Molecular detection of *Escherchia coli carrying* uropathogenic-specific-protein and typeIII secretion system from patients with urinary tract infection

Received :22/ 5 / 2018 **Accepted : 1/ 7 /2018**

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Abstract

To specify *Escherichia coli*, infects urinary bladder, whether it is a urospecific bacterium or not, this polymerase-chain-reaction (PCR)-based study was performed. Urine specimens from patients with urinary tract infection were subjected to a process of bacterial recovering 20 isolates of *E. coli*. The isolates were then used for PCR-based identification of uropathogenic specific protein (*usp*) and type III secretion system (*ETTT*) genes. The results revealed the presence of *usp* in 6 (30%) isolates and *ETTT* in 2 (10%) isolates. So these results give no-doubt confirmation that these isolates of *E. coli* are urospecific and pathogenic bacteria.

Keywords: *ETTT*, PCR, *usp*, UTI.

Introduction

The most well-known bacterium that more often causes UTIs in people is *E. coli*. This bacterium is highly recognized to induce UTI-based complication in high rate in those patients [1–3].The bacterium is responsible for causing asymptomatic bacteriuria in young women at high rates as 4-6%. These cases were previously studied in both genders for some factors that are important for the virulence of the bacterium. According to some of these studies,[4] found that hemolysin and mannose-resistant-based

adhesins were frequently linked to *E. coli* recovered isolates from women that encountered complicated UTIs. However, men and women have some di fferences regarding the effectiveness of *E. coli* via its virulent factors on the host-based clinical UTIs [5]. *Usp* gene was found to be associated with isolates of *E. coli* responsible for certain UTIs such as pyelonephritis, and it strongly affects cell lining of the urinary tract via the presence of *lmu*2 gene [6].Also, there are the *ETTT* (type III secretion system) genes which responsible for delivering these protein-based toxins to the target-host cells inducing more damages in UT[7].In the current work, focused was performed on the *usp* and *ETTT* genes of isolates belong to *E. coli* from patients that suffered UTIs in Al-Diwaniyah city, Iraq.

Materials and Methods

Bacterial cultivation

Twenty urine specimens of patients in Al-Diwaniyah Teaching Hospital, Diwaniyah, Iraq who suffered from urinary tract infection (UTI) were subjected to a process of bacterial recovering of 20 isolates of *E. coli* . Bacterial isolates were inoculated in nutrient broth and transmitted to a Laboratory in the college of veterinary medicine, University of Al-Qadisiyah, Diwaniyah, Iraq. These samples were stayed overnight at 37℃ to activate these bacteria. Then these isolates were cultivated on MacCkonkey and eosine methylene blue (EMB) agars for better identification. These bacteria were subjected to certain biochemical tests of catalase, citrate utilization, TSI agar, gelatin liquefaction, Indole production, nitrate reduction, urease, Voges-Proskauer, methyl red, and motility via sulfide indole motility (SIM) medium [9] .DNA extraction and PCR process

The bacterial growth was placed in 0.2ml of sterile distal water for molecular biology. The DNA was extracted from these bacteria after brief incubation, 10min at 95℃, and centrifugation[10].A PCR-based technique was used to target two specific regions of *usp*, 615bp, and *ETTT*, 783bp. Primers (table 1), mastermix preparation, and the conditions of PCR thermocycler were utilized from [8].These conditions were 1 cycle for primary denaturation at 94℃ for 10min, after that, 30 cycles of (main denaturation at 94℃ for 1min, annealing at 60℃, and main extension at 72℃ for 1.5min), and final extension at 72℃ for 7min.The PCR-based products were

electrophoresed on 2% agarose-based gel. The image of the PCR products in the gel was generated via the use of a UV-light-based imager.

Table 1: primer used in this study

primer	Forward	Reverse	Product size (pb)	Reference
usp	5′-CGGCTCTTACATCGGTGCGTTG-3′	5′-GACATATCCAGCCAGCGAGTTC-3′	615	[8]
ETTT	5′-GCGGAAGTTTTGTATGATTGCCG-3′	5′-ATCAACCAGGAAAGCCAGTACG-3′	783	[8]

Results

Patients that suffered from urinary tract infection (UTI) were subjected to a process of bacterial recovering of 20 *E. coli*. The results of the biochemical tests used in this study are shown in table 2.

Table 2: The results of the biochemical tests.

Test	Result
Catalase	+
Citrate utilization	-
TSI Agar	AG/A
Gelatin liquefaction	-
Indole Production	+
Nitrate Reduction	+
Urease	-
Voges-Proskauer	-
Methyl Red	+
Motility	+

The isolates of *E. coli* then used for PCR-based identification of *usp* and *ETTT* genes. The results revealed the presence of *usp* in 6 (30%) isolates and *ETTT* in 2 (10%) isolates. Figure 1 shows the bands of those two genes.

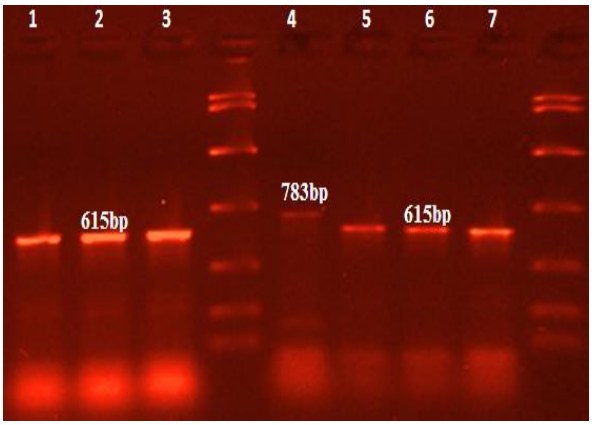

Figure 1: 2% agarose gel image after electrophoresis. Positive amplification of *ETTT* gene is in the lane 4 and *usp* gene in the lanes 1-3 and 5-7. Lanes without numbers are the ladders, 100-8000bp.

Discussion

E. coli is manifested by the frequent isolation from patients suffering UTIs. This bacterium induces high levels of complications regarding UTIs[1–3]. Asymptomatic bacteriuria is considered as a major health problem in young women that could occur at rates of 4-6%. These rates could be positively correlated with age of women to reach as high or higher than 20% [10]. The results of current study were encouraging because the reliable numbers of *E. coli* isolates of that were recovered via the use of cultivation techniques were relatively high. The biochemical test as usual increased confidence to confirm the presence of this bacterium in the samples collected for this study. Using conventional techniques such as biochemical test gave reliable identification of *E. coli* isolates from different sources [11,12].To identify whether these isolates were from the uro-specific pathogenic *E. coli*, PCR-based detection of two genes *usp* and *ettt* genes . The results revealed the presence of *usp* in 6 (30%) isolates and *ettt* in 2 (10%) isolates. According to these results, the presence of these two genes confirms that these isolates of *E. coli* are from the urospecific strains of this bacterium. *Usp* gene, contained in the DNA-pathogenicity island, indicates the uropathogenic strains of *E. coli* that could activate the pathogenicity of this bacterium incorporation with the presence of certain genes [13–14.The results that were recorded in the current study give interesting information about the strain types that causes UTIs in patients from Al-Diwaniyah city, Iraq. Concusion: Results of this study conclude that targeting these genes (*usp* and *ettt*) is useful way to rapid differentiate and confirm the diagnosis of uropathogenic strains of *E. coli*.

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