Identification & characterization of Shiga toxin-producing *Escherichia coli* isolates from patients with diarrhoea in Iran

M. Bonyadian, H. Momtaz*, E. Rahimi**, R. Habibian†, A. Yazdani++ & M. Zamani++

Faculty of Veterinary Medicine, Institute of Zoonoses Research, Shahrekord University, Shahrekord-Iran, "Department of Microbiology, Faculty of Veterinary Medicine, Islamic Azad University of Shahrekord Branch, ""Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University of Shahrekord Branch, ‘Department of Infectious Disease, Faculty of Medicine, University of Medical Sciences of Shahrekord & ""Faculty of Veterinary Medicine, Shahrekord-Iran

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**Background & objectives:** Verotoxigenic *Escherichia coli* are important serotypes of enterohaemorrhagic *E. coli* (EHEC) subgroup that cause attaching and effacing lesions in enterocytes by producing verotoxins or shiga-like toxins resulting in haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). The aim of this study was to detect these serotypes specially *E. coli* O157:H7 in stool samples of patients with diarrhoea and identification of virulence genes (STX1, STX2, Hly and EAE) in Shahrekord-Iran area using PCR technique.

**Methods:** Two hundred diarrhoeal stool samples of patients were collected through 2007-2008. Microbiological and biochemical examinations were done to detect the *E. coli*. Serological tests carried out to identify the O157 or O157:H7 serotypes.

**Results:** Of the 58 *E. coli* isolates, 16 (27.6%) were detected as STX1 carrying *E. coli*, four (6.9%) carrying STX2, eight (13.8%) carrying both STX1 and STX2, and 12 (20.7%) were Hly carrying *E. coli*, but none of the isolates contained EAE gene. None of the isolates were *E. coli* O157 or O157:H7 serotypes.

**Interpretation & conclusions:** Our results revealed that verotoxigenic *E. coli* isolates other than O157 serotype were involved in causing diarrhoea in Shahrekord-Iran.

**Key words** Human - Iran - Shiga toxin-producing *Escherichia coli* - virulence genes

It has been demonstrated that certain *E. coli* isolates produced a toxin, which was initially called verotoxin because of its distinct effect on vero cells. This family of toxins was subsequently also called Shiga-like toxins (SLT), and more recently Shiga toxins (Stx), because of the close relation to the Stx of *Shigella dysenteriae* type 1.

Enterohaemorrhagic *E. coli* (EHEC) is the main group of verotoxigenic strains which has emerged as the leading cause of haemorrhagic colitis and haemolytic uremic syndrome (HUS) in humans.

Shigatoxins are categorized into two main groups, *stxI* and *stxII*. The majority of *Stx* genes are bacteriophage-
borne, which may be important for the spread of shiga
toxin-producing *E. coli* (STEC)\(^3\). EHEC strains
are characterized by the ability to form attaching and effacing
(A/E) lesions on the surface of epithelial cells in the
gastrointestinal tract\(^4\), and the production of shiga toxins.
The first gene to be associated with A/E activity was
the intimin gene, *eae*, and its presence is often used as a
marker for the infections caused by EHEC\(^5,6\).

*E. coli* O157:H7 was the first serotype associated
with haemorrhagic colitis, although more than 100
STEC serotypes have been isolated from different
sources, such as food\(^6\) and recreational\(^7\) and drinking
water\(^8\). However, not all pathogenic STEC isolates
have been shown to produce intimin.

EHEC appears to be transmitted primarily through the
ingestion of faecal contaminated foods, particularly
undercooked beef\(^6\). However, a large number of
outbreaks of EHEC have also been associated with
consumption of contaminated drinking water or contact
with recreational water\(^8\).

*E. coli* O157:H7 infections as a cause of disease
have shown a marked increase in many countries. Many
serotypes other than O157:H7 are capable of producing
Shiga toxins and similar clinical manifestations, but
this serotype was the one most commonly isolated
in North America and Europe as a cause of illness\(^1,9\).
Some researchers described the infection by STEC
including STEC O157 in some parts of Iran\(^10-12\) also. We
undertook this study to identify the presence of these
verotoxigenic isolates of *E. coli* from stool samples
from patients with diarrhoea in Shahrekord, Iran and to
characterize the genes.

**Material & Methods**

**Sample collection:** A total of 200 (male 89, female 111)
diarrhoeal faecal samples from the patients affected
with diarrhoea were collected in Hajar hospital of
Shahrekord, Iran, during January 2007 - December
2008. The questionnaire was prepared by the team
containing information like sex, age, etc., and was filled
by patients. The patients were followed for 2 month
period to confirm the complications of infection.

**Isolation of E. coli:** Macconky agar (McA) and Sorbitol
Macconky agar (SMAC, Merck, Germany), were used to
detect *E.coli* and EHEC colonies. A swab of faecal
sample was cultured on McA and SMAC agar and
incubated for 24 h at 37 °C. Five lactose fermenting
colonies from McA and five non-sorbitol fermenting
(NSF) (colourless) colonies from SMAC agar were
transferred individually to fresh Luria-Bertani (LB)
broth and incubated at 37°C for 18 h. Complete
biochemical identification (Gram staining, oxidase
negative, indole positive, Simon’s citrate negative,
urease negative and hydrogen sulphide negative) was
used to confirm the *E. coli* species\(^13\). Bacteriological
examinations were done on non lactose fermenting
colonies to confirm major causes of diarrhoea e.g.
*Salmonellae* and *Shigella*.

**Serology:** All the 58 *E. coli* isolates from 200 patients
were examined by O157 and H7 antisera (MAST Co,
UK) to identify O157 or O157:H7 serotypes by using
plate agglutination method.

**Detection of O157 rfb, stx1, stx2, eae and Hly genes:**
Total DNA of the isolates were extracted using
Genomic DNA purification kit (Fermentas, Germany).
The isolated DNA was resuspended in 50 ul of Tris
EDTA (TE) buffer at pH 8. Two microlitre of elute
was used as DNA template in PCR assay. PCR was
performed using five primer sets (Cinagen, Iran) that
detect genes of O157, and the major known virulence
genes including *STX1*, *STX2*, *EAE* and *Hly*. PCR was
performed as described previously\(^14\) and amplified DNA
fragments were resolved by gel electrophoresis using 2
per cent agarose and stained with ethidium bromide.

DNA was extracted from *E. coli* O157:H7 strain
(Obtained from Department of Microbiology, Faculty
of Vet. Med, Tehran University) using Genomic DNA
purification kit (Fermentas, Germany) and used as
template for standard control in PCR.

The results were analyzed by Chi-square test with
95 per cent confidence level.

**Results**

STEC were detected in 28 (14%) [male 12 (13.5%),
female 16 (14.4%)] of the 200 cases investigated, no
other micro organisms responsible for diarrhoea were
isolated from these patients. All the STEC infections
detected were from sporadic diarrhoeal cases, most
of them (71.5%) were under 7 yr and the sources of
infection were not identified in most cases.

One hundred fifty two lactose fermenting and
NSF colonies were isolated on McA and SMAC
agar, of these 58 (38.0%) were confirmed as *E. coli*
by biochemical and serological tests. No isolates of
*E. coli* O157 were detected by serological and PCR
examinations. Of the 58 *E. coli* isolates, 16 (27.6%)
were detected as *STX1* carrying *E. coli* (Fig. 1). Four
(6.9%) carried *STX2* (Fig. 2), eight (13.8%) carried both
*STX1* and *STX2*, and 12 (20.7%) were *Hly* carrying *E.
coli* (Fig. 3). None of the isolates had intimin (*EAE*)
gene. No significant difference was observed between male and female, but most of the cases (71.5%) with verotoxigenic *E. coli* were <7 yr old (P<0.05). Most of the patients consumed dairy products (milk, ice cream) or ground beef and hamburger in their diet two days before manifestation of diarrhoea. None of the patients showed HUS syndrome during the follow up period.

**Discussion**

Our findings showed that non-O157 STEC were the major cause of human infections in this area of Iran. Non-O157 STEC were also isolated in other countries like Germany, Italy and Denmark with higher frequency than O157:H7 strains

Similar results were found in France and in Switzerland. Non-O157 STEC may also play a more important role in disease compared to STEC O157:H7 as shown in Argentina, Australia, Chile and South Africa.

In Canada, United States, Japan, England and Scotland, in contrast, the prevalence of non-O157 is very low. However, the prevalence of STEC in the United States was reported to be 1.3 to 4.0 per cent, with non-O157 strains 25 to 63 per cent of human isolates. Most information on risk factors associated with STEC especially *E. coli* O157:H7 infection has come from outbreak investigations. Among identified dietary risk factors, foods of bovine origin, particularly undercooked ground beef, have been a frequently implicated source. Non-dietary risk factors including person-to-person transmission in day-care settings or swimming in contaminated water have also been documented.

Results of this study supported the fact that the occurrence of *E. coli* O157:H7 was very low, but other STEC played the main role in human infection in central Iran.

In our study, about half of isolated *E. coli* belonged to STEC, and >40 per cent of STEC were positive for *Hly* gene, but none carried *eae* gene. It may be due to absence of any HUS cases among the patients infected with STEC. The *eae* gene, which has been shown to be necessary for attaching and effacing activity, encodes a 94 to 97 kDa outer membrane protein termed intimin. A strong association has been reported between carriage of the *eae* gene and the capacity of STEC isolates to cause severe human disease, especially HUS. This important virulence gene was detected in most of O157:H7 and in some of the non-O157 strains. Nevertheless, production of intimin is not essential for pathogenesis, because a number of sporadic cases of HUS have been caused by *eae*-negative non-O157 STEC.

A few reports about the prevalence of STEC in human and animals in the other parts of Iran are available. In Tehran, 15 per cent of the diarrhoeal children were
positive for verotoxigenic *E. coli* and 23 per cent of the isolates were *E. coli* O157:H7, in north of Iran 0.7 per cent of the population was infected with VTEC, however, none of the isolates belonged to O157:H7 serotype. In the west of Iran, 4.9 per cent of the faecal samples were found to be VTEC-positive but none belonged to the O157: H7 serotype. Our study revealed that the presence of STEC in diarrhoeal stool samples of patients emphasizing the need of using protocol for detection of all serotypes of STEC from human, animals and meat products in clinical and food microbiology laboratories. Until now, the detection of STEC isolates has not been possible in most laboratories in Iran. Now different PCR protocols for detection of STEC are available making diagnosis of STEC infections possible. However, as non-O157 STEC is more prevalent in animals and as contaminant in foods, humans are probably more exposed to these strains.

In conclusion, our results showed that verotoxigenic *E. coli* other than O157 serotype was the pathogen causing diarrhoea in this part of Iran and advanced detection methods like PCR need to be used in microbiology laboratories.

References


Reprint requests: Dr M. Bonyadian, Faculty of Veterinary Medicine, Institute of Zoonoses Research Shahrekord University, PO Box: 115, Shahrekord-Iran

e-mail: boniadian@vet.sku.ac.ir