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Genotypic assessment of Uropathogenic*Escherichia coli* isolated from urinary tract infections

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Abstract
Uropathogenic*Escherichia coli* is considered the most important infectious agent responsible for the occurrence of urinary tract infections. The present survey was conducted to assess the genotypic pattern of virulence factors amongst the 40 UPEC isolates of UTIs. Forty UPEC strains were isolated from patients with UTIs. Isolates were confirmed using different biochemical tests. The polymerase chain reaction assessed the genotypic distribution of virulence factors. Findings showed that *fimh* (90%), *pap* (85%), *cnf1* (70%), *hlyA* (65%), *afa* (60%), *iron* (57.5%), and *iuc* (50%) were the most commonly detected virulence factors, However, *tsh* (5%), *usp* (12.5%), *ompT* (20%), and *irp2* (32.5%) were the less commonly detected virulence factors amongst the UPEC strains. Statistically, a significant difference was found between the distribution of different virulence factors (P<0.05). Considering the high distribution of UPEC virulence factors amongst isolated bacteria, proper control of the virulent UPEC strains as an important cause of UTIs should perform in healthcare units. PCR method was defined as a property assay for rapid and sensitive detection of virulence factors.
Keywords: Uropathogenic*Escherichia coli*, Virulence factors, Urine, Urinary Tract Infections, PCR.

Introduction
The majority of urinary tract infections (UTIs) in humans are caused by *Escherichia coli* (*E. coli*) (1). UTIs comprise a range of disorders, including cystitis (infection of the bladder) and pyelonephritis (infection of the kidney)(2). About 50% of women suffered from UTIs during their life (3). Thus, it is essential to studyand control.
UPEC strains harboured several types of virulence characters encoded by the presence of diverse virulence factors. The most important virulence factors amongst the UPEC strains are afimbrial adhesin I (*afa*I), aerobactin (*aer*), P fimbriae (*pap*), S fimbriae (*sfa*), aerobactin (*aer*), hemolysin (*hly*), and cytotoxic necrotizing factor 1 (*cnf1*), (4). Otherwise, *ompT*,*iroN*, *kpsMT*,*iha*, *usp*,*astA*, *iutA*, *traT*, and *fimH* are other important virulence factors of the UPEC strains (5, 6).
Virulence factors are mainly associated with bacteria adhesion, growth, infection, invasion and metabolism. Otherwise, they are mainly important in UPEC infections' pathogenesis, including cystitis, pyelonephritis, and urethritis (7, 8). To help bacterial colonization in the urinary epithelium, these virulence genes cause severe acuity in UPEC strains responsible for causing severe diseases and even resistance against drugs. As a result, their detection is essential, especially in cases with UTIs (9).
To the best of our knowledge, scarce research is available about the Polymerase Chain Reaction (PCR)-based identification of virulence factors among UPEC strains in Iran. Thus, the present survey was conducted to assess the genotypic pattern of UPEC strains isolated from UTIs in Iran.

Materials and methods
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UPEC isolates
A total of 40UPEC strains were isolated from patients with UTIs in Summer 2020. UP[EC strains were biochemically confirmed using Gram-staining and various biochemical tests, including urease, oxidase, catalase, indole production, citrate and triple sugar iron utilization, and methyl red-Voges Proskauer (10).

DNA extraction
UPEC isolates were sub-cultured on TSB media (Merck, Germany) and further incubated for 48 h at 37 ^{o}C.According to the manufacturer's instruction, genetic DNA was extracted from bacterial colonies using the DNA extraction kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). Purity (A260/A280) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA) (11-15). The truth of the

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DNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany) (16-18).

Genotypic assessment of virulence factors

Table 1 represents the list of primers and PCR conditions used to amplifyvirulence factors amongst the UPEC strains isolated from UTIs(19).

Table 1. List of primers used to amplify virulence factors amongst the UPEC strains isolated from UTIs (19).

Virulence factors	Primer Sequence (5'-3')	Size of product (bp)
pap	GCAACAGCAACGCTGGTTGCATCAT AGAGAGAGCCACTCTTATACGGACA	336
cnf1	AAGATGGAGTTTCCTATGCAGGAG TGGAGTTTCCTATGCAGGAG	498
hlyA	AACAAGGATAAGCACTGTTCTGGCT ACCATATAAGCGGTCATTCCCGTCA	1177
afa	GCTGGGCAGCAAACTGATAACTCTC CATCAAGCTGTTTGTTCGTCCGCCG	750
iuc	ATGAGAATCATTATTGACATAATTG CTCACGGGTGAAAATATTTT	1482
fimH	GAGAAGAGGTTTGATTTAACTTATTG AGAGCCGCTGTAGAACTGAGG	559
iha	CTGGCGGAGGCTCTGAGATCA TCCTTAAGCTCCCGCGGCTGA	827
iroN	AAGTCAAAGCAGGGGTTGCCCG GACGCCGACATTAAGACGCAG	665
ompT	ATCTAGCCGAAGAAGGAGGC CCCGGGTCATAGTGTTCATC	559
usp	ACATTCACGGCAAGCCTCAG AGCGAGTTCCTGGTGAAAGC	440
irp2	AAGGATTCGCTGTTACCGGAC AACTCCTGATACAGGTGGC	413
tsh	ACTATTCTCTGCAGGAAGTC CTTCCGATGTTCTGAACGT	824

Table 2 shows the PCR circumstances used for the virulence factors detection (19).

Virulkence factors	PCR program	PCR volume (50 µL)
irp2, tsh	1 cycle: 95^{0C} ---------- 4 min. 32 cycle: 94^{0C} ---------- 60 s 56^{0C} ---------- 60 s 72^{0C} ---------- 2 min 1 cycle: 72^{0C} ---------- 6 min	5 µL PCR buffer 10X 1.5 mM $MgCl_2$ 200 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.5 U *Taq* DNA polymerase (Fermentas) 3 µL DNA template
usp	1 cycle: 94^{0C} ---------- 2 min. 30 cycle: 94^{0C} ---------- 30 s 58^{0C} ---------- 30 s 73^{0C} ---------- 30 s 1 cycle: 72^{0C} ---------- 10 min	5 µL PCR buffer 10X 2 mM $MgCl_2$ 200 µM dNTP (Fermentas) 0.4 µM of each primers F & R 1 U *Taq* DNA polymerase (Fermentas) 3 µL DNA template
iha, iroN, ompT	1 cycle: 94^{0C} ---------- 6 min.	5 µL PCR buffer 10X 1.25 mM $MgCl_2$

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	30 cycle: 94^{0C} ---------- 45 s 58^{0C} ---------- 60 s 72^{0C} ---------- 75 s 1 cycle: 72^{0C} ---------- 8 min	150 µM dNTP (Fermentas) 1 µM of each primers F & R 1.2 U *Taq* DNA polymerase (Fermentas) 3 µL DNA template
iuc, fimH	1 cycle: 94^{0C} ---------- 3 min. 40 cycle: 94^{0C} ---------- 60 s 58^{0C} ---------- 70 s 72^{0C} ---------- 70 s 1 cycle: 72^{0C} ---------- 6 min	5 µL PCR buffer 10X 1.25 mM $MgCl_2$ 125 µM dNTP (Fermentas) 0.5 µM of each primers F & R 1.2 U *Taq* DNA polymerase (Fermentas) 3 µL DNA template
pap, afa, hlyA, cnf1	1 cycle: 94^{0C} ---------- 1 min. 30 cycle: 94^{0C} ---------- 60 s 63^{0C} ---------- 30 s 72^{0C} ---------- 90 s 1 cycle: 72^{0C} ---------- 5 min	5 µL PCR buffer 10X 1.5 mM $MgCl_2$ 200 µM dNTP (Fermentas) 0.4 µM of each primers F & R 1 U *Taq* DNA polymerase (Fermentas) 4 µL DNA template

A programmable DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. Ten microliters of PCR product were exposed to electrophoresis in a 2% agarose gel in 1X TBE buffer at 80 V for 30 min, stained with SYBR Green. The UVI doc gel documentation systems (Grade GB004, Jencons PLC, London, UK) were applied to analyze images (21, 22).

Data analysis

Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact two-tailed test were used to assess any significant relationship between data obtained from the present study. *P*-value <0.05 was considered as a statistically significant level (23, 24).

Results

The present research was carried out to assess the genotypic pattern of virulence factors amongst the UPEC strains isolated from UTIs. Table 3 shows the genotypic assessment of virulence factors amongst the 40 UPEC isolates of UTIs. Findings showed that *fimh* (90%), *pap* (85%), *cnf1* (70%), *hlyA* (65%), *afa* (60%), *iron* (57.5%), and *iuc* (50%) were the most commonly detected virulence factors, However, *tsh* (5%), *usp* (12.5%), *ompT* (20%), and *irp2* (32.5%) were the less commonly detected virulence factors amongst the UPEC strains. Statistically, a significant difference was found between the distribution of different virulence factors (*P*<0.05).

Table 3. Genotypic assessment of virulence factors amongst the 20 UPEC isolates of UTIs.

No. UPECstrains	N. isolates harboured each virulence factors (%)											
	fimH	***tsh***	***usp***	***cnf1***	***hlyA***	***pap***	***iron***	***afa***	***iuc***	***iha***	***ompT***	***irp2***
40	36 (90)	2 (5)	5 (12.5)	28 (70)	26 (65)	34 (85)	23 (57.5)	24 (60)	20 (50)	18 (45)	8 (20)	13 (32.5)

Discussion

UTIs is one of the most common nosocomial infections caused by *E. coli*. This bacterium is located in the epithelium of urinary tract tissue and can cause inflammation of the urinary tract and inflammation of the ureters, bladder and kidneys. This bacterium can form biofilms. It is resistant to the third generation of antibiotics,which has made this disease a complex problem in the medical community (25).

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The present survey findings showed the high distribution of putative virulence factors among UPEC bacteria isolated from UTIs. Among all detected factors, *fimh* (90%), *pap* (85%), *cnf1* (70%), *hlyA* (65%), *afa* (60%), *iron* (57.5%), and *iuc* (50%)harboured the higher distribution.

Additionalresearchdesignated that *usp* and *iha* were present in 63.7% and 34.1% of all *E. coli* isolates of UTIs (26). A study on children showed thatthe distribution of*pap*, *sfa*, *hlyA*, and *cnf1* genes was 27.1%, 14.6%, 13.5% and 22.9%, respectively (27). A study in Brazilshowed that the distribution of*pap*, *sfa*and *afa* virulence factors amongst the UPEC strains was 32.0%, 19.0% and 11.0%, respectively (28). *Fimh*, *pap*, *cnf1*, *hlyA*, *afa*, *iron*, and *iuc*virulence factors were also predominant amongst the UPEC strains isolated from urine samples of previous researches (29-31).

Despite previous studies, our results showed that the UPEC strains in Iran have a different virulence profile. These differences in the prevalence of UPEC virulence genes showed that the virulence properties of UPEC strains are closely dependent on the geographic region and even the weather climate of each region. The epidemiology and prevalence of virulence factors of UPEC strains isolated from patients with UTI are different in Iran. Probably customs, food diets, the levels of public health, hospital's health and even methods of sampling have great rules in the prevalence of virulence genes of UPEC strains. In this present study, we tried to collect samples with the lowest rate of cross-contamination. On the other hand, the statistical analysis between the levels of other genes is not significant. Our results showed that the urine samples of patients with UTIs are a potential source of UPEC strains and their virulence factors.

The present survey was limited to the low number of isolated bacteria, the absence of the multi-group analysis of patients, and finally, the absence of the antimicrobial resistance properties or even molecular typing of isolated strains. However, working on diverse virulence factors is the most important aspect of the present research.

Conclusion

UPEC virulence factors are considered important causes for the pathogenesis of UTIs. *Fimh*, *pap*, *cnf1*, *hlyA*, *afa*, *iron*, and *iuc* virulence factors were also considered pathognomic and predominant genes of the UPEC activation in the vcases UTIs. Their detection in diverse kinds of UPEC-associated diseases can prepare different and novel epidemiological aspects of the UPEC strains in the UTIs. PCR is considered safe and sensitive for UPEC virulence factors detection.

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