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The Frequency of *Stx1* and *Stx2* Genes in Uropathogenic *Escherichia coli* Isolated From Patients in Kermanshah, Iran

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A B S T R A C T

1) Background: Uropathogenic *E. coli* (UPEC) is responsible for 70-90% of urinary tract infections. On the other hand, *E. coli* producing Shiga toxin (STEC), the so-called Verotoxin-producing *E. coli* (VTEC), has two *stx1* and *stx2* genes (Producing Stx1 and Stx2 toxins). Since these genes are plasmidal and can be transmitted between *E. coli* strains, it is likely that *stx1* and *stx2* genes are also found in the Uropathogenic *E. coli*. Studies in different parts of the world indicate some cases of dangerous syndromes such as Haemolytic-Uraemic Syndrome (HUS) following urinary tract infections. Also, the incidence of urinary tract infections caused by Verotoxigenic *E. coli* strains is increasing. Therefore, the present study was conducted to investigate the presence of verotoxin genes in Uropathogenic *E. coli*; 2) Methods: A total of 180 clinical specimens were collected during five months. After diagnostic tests and differential biochemistry tests, 100 samples were confirmed as *E. coli* and the presence of *stx2* and *stx1* genes was investigated by Muliplex-PCR; 3) Results: The results showed that the prevalence rates of *stx1* and *stx2* genes were 15% and 13%, respectively, in UPEC samples examined in this study, which is in agreement with the results of few similar studies in Iran; and 4) Conclusions: It seems that the frequency of verotoxin genes in *E. coli* causing urinary tract infections in Kermanshah is more than the other parts of Iran. Therefore, the potential risks of these bacteria could not be ignored.

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Introduction

The diagnosis and treatment of urinary tract infections (UTIs) are a major concern in the field of health care. Around 150 million people worldwide are diagnosed with UTI every year, with an annual economic cost of over $ 6 billion in the world (Flores-Mireles et al, 2015; Farshad et al, 2012).

Escherichia coli is one of the most common causes of UTI, often caused by uropathogenic strains of *E. coli*. The UTIs caused by this bacterial strain include a wide range of disorders, including cystitis, urethritis, and pyelonephritis. The prevalence of this infection is 1% in boys and 3-8% in girls (Turabian et al., 1996; Wagenlehner et al., 2008).

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Uropathogenic *E. coli* (UPEC) strains are responsible for 70-90% of UTIs. On the other hand, Shiga toxin-producing *E. coli* (STEC) or Verotoxigenic *E. coli* (VTEC) is a group of diarrheagenic *Escherichia coli* (DEC) that possesses one of *stx1* or *stx2* genes or both (producing stx1 and stx2 toxins) (Nazemi et al., 2012). Several studies have been carried out on the frequency of UPEC and the characteristics of its genes in Kermanshah (Jalilian et al., 2014; Amini et al., 2017). To the authors' knowledge, however, there have been few studies so far in Iran regarding the study of the VTEC genes in UPEC, and there is no report in Kermanshah. Therefore, such a study was necessary to investigate the presence of these genes and their possible frequency in Kermanshah city.

Materials and Methods

Sample collection and processing

A total of 180 clinical samples was collected during a period of 5 months (from February 2012 to June 2017). Bacteriological tests in laboratories were carried out by sampling the middle urine and cultured on blood agar and eosin methylene blue agar (EMB Agar). After performing differential biochemistry tests, 100 samples were diagnosed as *E. coli* bacteria. The isolates were kept at -20°C for further investigations (Foroughi et al., 2019).

DNA extraction

To extract the genomic DNA, a phenol-chloroform-isoamyl protocol was performed as described before. The extracted DNAs were refrigerated at 4 °C for one night, and then kept frozen at −20 °C for further steps (Moore et al., 2004).

The primers for *stx2* and *stx1* gene amplification were selected from Nazemi et al. (2012) and then purchased from CinnaGen (Iran, Tehran). The sequences of the primers are shown in Table 1.

Polymerase chain reaction

DNA samples diluted at 10 ng/µl concentration were used and amplified using selected primers. PCR was performed using a thermal cycler (BioRad, USA) in a volume of 20 µl for each reaction containing the materials mentioned in Table 2.

After completion of the cycles, samples were removed from the machine and kept at 4 °C until electrophoresis. For electrophoresis, agarose gel (2%) was used with half-percent TBE buffer, and 10 µl SafeView was used for staining. At first, 5 µl of loading buffer was added to each extracted DNA, and then 10 µl of each sample was poured into the wells created in the agarose gel. Ultimately, electrophoresis was run with a voltage of 90-120 for 1.5-2 hours. Gel documentation was then used to represent the bands (Akoachere et al., 2012).

Results

Figure 1 shows the electrophoresis of the stx1 gene with a molecular weight of 894 bp, indicating one of the two genes encoding Shiga toxin (ST or SLT) in *E. coli*. The results of gel electrophoresis of the stx2 gene with a molecular weight of 478 bp indicate the other Shiga toxin-encoding gene (Fig. 2). This toxin causes more severe clinical symptoms than the stx1 toxin.

Table 1- Primer Sequences Used in This Study.

Gene name	Primer sequences	Product size (bp)	Ref.
stx1	F:CAGTTAATTTGGTGGCGAAG R:CTGCTAATAGTTCTGCGAATC	894	8
stx2	F: CCTCGGTATCCTATTCCCGG R: GGATGCATCTCTGGTCATTG	478	8

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Table 2- Optimized Reaction Components.

Sample components	Volume (µl)
DD water	12.6
Buffer (10X) PCR	2
$MgCl_2$ (50 mM)	1.5
dNTPs (10 mM)	0.4
Primers	1.2 (from each one)
Taq DNA Polymerase (5 U/µl)	0.3
DNA (10 ng/µl)	2
Sum	20

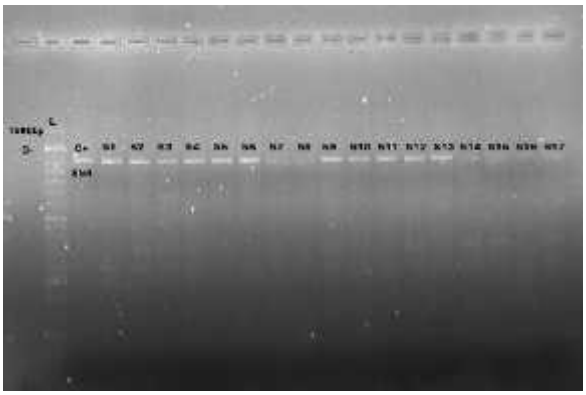

Fig. 1- Electrophoresis of PCR Products for the stx1 Gene: C-: Negative Control; L: Ladder (50-1500 bp); C+: Positive Control (*E. coli* O157: H7 ATCC No. 43895); S1-S6 and S9-S13: Positive Samples. S7-S8 and S14-S17: Negative Samples.

Fig. 2: Electrophoresis of PCR Products for the *stx2* Gene: C-: Negative Control; L: Ladder (50-1500 bp); C+: Positive Control (*E. coli* O157: H7 ATCC No. 43895); S1: Positive Samples. S2-S15: Negative Samples.

According to the PCR results, the prevalence rates of stx2 and stx1 genes were 13% and 15%, respectively (Fig. 3).

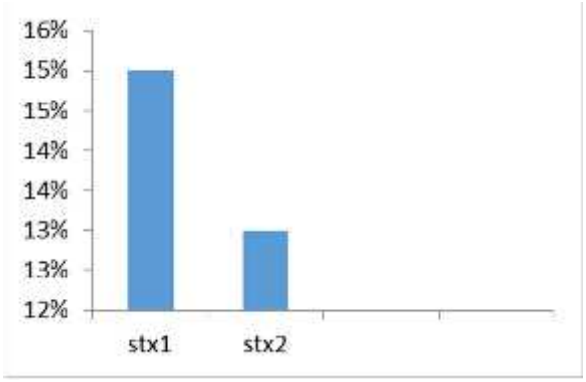

Fig. 3- Frequencies of the *stx1* and *stx2* Genes by PCR.

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Discussion

Studies in different parts of the world show some cases of dangerous syndromes such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC) following UTIs. Also, the incidence of UTIs is increasing due to verotoxigenic *E. coli* (Starr et al., 1998; Toval et al., 2014). Therefore, detection of STEC strains is very important. On the other hand, proper prevention of this infection depends on the rapid diagnosis of these bacteria using an accurate and rapid method.

In this regard, the results of this study indicate that the prevalence rates of *stx1* and *stx2* genes in UPEC samples were 15% and 13%, respectively. These findings are in agreement with those of a few studies on the frequency of these genes in *E. coli* causing UTIs in Iran.

In a study in Iran, only one urine sample (0.32%) was positive for verotoxigenic *E. coli* (stx2 harboring) among 311 ones (Naeb-Aghaee and Mansouri, 2006). Another study, however, showed frequencies of 10% and 6% for these two genes, respectively (Nazemi et al, 2012). In a study carried out in Tehran, only 2.3% of *E. coli* isolated from children with UTI was found to be enterohemorrhagic (Navidinia et al., 2012). In addition, the prevalence rates of *stx1* and *stx2* genes were reported to be 1% and 2%, respectively (Adeli et al., 2013). In contrast, these two genes were found in none of 146 *E. coli* isolated from UTIs in Khorramabad (Lorestan, Iran) (Mansouri et al., 2015).OIn the other hand, some researchers discovered *stx1* and *stx2* genes (2.63% and 1.31%), respectively, in two and one samples from 76 isolated UPEC in Shahrekord, Iran (Abbasi and Tajbakhsh, 2015). In a study conducted by Staji in Smnan (Iran), *stx1* and *stx2* genes were found in 16% and 10% of UPECs (Staji, 2017).

A review of relatively little research findings in Iran indicates variable and somewhat low prevalence of *stx1* and *stx2* genes in the UPEC. However, according to the percentages of 15% and 13% obtained in this study, it seems that the frequency of verotoxin genes in *E. coli* causing UTIs in Kermanshah is more than those in other parts of Iran. The reasons for this may be that Kermanshah province is the animal husbandry hub and the native people are interested in local dairy consumption. In addition, a large number of nomads live in this province, which in turn has an important role in the production and circulation of dairy products. On the other hand, *E. coli* harboring *Stx1* and *Stx2* genes are part of the ruminant intestinal flora, especially cattle. During milking or slaughtering, products or carcasses of the animals could be contaminated with this bacterium and transported to humans. Therefore, it may be argued that the mentioned issues are the reasons for the high frequency of these genes, as compared to other studies conducted elsewhere in Iran.

It should be noted that, although the detection and/or isolation of verotoxigenic *E. coli* from UTIs is done in limited cases, studies conducted in different parts of the world indicate some cases of serious syndromes such as HUS following UTIs. Furthermore, the potential risks of these bacteria should not be ignored due to the increasing incidence of UTIs caused by verotoxigenic *E. coli* (Scheutz et al., 2000; Page and Liles, 2013)..

Therefore, the presence of *stx1* and *stx2* genes in the UPEC is very important due to the possibility of simultaneous or subsequent infections. It causes the disease complexity, clinical misdiagnosis, and difficult and costly treatment of the disease. Also, the relatively high frequencies of these two genes in the present study indicate a high risk of simultaneous infection (e.g. HUS) in patients with UTI caused by verotoxigenic *E. coli* in Kermanshah, Iran.

Acknowledgements

Acknowledgements and Reference heading should be left justified, bold, with the first letter capitalized but have no numbers. Text below continues as normal.

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بررسی فراوانی ژن های stx1 و stx2 در ایکلای مسبب عفونت ادراری جداشده از بیماران در شهرستان کرمانشاه، ایران

آزاده فروغی[1*]، شروین رمضان قنبری[2]

چکیده

اشریشیاکلی های یوروپاتوژنیک (UPEC) مسئول ۹۰-۷۰٪ از عفونت های ادراری هستند. از طرف دیگر، *E. coli* تولیدکننده شیگاتوکسین (STEC) یا همان *E. coli* تولیدکننده وروتوکسین (VTEC) دارای دو دسته ژن stx_1 وstx_2 (تولیدکننده توکسین‌های Stx1 و Stx2) می‌باشد. از آنجایی که این ژن‌ها پلاسمیدی می‌باشند و قابل انتقال بین سویه های *E. coli* هستند، احتمال می‌رود که ژن‌های stx_1 و stx_2 در *ایکلای* یوروپاتوژنیک نیز یافت شود. مطالعات انجام‌شده در مناطق مختلف دنیا نشاندهنده مواردی از بروز سندروم‌های خطرناکی مانند اورمی همولیتیک متعاقب عفونت ادراری می‌باشد و نیز موارد بروز عفونت ادراری ناشی از سویه های *اشریشیاکلی* وروتوکسیژنیک رو به افزایش می‌باشد. لذا مطالعه حاضر جهت بررسی احتمال حضور ژن های وروتوکسیژنیک در ایکلاهای یوروپاتوژنیک انجام شد. تعداد ۱۸۰ نمونه بالینی طی مدت زمان ۵ ماه جمع‌آوری شد که پس از انجام آزمایش‌های تشخیصی و تست‌های بیوشیمیایی افتراقی تعداد ۱۰۰ نمونه آلوده به باکتری *اشریشیاکلی*، تأیید شد و حضور ژن‌های *stx2* و *stx1* در آنها با روش Muliplex- PCR مورد بررسی قرار گرفت. نتایج حاصل نشان داد که فراوانی ژن های stx_1 و stx_2 در نمونه‌های UPEC بررسی شده در این مطالعه، به ترتیب ۱۵٪ و ۱۳٪ است که در توافق با نتایج حاصل از مطالعات اندک بررسی فراوانی این ژن ها در *ایکلای* عامل عفونت ادراری در ایران می‌باشد. گرچه به‌نظر می‌رسد که فراوانی ژن‌های وروتوکسین در *ایکلای* مسبب عفونت‌های ادراری در شهرستان کرمانشاه، بیشتر از سایر نقاط ایران باشد. بنابراین، نباید خطرات بالقوه ناشی از این باکتری‌ها را نادیده گرفت.

واژگان کلیدی: یوروپاتوژنیک *اشریشیاکلی* (UPEC)، *stx1*، *stx2*، *VTEC*،*STEC*.

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