Identification of serotypes and virulence markers (stx) of *Escherichia coli* isolated from patients with diarrhea in Shiraz, Iran

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**Abstract**

**Background:** In developing countries diarrheal infections with bacterial species especially enterohemorrhagic *Escherichia coli* (EHEC) cause severe diseases including hemorrhagic colitis, hemolytic uremic syndrome and mortality in children less than five years of age. Such life-threatening infections are due to Shiga toxin (Stx) produced by EHEC including stx1 and stx2. stx1 is identical to shiga toxin produced by *Shigella dysenteriae* type1. However, stx2 is different from Stx1, in immunological properties, but similar to Stx1, in biological characteristics. Both of these toxins are encoded by stx genes of lysogenic bacteriophage (Stx phage) integrated into the genome of EHEC.

**Aims:** The aim of this study was to determine the prevalence and distribution of Stx toxins in O157:H7 E.coli and non-O157:H7 E. coli isolated from patients with diarrhea from 2013 to 2014 in Shiraz, Iran.

**Methods and materials:** Stool samples from 1050 children and adults patients collected from June 2014 to June 2015 were investigated for Shiga toxigenic *E. coli* by conventional and molecular methods.

**Results:** Of 1050 diarrheal specimens, 306 isolates (29.1%) was diagnosed as *E. coli*. Gene analysis for virulence factors showed that 38 (12.4%) isolates carried Stx1, 57 (18.6%) Stx2 and 13 (4.2%) harbored both Stx1 and Stx2.

**Conclusions:** This study showed that ETEC was potential pathogen in children aged less than five years and the relatively high prevalence of Stx1 and Stx2 producing ETEC in our region. Moreover, the higher detection rate of Stx2 indicated the important role of this gene in diarrheal diseases.

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1. Introduction

*E. coli* is a Gram negative bacteria, which is the major inhabitant of human gastrointestinal tracts and totally considered as normal flora (Alikhani et al., 2011). Some strains of *E. coli* can cause diarrhea especially diarrheagenic *E. coli* (DEC), which accounts for 30% of the total number of diarrheal pathogens and is now being recognized as emerging entero-pathogens in the developed countries (Yazdi et al., 2011; Alikhani et al., 2007). DEC is divided into five different categories based on distinct epidemiology, clinical syndromes, and virulence properties, including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteraggregative *E. coli* (EAEC), and Shiga toxin-producing *E. coli* (STEC), referred to enterohemorrhagic *E. coli* (EHEC) or verotoxin–producing *E. coli* (VTEC) (Korczak et al., 2005; Beutin et al., 2007). The latter group is characterized by production of one or more types of cytotoxins causing tissue damage in humans and animals (Beutin et al., 2007).

The clinical picture of STEC infection may vary from an asymptomatic state to bloody diarrhea and severe life-threatening complications such as hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) (Eklund et al., 2002). Transmission of STEC usually occurs through consumption of undercooked meat, unpasteurized dairy products, unwashed vegetables, or water contaminated by feces of carriers. Although STEC strains are found as part of the normal intestinal flora of the animals, person-to-person transmission has also been documented (Shima et al., 2006). The human disease is caused by STEC can result from ingestion of fewer than 100 viable cells (Loukiadis et al., 2006).

The ability of STEC strains to cause severe disease in humans is related to their capacity of secreting shiga toxin I (Stx1) and shiga toxin II (Stx2) (Leotta et al., 2008). Stx1 and Stx2 are two of the most important virulence factors encoded by lysogenic prophages integrated into the bacterial chromosome (Zhang et al., 2010). Stx1 with a three described variants (Stx1, Stx10, Stx12) is a homogenous group, whereas Stx2 with several subtypes (Stx2a, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g) and activatable

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**Abbreviations:** Stx, shiga toxin like gene; EHEC, enterohemorrhagic *Escherichia coli*; ETEC, enterotoxigenic *E. coli*; EPEC, enteropathogenic *E. coli*; EIEC, enteroinvasive *E. coli*; EAEC, enteraggregative *E. coli*; STEC, and Shiga toxin-producing *E. coli*; EHEC, referred to enterohemorrhagic *E. coli*; VTEC, verotoxin–producing *E. coli*; TTP, thrombotic thrombocytopenic purpura; HUS, hemolytic uremic syndrome; IBD, inflammatory bowel disease.

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Stx2Δ) is a heterogeneous group (Nguyen et al., 2011). Stx1 is almost identical and is closely related to shiga toxin (Stx) that is produced by *Shigella dysenteriae*. Stx2 shares 56% sequence homology with Stx1 (Gupta et al., 2007). STEC strains are divided into >200 serotypes according to their somatic (O) antigen reaction, of which >100 are linked to human diseases (Yaghobzadeh et al., 2011; Naidu et al., 2011). STEC O157:H7 is the most prevalent serotype and considered as an important cause of hemorrhagic colitis and hemolytic uremic syndrome in humans (Shima et al., 2004; Girardeau et al., 2005), while Non-O157:H7 Shiga toxin–producing *E. coli* strains have also been associated with human disease in relation to consumption of cattle, and beef production (Barcay-Gallagher et al., 2003). In addition to diarrhea, severe disease has also been associated with these strains. Several serogroups of non-O157 STEC have been described so far; of them, the serogroups O26, O45, O103, O111, O121, and O145 have been identified as the “big six” non-O157 STEC O serogroups, and have been associated with increasing frequency in patients with bloody diarrhea and HUS (Farfan and Torres, 2012; Mellmann et al., 2005; Rump et al., 2012). The importance of Stx is due to its association with the development of HUS but cannot be solely responsible for full pathogenicity (Farfan and Torres, 2012).

This study was conducted to determine the prevalence of O157:H7 STEC and non-O157:H7 STEC and the frequency of other O serotypes, among diarrheic patients referred to the teaching hospitals affiliated with Shiraz University of Medical Sciences during 2013–2014.

2. Materials and methods

2.1. Collection of samples

A total of 1050 fecal samples were collected from patients with diarrhea symptoms referred to the teaching hospitals affiliated with Shiraz University of Medical Sciences from November 2014 to September 2015. Relevant clinical information was collected on a standard questionnaire. This information included hospitalization status, age, sex, clinical symptoms such as prolonged diarrhea, polyph and inflammatory bowel disease (IBD) and immune disorders including acquired immunodeficiency syndrome (AIDS), chemotherapy for cancer or transplant. Samples of persons who had used antibiotics in the previous three days were excluded from the study. Considering our study criteria, a total of 850 (81%) samples were included in the study.

2.2. Bacterial isolation

All stool samples were examined for enteric pathogens especially *E. coli* according to conventional microbiology methods.

Collected samples were plated onto EMB and MacConkey’s agar media (Merck; Germany) and incubated at 37 °C for 20 h. Biochemical tests including indole, methyl red (MR), Voges-Proskauer (VP) and citrate (Merck; Germany) were then performed, on 2 to 3 lactose positive tests including indole, methyl red (MR), Voges-Proskauer (VP) and citrate reactions were carried out in a thermal cycler (Eppendorf; Germany) using PCR program including first denaturation at 95 °C within 10 min, followed by 35 cycles were programmed 1 min denaturation at 95 °C, 1 min annealing at 51–52 °C for stx2, stx2r respectively, 1 min extension at 72 °C, and a final 10 min extension cycle at 72 °C, before cooling at 4 °C. *E. coli* strain EDL933 (stx2Δ) (Tahamtan-Razi Vaccine and Serum Research institute-Shiraz-Iran) was used as positive control for PCR (Tahamtan and MM, 2010).

PCR products were analyzed by using 1.5% agarose gel electrophoresis, stained with ethidium bromide (0.5 μg/ml) and visualized with an UV transilluminator. A 100 bp DNA ladder (Fermentas) was used as a molecular size marker in all gels.

3. Results

Considering inclusion criteria, of 1050 collected specimens, 850 (81.6%) specimens were selected for our study. The primary IMViC assays identified *E. coli* in 310 (36.5%) specimens.

According to the API system results, of 310 presumptive diagnosed *E. coli* strains, 306 (98.7%) isolates confirmed to be *E. coli*. These were from 157 males and 149 females. Of these, 102 (33.3%) were outpatients and 204 (66.7%) were inpatients. The analysis of patients’ data showed that 238 (77.8%) patients were with prolong diarrhea, 21 (6.9%) with polyph, 15 (4.9%) with inflammatory bowel disease (IBD), 14 (4.6%) with cancer, 6 (2%) graft recipient and 12 (3.9%) were HIV positive.

The majority of patients with confirmed *E. coli* related diarrhea were males (157, 51.31%) and the patients’ age ranged was between 2 and 90 years with the mean age of 20 ± 3 years.

Based on the API results for sugar fermentation, 8 (2.5%) isolates showed no sorbitol fermentation after 24 h incubation and were considered as *E. coli* O157.

Of 306 confirmed *E. coli* isolates, 131 (42.8%) were type I, 102 (33.3%) type II and 73 (23.9%) type III according to Sfín serogroup typing method.

Among 8 presumptive *E. coli* O157 shown by biochemical test and type III *E. coli*, only one isolate was confirmed as *E. coli* O157 serotype, using OXOID anti-*E. coli* O157 antisera agglutination kit.

### Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Serogroups</th>
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<tbody>
<tr>
<td>I</td>
<td>O1a, O1b, O1c, O2, O4, O144</td>
</tr>
<tr>
<td>II</td>
<td>O9a, O9b, O10, O11, O128, O26, O175, O128</td>
</tr>
<tr>
<td>III</td>
<td>O48, O55, O78, O106, O126, O175, O128</td>
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</tbody>
</table>

For specific detection of O157, all *E. coli* isolates included in type III were assayed by additional serotyping test, using anti-*E. coli* O157 and H7 antisera agglutination kit (Oxoid DR620) according to the manufacturer’s instruction.