



Distribution of Pathogenicity Islands Among Uropathogenic *Escherichia coli* Isolates From Patients With Urinary Tract Infections

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Abstract

Background: Uropathogenic *Escherichia coli* (UPEC) is one of the most common etiologic agent of urinary tract infection (UTI). The ability of *Escherichia coli* to cause UTI is associated with specific virulence determinants, which are encoded by pathogenicity islands (PAIs).

Objectives: This study aimed to investigate the distribution of PAIs among the UPEC isolates collected from patients with UTIs.

Materials and Methods: In this study, a total of 100 *E. coli* isolates were collected from patients with UTIs using standard microbiological methods. Polymerase chain reaction (PCR) was used for the identification of the main PAIs of UPEC according to insertion sites and virulence markers.

Results: In total, PAI IV536, PAI III536, PAI I536, PAI, IICFT073, PAI ICFT073, PAI IJ96, PAI II536, and PAI IJ96 were detected in 23, 22, 17, 17, 13, 11, 11, and 8% of isolates. PAI combinations were identified in 15% of isolates.

Conclusion: The results showed that PAIs of UPEC are not strain-specific and some strains can carry the PAIs associated with the prototype strains of UPEC simultaneously.

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Background

Urinary tract infections (UTIs) are among the most common infections worldwide and Uropathogenic *Escherichia coli* (UPEC) bacteria are known to be the most important cause of UTI, which are involved in more than 80% of these infections.¹ The UPEC strains are a genetically heterogeneous group, which encode various types of virulence factors associated with bacterial colonization and survival of bacteria in the urinary tract.² The main virulence factors of UPEC are iron acquisition systems, adhesins, and cytotoxins. These factors help the bacterial colonization and invasion of bacteria to the urinary tract.³ Virulence factors are encoded by bacterial genes that are located on the plasmid or the chromosome of UPEC. Most of these genes are organized on certain genetic elements that are called "Pathogenicity Islands (PAIs)."⁴ An array of virulence factors were encoded on PAIs. PAI I536 is associated with the well-known virulence factors Alpha-hemolysin, F17-like fimbriae, and CS12-like fimbriae. PAI II536 contains genes for P-related fimbriae, another Alpha-hemolysin gene cluster, the Hek adhesion, and hemagglutinin-like adhesins. PAI III536 encodes S-fimbriae, Iron siderophore system, HmuR-like heme receptor, Sap adhesin, and TSH-like hemoglobin protease. PAI IV536 represents the core element of the

HPI of pathogenic *Yersinia* spp. It is encoded by the Yersiniabactin siderophore system.⁵ PAIJ96 contains a *hly* operon and a *pap* operon encoding a P-pilus. It is the assembly of the P-pilus and that of type 1 pili. PAI IJ96 contains a *hly* operon, as well as a *prs* operon encoding a different class of pilus and a gene encoding the cytotoxic necrotizing factor 1 (CNF1).⁶⁻⁸ PAI ICFT073, which carries an Alpha-hemolysin operon (*hly*), a *pap* operon encoding P-fimbriae, aerobactin, and genes related to iron transport systems and putative carbohydrate transport systems.⁹ PAI IICFT073 contains a *pap* operon, iron-regulated genes, mobile genetic elements, and a large number of unknown genes.⁸ PAIs are often associated with tRNA genes and usually located at the downstream of tRNA genes. The tRNA loci are a compatible site for the insertion of PAIs.¹⁰ Eight PAIs have been identified in three prototype strains of UPEC 536 (4 PAIs), UPEC J96 (2 PAIs) and UPEC CFT073 (2 PAIs). These PAIs are commonly associated with *selC* (PAI I536), *leuX* (PAI II536), *thrW* (PAI III536), *asnT* (PAI IV536), *pheV* (PAI IJ96 and PAI ICFT073), and *pheU* (PAI IJ96 and PAI IICFT073) tRNA sites.¹¹

Acquiring data about the genetic structure and the virulence factors which can be encoded by PAIs have a great value for fully understanding UPEC pathogenicity

potential. Moreover, the relationship between the pathogen and the host can differ when the different PAI was carried by UPEC strains. The studies on the distribution of PAIs of UPEC isolates can be epidemiologically important, and it can offer useful information for controlling isolates with significant pathogenicity by identifying the virulence of indigenous isolates. This study aimed to evaluate the distribution of PAIs among the isolates of UPEC from patients with UTIs.

Materials and Methods

Sample Collection and Identification of Isolates

In this descriptive and cross-sectional study conducted from January 2017 to September 2018, the midstream urine samples were obtained from patients with pyelonephritis and cystitis who referred to the Shahid Modarres Hospital of Tehran. The *E. coli* isolates were collected from these patients and confirmed by conventional biochemical tests (IMViC).

DNA Extraction, Primer Design, and Detection of Pathogenicity Islands of UPEC

The genomic DNA was extracted using the DNA extraction kit (Roche, Germany) according to the kit protocol. The DNA quality was evaluated based on the OD 260/280 ratio using a NanoDrop spectrophotometer (Quawell, USA). The presence of *selC*, *leuX*, *thrW*, *asnT*, *pheV*, *pheU* tRNA encoding genes was examined using the specific primers designed in this study by Allele ID 6.0 software (Premier Biosoft, USA). Forward primers were designed for the encoding regions of the tRNA genes and reverse primers were designed for the downstream of these genes. In this way, the insertion of PAIs disrupts the genes and results in negative polymerase chain reaction (PCR). Because of the presence of common insertion sites, the primers of *iucC* and *cnf1* virulence genes were used for differentiation of PAIs I and PAIs II J96/CFT073

strains, respectively. The PCR assay was performed in a final volume of 25 µL containing 2.5 µL of buffer (10X), 1 µL of (50 mM) MgCl₂, 1 µL (15 pM) of forward and reverse primers, 0.5 µL (5U/µL) of Super Taq DNA Polymerase, 2 µL (500 ng) of bacterial DNA, and 17 µL of sterile distilled water. Thermal conditions included the primary denaturation at 95°C for 5 minutes, followed by 35 cycles (denaturation at 95°C for 30 seconds, annealing at temperatures mentioned in Table 1, for 30 seconds and extension at 72°C for 1 minute). The final extension was carried out at 72°C for 5 minutes. *E. coli* strain K12 was used as a positive control. The PCR products were evaluated after electrophoresis on 1% agarose gel.

Statistical Analysis

Data of this study were statistically analyzed using the Statistical Package for Social Sciences (SPSS) software version 22.0 (IBM, USA).

Results

A total of 100 UPEC isolates were collected from patients with UTIs. Twenty-two (22%) of isolates were obtained from male patients and 78 (78%) of isolates were isolated from female individuals. Additionally, 7 patients were under 20 years, 40 patients were between 21 and 50, and 53 of the patients were over 50 years old.

The Frequency of Pathogenicity Islands

The tRNA encoding genes *leuX*, *selC*, *pheV*, *thrW*, *asnT*, and *pheU* were positive in 89%, 83%, 79%, 78%, 77% and, 72% of UPEC isolates, respectively (Figure 1). The presence of intact tRNA genes indicated that the relevant PAI was not inserted into this site and the genetic structure remained intact. On the other hand, the positive results of the PCR for the tRNA genes indicated the no insertion of the related PAI at the downstream of the tRNA gene. The *iucC* and *cnf1* virulence genes were positive in 72%

Table 1. Characteristics of Primers Used in the Study

Target	Primer Sequence (5'→3')	Amplicon Size	Annealing Temperature	Accession Numbers	References
<i>selC</i>	F: GGTGAGGCGGCTGGACTTC R: TGGCGATACAGTTGGCTGAGAG	324 bp	62°C	CP017979.1	This study
<i>leuX</i>	F: GAAGTGGCGAAATCGGTAGAC R: CATCGTATCCCTCCCTGTTGG	270 bp	61°C	CP014272.1	This study
<i>thrW</i>	F: TCGTAATGCGAAGGTCGTAGG R: AGGATGTATAGTGAGCGAAGCC	194 bp	63°C	LN832404.1	This study
<i>asnT</i>	F: TAATCCGTATGCTACTGGTTTCG R: CTCCAATTCTCTTCCTGATAGC	379 bp	60°C	CP000948.1	This study
<i>pheV</i>	F: CAGTCGGTAGAGCAGGGGATTG R: GGTGGTCATCAGCGGATAGC	408 bp	60°C	AP009048.1	This study
<i>pheU</i>	F: ACCAAGGACACGGGGATTTC R: GAAACGCAAACCGCCGAAC	526 bp	58°C	CP042184.1	This study
<i>iucA</i>	F: AAACCTGGCTTACGCAACTGT R: ACCCGTCTGCAATCATGGAT	269 bp	59°C	-	12
<i>cnf1</i>	F: AAGATGGAGTTTCTATGCAGGAG R: CATTAGAGTCTGCCCTCATTATT	498 bp	62°C	-	13

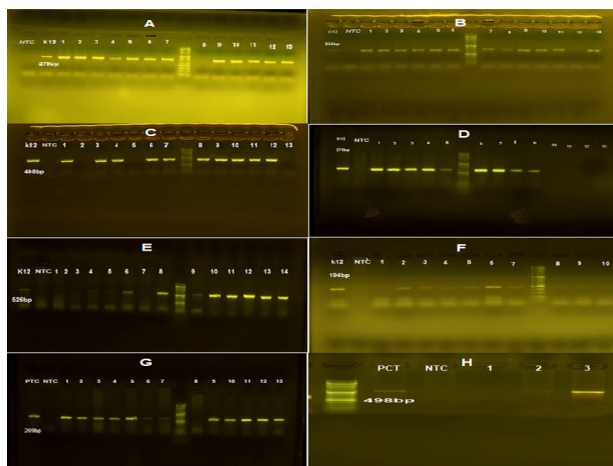


Figure 1. Results of PCR for the Identification of Insertion Sites and Marker Genes Used for Differentiation of PAIs of UPEC Isolates. A: *asnT*; insertion marker of PAI IV 536, B: *selC*; insertion marker of PAI I536, C: *pheV*; common insertion marker of PAI IJ96 and PAI ICFT073, D: *leuX*; insertion marker of PAI II536, E: *pheU*; common insertion marker of PAI IJ96 and PAI IICFT07, 3 F: *thrW*; insertion marker of PAI III536, G: *iucA*; differentiation marker of PAI IJ96 and PAI IICFT07, H: *cnf1*; differentiation marker of PAI IJ96 and PAI ICFT073. Each part of the figure includes (positive control (PCT) and no template control (NTC) in the first and second wells. The 100 bp DNA ladder was used for electrophoresis.

and 38% of *pheU* and *pheV* negative isolates, respectively (Figure 1). Both PAI IJ96/PAI ICFT073 and PAI IJ96/PAI IICFT073 have the same insertion site and they were inserted in the downstream of *pheV* and *pheU* tRNA genes, respectively. The *iucC* gene, an aerobactin encoding gene, was used for differentiation of PAI IJ96 (negative) from PAI ICFT073 (positive), and *cnf1* gene that encodes cytotoxic necrotizing factor 1 was used for separation of PAI IJ96 (positive) from PAI IICFT073 (negative). According to the aforementioned results, the frequency rates of PAIs in this study were reported to be 17, 11, 22, 23, 8, 11, 13, and 17% for PAI I536, PAI II536, PAI III536, PAI IV536, PAI IJ96, PAI IJ96, PAI ICFT073, and PAI IICFT073. The simultaneous presence of PAIs (PAI combination) was identified in 15% of UPEC isolates. More details about the distribution of UPEC PAIs have been mentioned in Table 2.

Discussion

UTI is recognized as one of the common infections, both in the hospital and in the community, which is associated with human health problems in the world.¹⁴ Various epidemiological studies on UTI have reported that UPEC is the cause of 80% of these infections.¹⁵ Different virulence factors are encoded by the UPEC PAIs. By overcoming the host defense mechanisms, these factors play important roles in the pathogenesis of UPEC strains and causing the disease.¹⁴ The PAIs usually enter the tRNA encoding gene loci such as *selC*, *pheU*, *pheV*, etc, and each locus is associated with a particular PAI.¹⁶ The UPEC isolates from patients with UTIs were examined in

terms of the distribution of PAIs in the current study. The identification of the PAIs was based on the detection of their insertion sites in tRNA loci. The forward primers were designed for the coding region of tRNA and reverse primers were designed for downstream of these sites in this study. The presence of PAIs in the downstream region of these tRNA genes was investigated. However, due to the failure of the identification of these sites by reverse primers, no amplification was observed in PCR. In some studies, the PAIs have been identified based on their insertion sites in tRNA loci.¹⁷⁻¹⁹

The UPEC 536 related PAIs were the most prevalent PAIs among isolates, while PAIs associated with J96 and CFT073 strains showed approximately similar abundance in this study. Moreover, 15% of isolates had PAI combination. Sabaté et al²⁰ reported PAI IV536, PAI ICFT073, PAI IICFT073, PAI IJ96, PAI I536, PAI II536, and PAI III536 in 89%, 73%, 46%, 34%, 33%, 20%, and 2%, respectively, and PAI IJ96 was not detected among 100 isolates of *E. coli* isolated from the urine of patients with UTI. PAI combination was shown in their study. In other words, different types of PAIs (536, J96, and CFT073) were found simultaneously in 75% of isolates. Moreover, in a study by Navidinia et al²¹ in 2013, on 100 isolates of *E. coli* collected from the urine of children with UTI, the prevalence rates were reported to be 19%, 11%, and 18% for PAI ICFT073, PAI II536, and PAI I536, respectively, which are very close to those found in the present study. Dobtindt et al⁵ reported in their study that 64.5% of the isolates caused UTI and 39.3% of the

Table 2. Distribution of Pathogenicity Islands in UPEC Isolates Collected from Patients with UTI

PAI Composition	Total Frequency (%)	Patients (n=100)	
		Female (78%)	Man (22%)
PAI I ₅₃₆	9	6%	3%
PAI II ₅₃₆	8	5%	3%
PAI III ₅₃₆	15	13%	2%
PAI IV ₅₃₆	13	11%	2%
PAI I _{J96}	8	3%	5%
PAI II _{J96}	11	8%	3%
PAI I _{CFT073}	9	7%	2%
PAI II _{CFT073}	12	11%	1%
PAI I536 + PAI II536	1	1%	-
PAI I536 + PAI III536	1	1%	-
PAI I536 + PAI IV536	2	2%	-
PAI III ₅₃₆ + PAI IV ₅₃₆	3	3%	-
PAI I _{CFT073} + PAI II _{CFT073}	3	3%	-
PAI IV ₅₃₆ + PAI II _{CFT073}	1	1%	-
PAI I ₅₃₆ + PAI III ₅₃₆ + PAI IV ₅₃₆	1	1%	-
PAI I ₅₃₆ + PAI II ₅₃₆ + PAI III ₅₃₆ + PAI IV ₅₃₆	2	1%	1%
PAI I ₅₃₆ + PAI IV ₅₃₆ + PAI I _{CFT073} + PAI II _{CFT073}	1	1%	-

non-pathogenic bacteria were PCR-positive for a specific region of PAIV536 and concluded that PAI III536 was more prevalent than PAI II536 or PAI I536, which, like our study, outlined the prevalence and frequency of these PAIs. However, the frequency rates in some studies were different from our study. For example, in a study conducted by Navidinia et al,²¹ the PAI III536 was present in 3% of isolates that were less than the frequency rate of PAI III536 (22%) in our study. Additionally, in the report of Sabaté et al,²⁰ the frequency rate of the PAI III536 was 2%, which is lower compared to our study (22%). A remarkable point in our study was the low prevalence of PAI IJ96, which is common in all the aforementioned studies.

Previous studies have identified strains 536, J96, and CFT073 as the UPEC prototype and some associated PAIs were identified in these strains.^{5,22,23} In the current study, it was found that some isolates carry PAIs of different UPEC prototypes simultaneously. Similar results were reported by other studies.^{11,20,21} The structure of the PAI is very similar to other mobile genetic elements such as bacteriophages and integrons.²⁴ Furthermore, the possibility of horizontal PAI transfer between different strains of UPEC is not unexpected. According to earlier studies, the highest number of PAI combination was associated with PAIs of 536 and CFT073 strains.^{20,21} In the present study, PAIs of these two strains were found simultaneously in some isolates, which may confirm the higher rate of genetic exchange between these two strains. Previous studies have shown that PAI IJ96/I CFT073 and PAI IJ96/IICFT073 have similar insertion sites in the bacterial genome.^{24,25} Pittard et al²⁶ reported that *E. coli* has only two target tRNA *pheV* and *pheU* genes. It can increase the level of competition between these two PAIs. Therefore, the results suggest that PAIs of CFT073 have a greater affinity than PAIs of IJ96 to be located in the target area. This may be the reason that PAIs of CFT073 are more prevalent than PAIs of J96 among UPEC clinical isolates. Mixed bacterial infection is not common in the sterile areas of the body and it is usually caused by one type of bacteria in these areas. However, evidence of infection with more than one bacterial species has been reported.²⁷ Given this condition, the simultaneous isolation of two strains of a species may not be unexpected. This may be a reason for the simultaneous identification of PAIs of different UPEC strains in a patient with a UTI.

Conclusion

The presence of PAI combination was shown among the UPEC isolates from patients with UTIs in our region. These results revealed that the PAIs of well-known strains of UPEC is not strain-specific and different PAIs can be present simultaneously in some clinical isolates. However, the possibility of mixed infection with two strains simultaneously should be refused, which requires further studies.

Authors' Contributions

EA: Sample collection, project administration; MM: Conceptualization, software, review & editing; MN: Original draft, review & editing.

Ethical Approval

This study was approved by the Ethics Committee of Shahed university (IR.Shahed.REC.1395.119).

Conflict of Interest Disclosures

The authors report no conflicts of interest.

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