

Original Article

Distribution of *Enterotoxigenic Escherichia coli* among *E. coli* isolates from diarrheal samples referred to educational hospitals in Tehran-Iran

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Abstract

Background: Introduction: Enterotoxigenic *Escherichia coli* (ETEC) is the most important bacterial cause of watery travelers' diarrhea in developing countries. Watery diarrhea is can cause serious life-threatening dehydration. ETEC was caused diarrhea by the secretion of two heat-labile enterotoxins (LTs) and the heat-stable enterotoxins (STs) which increase intestinal secretion. Routine laboratory methods are not appropriate to detect ETEC and other diarrheagenic *E. coli* pathotypes. The molecular techniques such as PCR are rapid and accurate methods that have been developed for detection of ETEC. We were recognized ETEC by PCR on *lt* and *st* genes from *E. coli* isolates from patients with diarrhea collected from selected Tehran educational hospitals.

Materials and Methods: The *E. coli* isolates were collected from total 140 patients with diarrhea and 110 patients without diarrhea using culture and IMViC test. DNA was extracted by boiling method and the presence of the *uidA*, *lt* and *st* genes was detected by PCR.

Results: Among 140 *E. coli* isolates from diarrheal stools 5 (3.6%) isolates were positive for, just *lt* gene, 3 (2.1%) co-amplified for both *lt/st* and 1 (0.7%) was positive for just the *st* gene which were considered as ETEC. In the *E. coli* isolates from non-diarrheal control samples just one (0.9%) isolate was positive for both *lt* and *st* genes.

Conclusion: The results showed that the ETEC as a significant cause of diarrhea, usually ignored by laboratories using traditional methods. Sometimes the ETEC causes severe diarrhea and can threaten for patient's life. Thus a rapid diagnostic test such as PCR can be very helpful in the treatment of patients.

Keywords: Enterotoxigenic *Escherichia coli*, Diarrhea, *lt*, *st*

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Please cite this article as: Mohammadzadeh M, Goudarzi H, Dabiri H, Fallah F. Distribution of *Enterotoxigenic Escherichia coli* among *E. coli* isolates from diarrheal samples referred to educational hospitals in Tehran-Iran. Novel Biomed. 2015;3(3):144-7.

Introduction

Enterotoxigenic Escherichia coli (ETEC) is the most important bacterial cause of watery travelers' diarrhea in all age groups in areas with poor sanitation especially developing countries^{1,2}. ETEC is the most

common bacteria which are detected in 30-70% of patients with traveler's diarrhea³. Watery diarrheas usually self-limiting, but can cause serious life-threatening dehydration. There are more than 650 million cases of ETEC infection, which result in 800,000 deaths in each year⁴. There are

virulent plasmids in ETEC strains that encode colonization factors (CFs), toxins, and other adhesins. The ETEC colonizes the surface of the small bowel via a group of surface structures such as colonization factor antigen I (CFA/I) and produces enterotoxins, that raise intestinal secretion^{5,6}. ETEC enterotoxins is classified to heat-labile enterotoxins (LTs) and the heat-stable enterotoxins (STs)^{7,8}. ETEC may contain one of LT, ST, or both simultaneously (9). LT is closely related in structure and function to *Vibrio cholera* enterotoxin (CT)⁹. These toxins are made of a single A subunit and five identical B subunits which B subunits mediate binding of the cell surface gangliosides such as GM1 and GD1b, and the A subunit has ADP-ribosyl transferase activity that upregulates adenylate cyclase. This function leads to increased levels of intracellular cAMP and results to increase intestinal secretion^{10,11}. STs are small, single-peptide toxins that include two classes STa and STb that are different in structure and mechanism. Only the STa is related to diarrhea. The STa toxin is a small peptide, with guanylate cyclase activity which increases intracellular cGMP and leads to intestinal secretion¹². Traditionally, the diagnosis of ETEC is based on the production of LT or ST in animal models and serological and immunological assays^{13,14}. These tests are difficult and time consuming with high rates of false results. Also, routine medical laboratories lack essential facilities to perform the mentioned assays and culture methods are not able to distinguish *E. coli* pathotypes such as ETEC. Thus, rapid and accurate methods are necessary to detect this critical pathogen. Recently, molecular techniques such as polymerase chain reaction (PCR) have been developed for detection of ETEC, which is based on amplification of *lt* and *st* genes. According to the importance of ETEC infections and weakness of traditional methods, further molecular studies are necessary to evaluate the ETEC in patients with diarrhea.

The current study aims to detect the ETEC in patients with watery diarrhea who were referred to selected Tehran educational hospitals during 2013-2014 using PCR.

Methods

Sampling and culture

In this descriptive study, a total of 140 stool samples from patients with diarrhea (Cases) and 110 stool samples from patients without diarrhea (Controls) collected from three Tehran educational hospitals (Emam Hossein, Loghman and Taleghani hospitals). Patients which have three or more watery stools were evaluated in this study. Sampling was performed during April 2013 to September 2013. Stool samples were cultured on MacConkey and Hekton enteric agar (Merck, Germany) and were incubated in 37°C overnight. Lactose positive colonies were selected and examined with IMViC tests to identify *E. coli* isolates. All stool samples were examined microscopically for WBC and RBC.

DNA extraction

DNA was extracted with boiling method. The *E. coli* isolates were cultured in 5 ml Luria Bertani broth (Merck, Germany) and incubated in 37°C overnight. All isolates were poured in 1.5 ml microtubes and centrifuged at 3000 rpm for 3 minutes. The supernatant was discarded and 100 µl distilled water was added to the pellets and the microtubes boiled for 10 minutes. The microtubes were centrifuged at 12000 rpm for 2 minutes and the DNA containing supernatant was collected and stored in -20°C for molecular process.

PCR assay

All *E. coli* isolates were tested for beta-D-glucuronidase encoding *uidA* gene as a *E. coli* housekeeping gene. *E. coli* K12 was used as positive control. The *uidA* positive isolates were evaluated for *lt* and *st* genes by PCR using specific primers (Table 1). ETEC H10407 was used as positive control. PCR amplification was performed in a 25 µl reaction mixture containing 2 µl of DNA template, 12 µl ready to use Mastermix (Fermentase, Germany), 9 µl of distilled water and 1 µl of 20 pmol forward and reverse primers.

DNA amplification was carried out the following PCR cycling program: initial denaturation at 95°C for 5 min was followed by 30 cycles of amplification (denaturation at 95°C for 30 Sec, annealing for 30 Sec at 67°C for *uidA*, 58°C for *lt* and 61°C for *st* extension at 72°C for 30 sec) and final extension at

72°C for 5 min.

Results

In total, 140 *E. coli* isolates from patients with diarrhea and 110 *E. coli* isolates from patients without diarrhea were collected for this study. The mean age of the patients was 38 years and in controls was 34. All *E. coli* isolates first were confirmed with IMViC tests and then isolates were tested genotypically by amplification of *uidA* gene that all isolates were positive PCR results. Among 140 *E. coli* isolates from diarrheal stools 5 (3.6%) isolates were positive for just *lt* gene, 3 (2.1%) co-amplified for both *lt/stand* 1 (0.7%) was positive for just the *st* gene which were considered as ETEC. In the *E. coli* isolates from non-diarrheal control samples just one (0.9%) isolate was positive for both *lt* and *st* genes. The calculated p value was 0.046 that was confirmed a significant association of ETEC with diarrhea among the studied patients. The mean age of patients with ETEC was 26 years. Also, all stool samples with ETEC isolates were negative for WBC and RBC.

Discussion

ETEC pathotypes are a major cause of diarrheal disease in childhood in developing countries and a frequent cause of traveler’s diarrhea among visitors to these countries¹⁶. About 20-60% of the peoples who travel to countries with poor sanitations, will suffer from travelers’ diarrhea, Which ETEC is the most common agent^{2,17}. The diarrhea caused by two heat-labile (LT) and the heat-stable (ST) enterotoxins. ETEC may contain only an LT, an ST, or both LT and ST. About 35% of ETECs express

ST, 35% express ST and LT, and the remnants express only LT¹⁸. Like other diarrheagenic *E. coli* pathotypes, The ETEC did not identify by routine laboratory culture methods, thus molecular methods such PCR are necessary to distinguish these pathotypes. Many studies use the PCR for detecting ETEC from diarrheal samples by amplifying *lt* and *st* genes. In a recent study, we detect ETEC in 6.4% of patients with diarrhea and 0.9% of control cases. Also *lt* present only in 5 (55.6%) isolates, both *lt* and *st* in 3 (33.3%), and *st* only in one (11.1%) isolate.

Aslani et al used *lt* and *st* gene to detect ETEC from 193 *E. coli* isolates from diarrheal samples which were collected from different urban and rural area of Iranian 2005. They showed that 14 (7.2%) of *E. coli* isolates were ETEC. The *lt* and *st* distribution in their ETEC isolates was 7 (50%) isolate only *st*, 6 (42.9%) only *lt* and one (7.1%) both *st* and *lt*¹⁹.

Alikhani et al. in 2009 detect ETEC in 3.7% of 187 adult and adolescent patients with diarrhea in western Iran, using PCR for *lt* and *st* gene. They explain that the 5 (71.4%) isolates produced ST, 1 (14.3 %) produced LT, and 1 (14.3%) of the isolates produced both ST and LT, respectively²⁰.

In another study in 2011 in Turkey Iseri et al reported ETEC in 4.9% of 245 patients with diarrhea by using passive latex agglutination and enzyme immunoassay methods. Of these, 5 (41.7 %) were ST and 7 (58.3%) were LT²¹.

Pourakbari et al in a study on children with and without diarrhea in an Iranian referral pediatric center reported ETEC in 26% of cases and 10% of controls²². The ETEC was reported by Oscar et al. among *Escherichia coli* isolates associated with childhood diarrhea in Colombia. A case-control study was conducted on 815 samples from children younger than

Table 1: Base sequences and product sizes of the oligonucleotide primers to amplify specific, fragments of the *uidA*, *lt* and *st* genes.

Primer	Sequence (5'–3')	Product size bp	Reference
<i>uidA</i>	5-GCGTCTGTTGACTGGCAGGTGGTGG-3	503 bp	15
	5- GTTGCCCGCTTCGAAACCAATGCCT-3		
<i>lt</i>	5-GCACACGGAGCTCCTCAGTC-3	218 bp	15
	5-TCCTTCATCCTTTCAATGGCTTT-3		
<i>st</i>	5-GCTAAACCAGTAGAGCTCTTCAAAA-3	149 bp	15
	5-CCCGGTACAGAGCAGGATTACAACA-3		

five years of age. The ETEC was detected in 17 (4.87%) in cases and 8 (2.29%) in controls. Based on the presence of enterotoxins, 11 (44%) ETEC isolates were LT positives, 10 (40%) were ST positive and only 4 (16%), were LT/ST positive²³. Because of differences in time, place and the study conditions seem to be reasonable for different reported results in reviewing studies. The reviewed results showed that the ETEC can be seen as a significant cause of diarrhea cases, which usually ignored by microbiology laboratories using traditional culture methods. Sometimes the ETEC causes severe watery diarrhea that can threaten the patient's life, thus a rapid diagnostic test such as PCR can be very helpful in the treatment process.

Conclusion

Sometimes the ETEC causes severe watery diarrhea that can threaten the patient's life, thus a rapid diagnostic test such as PCR can be very helpful in the treatment process.

Acknowledgements

Special thanks to Research Vice-Chancellor of Shahid Beheshti University of Medical sciences, school of medicine, Tehran, Iran for supporting this study.

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