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Original Article

Evaluation of pathogenicity islands in uropathogenic *Escherichia coli* isolated from patients with urinary catheters

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Abstract

Introduction: Uropathogenic *Escherichia coli* (UPEC), an important causative agent of urinary tract infections (UTIs), carries virulence factors which are clustered on pathogenicity islands (PAIs) .The goal of this study was to characterize the PAIs among the UPEC isolated from patients with urinary catheters.

Methodology: A descriptive cross-sectional study was designed and from December 2014 to April 2015, 78 non-duplicate *E. coli* were collected from hospitalized patients with UTIs, including patients with and without indwelling urinary catheters. Two multiplex polymerase chain reaction (PCR) assays were performed to evaluate the presence of the eight most studied PAIs (I_{536}, II_{536}, III_{536}, IV_{536}, I_{CFT073}, II_{CFT073}, PAI I_{J96}, and PAI II_{J96}).

Results: Of 78 patients with a UTI 31 (39.7%) used indwelling catheters. Of these 31 patients, 27 (87.1%) carried PAIs markers, including 25 (80.6%) PAI IV_{536}, 12 (38.7%) PAI I_{CFT073}, 6 (19.4%) PAI II_{CFT073}, 6 (19.4%) PAI III_{536} and 3 (9.7%) PAI II_{536}. PAI I_{536}, PAI I_{J96}, and PAI II_{J96} were not detected in the UPEC strains.

Conclusions: The findings of this study revealed that the frequency of PAI markers in UPEC isolates from patients with indwelling urinary catheters was high. The rate of multiple PAIs carriage was notable among those patients, suggesting that UPEC strains that colonize the indwelling urinary catheters have the potential to cause complicated urinary infections. PAI I_{CFT073}, which was found in association with pyelonephritis, prostatitis, and sepsis, could be considered as a target for medical interventions.

Key words: uropathogenic *Escherichia coli*; pathogenicity islands; urinary catheter

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Introduction

Catheter-associated urinary tract infections (CAUTIs) are among the most common nosocomial infections, and the presence of urinary catheters is a major risk factor for bacteriuria [1,2]. Over one million cases of CAUTIs are reported annually, and CAUTIs account for about 80% of all nosocomial UTIs cases [3]. *Escherichia coli* is the most common cause of UTIs, accounting for 80%–90% of all UTIs seen among outpatients [4]. Uropathogenic *Escherichia coli* (UPEC) strains are known as the most frequent causative agent of UTIs [5]. Several different virulence factors necessary for the establishment of those in the urinary tract and causing infection have been recognized in UPEC strains [6,7]. The virulence factors of pathogenic extraintestinal *E. coli* isolates are clustered on pathogenicity islands (PAIs) [8]. PAIs are generally identified in pathogenic strains and are seldom found in non-pathogenic strains [6]. PAIs structurally consist of a sequence of DNA (< 10 kb) in size. They encode mobility genes (*e.g.*, integrases and insertion sequences), and are generally associated with tRNA genes, flanked by short repeated sequences that have different guanine and cytosine content from the bacterial core genome [9,10]. PAIs were reported in UPEC strain 536 for the first time [9]. There are three major strains of UPEC, including CFT073, 536 and J96, and different types of PAIs have been documented for each one [11,12]. PAIs are mobile genetic elements that provide a strong route for horizontal transfer of virulence genes, which is why these DNA regions are involved in bacterial evolution, especially among pathogenic bacteria [9]. The study of PAI markers is useful in better understanding the virulence genes of pathogenic bacteria. However, very little is known about PAI markers of UPEC strains isolated from

patients who use indwelling urinary catheters. The objective of this study was to characterize the PAIs among the UPEC strains isolated from patients with urinary catheters.

Methodology

Bacterial isolates

In this cross-sectional study, a total of 78 *E. coli* strains were isolated from urine samples of patients who were hospitalized due to UTIs in Shahid Beheshi University Hospital in Kashan during December 2014 to April 2015. The UPEC isolates were collected from patients with and without indwelling urinary catheters. The patients were of both sexes (79.5% female, 20.5% male). UTI was defined by positive urine culture with bacterial counts $\geq 10^5$ colony-forming units (CFU)/mL. The UPEC strains were identified by conventional standard biochemical tests [13]. After confirmation as UPEC, the strains were cultured in tryptic soy broth (TSB) with 15% glycerol and stored at -20° for further study.

Detection of PAI markers by multiplex PCR

DNA extraction of each confirmed UPEC isolate was performed using the boiling method. Two multiplex PCR, no. 1 and no. 2 assays, were performed to evaluate the presence of the eight most studied PAIs (I_{536}, II_{536}, III_{536}, IV_{536}, I_{CFT073}, II_{CFT073}, I_{J96}, and II_{J96}). PAIs III_{536}, IV_{536}, and II_{CFT073} were detected by multiplex PCR no.1, producing 200, 300, and 400 bp PCR products, respectively. Multiplex PCR no. 2 was performed to identify PAI II_{J96}, PAI I_{536}, PAI II_{536}, PAI I_{CFT073}, and PAI I_{J96}, obtaining 2,300, 1,800, 1,000, 930, and 400 bp PCR products, respectively [11,14,15]. The amplifications were carried out using an Eppendorf master cycler (Eppendorf Mastercycler Gradient, Foster City, USA) in a total volume of 50 µL including 1U Taq DNA polymerase (Fermentase, Burlington, Canada), 5 µL of 10 × reaction buffer, 1.5 mM $MgCl_2$, 1 µL of 0.2 mM of dNTP (Fermentase, Burlington, Canada), 5 µL template DNA, 1 µL (10 pmol) forward and reverse primers, and nuclease-free water.

The following amplification program was used for both multiplex PCRs: an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 45 seconds, and extension at 72°C for 1 minute, with the final extension step at 72°C for 10 minutes. The amplification program was according to Sabate *et al.*'s study, although the annealing steps were changed for better results [14]. The electrophoresis of PCR products was performed on 1.5% agarose gel and stained with ethidium bromide (0.5 mg/mL). The UPEC clinical strains with defined PAIs markers obtained from the Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, were used as positive controls. Primers used in this study are shown in Table 1.

DNA sequencing analysis

The PCR products were sequenced by Macrogen (Macrogen Research, Seoul, Korea). Sequence data were analyzed using Chromas Pro version 1.7.5 Technelysium (www.technelysium.com.au) and compared with online BLAST software (http://www.ncbi.nlm. nih.gov/BLAST/).

Table 1. Primers used for polymerase chain reaction.

Multiplex PCR	Primer name	Target	Sequence (5'→3')	Amplification product (bp)	Reference
No. 1	sfaAI.1	PAI III_{536}	F-CCATGTCCAAAGCTCGAGC	200	11
No. 1	sfaAI.2	PAI III_{536}	R-CTACGTCAGGCTGGCTTTG	200	11
No. 1	IRP2 FP	PAI IV_{536}	F-CGGGCATGCATCAATTATCTTTG	300	14
No. 1	IRP2 RP	PAI IV_{536}	R-TGTGTAGATGCAGTCACTCCG	300	14
No. 1	cft073.2Ent1	PAI II_{CFT073}	F-AAGGATTCGCTGTTACCGGAC	400	14
No. 1	cft073.2Ent2	PAI II_{CFT073}	R-TCGTCGGGCAGCGTTTCTTCT	400	14
No. 2	I.9	PAI I_{536}	F-GCGGACGGGTGAGTAATGT	1,800	14
No. 2	I.10	PAI I_{536}	R-TCATCCTCTCAGACCAGCTA	1,800	14
No. 2	orf1up	PAI II_{536}	F-CATGTCCAAAGCTCGAGCC	1,000	14
No. 2	orf1down	PAI II_{536}	R-CTACGTCAGGCTGGCTTTG	1,000	14
No. 2	RPAi	PAI I_{CFT073}	F-GGACATCCTGTTACAGCACGCA	930	15
No. 2	RPAf	PAI I_{CFT073}	R-TCGCCACCAATCACAGCCGAA	930	15
No. 2	papGIf	PAI 1_{J96}	F-TCGTGCTCAGGTCCGGAATTT	400	15
No. 2	papGIR	PAI 1_{J96}	R-TGGCATCCCACATTATCG	400	15
No. 2	Hlyd	PAI II_{J96}	F-GGATCCATGAAAACATGGTTAATGGG	2,300	14
No. 2	Cnf	PAI II_{J96}	R-GATATTTTTGTTGCCATTGGTTACC	2,300	14

Statistical analysis

Statistical analysis was done using SPSS software version 18 (IBM, Armonk, USA). Chi-squared and Fisher's exact tests were used to evaluate associations of the variables. $P < 0.05$ was considered statistically significant.

Results

In total, 62 of 78 patients with UTIs (79.5%) were female and 16 (20.5%) were male, between 1 and 95 years of age; the mean age was 50 years. Of the 78 UPEC isolates, 31 (39.7%) were from patients with indwelling urinary catheters and 47 (60.3%) were from patients without urinary catheters. Of the patients with catheters, 24 were female (77.4%) and 7 (22.6%) were male.

Of the 31 strains recovered from catheters, 27 (87.1%) carried PAI markers. PAI markers present in UPEC isolates from patients with indwelling catheters were as follows: 25/31 (80.6%) PAI IV_{536}, 12/31 (38.7%) PAI I_{CFT073}, 6/31 (19.4%) PAI II_{CFT073}, 6/31 (19.4%) PAI III_{536}, and 3/31(9.7%) PAI II_{536}. PAI I_{536}, PAI I_{J96}, and PAI II_{J96} were not detected in UPEC strains. Of 27 UPEC isolates from patients with indwelling catheters that carried PAI markers, 16/27 (59.2%) carried more than one PAI marker simultaneously (Table 2). The patterns of PAI markers identified in UPEC strains isolated from patients with and without indwelling catheters are shown in Table 3.

The analysis of PAI patterns revealed that one UPEC strain isolated from patients with indwelling catheters carried five PAI markers at the same time and two of them carried four, whereas four UPEC isolates recovered from patients without catheters contained four PAI markers simultaneously (Table 3). The most prevalent pattern was PAI IV_{536}.

There was no correlation ($p > 0.05$) between detected PAI markers among patients with and without

Table 2. PAI markers identified in UPEC strains in patients with indwelling catheters as well their age, sex, and related infections.

	PAIs							
UPEC strain	**PAI II_{536}**	**PAI III_{536}**	**PAI IV_{536}**	**PAI I_{CFT073}**	**PAI II_{CFT073}**	**Infections**	**Sex**	**Age**
UPEC1	-	-	+	-	-	Sepsis	F	66
UPEC2	-	-	+	+	-	Sepsis	M	89
UPEC3	-	+	+	-	-	UTI	F	1
UPEC4	-	+	+	-	-	UTI	F	44
UPEC5	-	-	+	+	-	UTI	M	1
UPEC6	-	+	+	+	+	UTI	F	38
UPEC7	+	+	+	+	-	UTI	M	63
UPEC8	+	+	+	+	+	UTI	F	77
UPEC9	-	-	+	-	-	UTI	M	11
UPEC10	-	-	+	-	-	UTI	F	3
UPEC11	-	-	+	-	-	UTI	F	83
UPEC12	-	-	+	+	-	UTI	M	82
UPEC13	-	-	+	-	-	Sepsis	F	85
UPEC14	-	-	+	-	-	UTI	F	6
UPEC15	-	-	+	-	-	UTI	F	1
UPEC16	-	-	+	+	-	UTI	F	72
UPEC17	-	-	+	-	+	UTI	F	80
UPEC18	+	-	+	+	-	UTI	F	45
UPEC19	-	-	-	-	-	UTI	F	20
UPEC20	-	-	-	-	-	UTI	F	42
PEC21	-	-	-	-	-	UTI	M	63
UPEC22	-	-	-	+	-	Prostatitis	M	86
UPEC23	-	-	-	+	-	UTI	F	3
UPEC24	-	-	+	-	-	UTI	F	62
UPEC25	-	-	+	-	-	UTI	F	75
PEC26	-	-	-	-	-	UTI	F	75
UPEC27	-	+	+	-	-	UTI	F	82
UPEC28	-	-	+	-	-	UTI	F	21
UPEC29	-	-	+	-	+	UTI	F	1
UPEC30	-	-	+	+	+	Pyelonephritis	F	55
UPEC3	-	-	+	+	+	UTI	F	81

UPEC: uropathogenic *E. coli*; PAIs: pathogenicity islands; UTI: urinary tract infection

indwelling catheters (Table 4). The results of the PCR product sequencing were identical to those deposited in the GenBank.

Discussion

Uropathogenic *Escherichia coli* strains are among the most important causative agents of catheter-associated urinary tract infections [2,9]. The difference between UPEC and non-pathogenic *E. coli* strains is due to the production of several virulence factors that are coded by virulence genes [16]. These genes are generally located on PAIs [8]. In our study, 87.1% of patients with indwelling urinary catheters carried PAI markers. In a study conducted by Navidinia *et al.* in Tehran, Iran, of UPEC isolates from children between 2 and 12 years of age with UTIs, 89% appeared to carry PAI markers [17]. In Spain, PAI markers were found in 93 of 100 UPEC isolates, including 50 isolates from patients with pyelonephritis and 50 isolates from patients with urinary sepsis [14]. The 87.1% carriage of PAI markers, large pieces of DNA that code virulence genes with the potential of mobility among UPEC strains, is considerable and predicts the ability of these strain to generate complicated UTIs.

The most prevalent PAI marker detected among UPEC strains of patients with and without indwelling catheters was PAI IV_{536}. In a study by Sabate *et al*., PAI IV_{536} was found most frequently in both commensal and UPEC isolates from patients with pyelonephritis and urinary sepsis [14]. In most studies worldwide, the presence of PAI IV_{536} in the Enterobacteriace family is documented, and this marker is known as broad-host-range PAI or high-pathogenicity island (HPI) [11,18,19]. Our results also showed that one UPEC isolate from patients with an implanted catheter and four UPEC isolates from patients without urinary catheters suffering from sepsis carried only PAI IV_{536} as a single PAI marker. The high frequency of PAI IV_{536} has been documented in commensal isolates, although some studies have shown that HPI participates in pathogenicity of extraintestinal pathogenic *E. coli* [14,20]. The high frequency of PAI IV_{536} shows this

Table 3. The patterns of PAI markers identified in UPEC strains in patients with and without indwelling catheters.

PAIs (N)	UPEC strains in patients with catheter, N	PAIs patterns in patients with catheter	UPEC strains in patients without catheter, N	PAI patterns in patients without catheter
1 PAI	9	PAI IV_{536}	16	PAI IV_{536}
1 PAI	2	PAI I_{CFT073}	1	PAI II_{CFT073}
2 PAIs	3	PAI III_{536}, PAI IV_{536}	1	PAI I_{CFT073}, PAI II_{CFT073}
2 PAIs	4	PAI IV_{536}, PAI I_{CFT073}	7	PAI IV_{536}, PAI I_{CFT073}
2 PAIs	3	PAI IV_{536}, PAI II_{CFT073}	5	PAI IV_{536}, PAI II_{CFT073}
3 PAIs	1	PAI II_{536}, PAI IV_{536}, PAI I_{CFT073}	1	PAI II_{536}, PAI IV_{536}, PAI I_{CFT073}
3 PAIs	2	PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}	7	PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}
3 PAIs	-	-	1	PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}
4 PAIs	1	PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}	2	PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}
4 PAIs	1	PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}	3	PAI II_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}
5 PAIs	1	PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073}	-	-
Total	**27**		**44**	

UPEC: uropathogenic *E. coli*; PAIs: pathogenicity islands

Table 4. Association between detected PAI markers and UPEC isolates in patients with and without urinary catheter.

PAI markers type		Patients with catheter (N = 31) n (%)	Patients without catheter (N = 47) n (%)	P value
II_{536}	Positive	3 (9.7)	7 (14.9)	0.50
II_{536}	Negative	28 (90.3)	40 (85.1)	
III_{536}	Positive	6 (19.4)	3 (6.4)	0.079
III_{536}	Negative	25 (80.6)	44 (93.6)	
IV_{536}	Positive	26 (83.9)	42 (89.4)	0.478
IV_{536}	Negative	5 (16.1)	5 (10.6)	
I_{CFT073}	Positive	11 (35.5)	23 (48.9)	0.241
I_{CFT073}	Negative	20 (64.5)	24 (51.1)	
II_{CFT073}	Positive	7 (22.6)	17 (36.2)	0.203
II_{CFT073}	Negative	24 (77.4)	30 (63.8)	

UPEC: uropathogenic *E. coli*; PAIs: pathogenicity islands.

marker is stable in UPEC strains. The presence of this marker as the single PAI marker in isolates from urinary sepsis indicates the probable role of PAI IV_{536} in the pathogenicity of some UPEC strains.

The second most prevalent PAI marker in our study was PAI I_{CFT073}. In a study in Mofid Children's Hospital in Tehran, PAI I_{CFT073} was documented as the most prevalent PAI marker among patients with UTIs, after PAI IV_{536} [17]. Sequencing results revealed that our PAI I_{CFT073} carried *hly* and *pap* genes. Three cases of complicated infections such as pyelonephritis, prostatitis, and sepsis were associated with PAI I_{CFT073}. In other studies, these virulence genes and markers were also identified among patients with complicated UTIs and isolates from women with pyelonephritis [21,22], which could explain why this marker is found more frequently in extremely virulent strains.

Similar to our study, Sabate *et al.* reported PAI IV_{536} as the most prevalent PAI among UPEC and commensal strains, followed by PAI I_{CFT073} and PAI II_{CFT073}. In contrast to the present study, PAI II_{J96} and PAI I_{536} were detected at a relatively high frequency, and PAI III_{536} showed the lowest frequency in UPEC strains and was not detected in commensal isolates [14]. Dobrindt *et al.* reported that PAI III_{536} was more common than PAI II_{536} or PAI I_{536} in UPEC strains, including isolates from women with chronic UTIs, in contrast with our UPEC isolates from patients without catheters [11]. In other reports [20], PAI III_{536} and PAI II_{536} were reported as unstable PAIs, which could be the reason for the difference between our results and Sabate *et al.*'s findings. PAI I_{J96}, and PAI II_{J96} were not identified in our UPEC isolates. In a study conducted by Samei *et al*., the frequency of these markers was low in both commensal and uropathogenic *Escherichia coli* isolates [23]. In other studies, in accordance with our results, PAI I_{J96} was shown not to play a major role in the pathogenesis of UTIs [14].

The presence of multiple PAIs has been found in other studies [14, 23]. In a study in three major university hospitals in Zanjan, Iran, it was reported that 79.3% of UPEC strains harbored ≥ 2 PAI markers in comparison with 6% in commensal isolates [23]. Our results showed 51.6% and 57.4% of UPEC strains of patients with and without indwelling catheter carried two or more PAIs, respectively, showing that our UPEC strains have high pathogenic potential.

Conclusions

The findings of the present study revealed that PAIs markers were highly prevalent in UPEC isolates from both patients with and without indwelling urinary catheters. The PAI I_{CFT073} was found in association with pyelonephritis, prostatitis, and sepsis, especially in patients with catheters, whereas PAI IV_{536} were detected as a single PAI marker among most of the sepsis cases in patients without catheters. These findings could be considered for future medical interventions.

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