

Detection of *fim*, *pap*, *sfa* and *afa* Adhesin-Encoding Operons in *Escherichia coli* Strains Isolated from Urinary Tract Infections

Mahsa Yazdi (PhD)

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Majid Bouzari (PhD)

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Ezzat Allah Ghaemi (PhD)

Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Corresponding authors: Majid Bouzari

Email: mahsayazdi92@gmail.com

Tel: +989133130546

Address: Department of Biology, Faculty of Sciences, University of Isfahan, Hezar Jereeb Street, 81746-73441, Isfahan, Iran

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ABSTRACT

Background and objectives: Urinary tract infections (UTIs) are one of the most common infectious diseases caused by bacteria. The primary etiologic agent of UTIs is *Escherichia coli*. Uropathogenic *E.coli* (UPEC) strains have a number of specific virulence factors, which can worsen UTIs. This study was performed to detect *fim*, *pap*, *sfa* and *afa* genes among *E.coli* strains isolated from UTIs.

Methods: A total of 100 *E. coli* isolates from patients with UTI was collected between June and December 2015 from Mosavi and Sayyad Shirazi hospitals in Gorgan, Iran. All bacterial isolates were identified via standard biochemical testing and Gram staining. Presence of the genes was assessed by polymerase chain reaction.

Results: The frequency of the *fim*, *pap*, *sfa* and *afa* genes was 100%, 79%, 69% and 8%, respectively. All isolates contained at least one virulence gene. Prevalence of multiple adhesion genes was 6% for all genes and 65% for three genes (*fim*, *pap* and *sfa*) together. In addition, the frequency of the *fim* gene was significantly higher than that of the other genes ($P<0.0001$).

Conclusion: The results of this study indicate the high prevalence of virulence factors that can enhance pathogenicity of *E. coli*. Therefore, these factors could be used as diagnostic markers or vaccine targets.

Keywords: Virulence factors, Urinary tract infection, Uropathogenic *Escherichia coli*.

INTRODUCTION

Urinary tract infections (UTIs) are inflammatory diseases caused by various pathogens, which alter the correct functioning of the urinary system (1). *Escherichia coli* is the major cause of UTI and the predominant facultative member of the normal human intestinal flora. The bacterium accounts for more than 80% of all UTI incidents (2). UTI comprises a range of disorders including cystitis and pyelonephritis, which are characterized by presence of microorganisms such as *E. coli* in the urinary tract (3, 4). Only a few *E. coli* strains have the potential to cause UTIs, which are classified as uropathogenic *E. coli* (UPEC) strains (5). UPECs express a multitude of virulence factors such as pili or fimbriae, which break the inertia of the mucosal barrier (6). The ability of these bacteria to adhere to host epithelial cells is considered as a prerequisite for the establishment of infection (7). Pyelonephritis-associated pili or P fimbriae (*pap*), type 1 fimbriae, afimbrial adhesin I (*afaI*), hemolysin (*hly*), cytotoxic necrotizing factor 1 (*cnf 1*), aerobactin (*aer*) and S fimbriae adhesion (*sfa*) are the most important virulence genes in UPEC strains, which are associated with severe UTI (8, 9). These virulence factors help the microorganism colonize host surfaces, avoid and/or subvert host defense mechanisms, damage and/or invade host cells and tissues and incite a noxious inflammatory response, thereby leading to a clinical disease (10). UPECs generally possess type 1 and P fimbriae (11). The adhesive subunit of type 1 fimbriae, FimH, is a major determinant, which has high tropism for urinary tract receptors; thus, FimH adhesion is important for colonization of *E. coli* in different niches (1). *E. coli* P fimbriae are mannose-resistant hemagglutinins predominant at the cell surface, which are associated with colonization of upper urinary tract and binding to vascular endothelium of the kidney, causing pyelonephritis. The S fimbriae is a mannose-resistant adhesin encoded by the *sfa* operon that recognizes α -sialyl- β -2, 3-galactose receptors in humans. Presence of S fimbriae is correlated with pathogenicity of *E. coli* in human meningitis and septicemia (12, 13). Pathogenic *E. coli* strains associated with intestinal and extra intestinal infections in humans and animals are reported to express operons of the *afa* family (14). The afimbrial

adhesin is a mannose-resistant, P-independent, X-binding adhesin, encoded by the *afa-1* operon that mediates the specific binding to uroepithelial cells and human erythrocyte receptors. The *afa* operon shows a high degree of heterogeneity among UPEC strains with certain subtypes being predominantly present in pyelonephritis as well as in other UTIs (15). The aim of this study was to detect the virulence genes of *E. coli* strains isolated from UTIs in Gorgan, Iran.

MATERIAL AND METHODS

A total of 100 *E. coli* strains from patients with UTI were collected between June and December 2015 from Mosavi and Sayyad Shirazi hospitals in Gorgan, Iran. The bacterial isolates were identified by Gram staining and biochemical tests, such as catalase, oxidase, indole production, citrate utilization, triple iron sugar, and methyl red-Voges Proskauer as described previously (16). The bacteria were maintained in brain heart infusion broth (Merck, Germany) with glycerol and stored at -70°C for further studies.

Bacterial strains were sub-cultured overnight in brain heart infusion broth and genomic DNA was extracted from typical *E. coli* colonies using the phenol chloroform extraction method. To extract the DNA from *E. coli* strains, 1 ml cell suspension was centrifuged and the pellet was resuspended in lysis buffer (Sinnagen, Iran) containing proteinase K and SDS. After incubating the mixture at 65°C for 60 min, phenol: chloroform: isoamyl alcohol (25:24:1) (Merck, Germany) was added and the suspension was shaken and centrifuged at 12000 g at 22°C for 10 min. The supernatant was transferred to a new tube and then mixed with phenol-chloroform. The mixture was centrifuged at 9000 g for 10 min and the supernatant containing DNA was precipitated with 95% ethanol and centrifuged as described above. The pellet was washed twice with 70 % cold ethanol (-20°C). Finally, the precipitate containing DNA was air-dried and dissolved in 50 μl of deionized distilled water and was stored at -20°C as a template DNA stock (17). Specific primers were used to detect some virulence factors (*fim*, *pap*, *sfa*, and *afa*) in *E. coli* isolates from patients with UTI. Primers for the adhesin genes were first established individually using a template DNA from

appropriate positive control strains obtained from the Department of Microbiology of the Golestan University of Medical Sciences. Table 1 shows the primers used for the detection of UPECs' virulence genes. Thermocycling conditions for the polymerase chain reaction process (PCR) are shown in table 2. Amplified DNA products were analyzed by standard submarine gel electrophoresis using 10 μ L of the final reaction mixture on a 1.5% agarose gel in TBE buffer. Amplified DNA fragments of specific sizes were visualized by UV fluorescence with SYBR Green I dye (Sinaclone, Iran). A 50 bp ladder (Sinaclone, Iran) was used for determining the molecular size of the PCR products (7). Data were analyzed using GraphPad Prism 6.1 software (GraphPad Software Inc., USA). The Fisher's exact test

was used for statistical analysis and P-values less than 0.05 were considered statistically significant.

RESULTS

Figure 1 shows the results of gel electrophoresis for the detection of the adhesin genes (*fim*, *pap*, *sfa* and *afa*). The most common virulence genes in the isolates were the *fim* gene (100%) followed by the *pap* gene (79%), the *sfa* gene (69%) and the *afa* gene (8%). The frequency of the *fim* gene was significantly higher than that of the other genes ($P < 0.0001$).

All isolates harbored the adhesin genes either singly or in combination. Six isolates (6%) were positive for all genes and 65 isolates (65%) were positive for the *fim*, *pap* and *sfa* genes (Table 3).

Table 1-The specific primers used for amplification of fimbriae genes in *E. coli* strains isolated from UTI

Gene	Sequence (5' to 3')	Size, bp	Reference
<i>fim</i>	GCTGTGATGTTTCTGCTCGT AAAACGAGGCGGTATTGGTG	167	(18)
<i>sfa</i>	CTCCGGAGAAGTGGGTGAT CGGAGGAGTAATTACAAACCTGGCA	410	(19)
<i>afa</i>	GCT GGG CAG CAA ACT GAT AAC TCT C CAT CAA GCT GTT TGT TCG TCC GCC G	750	(12)
<i>pap</i>	GAC GGC TGT ACT GCA GGG TGT GGC G ATA TCC TTT CTG CAG GGA TGC AAT A	328	(20)

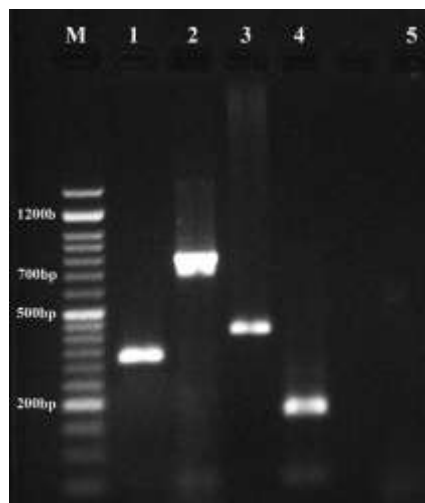
Table 2- PCR thermocycling conditions for detection of the virulence (fimbriae) genes in UPEC strains

Gene	PCR program	PCR reaction mixture (25 μ l)
<i>fim</i>	1 cycle: 95°C ----- 5 min	2.5 μ l PCR buffer 10X 2 mM MgCl ₂
	35 cycle: 94°C ----- 30 s 60°C ----- 60 s 72°C ----- 30 s	0.2 mM dNTP 10 Pmol of each primers F & R 1.25 U <i>Taq</i> DNA polymerase 3 μ l DNA template
	1 cycle: 72°C ----- 8 min	
	1 cycle: 95°C ----- 4 min	2.5 μ l PCR buffer 10X 2 mM MgCl ₂
	35 cycle: 95°C ----- 30 s 53°C ----- 30 s 72°C ----- 40 s	0.4 mM dNTP 10 Pmol of each primers F & R 1.25 U <i>Taq</i> DNA polymerase 3 μ l DNA template
<i>sfa</i>	1 cycle: 72°C ----- 3 min	
	1 cycle: 95°C ----- 4 min	2.5 μ l PCR buffer 10X 2.3 mM MgCl ₂
	35 cycle: 94°C ----- 1 min 60°C ----- 30 s 72°C ----- 3 min	0.2 mM dNTP 20 Pmol of each primers F & R 1.25 U <i>Taq</i> DNA polymerase 3 μ l DNA template
	1 cycle: 72°C ----- 7 min	
	1 cycle: 95°C ----- 4 min	2.5 μ l PCR buffer 10X 1.5 mM MgCl ₂
<i>afa</i>	35 cycle: 94°C ----- 1 min 65°C ----- 1 min 72°C ----- 1 min	0.2 mM dNTP 10 Pmol of each primers F & R 1.25 U <i>Taq</i> DNA polymerase 2 μ l DNA template
	1 cycle: 72°C ----- 4 min	

Table 3- Frequency of the virulence genes alone or in combination in UPEC strains isolated from UTIs

Virulence genes (alone or in combination)	Number of positive strains
<i>fim</i>	100
<i>pap</i>	79
<i>sfa</i>	69
<i>afa</i>	8
<i>fim, pap</i>	79
<i>fim, sfa</i>	69
<i>fim, afa</i>	8
<i>pap, sfa</i>	65
<i>pap, afa</i>	6
<i>Sfa,afa</i>	6
<i>pap, fim, sfa</i>	65
<i>pap, fim, sfa, afa</i>	6

Figure 1- Results of gel electrophoresis for the detection of virulence genes in *E. coli* strains isolated from UTIs. Column M: 50 bp DNA ladder; column 1: amplified *pap* gene (328 bp); column 2: amplified *afa* gene (750 bp); column 3: amplified *sfa* gene (410 bp); column 4: amplified *fim* gene (167 bp); column 5: negative control.



DISCUSSION

E. coli is the most common cause of UTI in both ambulatory and hospitalized patients (7). Severity of the infection depends on the virulence of the responsible strains and susceptibility of the host, particularly if there is a concomitant urological disease. A better knowledge of the bacterial virulence characteristics assists physicians in diagnosis and treatment of infections. This study was performed to evaluate the frequency of adhesin-encoding operons in *E. coli* strains isolated from UTIs in Gorgan, northeast of Iran. Several virulence determinants contribute to the pathogenicity of *E. coli* in UTI (7, 21), and PCR allows detection of the genes encoding these determinants (13, 22). The adhesive systems are the most common virulence factors in UPECs. They play an important role in colonization and invasion of the bladder epithelial cells by UPECs (5, 18). PCR is a highly specific, informative and a powerful genotypic assay used for the

detection of virulence factors. In this study, *fim* was the predominant gene (100%) in the *E. coli* isolates compared to the *pap*, *sfa* and *afa* genes. These results are consistent with results of previous studies (5, 19, 20). Garofalo et al. studied 18 UPEC isolates collected from females and found that the *fim* gene was present in all isolates (23). In another study, Watts et al. reported presence of the *fimH* gene in 98% of *E. coli* strains isolated from patients with UTI (24). Rahdar et al. evaluated the distribution of virulence genes among UPEC strains and reported that 95% of the isolates contained the *fim* gene (7). These findings may indicate the crucial role of this gene in the virulence of *E. coli* strains causing UTI. In this study, the frequency of the *pap* and *sfa* virulence genes was 79% and 69%, respectively. Saki et al. also indicated the high frequency of these virulence genes in patients with UTI in Iran (5). Some studies reported the high frequency of combined *pap* and *sfa* genes

(24, 25). The high number of samples containing the *pap* and *sfa* genes together could be due to the localization of these genes on the same pathogenicity island in UPEC strains (15, 26).

Similar to our results, previous studies on the frequency of the *sfa* and *afa* gene combination among UPEC isolates reported that this gene combination is either absent or less documented (15, 19). In the present study, 65% of the strains carried the *fim*, *pap* and *sfa* genes together. This is in agreement with findings of a recent study by Shetty et al., which stated that presence of strains with multiple adhesin genes might further increase the risk for development of UTI, particularly in women (15).

CONCLUSION

We speculate that the high prevalence of the *fim*, *pap*, and *sfa* operons may be

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responsible for UTIs in the study area. High prevalence of virulence factors can enhance the capacity of UPEC strains for colonization of the urogenital tract, resulting in increased bacterial attachment to target cells and increased pathogenicity. Thus, these genes could be targeted for vaccine production for prevention of *E. coli* infections. Further studies are required to identify virulence factors of *E. coli* strains causing UTIs and to determine the pathophysiology of these infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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