

Prevalence of *Escherichia coli* O157:H7 in Children with Bloody Diarrhea Referring to Abuzar Teaching Hospital, Ahvaz, Iran

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ABSTRACT

Introduction: *Escherichia coli* O157: H7 are recognized as important aetiological agents of diarrhea in children, particularly in developed countries.

Aim: The aim of the study was to determine the rates of detection of *E. coli* O157: H7 strains among children in Ahvaz, Iran.

Materials and Methods: From June 2010 to December 2010, 137 diarrheal stool samples of children were collected. *E. coli* was identified by standard microbiological techniques. O157 or

O157:H7 subtypes discerned by serological tests.

Results: Of the 137 *E. coli* isolates, enteropathogens were found in 53 (38.7%) of the patients as follow: *Shigella* spp. (75.5%), EPEC (enteropathogenic *E. coli*) (16.9%), *Campylobacter* spp. (3.8%) and *Salmonella* spp. (3.8%). None of the isolated *E. coli* was O157:H7 serotype.

Conclusion: This shows that non-O157:H7 *E. coli* are the major cause of paediatric infections in this region of Iran.

Keywords: Diarrheal stools, Gastroenteritis, Paediatric infections, Stool samples

INTRODUCTION

In developing countries morbidity and mortality among infants and young children due to diarrhea is prevalent. The annual mortality rate in Asia, Africa, and America is about 4.6-6 million [1,2]. The different diarrheal syndromes can be caused by bacterial, viral and parasitic infections of either single or multiple aetiology [3]. *E. coli* is responsible for 30% of gastroenteritis [4]. Six groups are known for *E. coli* correlated with diarrhea: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAaggEC) and diffusely adherent *E. coli* (DAEC) [5].

The predominant EHEC serotype associated with human infection and death in the United States is O157:H7 [6]. STEC virulence factors are as follow: Shiga toxins (Stx1 and Stx2), intimin, enterohaemolysin, and the Shiga toxin-producing *E. coli* (STEC) autoagglutinating adhesin (Saa) [7]. The cattle are mostly reservoir of STEC. The ingestion of contaminated food or water, person-to-person spread, and contact with animals are the main transmission modes [8]. It is known as the third common bacterial pathogens of human intestinal after *Salmonella* and *Campylobacter* spp. [9]. Serogroup O157 from EHEC was recognized as the causative agent following two outbreaks of haemorrhagic colitis (HC) in 1982 [10]. Human diseases ranging from mild diarrhea to haemorrhagic colitis and haemolytic uremic syndrome (HUS) can be caused by STEC typically affecting children, the elderly and immunocompromised patients [6]. Almost 1 week after diarrhea, a thrombotic microangiopathy begins in nearly 15% of infected children with *E. coli* O157:H7 [11]. Some sporadic outbreaks of infection with *E. coli* O157 correlated with ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water [12]. Infections can also be acquired by direct contact with animals and by person-to-person spread [13]. However, some studies have shown that *E. coli* O157 is not causal agent of diarrheal stools in Iran [14,15].

AIM

The aim of this study was to detect *E. coli* O157:H7 in stool samples of children with bloody diarrhea in Ahvaz, Iran.

MATERIALS AND METHODS

The study was conducted from June 2010 to December 2010. In this study all children (ages 0–10) with gastroenteritis referred to Abuzar hospital, Ahvaz, Iran, in a 6 month period were investigated. The questionnaire containing demographic information including sex, age, antibiotic use and clinical finding including fever, vomiting, abdominal pain was prepared. All patients had diarrhea. The mean age of the patients was 5 years (range, 1 year to 10 years). The stools samples were only collected from patients who had not received antibiotic treatment at the time of investigation. Fecal samples were collected in sterile plastic containers and immediately transported to laboratory in less than two hours. A swab of fecal sample was cultured on MacConkey agar (Merck, Germany), Xylose-lysine-deoxycholate (XLD) agar (Merck, Germany) and Sorbitol MacConkey agar (SMAC) (Merck, Germany). The plates incubated for 24 hours at 37 °C under aerobic conditions. A swab from each stool sample was placed in Selenite-F broth and then incubated at 37°C for 24 hours in aerobic environment. After overnight incubation, Selenite-F broth was subcultured on XLD agar. The samples were cultured on Preston agar (Hi-Media, Mumbai, India) and incubated at microaerophilic condition. Suspected colonies were identified by further standard identification test [16]. The stool specimens were also examined by light microscope for the presence of pus cells and red blood cells (RBC). The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on XLD agar. On XLD agar, reddish pink colonies with and without black centre suspected to *Salmonella* & *Shigella* were identified by biochemical tests. Colonies with black centre suspected to *Salmonella*. Serogrouping was performed by slide agglutination with polyvalent antisera. (Baharafshan, Iran). Suspected colonies on SMAC media that had remained colorless due to the absence of sorbitol fermentation, were tested by other biochemical tests (oxidase, indole production, Simmon's citrate, urease and hydrogen sulphide production to confirming as *E. coli*. The isolated *E. coli* colony was then serotyped using specific antisera (anti-O157 and anti-H7), (Mast Co, UK) according to the instruction of manual.

RESULTS

A total of 137 stool samples from paediatric patients under the age of 10 years with presentation of gastroenteritis were cultured. Overall, 74 (54.01%) patients were male and 63 (45.99%) were female. Of the 137 stool cultures, enteropathogens were found in 53 (38.7%) of the patients. The [Table/Fig-1] summarizes the frequency of enteropathogens isolated from bloody diarrhea. The most frequent isolate from bloody diarrhea was *Shigella* spp. (75.5%) followed by EPEC (16.9%), *Campylobacter* spp. (3.8%) and *Salmonella* spp. (3.8%). Out of all isolates which were recovered from 137 stool samples, 4 strains were non-sorbitol fermenting (NSF) colonies isolated on SMAC. They were confirmed as *E. coli* by biochemical and serological tests. These colonies were then serotyped using specific antisera (anti-O157 and anti-H7) (Mast co, UK). None of the isolated *E. coli* was O157:H7 serotype.

Enteropathogen	Number	Percentage
<i>Shigella</i> spp.	40	75.5
EPEC	9	16.9
<i>Campylobacter</i> spp.	2	3.8
<i>Salmonella</i> spp.	2	3.8
<i>E. coli</i> O157H7	0	0
Total	53	100

[Table/Fig-1]: Distribution of enteropathogens of 53 patients in bloody diarrhea, Ahvaz, Iran

DISCUSSION

Diarrheal diseases are one of the major health problems in the world. Every year more than two million deaths from diarrhea in children under five in developing countries occur [17]. In the present study *Campylobacter* spp. was isolated in 3.8% of bloody diarrhea patient's samples. Our finding had a lower rate than studies from different parts of Iran. In one study 8% *Campylobacter* spp. was isolated from diarrheic children in Tehran, Iran [18]. The prevalence of *Campylobacter* spp. in other studies from Iran were 9.8% [19], 4.6% [20], 5.4% [21], and 9.6% [22]. *Shigella* spp. was isolated as causative enteropathogen among 53 of patients in current study. Some studies from south of Iran detected *Shigella* from bloody diarrhea as most frequent agent [17,23,24]. Few studies have been conducted on children in Iran for *E. coli* O157 infection. In present study, none of the four sorbitol-negative *E. coli* was of O157:H7 serotype. Our finding was in concordance with that of reported by Alborzi et al., [25]. In their study, none of the *E. coli* species isolated from stool samples in children was of O157:H7 serotype by culture and PCR. They showed that *E. coli* O157: H7 is not a cause of bloody diarrhea in southern Iran. Similarly, in Aslani et al., studies undertaken in three provinces of Iran, no *E. coli* O157:H7 serotype was detected [14,15]. They reconfirmed this result in another study by PCR-RFLP analysis [26].

In Bonyadian et al., study verotoxigenic *E. coli* isolates from patients with diarrhea contain stx1, stx2 genes but none of them belonging to serotype O157 in Shahrekord, Iran [27]. In another study on enteropathogenic and Shiga toxin-producing *Escherichia coli* among children with and without diarrhea in Iran none of the strains produced stx1 or stx2 were O157 serotype [28]. Several studies have detected *E. coli* O157:H7, at low rates in animal sources such as ground beef samples [29] and dairy farms [30], which imply the source of *E. coli* O157:H7. Most human epidemiological studies in Iran have defined that the prevalence of STEC (Shiga toxin-producing strains of *Escherichia coli*) infection ranges between 0.7 to 15%, but none of them belonged to the O157:H7 serotype [14,31]. Rahimi et al., collected 290 cheese, ice cream and yoghurt. These samples tested by PCR. *E. coli* O157: H7 was not detected in any samples [32]. Fard et al., examined 322 collected stool samples but only 0.6% of the total was identified as *E. coli* O157: H7 serotype [17]. Some studies in another countries noted similar findings. Stephan et al., in Switzerland examined 5590 samples from healthy personnel

working in the beef industry and isolated only one strain of *E. coli* O157:H7 [33]. Another serotypes of *E. coli* was reported the main causative agent of acute diarrhea in children in North of Iran [34]. This survey showed that *Shigella* is the main bacteria among enteropathogens isolated from bloody diarrhea in children in Ahvaz southwestern Iran. Our findings are in agreement with the results of other studies from Nepal [35] and Uruguay [36].

LIMITATIONS

One of the major limitations of this study was inadequate demographic information about patients. In addition, previous use of antibiotics in some patients before hospitalization was another problem.

CONCLUSION

This study showed that non-O157:H7 are the major cause of paediatric infections in this region of Iran. Causative agent of diarrhea in children in each area must be clearly identified. Determining these factors will help to control the disease. Appearance of resistant strains can be avoided with proper treatment.

ACKNOWLEDGEMENT

This study is conducted and financially supported by Infectious and Tropical Diseases Research Center of Ahvaz Jundishapur University of Medical Sciences, Iran.

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Date of Submission: **Sep 08, 2015**
Date of Peer Review: **Oct 15, 2015**
Date of Acceptance: **Nov 16, 2015**
Date of Publishing: **Jan 01, 2016**

FINANCIAL OR OTHER COMPETING INTERESTS: As Declared above.