to February 2015, 435 non-duplicative diarrheal stool samples were collected from the Imam Reza, Besat Nahaja general, Khanevadeh Artesh and Imam Khomeini Medical Center Hospitals. For separation and differentiation bacterial species selective and differential medium was used. Of the 435 cases, 204 (46.9%) and 231 (53.1%) were female and male, respectively. The prevalence of *Shigella*, *C. jejuni*, *Salmonella* and EHEC were 98 (22.5%), 41 (9.4%), 21 (4.8%) and 6 (1.8%), respectively. The frequency of *S. flexneri* and *S. sonnei* were 56.1% and 4.9%, respectively. The prevalence of bacterial infection among cases of diarrhea was 22.5%, and the most infection rate was related to *S. flexneri*.

1. Introduction

Bacterial gastroenteritis occurs when microorganisms cause an infection in the gastrointestinal tract (GI). This condition causes inflammation in GI, and may experience disagreeable signs such as vomiting, severe abdominal cramps and diarrhea. Diarrheal disease is caused by increased secretion of liquid, reduced absorption of fluid or quick passage of feces through the intestine (Samie et al., 2009; Jafari et al., 2008). Indications related to this disease include abdominal pain, particularly cramping. Other symptoms depend on the cause of the diarrhea (Jafari et al., 2008). Bacterial gastroenteritis can result from poor sanitation (Neal et al., 1997). This disease is a major public concern and responsible for 3 million deaths per year worldwide. *E. coli*,

Shigella, *Campylobacter* and *Salmonella* species are the main pathogenic bacteria that causing gastrointestinal infection in the world (Sackey et al., 2001; Cunningham et al., 2010). In general dysentery caused by *Shigella* spp., is a main global alarm worldwide and responsible for approximately 5 to 10% of diarrhoeal illnesses in many areas (Schroeder et al., 2008). Recently in Asia the frequency of dysentery was estimated to be 91 million and 414,000 each year (Girard et al., 2006). *Shigella* spp., are categorized in four serogroups, including *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei* (Ashkenazi et al., 2004). Between these serotypes*, S. flexneri* is the most common serotype, followed by *S. sonnei* (Hale et al., 1983). *Campylobacter* infections are among the most common bacterial infections in humans. They cause both diarrheal and systemic diseases.

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In industrialized regions, enteric *Campylobacter* infections cause an inflammation and bloody or watery diarrhea. Infection by thermophilic species of *Campylobacter* especially *C. jejuni* is one of the most common bacterial causes of diarrhea in human. This bacterium has been frequently the most common cause of community-acquired inflammatory enteritis (Schonberg-Norio et al., 2004; Acheson et al., 2001). In developing areas, the diarrhea may be watery. *Salmonella* is one of the most common bacteria causing GI infections. *Salmonella* spp. are facultative intracellular pathogens. Many infections are a result of digestion of contaminated food. *Salmonella* serovars can be divided into two main groups typhoidal and nontyphoidal *Salmonella* (Scallan et al., 2011; Acheson et al., 2001). Nontyphoidal serovars are more common, and usually cause self-limiting GI disease. So, the serovars transmitted by contaminated water, food, poultry and eggs (Acheson et al., 2001). Food contaminated with *Salmonella* is a significant threat to the health care system and economy. Typhoidal serovars include *S. typhi* and *S. paratyphi A*, which are adapted to humans and do not occur in other animals (McClelland et al., 2004). The World Health Organization (WHO) in 2006 estimated that between 15 and 33 million typhoid fever occur annually worldwide, leading to 500,000 to 600,000 deaths. Verocytoxin-producing *Escherichia coli* (VTEC), also known as Shiga-toxin producing *E. coli* (STEC) or enterohemorrhagic *Escherichia coli* (EHEC), is a food-borne zoonotic pathogen related to outbreaks worldwide that poses a thoughtful public health concern. Characteristics of EHEC infection include; abdominal cramps and bloody diarrhea (dysentery), as well as the life-threatening complication hemolytic uremic syndrome (HUS) (Karch et al., 2001). There are various reports of the bacterial causes of diarrhea in Iran. Several studies showed that the incidence of bacterial causes of diarrhea are 45.6%, 38.8%, 13.8% and 5.4% for *Shigella*, *E. coli*, *Salmonella* and *Campylobacter*, respectively (Karch et al., 2001; Jafari et al., 2009). Due to the lack of awareness about the incidence of bacterial infectious diarrhea in the region and to determine the frequency of GI infections caused by *Shigella* spp. *Salmonella* spp. *E. coli* and *C. jejuni*, the study was performed on the patients with diarrhea referring to Imam Reza, Besat Nahaja general and Khanevadeh Artesh hospitals in 2014-2015 in Tehran, Iran.

2. Materials and Methods

This cross-sectional study was conducted from June 2014 to February 2015. The study population of patients with diarrhea referring to Imam Reza, Besat Nahaja general, Khanevadeh Artesh and Imam Khomeini Medical Center Hospitals that had not taken antibiotics in the past two weeks. All diarrheal samples were transferred to the Microbiology Laboratory of Imam Khomeiny Hospital using the Cary Blair transport medium (Merck Co., Germany). Microscopic test was performed in order to detect of RBC and WBC. Xylose lysine deoxycholate (XLD agar) and MacConkey agar were used for identification of *Salmonella* and *Shigella* species and Sorbitol MacConkey agar (SMAC) (Merck Co., Germany) used for detection of EHEC strains. After incubation at 37°C for 24h, all suspected grown colonies were identified by conventional biochemical and culture base microbiological, API 20E kit (bioMerieux, Marcy l'Etoile, France) and *Shigella* polyvalent agglutinating sera provided by Bio-Rad (Marnes la Coquette, France). So, *Salmonella* serotyping was achieved agreeing with Kaufmann-White scheme using O antisera (Difco, USA) and flagellar antigens were detected by a technique of utilizing microtitre plates. To isolate *C. jejuni,* each sample was streaked onto a Campylobacter selective agar (Merk Co., Germany) containing supplement (50 μg mL^{-1} polymyxin B, 1 μg mL^{-1} trimethoprim and 2 μg mL^{-1} vancomycin) and 7% defibrinated sheep blood. These plates were incubated for 24-72 h at 42°C in microaerophilic condition. Typical colonies from each suspected cultures were selected. Conventional biochemical and bacteriological tests were performed (catalase, oxidase, hippurate hydrolysis, urease, H$_2$S production, susceptibility to nalidixic acid and cephalothin) to confirm the genus and differentiate at the species level (Jafari et al., 2009).

3. Results

In the present study, 435 diarrhea samples were collected. 204 women (46.9%) and 231

men (53.1%) were recruited in this study. The mean age of patient was 20. 8 ± 23.9 years, and their age range were 1 to 72 years. Overall, 98 (22.5%) patient were positive culture for bacteria that were 41 (9.4%) *Shigella* spp. 30 cases (9.6%) *Campylobacter jejuni*, 21 cases (4.8%) *Salmonella* spp. and 6 cases (4/1 %) Entro hemorrhagic *Escherichia coli*. Out of 204 women, 47 patients (23%) and out of 231 men, 51 cases (22.1%) cases were positive. There were not found significant difference between gender and bacterial infection (p=0.81, OR=1.057 (0.673-1.66). The *Shigella* infection was observed more amongst women and men which was 11.8% and 8.2%, respectively. A significant difference correlation was not observed between patients with diarrhea infection and gender (P= 0.72) (Table 1).

33 cases (33.7%) were in the age group under 14 years, 35 patients (35.7%) in the age group 15 to 40 years, 30 patients (30.6%) were older than 40 years.

Statistically, there is not a relationship between age and infection (P=0.84). Among patient under 14 years 12 cases were *Shigella* spp. positive, 13 *Campylobacter jejuni* positive, 5 *Salmonella* spp. positive and 3 Entro hemorrhagic *Escherichia coli* positive. The frequency of bacteria in patients with age group of 15 to 40 years were 14 cases with *Shigella* spp, 10 cases with *Campylobacter jejuni*, 9 cases with *Salmonella* spp. and 2 cases with Entro hemorrhagic *Escherichia coli*. The distribution of pathogenic bacteria in patient over 40 years were 15 cases with *Shigella* spp. 7 cases with *Campylobacter jejuni*, 7 cases with *Salmonella* spp. and one cases with Entro hemorrhagic *Escherichia coli*. There was no statistically significant difference between genders and bacterial infection (P=0.68).

Among the 435 collected stool samples, persistent diarrhea was observed in 76 cases and acute diarrhea in 359 cases. Diarrheic stool in 98 cases were culture positive for bacteria, 7 cases of persistent diarrhea and 91 cases of acute diarrhea. There were not found significant difference between a bacterial infection and persistent diarrhea or acute diarrhea (P=0.07). Our result showed *S. flexneri* (56.1%) and *S. sonnei* (4.9%)

had the highest and the lowest rate amongst different species of *Shigella.*

4. Discussion

In this study, 435 stool samples were tested from patients with gastroenteritis referred to Imam Reza, Besat Nahaja general hospital and Khanevadeh Artesh. In this study, 204 patient was women (46.9%) and 231 case was men (53.1%). Among the 435 samples, 98 (22.5%) were positive for the *Shigella* spp. bacteria. Gastroenteritis is one of the significant problems the world is especially third world countries. This infection is one of the main causes of morbidity and mortality, especially in children (Feigin et al., 1998). In the acute phase of disease patients would loss fluids and electrolyte and in the form of chronic it can cause mental retardation. In a study by Salary et al, was conducted in Tehran hospitals, 2517 stool samples of patients with enterocolitis were tested and only 193 samples were positive which the positive rate in this study was very low. Due to the studies were detected only on bacterial agents, it seems that in Tehran the non-bacterial agents, including viruses cause the majority of intestinal infections (Salary et al., 1997). In our study the relation between bacteria and gender was evaluated. The frequency of all species except *Shigella* is higher in men than women.

This result suggests that women are more sensitive for *Shigella* infection than men, but the scientific justification for not disclosed. *Shigella* is one of the most dangerous intestinal pathogens in developing countries (Shuval et al., 1986). Moreover our results indicated higher levels of different species of bacteria isolated cases of diarrhea. The most common species of *Shigella* was *S. flexneri* with the frequency of 56.1%. In a study by Jafari et al, (2009) 808 samples of patients with diarrhea, the number of 369 cases of intestinal pathogens were shown to be *Shigella* positive. Our results was in agreement with Jafari et al study (Jafari et al., 2009). Throughout the world, many studies have been done on the prevalence of diarrhea-causing bacteria (Zhao et al., 2001). These studies illustrate the importance of different strains of *E. coli* bacteria isolated from patients with acute diarrhea as the largest causative agent in the world.

Table 1. Distribution of pathogenic bacteria isolated in terms of gender.

Type of bacteria	Men	Women	Total
Shigella spp.	19 (8.2)	22 (11.8)	41 (9.4)
Campylobacter jejuni	17 (7.4)	13 (6.4)	30 (6.9)
Salmonella spp.	11 (4.8)	10 (4.9)	21 (4.8)
EHEC	4 (1.7)	2 (1)	6 (1.4)
Negative cases	180 (77.9)	157 (77)	337 (77.5)
Total	231	204	435

Table 2. Distribution of pathogenic bacteria isolated from patients with diarrhea in different age group.

Type of bacteria \ Age	14 years and under	15 to 40 years	41 years and older	Total
Shigella spp	12 (7.6)	14 (9.5)	15 (11.5)	41 (9.4)
Campylobacter jejuni	13 (8.3)	10 (6.8)	7 (5.4)	30 (6.9)
Salmonella spp	5 (3.2)	9 (6.1)	7 (5.4)	21 (4.8)
EHEC	3 (1.9)	2 (1.4)	1 (0.8)	6 (1.4)
Negative cases	124 (79)	113 (76.4)	100 (76.9)	337 (77.5)
Total	157	148	130	435

Table 3. Distribution of pathogenic bacteria based on the type of diarrhea

Type of bacteria	Acute diarrhea	Persistent diarrhea	Total
Shigella spp	38 (41.8)	3 (42.9)	41 (41.8)
Campylobacter jejuni	29 (31.9)	1 (14.3)	30 (30.6)
Salmonella spp	20 (22)	1 (14.3)	21 (21.4)
EHEC	4 (4.4)	2 (28.6)	6 (6.1)
Negative cases	268	69	337
Total	91	7	98

Salmanzadeh et al (2006) was reported 84 STEC strains with a high incidence of diarrhea in children (Salmanzadeh et al., 2005). Jafari et al (2009) showed that 69 (44.7%) *Shigella flexneri* as a common etiological agent of diarrhea and this result were in consistent with our study (Jafari et al., 2009). In a study conducted between 1997-2006 in the United States, *Shigella sonnei* was the most common causative etiologic agent among *Shigella* spp. which had a frequency rate of 6% and *Shigella flexneri* was the second causative agent of infection (Petersen et al., 1998; King et al., 2003). In Sari, north of Iran, Nasrollahi et al, showed that, in all 400 children cases, *E. coli* and *Shigella* were the dominant species (Nasrolahi et al., 2000). In a study by Khalili et al, in Yazd (Khalili et al., 2004) *E. coli* was the predominant pathogen with the incidence of 43%. Moreover the incidence of both *Giardia* and *Shigella* and only *C. jejuni* were 18.8% and 5.9%, respectively (Petersen et al., 1998; King et al., 2003). The rate of Enterohemorrhagic *E.*

coli in our study was 1.4%, which reflects the low prevalence of this pathogen in this area (Kaper et al., 1998). The prevalence of *Campylobacter jejuni* is the leading cause of gastroenteritis in industrialized countries in the study was 9.6 %. The incidence rate of gastroenteritis in Iran is much less than report published from America and Europe. *C. jejuni* is a commensal in the intestines of animals, particularly cattle and has an optimal growth in the humidity environment (Sails et al., 2003). Because of the high rate of infection with these bacteria may be an extensive livestock farming source (Tezcan et al., 1984). This finding is consistent with previous information that the low prevalence of *Campylobacter* as a cause of diarrhea in developing countries. In the current study, the prevalence of the Salmonella was 4.8%. In comparison to our country, in developed countries, the prevalence of *Salmonella* is much higher than other diarrhea-causing pathogens (i.e.; in the America, 22%). The result of this study indicates that the most

common cause of gastroenteritis pathogens is *Shigella* spp. The *S. flexneri* serotype is high prevalence and according to the characteristics of the bacteria resistance, pathogenicity and side effects such as HUS syndrome and septicemia, it has concern for doctors and health practitioners (Goldwater et al., 2012). Annual epidemics of diarrhea caused by *Shigella* can affect thousands of people and damage to the health of individuals and society. Methods for rapid detection of important tools that can improve population health and reduce the public health burden of this disease on health. According to the multiple factors because diarrhea, including microorganisms other than bacteria, such as viruses, fungi, etc., as well as predisposing factors such as diarrhea, inflammatory diseases, autoimmune proposed that more studies to be done.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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