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Prevalence of *Shigella* and Other Pathogenic Gram-negative Bacteria in the Patients with Diarrhea in Kashan City, Iran During 2015-2017

Ehsan Moghanloo ^a | Ahmad Khorshidi ^a | Parisa Badameh ^b | Ali Ghadirian Abarghuie ^c | Mehdi Valipour ^a | Hossein Akbari ^{d,*}

^a Department of Microbiology and Immunology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran.

^b Department of Microbiology, College of Basic Science and Biology, Varamin Pishva Branch, Islamic Azad University Varamin, Iran.

^c Medical Student of Kashan University of Medical Sciences, Kashan, Iran.

^d Department of Biostatistics and Public Health, School of Public Health, Kashan University of Medical Sciences, Kashan, Iran.

*Corresponding author: Hossein Akbari

Department of Biostatistics and Public Health, School of Public Health, Kashan University of Medical Sciences, Kashan, Iran, 4719115875. Tell: +98-9366883257.

E-mail address: akbarih068@gmail.com

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ABSTRACT

Background: Diarrhea is an important cause of morbidity and mortality, particularly in developing countries. Enteric gram-negative bacteria, especially *Shigella*, *Salmonella*, *Escherichia*, and *Campylobacter* plays a key role in the occurrence of diarrhea. The present study aimed to determine the prevalence and importance of four bacterial genera in the incidence of diarrhea in Shahid Beheshti Hospital in Kashan, Iran.

Methods: This cross-sectional study was conducted on 528 diarrheal stool samples during March 2015-February 2017. The samples were collected for the isolation of the bacterial agents to appropriate selective and differential culture media. Laboratory diagnosis was performed using proper bacteriological and serological tests.

Results: Among 528 stool samples, 38.6% of the specimens belonged to women, and 61.4% belonged to men. In total, 233 specimens (44.1%) were positive for the mentioned bacteria. In 98 (18.5%) and 15 cases (2.8%), *Shigella* spp. and *Campylobacter* spp. had the highest and lowest frequency, respectively.

Conclusion: Shigellosis has been reported to be more prevalent in developing and industrialized countries. Our findings and similar studies in this regard have denoted the epidemiological patterns of shigellosis in some regions in Iran toward the epidemiological pattern of the disease in industrialized countries.

1. Introduction

Diarrhea generally refers to the increased time of fecal excretion to more than three times a day and disorders in fecal consistency. It is often associated with urgency in fecal excretion, fecal incontinence or a combination of both. Infectious diarrhea is among the main public health concerns

concerns across the world. Reports have suggested that the annual mortality rate of the disease is approximately three million cases worldwide [1].

Infectious bacterial diarrhea is the most common and acute type of diarrhea, the two forms of which are watery diarrhea and bloody diarrhea, also known as dysentery.

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In watery diarrhea, large portions of water and minerals are excreted from the patient's body, thereby leading to dehydration. This type of diarrhea is mainly noninvasive; in other words, microorganisms do not enter the blood flow in watery diarrhea. In addition, it is automatically limited, and the patient often recovers by replacing the water and electrolytes in the body. On the other hand, dysentery is an invasive diarrhea associated with fever, abdominal pain, demolition of the intestinal tissues, and bleeding. This disease begins as watery diarrhea with average intensity. Among pathogenic bacteria, genus members such as *Shigella*, *Campylobacter*, *Salmonella*, and *Escherichia* are considered to be the etiological factors associated with gastrointestinal infections [2]. *Shigella* is a gram-negative bacterium that belongs to the Enterobacteriaceae family and plays a key role in the occurrence of dysentery and inflammatory diarrhea. The mentioned bacteria are considered to be a significant public health threat throughout the world. Some annual reports have estimated that approximately 165 million individuals are affected by shigellosis, 200,000 of whom die due to the disease in various regions in the world [3].

Various thermophilic *Campylobacter* have also been denoted as the most common factors involved in bacterial diarrhea in human, especially the *Campylobacter jejuni* species. The gastrointestinal infections caused by these gram-negative, spiral bacteria may appear as dysentery or watery diarrhea [4, 5].

Similar to *Shigella*, *Salmonella* are among the classical pathogens of the Enterobacteriaceae family and play a major role in food poisoning and gastrointestinal infections. These bacteria are often transmitted to human through infected water, poultry, and eggs [6]. *Escherichia* is a well-known genus of the Enterobacteriaceae family, the most common species of which is *Escherichia coli*. *E. coli* plays a key role in the occurrence of gastrointestinal infections. In addition, its pathotypes (e.g., various enterotoxigenic, enteropathogenic, and enterohemorrhagic agents) have been reported to cause pseudo- cholerrheic watery diarrhea, severe dysentery or inflammatory diarrhea in humans [7].

The prevalence of the influential factors in dysentery varies in different regions in Iran, and of the frequency of the gram-negative bacteria that cause diarrhea varies at different periods [8-11]. The present study was conducted to investigate the frequency of infectious diarrhea due to a variety of *Shigella* among the referents to Kashan Shahid Beheshti Hospital and compare it to the cases due to other Gram negative bacteria.

2. Materials and Methods

This cross-sectional study was conducted during March 2015-February 2017. The sample population included the patients with diarrhea referring to Shahid Beheshti Hospital in Kashan, Iran. The inclusion criterion was no antibiotic use

within the past two weeks. Diarrhea samples were collected in clean plastic containers and swabs using Carly Blair's transitional medium (Merck, Darmstadt, Germany) and transferred to the microbiology laboratory of Kashan University of Medical Sciences.

The collected samples were cultured for two hours after transfer to the laboratory. In addition, the fecal samples were smeared and examined in terms of the red blood cell count (RBC) and white blood cell count (WBC) using a microscope (Model BX50, Olympus Company Ltd.). In order to isolate *Shigella* and *Salmonella* from the diarrhea samples, we used xylose lysine dicarboxylic agar selective-differential culture medium (XLD agar; Difco Company, USA). The inoculated plates were placed in an incubator for 24 hours at the temperature of 37°C, and the grown colonies were studied.

Fecal swabs were placed in the gram-negative broth enrichment medium for six hours (Difco Company, USA) at the temperature of 37°C and cultivated on XLD agar. After 24 hours of incubation, the biochemical tests of IMViC, ONPG, lysine decarboxylation, and TSI agar culture medium (Merck, Darmstadt, Germany) were used to distinguish *Salmonella* from *Shigella*. Moreover, the species and serogroups of *Shigella* were identified by adjoining the *Shigella* colonies grown on the TSI agar medium with proprietary monovalent antisera (Baharafshan Company, Iran) [8].

E. coli was isolated from the diarrhea samples using McCanky agar (Merck, Darmstadt, Germany) and EMB agar culture media (Difco Company, USA). After 24 hours of incubating the plates at the temperature of 37°C, IMViC confirmation tests, urea hydrolysis, lysine decarboxylation, and TSI agar culture medium were performed. The final identification of the enterotoxigenic (ETEC) and enteropathogenic (EPEC) strains of *E. coli* was performed using specific antigens of O and H, and the final diagnosis of the enterohemorrhagic strains (EHEC) was confirmed based on the bacterial culture on sorbitol medium (Merck, Germany) and proprietary antiserum O₁₅₇H₇ (Baharafshan Company, Iran) [9].

In order to isolate the *Campylobacters*, the *Campylobacter* selective agar medium with 5% defibrinated sheep blood (Merck, Darmstadt, Germany). Following that, incubation was performed for 48 hours at the temperature of 42°C under microaerophilic conditions. In order to identify these bacteria, we employed oxidase tests, catalase and hydrogen sulfide production, sensitivity testing of nalidixic acid, cephalothin disks, nitrate recovery, and hydrolysis of hypothyroidism [8].

2.1. Statistical Analysis

Data analysis was performed in SPSS version 20 using Chi-square and Fisher's exact test with 95% confidence interval.

3. Results and Discussion

Out of 528 diarrhea samples, 38.6% were collected from women, and 61.4% were obtained from men. Mean age of the patients was 34.9 ± 2 years (age range: 1-69 years). In 233 cases, 44.1% of the samples were positive for *Shigella*, *E. coli*, *Salmonella*, and *Campylobacter*. No statistically significant

association was observed between gender and type of bacteria ($P = 0.823$) (Table 1).

Table 1: The frequency distribution of Gram-negative bacteria causing diarrhea in terms of gender

Gender Type of bacteria	Number of females	Number of males	Total (%)
<i>Shigella</i>	53	45	98 (18.5)
<i>Escherichia</i>	34	37	71 (13.4)
<i>Salmonella</i>	22	27	49 (9.3)
<i>Campylobacter</i>	6	9	15 (2.8)
Total	107	126	233 (44.1)

The analysis of various age groups indicated no significant correlation between the type of the bacteria and age of the patients with diarrhea ($P = 0.682$). However, only in the case of *Shigella*, the number of the infected women was higher compared to men. The frequency distribution of the gram-negative bacteria causing diarrhea based on the age of the infected patients is presented in Table 2.

Table 2: The frequency distribution of Gram-negative bacteria causing diarrhea based on the age of the infected

Type of bacteria	Age			Total (%)
	14 and less	15-40	41 and more	
<i>Shigella</i>	23	39	36	98 (18.5)
<i>Escherichia</i>	34	26	11	71 (13.4)
<i>Salmonella</i>	10	21	18	49 (9.3)
<i>Campylobacter</i>	4	6	5	15 (2.8)
Total	71	92	70	233 (44.1)

Among 233 positive diarrhea samples, 195 cases were watery diarrhea, and 38 cases were obtained from patients with dysentery. However, no statistically significant association was observed between the *Campylobacter* genus and watery diarrhea ($P = 0.147$), while a significant correlation was denoted between *Shigella* and Dysentery (Table 3) ($P = 0.004$).

Table 3: The frequency distribution of Gram-negative bacteria causing diarrhea based on the type of diarrhea

Type of bacteria	Watery diarrhea	Dysentery	Total (%)
<i>Shigella</i>	69	29	98 (18.5)
<i>Escherichia</i>	65	6	71 (13.4)
<i>Salmonella</i>	49	0	49 (9.3)
<i>Campylobacter</i>	12	3	15 (2.8)
Total	195	38	233 (44.1)

Among various species of *Shigella*, *Shigella sonnei* and *Shigella boydii* were least significantly involved in the occurrence of diarrhea in the patients referring to Shahid Beheshti Hospital in Kashan, Iran. The frequency distribution of various *Shigella* species is depicted in Figure 1.

Among various pathotypes of *E. coli*, EPEC was most significantly involved in the incidence of diarrhea, while EHEC was least significantly involved in the patients referring to Shahid Beheshti Hospital in Kashan, Iran. The frequency distribution of various pathotypes of *E. coli* is depicted in Figure 2.

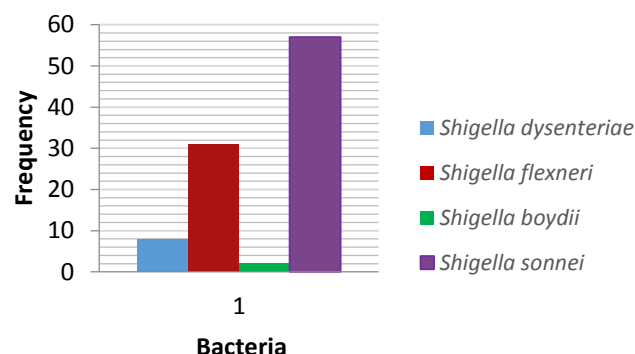


Figure 1: The frequency distribution of different *Shigella* species (n = 98)

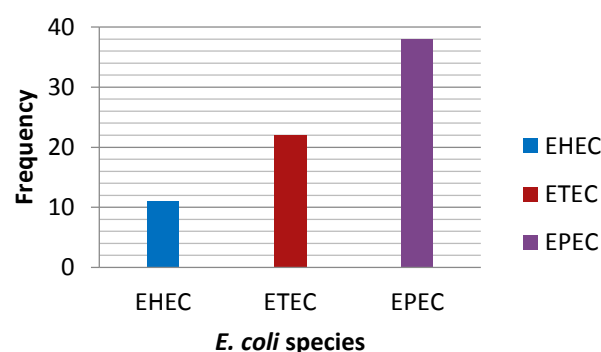


Figure 2: The frequency distribution of different *E. coli* species (n = 71)

In general, gastroenteritis is a critical clinical issue across the world, as well as a major cause of mortality in children. In addition to classical pathogen (e.g., *Shigella*), factors such as antibiotics could give rise to gastroenteritis as a common manifestation of diarrhea. Antibiotics may disturb the natural intestinal microflora population, thereby leading to the possibility of the excessive proliferation of opportunistic pathogens, such as *E. coli* and *Clostridium difficile* [12].

In a study conducted by Salary (1997) in the hospitals in Tehran (Iran), 2,517 fecal samples obtained from the patients with enterocolitis were investigated, and the cultures of only 193 specimens were positive for bacterial infection. The reported rate is significantly lower compared to the findings of the present study [13]. It seems that in Tehran, where there is a higher health level compared to desert areas and areas deprived of healthy drinking water, non-bacterial agents (e.g., viruses) may be the cause of most intestinal infections.

In the current research, the correlations between the isolated bacteria with the age and gender of the patients were also evaluated, and the findings showed the correlations to be more significant in men in terms of other genera, with the exception of *Shigella*. Therefore, it could be inferred that women are more susceptible to *Shigella* infection compared to men despite the lack of scientific justification in this regard. *Shigella* is among the most threatening intestinal pathogens in developing countries. The results of the present study clearly indicated the high incidence of the isolated bacteria causing diarrhea.

In the present study, *Shigella* was isolated from the feces of the patients with diarrhea more frequently than other gram-negative bacteria, and the frequency of *Shigella sonnei* was significantly higher. In a study by Fletcher et al. (2013), out of 808 fecal samples collected from the patients with diarrhea, 369 intestinal pathogenic bacteria were isolated, and *Shigella* was the most frequent (155 cases; 45%) [14]. However, in the present study, *Shigella flexneri* isolates predominated compared to the other isolates. In most of the studies performed in Iran, *Shigella flexneri* has been reported to be dominant as well [13-16]. On the other hand, the results obtained by Ranjbar et al. (2008) are inconsistent with our findings. In developing countries, the epidemiologic pattern of *Shigella* is often focused on *Shigella sonnei*, while in industrialized countries, *S. flexneri* has been reported to be dominant [17]. It is also notable that most of the shigellosis cases in the present study were presumably associated with multi-month epidemic of the disease with a significant correlation with the consumption of the vegetables that are irrigated with polluted water. In addition, the findings of the current research highlighted the importance of *E. coli* pathotypes, especially EPECs, in the incidence of diarrhea.

In the study by Salary (1997), among 558 fecal samples of children with diarrhea, *Shigella* were reported to be the dominant pathogens of gastroenteritis (36.4%), followed by *E. coli* [13]. This is in congruence with the results of the present study. In contrast, Salmanzadeh-Ahrabi et al. (2005), reported EHEC to be a common strain of diarrhea in children [18]. In another research performed in the United States during 1997-2006, *S. sonnei* had the highest prevalence (6%) among various *Shigella* strains, followed by *S. flexneri*. This comparison indicated that in various regions, *Shigella* strains could interfere with the incidence of diarrhea [19]. Another study in this regard was conducted by Nasrallahi and Sharif (2000) in Sari (Iran) on 400 children, and the obtained results demonstrated that the predominant species were EPECs and *Shigella* [20].

In a study by Khalili et al. (2004) in Yazd (Iran), 470 samples were investigated, and *E. coli* was reported to be the most dominant pathogen (43%), while *Shigella* accounted for 18.8% of the cases, and *Campylobacter jejuni* constituted 5.9% of the pathogens [21]. *C. jejuni* is a gram-negative spirillum found in the intestines of some livestock, especially cows. This pathogen grows abundantly in humidity. As a result, high levels of infection with this bacterium may be due to the wide range of livestock and poultry breeding centers. In the present study, *C. jejuni* was another important pathogen in terms of frequency, which is in line with the previous studies regarding the low prevalence of *Campylobacter* in developing countries as a diarrhea-causing agent [22]. In the current research, *Salmonella* species were in the third order of dominance with the frequency of 9.3% among the isolated pathogens. In industrial countries, unlike Iran, the prevalence of *Salmonella* is significantly higher, so that a study in the United States reported the prevalence of *Salmonella* to be 22% among various diarrhea-causing pathogens [23].

4. Conclusion

Annual *Shigella*-induced diarrhea epidemics affect thousands of individuals, adversely affecting their health, as well as that of communities. Since most of the studies in this regard have been focused on children, data is scarce regarding the prevalence of intestinal pathogens in adults in Iran. Therefore, the accurate study of these factors is essential to promoting community health. Due to the effects of several factors in the incidence of diarrhea, including microorganisms other than bacteria (e.g., viruses and fungi), as well as predisposing factors (e.g., inflammatory and autoimmune diseases), it is recommended further investigation be conducted in a multicentric manner in order to consider the effects of various factors.

Authors' Contributions

E.M. and A.Kh., designed the manuscript; H.A., Performed the statistical analysis; A.Gh.A. and M.V., wrote the protocol; E.M. and P.B., wrote the manuscript; E.M. and P.B. and A.Gh.A., Managed the analyses of the study and managed the literature searches.

Conflict of Interest

The author report no conflict of interest.

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