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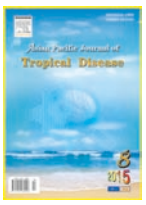

Clinical research doi: 10.1016/S2222-1808(15)60909-4 ©2015 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Prevalence of pathogenicity island markers genes in uropathogenic *Escherichia coli* isolated from patients with urinary tract infectious

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ARTICLE INFO

Article history:
Received 30 Mar 2015
Received in revised form 8 Apr, 2nd revised form 13 Apr, 3rd revised form 20 Apr 2015
Accepted 10 May 2015
Available online 8 Jul 2015

Keywords:
Escherichia coli
Urinary tract infection
Pathogenicity island markers
PCR

ABSTRACT

Objective: To investigate virulence associated characteristics in uropathogenic *Escherichia coli* (*E. coli*) isolates derived from urine specimens, and to investigate the distribution of the pathogenicity islands virulence markers (PAIs) among the isolates.

Methods: In this study, 63 *E. coli* strains isolated from patients with symptoms of urinary tract infections were collected and all of them were confirmed by biochemical tests and molecular techniques. PCR was conducted by specific pair primers in order to determine the prevalence of pathogenicity islands markers.

Results: Among the 63 examined isolates, PAI IV_{536} was reported in 53 strains (84.12%) showing the highest prevalence. The distribution of PAI II_{CFT073}, PAI III_{536}, PAI I_{536}, PAI II_{536}, PAI J_{196}, PAI IIJ_{196}, PAI I_{CFT073} were 41.26%, 4.76%, 34.92%, 20.63%, 1.57%, 31.74% and 72.01%, respectively.

Conclusions: Pathogenicity island markers cause horizontal transfer of genes. Many virulence genes are present in the pathogenicity islands regions in such a way that by deletion and/or mutation of these genes, the bacteria would lose its pathogenicity. Knowledge of the molecular details of uropathogenic *E. coli* is useful to develop successful strategies for the treatment of urinary tract infection and complications associated with urinary tract infections in human.

1. Introduction

Escherichia coli (*E. coli*), a versatile microbe, colonizes the intestinal tract of humans and animals with no harmful effects to the host, but opportunistically, it causes devastating and life-threatening diseases[1,2]. Based on the part of the host where pathogenic effect is elicited, an *E. coli* isolate is classified into one of these groups: (i) commensal (nonpathogenic) *E. coli*, (ii) intestinal pathogenic (diarrheagenic) *E. coli*, or (iii) extraintestinal pathogenic *E. coli* (ExPEC). Phylogenetically, *E. coli* has been classified into four groups namely A, B1, B2 and D[3].

ExPEC includes uropathogenic *E. coli* (UPEC), sepsis-associated *E. coli*, and neonatal meningitis-associated *E. coli* capable of eliciting pathogenic effects outside of the intestinal tract[1,4]. In humans, ExPEC colonizes the intestinal tract without causing any disease. ExPEC disseminates and elicits pathogenic effects to other body sites such as the urinary tract, bloodstream and central nervous system[4].

Urinary tract infections (UTIs) are among the most common type of bacterial infection in humans globally. They affect all age groups causing morbidity with chronic and recurrent infections resulting in enormous healthcare spending[5,6].

More than 80% of community-acquired UTIs are caused by UPEC[7]. Pathogenic extraintestinal *E. coli* isolates harbours specialized virulence factors (traits that confer pathogenic potential) which are often not found in commensal isolates[5]. The ability of UPEC to cause symptomatic UTIs is associated with the expression of a spectrum of virulence factors with adhesive molecules arguably being the most important determinant of its pathogenicity[7]. UPEC strains elaborate a number of virulence factors (such as fimbriae, toxins and siderophores), which enables the bacteria to colonize urinary tract, persist in the face of highly effective host defense and

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Foundation Project: Support by the Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (Grant No. T/06/1393).

elicit pathogenic effects[7-9].

Genes encoding for these virulence factors are located on transmissible genetic elements and/or in particular regions on the chromosome called the pathogenicity islands (PAIs)[5,9]. These PAIs encode several virulence factors, including adhesins, bacterial secretion systems, invasins, toxins, proteases, lipases, and iron uptake systems[1]. PAIs associated with the genome of pathogenic bacterial strains possess coordinate horizontal transfer of virulence genes between strains of one species or related species[9].

Identification of virulence factors in bacterial organisms can be useful for diagnosis and empirical treatment of bacterial infections associated with these organisms. The aim of this study was to investigate the distribution of the PAIs virulence markers among UPEC isolates from non-hospitalized patients in hospitals located in Shahrekord, Iran.

2. Materials and methods

2.1. Sampling, isolation and identification of UPEC isolates

Ethical approval for sampling of patients was obtained from the Research Ethics Committee of the Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

A total of 63 mid-stream urine samples (51 samples from females and 12 samples from males) from non-hospitalized patients with suspected cases of UTIs were collected from various clinical laboratories in Shahrekord, Iran. Samples were transported aseptically and processed within 30 min of collection in the Microbiology Laboratory, Islamic Azzad University, Shahrekord, Iran.

The samples were inoculated onto sterile MacConkey agar (Merck, Germany) plates, incubated at 37 ℃ for 24 h aerobically. Lactose-fermenting (pinkish) colonies were purified by subculturing on fresh sterile MacConkey agar, and incubated accordingly. The lactose-fermenting (pinkish) colonies were identified as *E. coli* by subjecting them to Gram staining, triple sugar iron agar, indole, methyl-red, Voges-Proskauer and Simmon's citrate tests following standard methods[10]. Stock cultures of the isolates were kept at -20 ℃ in 50% glycerol until needed.

2.2. DNA extraction

Stock culture of each isolate was subcultured by inoculated into 5 mL of sterile trypticase soy broth, incubated at 37 ℃ for 24 h. Then, bacterial DNA extraction was performed by boiling method according to the previously described protocol[11] with some modifications.

2.3. PCR components and amplification profile

PCR was performed using the DNA of each isolate to identify virulence related genes. Each reaction mixture contained 2.5 μL buffer (10×), 1.5 μL MgCl (50 mmol/L), 1 μL diethyl-nitrophenyl thiophosphate (10 mmol/L), 1 μL (50 pmol) each for the forward and reverse primers (primers are listed in Table 1), 1 μL *Taq* DNA polymerase, (Fermentas, USA) (1 IU/μL), 1 μL template DNA (200 ng), and 16 μL H_2O in a final volume of 25 μL. All reaction mixtures were overlaid with 30 μL of mineral oil.

The amplification conditions were as follows: 94 ℃ for 5 min, followed by 30 cycles of 94 ℃ for 1 min, 55 ℃ for 1 min and 72 ℃ for 1 min, with a final extension step at 72 ℃ for 10 min. Amplified PCR products were detected using 1% agarose gel electrophoresis. The electrode buffer used was Tris-borate-EDTA (ethylene diamine tetraacetic acid), which consists of Tris-base 10.8 g, 89 mmol/L, boric acid 5.5 g, 2 mmol/L, EDTA (pH 8.0) 4 mL of 0.5 mol/L EDTA (pH 8.0) (all components were combined in sufficient H_2O and aliquots 10 μL of PCR products were loaded to the gel). A constant voltage of 80 V for 30 min was used for product separation. After

Table 1
Primers used for amplification in this study.

Gene	Primer name	Primer Sequence (5' to 3')	Fragment base pair (bp)	Reference
16S rRNA	16S-F	F-GCGGACGGGTGAGTAATGT	200	[12]
	16S-R	R-TCATCCTCTCAGACCAGCTA		
PAI I_{536}	I.9	F-TAATGCCGGAGATTCATTGTC	1 800	[13]
	I10	R-AGGATTTGTCTCAGGGCTTT		
PAI II_{536}	orf1 up	F-CCATGTCCAAAGCTCGAGC	1 000	[13]
	orf1 down	R-CTACGTCAGGCTGGCTTTG		
PAI III_{536}	sfaAI.1	F-CGGGCATGCATCAATTATCTTTG	200	[14]
	sfaAI.2	R-TGTGTAGATGCAGTCACTCCG		
PAI IV_{536}	IRP2 FP	F-AAGGATTCGCTGTTACCGGAC	300	[15]
	IRP2 RP	R-TCGTCGGGCAGCGTTTCTTCT		
PAI II_{CFT073}	cft073.2Ent1	F-ATGGATGTTGTATCGCGCP	400	[13]
	cft073.2Ent2	R-ACGAGCATGTGGATCTGC		
PAI J_{196}	papGIf	F-TCGTGCTCAGGTCCGGAATTT	400	[5]
	papGIR	R-TGGCATCCCACATTATCG		
PAI IIJ_{196}	Hlyd	F-GGATCCATGAAAACATGGTTAATGGG	2 300	[5]
	Cnf	R-GATATTTTTGTTGCCATTGGTTACC		
PAI I_{CFT073}	RPAi	F-GGACATCCTGTTACAGCACGCA	930	[5]
	RPAf	R-TCGCCACCAATCACAGCCGAAC		

electrophoresis, images were obtained in UVIdoc gel documentation systems (UK).

2.4. Detection of PAI markers by multiplex PCR

All isolates were screened for the presence of PAI markers (PAI I_{CFT073}, PAI II_{CFT073}, PAI I_{536}, PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI IJ_{196}, and PAI IIJ_{196}) using PCR method. Primers used in this study are shown in Table 1.

2.5. Statistical analysis

Analysis of data was performed using SPSS version 18.0 computer software (SPSS, Chicago, IL) and statistical analysis was performed by using the Fisher exact and *Chi*-square tests. $P < 0.05$ was considered as statistically significant.

3. Results

A total of 63 (100%) *E. coli* isolates were analyzed from 63 patients with UTI. Urine samples were isolated from patients of both sexes; 51 (80.95%) from females and 12 (19.04%) from males. After PCR test for the final identification of *E. coli* bacteria and the presence of sequence of 16S rRNA gene, all samples with 200 base pairs and positive were identified. Results are shown in Figure 1.

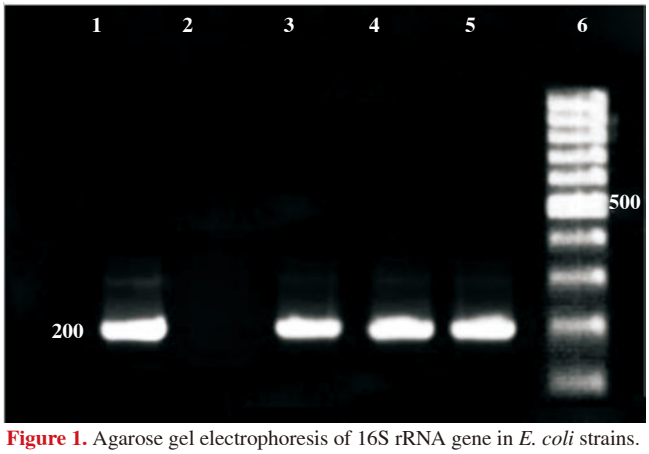

Figure 1. Agarose gel electrophoresis of 16S rRNA gene in *E. coli* strains. Line 1: Positive control; Line 2: Negative control; Lane 3-5: Positive samples (200 bp); Lane 6: 100 bp DNA ladder.

The distribution of various PAIs showed that the most prevalent PAI were PAI IV (84.12%) and PAI I_{CFT073} (73.01%), respectively. The prevalence of other PAIs was as follows: PAI II_{CFT073} (41.26%), PAI I_{536} (34.92%), PAI II J_{196} (31.74%), and PAI II_{536} (20.63%). PAI III_{536} and PAI IJ_{196} were detected only in 4.76% and 1.57% of UPEC, respectively. The percentage of the PAIs markers genes distribution in UPEC isolates from patients with UTI was shown in Table 2 and the percentage of the PAIs markers genes in various genders was shown in Table 3. The PCR for detection of PAIs markers was shown in Figures 2 and 3. A statistical analysis by Fisher exact test showed that there was no significant relationship between gender and the PAI III_{536}, PAI II_{CFT073}, PAI I_{536}, PAI II_{536}, PAI J_{196} and PAI IIJ_{196} ($P > 0.05$). A statistical analysis by Fisher exact test showed that there was a significant relationship between gender and the PAI IV_{536} and PAI I_{CFT073} ($P < 0.05$).

Table 2
The PAIs marker genes distribution in UPEC isolates from patients with UTI. *n* (%).

PAIs marker	UPEC (*n* = 63)
PAI III_{536}	3 (4.76%)
PAI IV_{536}	53 (84.12%)
PAI II_{CFT073}	26 (41.26%)
PAI I_{536}	22 (34.92%)
PAI II_{536}	13 (20.63%)
PAI J_{196}	1 (1.57%)
PAI IIJ_{196}	20 (31.74%)
PAI I_{CFT073}	46 (73.01%)

Table 3
The PAIs marker genes distribution in UPEC isolates from patients with UTI in both sexes. *n* (%).

PAIs marker	Female (*n* = 51)	Male (*n* = 12)	*P* value
PAI III_{536}	3 (5.9)	0 (0)	1.000
PAI IV_{536}	47 (92.2)	6 (50.0)	0.002
PAI II_{CFT073}	21 (41.2)	5 (41.7)	0.345
PAI I_{536}	20 (39.3)	2 (16.7)	0.188
PAI II_{536}	12 (23.5)	1 (8.3)	0.431
PAI J_{196}	1 (2.0)	0 (0)	1.000
PAI IIJ_{196}	17 (33.3)	3 (25.0)	0.737
PAI I_{CFT073}	41 (80.4)	5 (41.7)	0.012

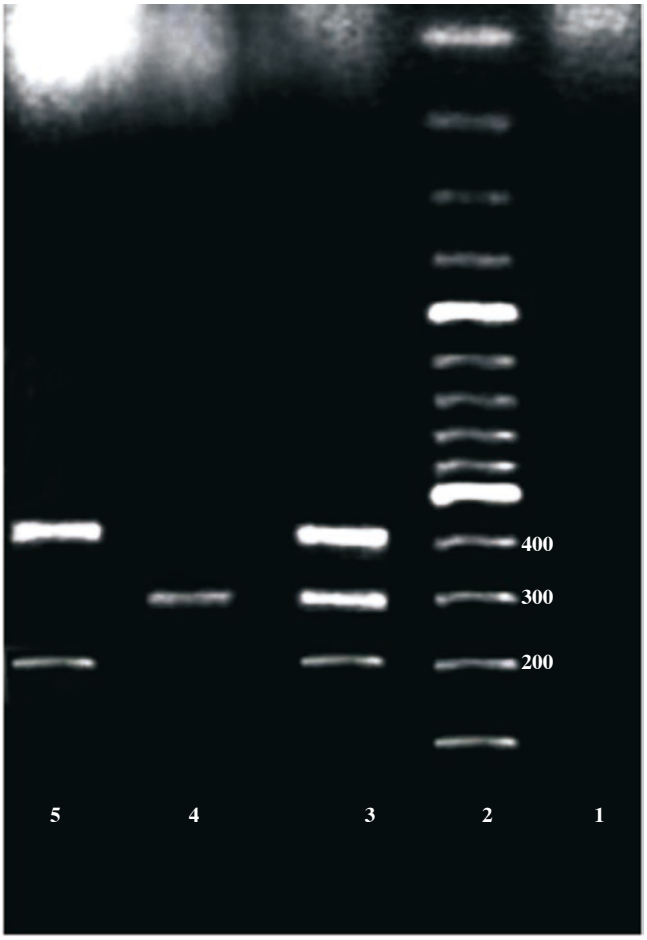

Figure 2. PCR for detection of PAI markers PAI III_{536}, PAI IV_{536}, PAI II_{CFT073}. Lane 1: Negative control; Lane 2: 100 bp marker; Lane 3: PAI III_{536} (200 bp), PAI IV_{536} (300 bp) and PAI II_{CFT073} (400 bp); Lane 4: PAI IV_{536} (300 bp); Lane 5: PAI III_{536} (200 bp) and PAI II_{CFT073} (400 bp).

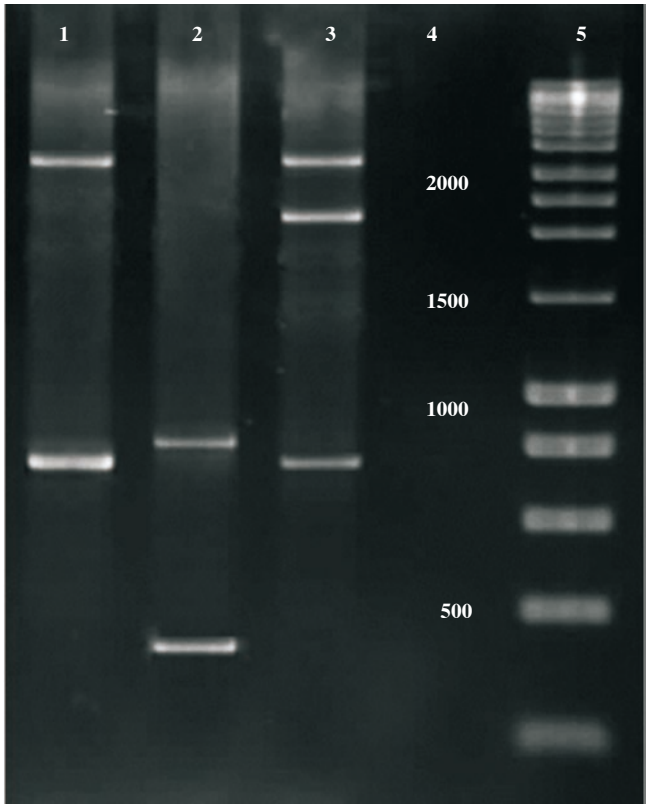

Figure 3. PCR for detection of PAI marker PAI I_{536}, PAI II_{536}, PAI J_{196}, PAI IIJ_{196} and PAI I_{CFT073}.
Lane 1: PAI I_{CFT073} (930 bp) and PAI J_{196} (400 bp); Lane 2: PAI J_{196} (400 bp) and PAI II_{536} (1 000 bp); Lane 3: PAI I_{CFT073} (930 bp), PAI II J_{196} (2 300 bp) and PAI I_{536} (1 800 bp); Lane 4: Negative control; Lane 5: 1 000 bp marker.

4. Discussion

UTI is usually treated empirically without culturing the urine sample, but this type of infection accounts for about 10% to 15% prolongation of hospitalization due to the emergence of resistance to antibiotics among the causative bacteria, particularly among UPEC strains. This may result in the spread of antibiotic resistant bacteria which led us to investigate this possibility by testing the effects of a range of functionally distinct antibiotics on UPEC to reduce morbidity and the duration of hospitalization in patients with UTI[5,16]. Accordingly, we considered *E. coli* strains isolated from urine and examined PAIs.

Pathogenicity-associated islands (PAIs) are particular regions on the bacterial chromosome where virulence genes have accumulated. PAIs and their associated virulence genes, spread among bacterial populations by horizontal transfer[17]. Several PAIs were previously identified in UPEC strains such as *E. coli* 536, *E. coli* J96, and *E. coli* CFT073. PAIs I to IV from strain 536 (I_{536} to IV_{536}) encode a range of virulence factors, including P-fimbriae, P-related fimbriae, α-hemolysin, S-fimbriae, and the yersiniabactin siderophore system. PAI IJ_{96} and IIJ_{96} encode P-fimbriae, P-related fimbriae, and α-hemolysin; PAI I_{CFT073} and II_{CFT073} encode P-fimbriae, α-hemolysin, and aerobactin[5,13,17].

Many studies have shown that urine isolates collectively differed dramatically from normal flora isolates with respect to phylogenetic background and virulence gene content profiles, suggesting an increased virulence potential for the urine isolates[18,19].

Navidinia *et al*. reported that phylogenetic background and to assess *hlyD* (involved in the secretion of haemolysin A) and *intI1* (encoding a class 1 integrase) in *E. coli* isolates derived from urinary and fecal specimens by PCR[8].

Herzer *et al.* reported that PAIs were enriched among *E. coli* and caused extraintestinal infections[20]. Here, we also confirmed the prevalence of eight PAI markers in *E. coli* strains isolated from the urine of children with UTI.

Dobrindt *et al*. in 2002 studied the genetic structure and distribution of four pathogenicity islands (PAI I_{536} to PAI IV_{536}) of UPEC strain 536[14]. In UPEC, multiple PAI sequences (PAI I_{536} to PAI IV_{536}) were studied and their presence in several wild types *E. coli* isolates was extensively investigated. Their results suggested PAIs are detectable in pathogenic *E. coli* isolates. We determined that the acquisition of large DNA regions, such as PAIs, was an important factor in the evolution of bacterial pathogens. Sabaté *et al*. in 2006 studied the PAI markers in commensal and UPEC isolates[13]. Their results showed the mean number of PAIs per isolate was high among UPEC. Similar with Brzuszkiewicz *et al*. results genomic differences between UPEC strains were mainly restricted to large pathogenicity islands[21]. Bingen-Bidois *et al.* provides new data on the prevalence and variability of physical genetic linkage between pap and certain virulence-associated genes that are consistent with their colocalization on archetypal PAIs[22].

The PAI marker showed a frequency of 61.3% in the study by Neamati *et al.*[9]. Johnson *et al.* reported a frequency of 71% for PAI markers among UPEC isolated from the patients with urosepsis in the USA[23]. In a survey conducted by Navidinia *et al.*[5], during September 2009 to August 2010, a total of 50 *E. coli* isolates were analyzed from 50 patients of both sexes with UTI who have admitted to the nephrology ward of Mofid children hospital, Tehran, Iran. Their results showed the distribution of the PAI genes in UPEC as follows: in total, 89% of UPEC isolates contained PAIs. The PAI IV_{536} (86%) and PAI I_{CFT073} (74%) isolates were the most among the UPEC, whereas PAI III_{536} (6%) and PAI I_{J96} (4%) were the least. We found a high number of PAI markers such as PAI I_{CFT073}, PAI II_{CFT073}, PAI I536, PAI IV536, PAI II J_{96}, and PAI II_{536} to be significantly associated with UPEC[5].

In another study by Parham *et al.*[24], it was reported that the presence of PAI II is associated with extraintestinal group B2 isolates of *E. coli*. Parreira and Gyles identified a gene encoding a serine protease autotransporter protein (Vat)[25], which was responsible for the vacuolating activity of a strain of *E. coli* (Ec222) that had been isolated from a septicemic chicken. Vat is encoded on a PAI, which is inserted adjacent to the *thrW* tRNA gene. The presence of Vat at this position has been demonstrated for UPEC strain CFT073 and the neonatal meningitis strain *E. coli* RS218[26].

It seems reasonable that UPEC might show similar adaptations for an extraintestinal lifestyle, which, in turn, enables them to cause extraintestinal disease in humans. Presentation of the molecular details of UPEC is necessary to develop successful strategies for the

prevention of human UTIs and urological complications associated with UTIs.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The support of the Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (Grant No. T/06/1393), is acknowledged. The authors are also grateful to Dr. Faham Khamesipour, Young Researchers and Elite Club, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran and Dr. Madubuike Umunna Anyanwu, Department of Veterinary Pathology and Microbiology, University of Nigeria, Enugu State, Nigeria.

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