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DISTRIBUTION OF THE *USP* GENE IN UROPATHOGENIC *ESCHERICHIA COLI* FROM PATIENTS WITH URINARY TRACT INFECTION AND INVESTIGATION OF ANTIBIOTIC RESISTANCE

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Abstract:
Urinary tract infection (UTI) is one of the most frequent infectious diseases around the world that caused by uropathogenic Escherichia coli (UPEC). Uropathogenic-specific protein (usp) is homologous to the Vibrio cholerae zonula occludens toxin gene wich is found in all common serogroups of UPEC. this study was performed to detect the distribution of usp and antibiotic resistance properties in E. coli strains isolated from patients with UTIs.
For this purpose, samples were collected from patients and the strains which were biochemically confirmed as E. coli- positive used for detection of usp gene by PCR. Also the standard disc diffusion method of Kirby-Bauer was performed for determination of antibiotic resistance.
The PCR assay results identified about 57.76 % of the urine samples and 7.46 % of the stool samples contained usp gene. And our results showed that ceftizoxime (78 %) and imipenem (70 %) are the best antibiotic for UTI treatment.

Keywords: *Escherichia coli, Uropathogenic Escherichia coli, Urinary tract infections, usp gene, Antibiotic resistance, PCR*

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INTRODUCTION:

Escherichia coli (*E. coli*) is the most common pathogen in acute uncomplicated urinary tract infection [1]. Urinary tract infections (UTIs) are one of the most frequent infectious diseases around the world that despite all advances in medical sciences, remains as one of the most prevalent infectious diseases leading pyelonephritis (infection of the kidney) and cystitis (infection of the bladder) [2 ,3]. UTI occurs not only among humans, but is also observed in companion animals such as dogs and cats [4].

E. coli strains associated with urinary tract infections, known as uropathogenic *Escherichia coli* (UPEC), possess traits that distinguish them from commensal strains of *E. coli*. These strains have shown certain virulent properties, including iron acquisition systems (genes: *iuc*, *iroN*, and *irp2*), adhesins (P fimbriae, type 1 fimbriae, S and F1C fimbriae, afimbrial adhesion; genes: *pap, papG* allele I, *papG* allele II, *papG* allele III, *sfa, afaI, fim, iha*, and *tsh*), synthesis of toxins (hemolysin and cytotoxic necrotizing factor; genes: *set, astA cnf1, hlyA, vat, usp,* and *cva/cvi*) and specific O: K (polysaccharide coatings (group II capsules)): H serotypes [5- 10]. Also UPEC strains are serotyped by O antisera and the serogroups O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75, and O83 are specifically represented in UPEC strains [8, 11- 14].

UPEC strains contain two or more large DNA blocks termed as pathogenicity islands (PIs) that encode a set of virulence determinants [15, 16]. Also was identified a 4167-bp putative PI commonly associated with UPEC strains [17]. This PI consists of a gene that encodes a 346 amino acid protein, which was named uropathogenic-specific protein (USP), and three small open reading frames (ORFs; *orfU1-3*) of 98, 97 and 96 amino acids [1, 17, 18]. *usp* is homologous to the *Vibrio cholerae* zonula occludens toxin gene [17]. *usp* is detected significantly more often in UPEC strains than in fecal E. coli strains from healthy individuals, and found in all common serogroups of UPEC [1, 18].

UTI treatments often need antibiotic therapy, while studies showed that antibiotic resistance in UPEC is increasing nowadays. Therefore, identification of bacterial resistance is very necessary [19].

The aim of this study was to detect the distribution of *usp* and antibiotic resistance properties in *E. coli* strains isolated from patients with UTIs.

MATERIAL AND METHODS:

Samples Collection and *Escherichia coli* Identification

235 urine samples were collected from patients with UTIs (185 women and 50 men) from Imam Khomeini and Golestan hospital in Ahwaz, Iran. Midstream urine was collected in sterile condition and immediately transferred to the microbiology laboratory.

The samples were cultured on Eosin Methylene Blue agar (EMB agar) plate (Merck, Germany) and incubated at 37 ℃ for 24h. A metallic green colony from each plate with typical *E. coli* morphology was selected and streaked on Nutrient Agar (Merck, Germany) for earning single colony. The identification of *E. coli* was performed by IMViC tests (Table 1), and hemolysin test was done by culturing on Blood Agar (Merck, Germany) (incubated at 37 °C for 24h with 5% CO_2) [20].

Also stool samples of patients that *E. coli* isolated from their urine were collected and examined for *E. coli* isolation.

The strains which were biochemically confirmed as *E. coli*- positive, were kept in Luria-Bertani /glycerol at −70°C.

Table 1: Biochemical Characteristic of *E. coli*

Test	MacConkey growth	Indole production	Methyl red	Voges-Proskauer	Hydrogen sulfide(TSI)	Motility	Oxidase	Urea hydrolysis	Citrate (simmons)
Result	Positive	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Negative

Antibiotic susceptibility testing

The standard disc diffusion method of Kirby-Bauer by using Mueller Hinton agar (Merck, Germany) was performed to evaluate antibiotic susceptibility, according to the Clinical and Laboratory Standards Institute guidelines.

The following antibiotics were used at the indicated concentrations: Imipenem (IPM) 10 µg, Ceftizoxime (CT) 30 µg, Nalidixic acid (NA) 30 µg, Tetracycline (TE) 30 µg, Ciprofloxacin (CIP) 5 µg, Sulfamethoxazole trimethoprim (SXT) 30 µg and Ceftriaxone (CRO) 30 µg.

After incubating the inoculated plates at 37°C for 18–24 h in an aerobic atmosphere, the susceptibility of the *E. coli* isolates was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006). For each test, *E. coli* ATCC 35218 was used as the control strain [2].

PCR

Bacterial strains were subcultured overnight in Luria-Bertani broth (Merck, Germany) and genomic DNA was extracted from typical colonies of *E. coli* using DNA extraction kit (Metabion, Germany) according to manufacturer's instruction.

All of the positive colonies were confirmed using the polymerase chain reaction (PCR) technique. Table 2 shows the primers used for detection of *usp* gene of *E. coli* strains isolated from patiants.

PCR was performed with a total volume of 25 µL including 0.75 µM $MgCl_2$, 1 µM of forward primer, 1 µM of reverse primer, 2.5 µL PCR buffer 10X, 0.5 µM dNTP (Fermentas), 0.2 U Taq DNA polymerase (Fermentas) and 5 µL DNA template. The DNA was then amplified by 32 successive cycles of denaturation at 94°C for 30s, primer annealing at 68°C for 60s, and DNA chain extension at 72°C for 60s, also initial denaturation was 95°C for 150s and final extension was 72°C for 7 min.

The amplified products were visualized by ethidium bromide staining after gel electrophoresis of 10 µL of the final reaction mixture in 1.5% agarose.

Table 2: Primers Used for Detection of *usp* Gene of *E. Coli* Strains

Primers	Sequence
Forward	ATGCTACTGTTTCCGGGTAGTGTGT
Rivers	CATCATGTAGTCGGGGCGTAACAAT

Table 3: Sex and Marital Status of Patients

Patients	Culture Positive, No. (%)	Single, No. (%)	Married, No. (%)
Female	106 (91.38 %)	13 (12.27 %)	93 (87.73 %)
Male	10 (8.62 %)	3 (30 %)	7 (70 %)
Total	116 (100 %)	16 (13.79 %)	100 (86.21 %)

RESULT:

116 of two hundred and thirty-five urine samples (49.36 %) of patients with UTIs were identified as *E. coli* by using standard biochemical tests, including 10 males (8.62 %) and 106 females (91.38 %) (Table 3).

Also after the fecal biochemical examination of these patients, 67 samples were positive for *E. coli* bacterium.

95 isolated bacteria from urine samples (81.89 %) and 27 isolated bacteria from stool samples (40.29 %) were positive for hemolysis.

Distribution of *usp* in *E. coli* Strains from patients with UTI

To determine the distribution of *usp* in uropathogenic *E. coli*, all strains obtained from the urine and stool of subjects with urinary tract infection were examined by PCR. Two types of size variations (1 and 2.5 kb) for *usp* were detected (Figure 1). The PCR assay results identified about 57.76 % (n= 67) of the urine samples and 7.46 % (n= 5) of the stool samples contained *usp* gene (Table 4).

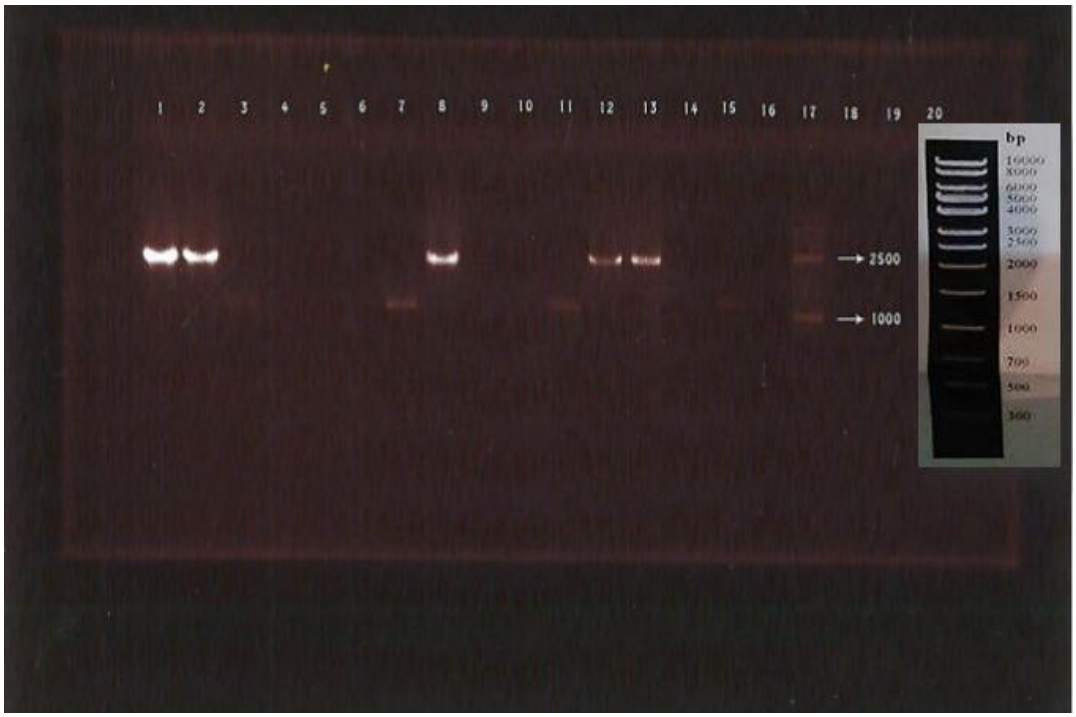

Fig1: PCR amplification of *usp* UPEC strains from patients

Table 4: Distribution of *usp* in Isolated Strains from The Urine and Stool of Patients

Samples	Number of Isolated Strains	Number of *usp* Gene Positive	Percent of *usp* Gene Positive
Urine	116	67	57.76 %
Stool	67	5	7.46 %

Investigation of antibiotic resistance

Our results showed that ceftizoxime (78 %) and imipenem (70 %) are the best antibiotic for UTI treatment, and ceftriaxone (64 %) is appropriate, too. These three antibiotics were suitable for *E. coli* strains isolated from the stool samples of patients (Table 5).

Table 5: Antibiotic Resistance Properties in Uropathogenic *E. coli* Serogroups Isolated from Urinary Tract Infections

Antimicrobial Agent	Disc concentration	Sensitivity (Urine sample)	Sensitivity (Fecal specimen)
Imipenem (IPM)	10 µg	70 %	80 %
Ceftizoxime (CT)	30 µg	75 %	78 %
Nalidixic acid (NA)	30 µg	25 %	25 %
Tetracycline (TE)	30 µg	18 %	22 %
Ciprofloxacin (CIP)	5 µg	32 %	45 %
Ceftriaxone (CRO)	30 µg	64 %	61 %
Sulfamethoxazole trimethoprim (SXT)	30 µg	48 %	24 %

DISCUSSION

Urinary tract infection is the most frequently diagnosed kidney and urological disease, and *E. coli* strains associated with urinary tract infections, known as uropathogenic *E. coli* (UPEC) [6, 18]. Based on their components and products called virulence factors, *E. coli* cells attach selectively to the mucosa uro-epithelium, promoting colonization and persisting in the urinary tract, inducing, then, a local inflammatory response and sometimes to promote tissue lesions [21, 22].

While current technology has made the identification of potentially new virulence genes a relatively simple endeavor. PCR method is highly specific, informative and a powerful genotypic assay, used for detection of virulence factors that can also contribute to virulence in UTI [9, 23, 24].

In this study, we confirmed the prevalence of *usp* among UPEC strains that isolated from patients with UTI. Yamamoto and et al. indicated that *usp* may contribute to the causation of urinary tract infection and may be considered a major virulence determinant of uropathogenic *E. coli* [1]. Nipič and et al. expressed *usp* is a novel *E. coli* genotoxin active against mammalian cells and told numerous studies have investigated the prevalence of *usp* nucleotide sequences among various collections of *E. coli* strains. Also they showed that Usp is released from bacterial cells and, in vitro, cleaves DNA [25]. In other study Nakano and et al revealed the presence of *usp* sequence in six UPEC strains and in an *usp*-positive *E. coli* strain isolated from the stool of a healthy individual [18]. As well as, Kurazono and et al examined the distribution of *usp* in *E. coli* strains isolated from dogs and cats with and without UTI. About 52.5% and 60% of the *E. coli* isolates from dogs and cats with UTI, and only 8.8% and 35.5% of *E. coli* isolates from dogs and cats without UTI showed *usp* gene [26].

Our experiment demonstrated 57.76 % of urine samples and 7.46 % of fecal samples were positive for *usp* gene wich is deferent from Paniagua-Contreras and et al. 's result (87.1 %) from Mexico [27] and Yamamoto and et al. 's result (71.7% to 100%) from Japan [1]. While the prevalence of *usp* gene in Tiba and et al. 's study from Brazil was 22.2 % [9].

In our study, about 91.38 % of patients were female and 8.62 % were male, that Similar results were re-ported previously [28- 30]. It is because of the relatively short, straight anatomy of the urethra in women. In addition, retrograde ascent of bacteria from the perineum is the most common cause of UTIs in women [31].

The increasing of antibiotic resistance of UPEC strains is a public health concern [32]. The most common antibacterial drugs in UTIs' treatment were trimethoprim-sulfamethoxazole, cephalosporin, semisynthetic penicillin with or without beta-lactamase inhibitors and quinolones [33, 34], but the results indicate that UPEC strains has been resistant to them [2, 35].

We examined antibiotic resistance properties in uropathogenic *E. coli* for several antibiotic include: Imipenem (IPM), Ceftizoxime (CT), Nalidixic acid (NA), Tetracycline (TE), Ciprofloxacin (CIP), Sulfamethoxazole trimethoprim (SXT) and Ceftriaxone (CRO). The highest resistance was against Nalidixic (75 %) acid and Tetracycline (82 %) and least against Ceftizoxime (25 %) and Imipenem (30 %). Likewise, Farshad et al. showed the high prevalence of resistance genes to ampicillin (80.2%), cotrimoxazole (76%) and tetracycline (70.8%) in Iran [36]. So, unfortunately in a very short period of time, antibiotic resistance will appear and is rising rapidly.

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