

# Virulence Factors of Shiga-Toxigenic *Escherichia coli* in Drinking Water of Shahrekord, Iran

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## Research Article

### Abstract

**Background:** Shiga toxigenic *Escherichia coli* are a substantial cause of water-borne diseases. The current research was done to study the microbial quality of the drinking water of Shahrekord with respect to the recognition of virulence factors of Shiga toxigenic *Escherichia coli*.

**Methods and findings:** A total of 200 drinking water samples were collected and positive strains were analyzed for presence of STEC strains and virulence factors by PCR technique. Ten out of 200 drinking water samples (5%) were positive for *E. coli*. Seven samples (70%) were STEC. All of the EHEC strains were simultaneously positive for *stx1*, *eaeA* and *ehly* genes. The prevalence of *stx1* and *eaeA* genes, *stx2* and *eaeA* genes and finally *stxs* and *eaeA* genes in the AEEC strains were 50%, 25% and 25%, respectively.

**Conclusion:** The bases of animal and human intestinal contaminations should be control to decrease the menace of *E. coli* in drinking water of Shahrekord.

**Keywords:** Shiga toxigenic *Escherichia coli*; Drinking water; Shahrekord; Virulence factors.

### 1. Introduction

In a day, millions of people use from drinking water. The safety and health of drinking water are major concerns throughout the world. Health risks may arise from consumption of water contaminated with infectious agents, radiological hazards and toxic chemicals. It has been estimated that water-borne diseases were the causative agents of more than two million deaths and four billion cases of diarrhea annually [1]. In keeping with this, there are several reports concerning the contamination of drinking water with dangerous pathogens such as *Escherichia coli* [2].

*E. coli* is a gram-negative, flagellated, non-sporulating, facultative anaerobic and rod-shaped bacterium

which belongs to Enterobacteriaceae family [2-5]. *E. coli* is characteristically classified into enterotoxigenic *E. coli* (ETEC), enteroadherent *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) [2-5]. EHEC strains are a portion of Shiga-toxigenic *E. coli* (STEC) strains. STEC strains are accountable for intensive clinical symptoms like bloody diarrhea, hemorrhagic colitis (HC), gastroenteritis and Hemolytic Uremic Syndrome (HUS) [2-5]. The most substantial genes which are related to STEC infections are intimin protein (*eaeA*), Shiga toxins (*stx1* and *stx2*) and hemolysin (*hlyA*) [2-6].

Based on the uncertain epidemiological and microbiological aspects of the drinking water in the Shahrekord, Iran, the current examination was performed to study the incidence of STEC strains and their virulence factors in the eating water samples of Shahrekord, Iran.

### 2. Materials and Methods

#### 2.1 Samples and *E. coli* identification

From March 2015 to May 2015, a total of 200 drinking water samples were arbitrarily recovered from the numerous locations of Shahrekord, Iran. Dechlorination of water samples was done using the 0.5 g of sodium thiosulphate which was added to the empty sterile bottles of sampling. To diminish the menaces of contamination, water samples were taken about 30 s after opening of faucet. Water samples were directly transported to the workroom in cooler with ice-packs. All procedures were used to deterrence of cross contamination in the process of sampling.

Typical Total Coliform Multiple-Tube (MPN) Technique was used for inspection of water samples. Tubes with typical growth were subjected to blood and MacConkey agar (Merck, Germany) media and incubated for 24 h at 37°C. Typical colonies of *E. coli* were transferred to the EMB agar (Merck, Germany) media and incubated at 37°C for 24 h. Typical *E. coli* bacteria were approved using the biochemical tests introduced by Dehkordi et al. [3].

**2.2 Genomic DNA purification and PCR amplification**

Typical *E. coli* colonies were sub-cultured on nutrient agar broth (Merck, Germany) and then incubated 24 h at 37°C. Then, Genomic DNA was extracted from the bacterial colonies using the DNA extraction and purification kit (Fermentas, Germany) according to the manufacture’s instruction. Concentration of extracted DNA samples have been determined by determining absorbance of DNA at 260 nm using spectrophotometer (SQ 2800, Unico, USA) [7]. Genomic DNA samples of each colony were further approved using 16S rRNA gene-based PCR amplification according to the method described by Woo et al. [8].

**2.3 Identification of virulence factors**

Set of primers and programs of PCR reactions applied for determination of virulence factors are shown in Table 1 [2-6]. Totally, 10 µl of PCR products were committed on a 2% agarose gel consisting of 0.5 mg/ml of CYBR Green (Fermentas, Germany) at 90 V for 20-30 min. Products were interpreted using ultraviolet illumination. *E. coli* ATCC 8739 and sterile distilled water were applied as positive and negative controls of PCR reactions.

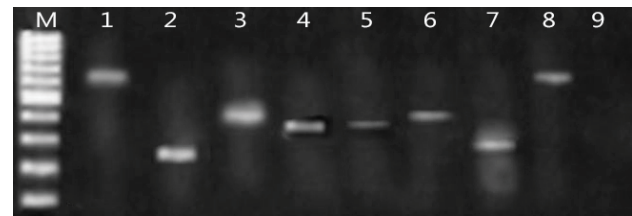
**2.4 Arithmetical examination**

Data recovered from all tests were subjected to

SPSS/19.0 software and  $P < 0.05$  was determined a significance level.

**3. Results**

A total of 200 drinking water samples were test for occurrence of STEC strains. Table 2 displayed the incidence of *E. coli* in the eating water samples of Shahrekord, Iran. Of 200 samples studied, 10 samples (5%) were positive for *E. coli*. Figure 1 shows the gel electrophoresis of PCR products for determination of virulence genes in the STEC strains of drinking water samples of Shahrekord, Iran. Frequency of virulence genes in the *E. coli* subtypes isolated from drinking water samples of Shahrekord, Iran is shown in Table 3. Of 10 *E. coli* strains detected, 7 strains (70%) were STEC. Arithmetical important alteration



**Figure 1.** Gel electrophoresis of PCR products for amplification of virulence factors in the STEC strains of drinking water samples of Shahrekord, Iran.

M: 100 bp ladder, 1-4: Positive samples for virulence factors and 5-8: Positive controls and 9: Negative control

**Table 1.** Oligonucleotide primers and PCR programs used for amplification of virulence factors in the *Escherichia coli* isolates of drinking water.

Target Gene	Primer Sequence (5'-3') *	PCR Product (bp)	PCR Programs	PCR Volume (50 µL)
<i>stx1</i>	F: AAATCGCCATTCGTTGACTACTTCT R: TGCCATTCTGGCAACTCGCGATGCA	366	1 cycle: 95°C - 3 min 34 cycle: 94°C - 60 s 56°C - 45 s 72°C - 60 s 1 cycle: 72°C - 10 min	5 µL PCR buffer 10X 2 mM MgCl <sub>2</sub> 200 µM dNTP (Fermentas) 0.5 µM of each primers F&R 1.5 U Taq DNA polymerase (Fermentas) 5 µL DNA template
<i>stx2</i>	F: CGATCGTCACTCACTGGTTTCATCA R: GGATATTCTCCCCACTCTGACACC	282		
<i>eaeA</i>	F: TCGGACACAACAGGCGGCGA R: CGGTCGCCGACACAGGATTC	629		
<i>ehly</i>	F: CAATGCAGATGCAGATACCG R: CAGAGATGTCGTTGCAGCAG	432		

\*The Oligonucleotide primers and PCR programs were obtained from previous studies [3,4]

**Table 2.** Total prevalence of *Escherichia coli* in the drinking water samples of Shahrekord, Iran.

Samples	No. samples collected	No. positive (%)
Drinking water	200	10 (5)

**Table 3.** Distribution of virulence factors in *Escherichia coli* subtypes isolated from drinking water samples of Shahrekord.

Samples (No. positive)	Subtypes	No. positive samples	Virulence genes
Water (10)	Non detected	2 (28.57)	-
	EHEC	1 (14.28)	<i>stx1, eae, ehly</i> : 1 (100)
	AEEC	4 (57.14)	<i>stx1</i> : 4 (100) <i>stx2</i> : 1 (25) <i>eaeA</i> : 3 (75) <i>stx1, eaeA</i> : 2 (50) <i>stx2, eaeA</i> : 1 (25) <i>stx1, stx2, eaeA</i> : 1 (25)
	Total	7 (70)	

was perceived amongst the incidence of AEEC and EHEC subtypes ( $P < 0.05$ ). All of the EHEC strains were simultaneously positive for *stx1*, *eaeA* and *ehly* genes. The prevalence of *stxs*, *stx2* and *eaeA* genes in the AEEC strains were 100%, 25% and 75%, respectively. Besides, the prevalence of *stx1* and *eaeA* genes, *stx2* and *eaeA* genes and finally *stx1*, *stx2* and *eaeA* genes in the AEEC strains were 50%, 25% and 25%, respectively.

#### 4. Discussion

The current research was focused on the detection of STEC strains and virulence genes in the drinking water samples of Shahrekord, Iran. Totally, 10% of drinking water samples were contaminated with *E. coli*. Ahmad et al. [9] reported that 26 out of 86 water samples (30.23%) collected from various parts of Pakistan were contaminated with *E. coli*. Patoll et al. [10] showed that the incidence of *E. coli* in drinking water samples was 64.29%. Previous study which was performed in Iran showed that 26.38% of tap water and 10.26% of bottled mineral water samples of Isfahan province were positive for *E. coli* [5]. The prevalence of *E. coli* in the surface water and waste water samples of Netherland were 88.88% and 100%, respectively [11]. EL-Jakee et al. [12] reported that total prevalence of *E. coli* in the water samples collected from the sources of agriculture, treated sewage, well, canal, untreated sewage and underground samples were 42.9%, 33.3%, 33.3%, 25, 25% and 7.7%, respectively.

A conceivable cause for the high incidence of *E. coli* in the drinking water of Shahrekord is due to primary contamination of the Dimeh fountain. Dimeh fountain is the main source of drinking water supply of the Shahrekord. Water of this fountain is transferred through pipelines to the Shahrekord refinery room. Several sources of pollution including primary contamination of Dimeh water, possibility of the linkage of polluted sources like waste waters of factories, cities and agricultural lands through pipes into the Dimeh water and presence of microbial biofilms in the pipelines can contributed in the contamination of drinking water of Shahrekord. Considerable incidence of *E. coli* in the urban, rural and agricultural waste waters has been reported previously [13,14]. Poor obedience to the principles of water purification and disinfection in the Shahrekord water purification is another cause for the considerable incidence of *E. coli* in the drinking water samples of this city. Considerable incidence and existence of *E. coli* biofilm in drinking water supply systems have been reported previously [9,15-17]. Another object for the considerable incidence of *E. coli* in Shahrekord is that it has open water accumulation sources. Therefore, there were the high possibilities for entrance of foreign material, dust, animals, birds and even human waste into these open water accumulation sources.

Additional significant result of our examination is the considerable incidence of *stx1*, *stx2*, *eaeA* and *ehly*

virulence markers in the *E. coli* strains of drinking water samples of Shahrekord. The most frequent virulence markers were *stx1* and *eaeA*. In a study which was conducted on Egypt, the *stx1* and *eaeA* genes were detected in the numerous samples of drinking water [18]. Ram et al. [19] reported the occurrence of different types of virulence markers of *E. coli* from water samples. Total prevalence of *stx* genes in the *E. coli* isolates of Dhaka city of Bangladesh was 60% [20]. In a study which was conducted on South Africa, the prevalence of *eaeA* and *stx* genes in the Plankenburg and Berg River systems were 15% and 0% and 8% and 15%, respectively [21]. Our results showed that all of the EHEC strains and majority of the AEEC strains harbored the *stx1* and *eae* genes. Considerable incidence of these factors in the water samples of Shahrekord city exposed an imperative community health matter.

Our discoveries are in contract with a beforehand recorded results of expressively advanced occurrence of the *eaeA* gene (96%) in surface water [22,23]. EHEC strains causes HC and HUS in humans, and main virulence marker consist of *eaeA* and Shiga toxins genes. The moderately considerable incidence of the *stx1* gene compared to *stx2* in the *E. coli* isolates of drinking water recommends that *E. coli* strains which harbored mixture of the *stx1* and *eaeA* genes is more routine than the mixture of *eaeA* and *stx2* genes. Such of our *E. coli* isolates had all *stx1*, *stx2* and *eaeA* genes which increased the importance of matter. The standing of these information lies on the point that *eae*-positive STEC strains are recognized more virulent for humans than *eae*-negative one [24]. It has been documented that combination of several virulence markers in such strains of *E. coli* may cause more sever diseases in humans [25,26].

#### 5. Conclusion

In conclusion, we recognized a large proportion of *E. coli* bacteria with distinct pathotypes which derived mostly from bases of animal and human in drinking water samples of Shahrekord. We also recognized numerous *E. coli* strains positive for *stx1* and *eaeA* genes. Concurrent attendance of *stx1* and *eaeA* and *stx2* and *eaeA* virulence markers in such *E. coli* strains specified the imperative community health concern regarding consumption of water. Application of bacteriological examination can diminish the bacterial load of drinking water. Monitoring of the bases of human and animal intestinal contamination, including management of municipal, industrial and agricultural wastewater is additional method to decrease the occurrence of *E. coli* in drinking water. It is essential to study the role of biofilms in survival of pathogenic bacteria in water pipes of Shahrekord.

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