Is *Escherichia coli* O157:H7 a common pathogen in children with bloody diarrhea in Shiraz, Iran?

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SUMMARY: Alborzi A, Aelami MH, Astaneh B, Pourabbas B, Farshad S, Kalani M, Nasiri J, Rashidi M. Is *Escherichia coli* O157:H7 a common pathogen in children with bloody diarrhea in Shiraz, Iran? Turk J Pediatr 2008; 50: 349-353.

***Escherichia coli* (E. coli) O157:H7 is a common cause of bloody diarrhea in developed countries. The aim of this study was to determine whether *E. coli* O157:H7 is a possible pathogen of bloody diarrhea in southern Iran. Out of 719 children with diarrhea, 243 (34%) patients with positive occult blood took part in our study. The polyclonal antibody test and polymerase chain reaction (PCR) were used to identify *E. coli* O157:H7. Stool cultures showed enteropathogens in 107 patients (44%). Shigella (34.3%) was followed by *E. coli* (8.6%), campylobacter (2%) and salmonella (0.4%). None of the *E. coli* species was of O157:H7 serotype. Antibiotic sensitivity of shigella species was 100% to ceftriaxone, ciprofloxacin and ceftazidime, 94% to nalidixic acid and 13% to co-trimoxazole. The results of the study showed that *E. coli* O157: H7 is not a cause of bloody diarrhea in our area.**

Key words: Escherichia coli O157:H7, bloody diarrhea, children, Iran.

Acute diarrhea is a major cause of morbidity and mortality among children, and bloody diarrhea, which can often occur in different outbreaks of shigellosis, amebiasis and *Escherichia coli* (*E. coli*) O157:H7 infections, is a public health hazard. *E. coli* O157:H7 is a common cause of bloody diarrhea in developed countries[1,2] but its incidence in developing countries including Iran is not clear. There are only two studies on *E. coli* O157:H7 carried out in randomly selected populations of three provinces in Iran[3,4]. The main goal of this study was to determine whether *E. coli* O157 H7 is a possible pathogen of bloody diarrhea in southern Iran.

Material and Methods

In this prospective study, all children with diarrhea referred to three hospitals affiliated with Shiraz University of Medical Sciences from April to October 2003 were investigated. Selection of the children was based on two criteria: age between 2 months and 14 years and presence of diarrhea with positive occult blood in stool examination. Having obtained verbal consents from the parents, the physician and health care staff collected demographic and clinical data as follows: patient age, sex, information about the disease including contact with diarrhea index case, history of eating out, antibiotic use, fever, vomiting, abdominal pain, and convulsion. The severity of dehydration was assessed by a physician on admission. All stool samples were transported immediately (maximum 2 hours) to the clinical microbiology research center where all specimens were tested for occult blood using the guaiac test. The stool specimens were also examined by light microscope for the presence of pus cells and red blood cells (RBC). Those that were positive for blood were cultured using standard methods: Sorbitol MacConkey agar with Cefixime and Tellurite (CT-SMAC), Shigella-Salmonella (SS) agar, xylose-lysine-deoxycholate (XLD) agar, eosin-methylene blue (EMB), MacConkey agar (Mac) and campylobacter blood agar plate (campy-BAP). All isolated shigella and salmonella species were confirmed biochemically, and then shigella serotypes were identified using specific antisera (Mastco).

To identify *E. coli* O157:H7, all colorless colonies on CT-SMAC were confirmed biochemically for *E. coli* and were tested with O157:H7 antisera (Difco) by tube agglutination method. For more accuracy, Multiplex polymerase chain reaction (PCR) was also used to detect O157 and O111 E. coli strains. The primers sequences were previously reported[6] and obtained from TIB MOLBIOL Syntheselabor GmbH (Berlin, Germany). Amplification was performed in a thermal cycler (Eppendorf, Germany) according to the method described by Paton and Paton[5]. Expected sizes of the amplicons were ascertained by electrophoresis in 1.5% agarose gel with an appropriate molecular size marker (100-bp DNA ladder, MBI, Fementas, Lithuania).

The diagnosis of campylobacter was confirmed by best growth at 42° C and positive biochemical tests. For all isolated shigella species, antibiotic sensitivity was done for gentamicin, cephalexin, amikacin, ceftriaxone, ceftazidime, nalidixic acid and ciprofloxacin by disk diffusion method.

Statistical Analysis

The results were analyzed using SPSS software version 11.5 and the data were presented as medians with interquartile ranges. Odds ratios (OR) and 95% confidence interval (CI), as appropriate for the tests, were used. P value <0.05 was considered as statistically significant. Multiple logistic regression analysis was used to evaluate the association between shigellosis and clinical and laboratory factors.

Results

In a six-month period from April to October 2003, 719 stool samples from children aged between 2 months to 14 years (71% <5 years, mean: 4 years) were tested for the presence of blood, out of which 243 (34%) samples were included in our study.

One hundred and eighteen (48.6%) patients were admitted and 125 (51.4%) patients were followed in outpatient departments (OPD). Table I shows the clinical features of the children who presented with bloody diarrhea.

Of the 243 stool cultures, enteropathogens were found in 107 (44%) patients and the most common pathogen was shigella (34.3%) followed by *E. coli* (8.6%), campylobacter (2%) and salmonella (0.4%). Twenty-seven percent of the patients had convulsions, and this ratio increased to 41.2% in patients with shigellosis ($p<0.05$). Shigella species, more prevalent in the 49-60 months age group, were isolated from the stool cultures of 29% of those who were followed up in OPD and in 42% of the admitted patients.

Table I. Clinical Characteristics of the Children with Bloody Diarrhea

	n_1 (%)
Fever	228 (94)
Vomiting	174 (71.6)
Abdominal pain	167 (68.8)
Convulsion	65 (27)
Lethargy	20 (8.2)
History of antibiotics use	98 (40.6)
Contact with diarrhea index case	34 (14.2)
History of eating out	58 (24.2)
Severe dehydration	22 (9.1)

n_1 : positive cases.
(%): n_1 n (total no of cases n: 243).

Multiple logistic regression analysis was also used to evaluate the association between shigellosis and various characteristics of the children (fever, vomiting, abdominal pain, convulsion, history of antibiotic use, history of contact with diarrheal index case, history of eating out, more than 10 RBCs per high-power field, and more than 10 white blood cells [WBCs] per high-power field) (Table II). Eighty out of 85 shigella species were serotyped. Shigella sonnei was the most prevalent isolated serogroup (65%), followed by *S. flexneri* (25%), *S. boydii* (5%) and *S. dysenteriae* (5%). Shigella detection was highest in July and lowest in April and October.

Stool specimens of 22 patients formed colorless colonies on the CT-SMAC culture medium, and *E. coli* was confirmed by biochemical tests, but none of them was of *E. coli* O157: H7 using both PCR and O157:H7 antisera. Campylobacter species, mostly *C. jejuni*, were isolated from five stool cultures. Six patients had dual infections: 4 had shigella and *E. coli*, 1 had salmonella and *E. coli*, and 1 had shigella and campylobacter.

Table III shows antibiotic sensitivity of shigella species. The isolated shigella species were mostly (87%) resistant to trimethoprim–sulfamethoxazole (TMP-SMZ).

Table II. Logistic Regression Analysis Testing the Relationship Between Some Clinical and Laboratory Factors and Positive Shigella Stool Culture

	Unadjusted		Adjusted[a]	
Variable	OR (95% CI)	p value	OR (95% CI)	p value
Fever	3.47 (0.75-16)	NS	1.70 (0.6 5-7.55)	NS
Vomiting	1.83 (0.64-2.20)	NS	1.32 (0.60-2.96)	NS
Abdominal pain	2.34 (1.08-5.06)	0.009	3.61 (1.38-9.48)	0.009
Convulsion	3.68 (1.78-7.59)	0.000	3.56 (1.58-8.03)	0.002
History of antibiotic use	0.57 (0.32-1.02)	NS	0.54 (0.25-1.14)	NS
Contact with diarrheal index case	1.04 (0.44-2.41)	NS	1.04 (0.36-3.02)	NS
History of eating out	1.54 (0.78-3.03)	NS	1.56 (0.66-3.65)	NS
More than 10 RBCs per HPF	2.72 (1.27-5.84)	0.000	2.51 (1.03-6.11)	0.043
More than 10 WBCs per HPF	2.26 (1.01-5.08)	0.000	2.78 (1.09-7.09)	0.033

[a] Variables were adjusted for all remaining parameters listed in the Table.
RBC: Red blood cells. WBC: White blood cells. HPF: High-power field. NS: Not significant.

Table III. Antibiotic Susceptibility Profiles for Shigella Species

Resistant n_1 (%)	Intermediate susceptibility n_1 (%)	Susceptible n_1 (%)	Antibiotic
4 (6)	–	64 (94)	Nalidixic acid
59 (87)	1 (1.5)	8 (11.5)	Co-trimoxazole
–	–	68 (100)	Ciprofloxacin
–	–	68 (100)	Ceftriaxone
1 (1/5)	1 (1/5)	66 (97)	Ceftazidime
5 (7)	8 (11.5)	55 (80.5)	Gentamicin
–	2 (3)	66 (97)	Amikacin
5 (7)	14 (21)	49 (72)	Cephalexin
13 (19)	2 (3)	53 (78)	Co-amoxiclav

n_1 : positive cases.
(%): n_1 n (total no of isolates n: 68).

Discussion

This is the first prospective clinical study about the etiology of acute bloody diarrhea that focused on *E. coli* O157:H7 in Iran. All specimens with positive occult blood test were included in the study, similar to the recent research in the United States[6]. Stool cultures showed enteropathogens in 44% of patients, 50% of whom were hospitalized. In the only two studies on *E. coli*, which were conducted on randomly selected populations in Iran, fecal samples from 5,226 inhabitants of three provinces were screened, but none of the isolated *E. coli* was of O157:H7 serotype[3,4]. *E. coli* O157:H7 was isolated only from a vegetable among 2,000 foodstuffs in Iran between 1998 and 1999[7]. In our study, none of the 22 sorbitol-negative *E. coli* was of *E. coli* O157:H7 using both PCR and O157: H7 antisera. The rate of *E. coli* O157:H7 in developed countries as reported is similar to that of shigella in some studies[8], higher in other studies[9,10] and lower in the remaining[6,11]. As an example, in Japan, rates of symptomatic and asymptomatic infection of *E. coli* O157: H7 ranged from 0.5 to 4 cases per 100,000 people in 2000[12]. In a large study in the United States, *E. coli* O157:H7 was isolated from 118 patients (0.39%) and was the most common pathogen isolated from visibly bloody stool specimens (39%)[1].

A variety of factors may be responsible for undetected *E. coli* O157:H7 in Iran, including the following:

- In comparison with the western diet, in which much fast food is consumed, the Iranian diets chiefly consist of cooked foods, especially home-cooked meals. - Food products prepared from beef (cow meat), like burgers, are responsible for food-borne outbreaks in many countries, but such foods are consumed less in towns and rural areas in Iran.

- Lamb and goat meat are consumed more than beef in our country. The latter is the most important animal reservoir of the mentioned bacteria[13].

- Crowded conditions in industrialized slaughterhouses increase the carriage rate of Shiga toxin-producing *E. coli* (STEC) and also facilitate meat contaminations with *E. coli* strains[13]; however, since there are few industrialized slaughterhouses in Iran, this route of contamination is of less concern.

In our study, shigella species were isolated from the stool cultures of 29% of those who were followed up in OPD and of 42% of the admitted patients. In two studies in Iran, shigella was isolated from 16.8% and 5.2% of the stool specimens from children with acute diarrhea[14,15]. Shigella was isolated from 19.1% of stool specimens of the patients with bloody diarrhea in Pakistan[16] and 0.13% of stool specimens of the patients with diarrhea in Jordan[17]. In another prospective study in the United States from 1996-1998, STEC (2.6%) was the fourth isolated pathogen after shigella (15.3%), campylobacter (6.2%) and salmonella (5.8%)[6].

We suggest that there are some relations between positive culture of shigella and a few risk factors: abdominal pain (OR: 2.34, 95%CI, 1.08–5.06), convulsion (OR: 3.68, 95%CI, 1.78-7.59), presence of more than 10 RBCs per high-power field (OR: 2.72, 95%CI, 1.27-5.84) and presence of more than 10 WBCs per high-power field (OR: 2.26, 95%CI, 1.01-5.08), which have had higher correlation with shigellosis than the other factors. Using multiple logistic regression analysis, we could predict shigellosis in children with bloody diarrhea with the probability of 80%, provided all four of the above-mentioned risk factors are present in a patient. Fever was present in approximately 94% of children with bloody diarrhea; therefore, we could not use this factor as a criterion for differentiation between shigella and other pathogens.

S. sonnei was the most common serotype in our study, although *S. flexneri* is the predominant serotype in developing countries[18], and had been the most common type in previous studies in Iran[14,15]. Eighty-five percent of shigella species in our study were resistant to TMP-SMZ, with some other studies in the other countries showing nearly the same results[19,20]. Therefore, TMP–SMZ should no longer be considered as an appropriate empirical therapy for shigellosis. All isolated shigella organisms were sensitive to ceftriaxone, ciprofloxacin and ceftazidime. Six percent of the isolated shigella organisms in our study were resistant to nalidixic acid, while there was no resistance to nalidixic acid as reported in a previous study in Shiraz[15]. It seems that ceftriaxone is the drug of choice for inpatient empirical therapy in children with bloody diarrhea and nalidixic acid is the preferred drug for outpatient children older than 3 months with bloody diarrhea.

E. coli O157:H7 was not found to be a cause of bloody diarrhea in our study. Shigella is the most common cause of bloody diarrhea in our area with *S. sonnei* as the most common serotype. We recommend that all patients with diarrhea accompanied by all four of the above-mentioned criteria (abdominal pain, convulsion, 10 or more RBCs and WBCs per high-power field) should be considered as having shigellosis and be managed empirically until stool culture shows the specific causative enteropathogen.

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