Uropathogenic *Escherichia coli* and the related virulence factors

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Abstract

Uropathogenic strains of Escherichia coli (UPEC) are the most common cause of non-hospital-acquired urinary tract infections (UTIs). The most common UTIs occur mainly in women and affect the bladder and urethra, leading to infections of the bladder (cystitis). Uropathogenic Escherichia coli have genes and virulence factors in association with adhesion, biofilm formation and colonization. These genes are: mrk, kpsM, foc, auf, C, Kps, chuA, hma, ireA, iha, iutA, fliC, ompA, upab, upaC, upaG, and upaH. Doctors typically treat UTIs with a wide range of different antibiotics, such as fosfomycin, nitrofurantonin, pivmecillinam, trimethoprim, sulfamethoxazole, ciprofloxacin, levofloxacin and prulifloxacin. However, some strains of E. coli, called extended-spectrum beta-lactamase (ESBL) E. coli, are resistant to most antibiotic treatments.

Keywords: *urinary tract infections, Escherichia coli, ESBL*

Rezumat

Tulpinile uropatogene de Escherichia coli sunt cea mai frecventă cauză de infecții non-intraspitalicești ale tractului urinar. Infecțiile de tract urinar apar mai frecvent la femei și afectează vezica urinară și uretra. Infecția vezicii urinare poartă denumirea de cistită. Escherichia coli uropatogen prezintă factori de virulență și gene responsabile de aderare, formarea biofilmului și colonizare. Principalele gene sunt: mrk, kpsM, foc, auf, C, Kps, chuA, hma, ireA, iha, iutA, fliC, ompA, upab, upaC, upaG și upaH. Pentru tratarea infecției, medicii apelează în mod obișnuit la o gamă largă de antibiotice diferite, cum ar fi fosfomicină, nitrofurantonină, pivmecilinam, trimetoprim, sulfametoxazol, ciprofloxacină, levofloxacină și prulifloxacină. Cu toate acestea, unele tulpini de E. coli, denumite beta-lactamaze cu spectru extins (ESBL) E. coli, sunt rezistente la majoritatea tratamentelor cu antibiotice.

Cuvinte-cheie: *infecții de tract urinar, Escherichia coli, ESBL*

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Introduction

Despite many progresses in the field of medical microbiology, urinary tract infections (UTIs) are still known as a big concern for microbiologists and stay as the second ranking infectious diseases all over the world. The most important problem with the UTIs is related to their multimicrobial spectrum. Either bacterial or fungal agents involve lower part (cystitis) and/or upper part (pyelonephritis) of urinary tract. *Escherichia coli* strains and in particular the uropathogenic pathotypes are recognized as the most important bacterial etiology of the UTIs, while the fungal agent of *Candida albicans* has the same role; however, *C. albicans* may cause different types of candidiasis, including UTIs.

E. coli bacteria are divided into two groups of intra-intestinal and extra-intestinal strains. The intra-intestinal strains may be categorized into two subgroups of commensal strains and the pathotypes. On the other hand, the extra-intestinal strains include different types of pathotypes such as uropathogenic *E. coli* (UPEC). UPEC pathotypes are known as invaluable genomic treasures of a wide range of diverse virulence factors which each one has its own importance regarding UTIs. Antigens of Flagella (H), Soma (O) and Capsule (K) are common criteria for UPEC strains classification. In addition to varieties of virulence factors, the feature of antibiotic resistance in UPEC pathotypes is a big deal to be concerned for. The progression of multi-drug resistant (MDR) and extensively drug resistant (XDR) strains of UPEC worldwide has complicated the treatment of the UTIs, too[1-12].

As mentioned before, the treasure of virulence factors and genes within the pan-genome of UPEC strains is amazingly widespread. But, in this review, some of them which are involved in biofilm formation have been studied (Table 1 and Figure 1)[6,11-13].

Materials and method

In silico studies have been done for this article. Databases such as NCBI and GenBank in parallel with Google Scholar were searched to find and study various review articles about UPEC genes from the year 2000 to 2018. We have also used reference books to improve our data.

Genes involved in biofilm formation

As indicated in Table 1, genes which have contributed in biofilm formation can be divided into two groups: biofilm formation in catheher and the host's body. Some

Table 1 Uropathogenic *Escherichia coli* (UPEC) genes and virulence factors in association with adhesion, biofilm formation and colonization

Genes	Virulence factors	References
Mrk	Type 3 fimbriae	(13)
kps	Capsule	(13, 14)
Foc	F1C	(15)
Auf	Auf fimbriae	(13)
C	F9 fimbriae	(13)
chuA, hma, ireA, iha, iutA	Hemin uptake system	(15)
flic	H antigen (Flagella protein)	(13)
ompA, upab, upaC, upaG, upaH		(16)

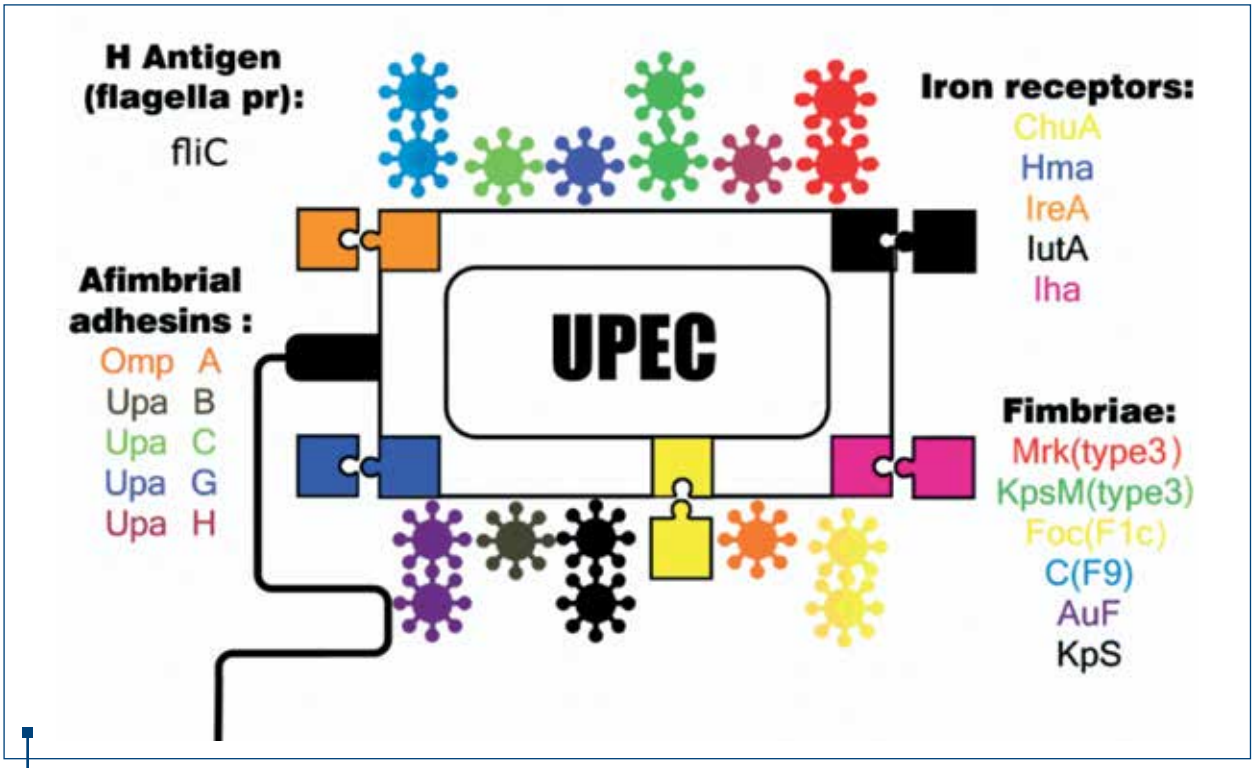

Figure 1. *Uropathogenic Escherichia coli (UPEC) genes and virulence factors*

of the most important virulence genes of UPEC strains which are associated with biofilm formation are, *mrk*, *kpsM*, *foc*, *auf*, *C*, *Kps*, *chuA*, *hma*, *ireA*, *iha*, *iutA*, *fliC*, *ompA*, *upab*, *upaC*, *upaG* and *upaH*. Among them, *mrk* and *kpsM* can form biofilms on indwelling catheters and others in the host's body. Specific virulent properties of each gene have been analyzed in this study.

1. *mrk*

Catheter-associated urinary tract infection (CAUTI) had been relatively abandoned in clinical research until recently. CAUTIs occur from the growth of bacterial biofilms on the inner surface of the urinary catheter. The urinary catheters are tubular latex or silicone devices, which when inserted, biofilms can be formed on

the inner or outer surfaces[17]. Biofilm formation is typically facilitated by fibrillar structures such as fimbriae or pili. In *Escherichia coli*, the production of determined types of fimbriae (e.g., type 3 fimbriae) increases biofilm formation. One of the genes that are deeply associated with biofilm formation has been named as *mrk* gene. The genetic structures in a cell which can replicate independently of the chromosomes are called plasmids. Plasmids are circular DNA in the cytoplasm of a bacterium. *Mrk* gene located in plasmid (pMAS2027) encodes the type 3 fimbriae. The precise position of *mrk* gene is on a segment (5,536-bp) with G+C content of 56.6%. It has been demonstrated that *mrk* genes are located on a mobile genetic element and on upstream and downstream of the *mrk* cluster; they are genes encoding proteins associated with transposition[18].

2. *kpsM*

Capsule (also known as K antigen) is a large structure of some prokaryotic cells such as bacteria. It is a polysaccharide layer that lies outside the cell envelope of bacteria, and is thus deemed part of the outer envelope of a bacterial cell. It is a layer which is not easily washed off, and it can be the cause of various diseases. Capsule is located immediately exterior to the peptidoglycan layer of Gram-positive bacteria and the Lipopolysaccharide layer of Gram-negative bacteria[19]. In recent years, several studies have examined the role of polysaccharide capsules in the pathogenesis of UTI and polysaccharide capsules have been identified, and they have been divided into three groups[20]. Remarkably, *kpsM* gene encodes a capsule transport protein which is one member of the gene cluster responsible for type 2 capsular polysaccharide synthesis. This gene is one of the reasons of biofilm formation. It is recognized that the deletion of the gene *kpsM* would cause a huge decrease in the virulence of the UPEC strain both *in vitro* and *in vivo*. Many studies show that *kpsM* was relatively highly conserved during the evolution process of bacterial species, so we can conclude that this gene is probably a molecular clock[21].

3. *foc*

Biofilm formation in bacteria including UPEC strains has determined increase survival in natural environments and in the host's body[22]. *Foc* gene encodes F1C fimbriae which is located on the bacterial surface and is capable of adhesion to mucosal and endothelial cells[23]. F1C fimbriae are essential for biofilm formation on an inert surface. Among three genes encoding for fimbrial adhesive systems (*fimH*, *pap* and *sfa/foc*), the prevalence of *sfa/foc* gene had been found 23%[24]. Many antibiotics have proven to be effective for the clinical symptoms of UTIs, but recurrent and chronic infections continue to afflict many people. Saira Bashir et al.[25] found that, due to indiscriminate use of drugs in developing countries, the pathogenic bacteria are more battle-hardened as compared with developed countries. Studies show that the resistance of nalidixic acid can be related to considerably decreased prevalence of the gene *sfa/foc* (S and F1C fimbriae)[26]. Daniël J. Wurpel et al. observations along with the binding specificity of these organelles proved that F1C pili may impact the pathogenesis of a remarkable number of UTI cases. β-GalNac-1, 4β-Gal residues on glycolipids expressed by epithelial cells of the distal tubules and collecting ducts of the kidney and also by bladder and kidney endothelial cells. F1C pili are encoded by approximately 14% of UPEC isolates and can bind β-GalNac-1 and 4β-Gal residues[27].

4. *auf*

Buckles et al.[28] determined that *auf* gene cluster was significantly associated with uropathogenic *E. coli* isolates. The role of *auf* genes is in biofilm formation and adhesion. *Auf* fimbriae (encoded by auf gene) are encoded by 67% of UPEC strains and 27% fecal *E. coli* strains (commensal isolates)[29]. Furthermore, *auf* can cause all types of UTIs. Each type of UTI may result in more-specific signs and symptoms, depending on which part of the urinary tract is infected. Acute pyelonephritis usually occurs with upper back and side (flank) pain, high fever, shaking and chills, nausea and vomiting signs[30]. Cystitis can be recognized with signs and symptoms such as pelvic pressure, lower abdomen discomfort, frequent, painful urination, and blood in urine[31]. Urethritis are often seen with burning with urination and discharge[32].

5. *C*

F9 Fimbriae of uropathogenic *Escherichia coli* mediates biofilm formation which encodes by *C* gene. F9 fimbriae expression was indicated at 20c, representing the first proof of functional F9 fimbriae expression by wild-type *E. coli*. However, for binding directly to the *f9* promoter, the *f9* gene expresses at 37c[27]. Besides of UTIs, this fimbriae can be effective at the clinical symptoms of pyelonephritis. Pyelonephritis is a common infection in adult women, but there is a paucity of controlled trials of its treatment and the optimum duration of antibiotic treatment has not been properly defined[33]. UPEC strains have different specific genomes, for example the UPEC strain CFT073 genome contains ten gene loci that share sequence identity with the chaperone-usher class of fimbriae. In this strain, the *f9* has c number named *c1931-c1936* and the genes are *c1936-34-ydeSRQ*, and these information in other strains can be various[34].

6. *Kps*

Biofilms are the microbial communities of the surface-attached cells which are embedded in a self-produced extracellular polymeric matrix. *Kps* genes encode K1, K2, K3, K5, K12, K13, K20 and K51/KspMT capsular polysaccharides. Among the K group, k1 and k2 are more outstanding. The K1 capsule on the surface of UPEC strains is a key virulence factor and its expression may be important in the beginning and development of UTIs and cystitis[35]. Similar to other bacterial polysaccharide capsules, K1 capsule has two classical roles which contain inhibition of phagocytosis by granulocytes/monocytes and serum resistance. It has been recognized that the polysialic acid K1 capsule may not only protect UPEC from natural immunity, but also form an IBC matrix component which facilitates the aggregation of the bacterial communities, which in turn precludes infiltration of host

inflammatory mediators and environmental stressors[36]. While K1 played an insignificant role in conferring serum resistance, the K2 antigen is mildly important. In the presence of whole blood, both K1 and K2 antigens provided a survival advantage to the UPEC strains tested[37].

7. *chuA*, *hma*, *ireA*, *iha*, *iutA*

UPEC, the famous cause of urinary tract infection, uses specific outer membrane receptors, facilitating the import of iron-chelating siderophores and iron from host cells. For the colonization of the urinary tract by uropathogenic *Escherichia coli*, iron attainment mediated by special outer membrane receptors is essential. Heme is an essential source of iron for UPEC in the kidney. Siderophores are small, iron-chelating molecules which bind and transport iron in microorganisms. Many distinguished types of siderophores are produced by bacteria. For the synthesis of a siderophore after many steps in *E. coli*, the completed molecules are discharged instantly from the cytoplasm to the extracellular space through a complex named TolC. *ChuA*, *hma*, *ireA*, *iha*, and *iutA* genes work together for UPEC iron uptake system and *chuA* performs the task of heme transport. The transcriptional regulators influence on the expression of iron uptake genes. The expression of *chuA* is also regulated by RfaH[38]. *Iha* is an abbreviation of the IrgA homolog adhesin which transport both enterobactin and dihydroxy benzoylserine (DHBS) and also help UPEC to fitness in the urinary tract[39].

8. *filC*

The structure of the flagellum is free at one end and attached to the cell at the other end. The bacterial flagella with a diameter of about 20 nm move the bacteria towards nutrients and other attractants. The flagellated *Escherichia coli* spp. are common causes of urinary tract infections and the flagella help the bacteria by propelling up the urethra into the bladder. There are four types of flagellar arrangement: monotrichous, amphitrichous, lophotrichous and peritrichous[40]. *FliC* gene encodes the H antigen or flagella protein. The expression of *fliC* gene *in vitro* is optimal and increasing osmolarity combined with lowering pH represses *fliC* activity which is the similar condition to the environment of the bladder[41]. According to a study by M.R. Karam Asadi et al.[42], about 70% of clinical isolates obtained from patients with UTI harbored *fliC* gene, so we conclude that *fliC* is another conserved gene among UPEC strains.

9. *ompA*, *upab*, *upaC*, *upaG*, *upaH*

The pathogenic roles of OmpA proteins, including adhesion, invasion or intracellular survival, have been expected. Features of the outer membrane protein A (OmpA) encoded by *ompA* gene are monomeric, main, integral, porin and heat-modifiable component of the bacteria. The *ompA* functions as an intracellular virulence for UPEC and also within bladder epithelium it causes persistent infection. It has been demonstrated that the deletion of the *ompA* gene did not disrupt the initial epithelial binding and invasion by UPEC, whereas it did preclude completion of the intracellular bacterial community (IBC) pathway[43].

The formation of the extracellular matrix (ECM) requires cells to secrete ECM proteins[44]. *UpaB* is located at the bacterial cell surface. The function of *upaB* is cell adhesion and *upaB* can mediate the adherence to several ECM proteins, so the deletion of *upaB* can reduce the early colonization of the bladder.

The *upaB* gene is common among UPEC strains and is present in all available UPEC genomes, but absent or disrupted in all diarrheagenic *E. coli* genomes[45]. UpaC AT-encoding gene is common in *E. coli*. Autotransporter (AT) proteins have their independent transport across the bacterial membrane system and final routing to the cell surface which facilitates by special structural properties. Several AT proteins have been characterized from UPEC, and these include the upaC protein which encodes by *upaC* gene[46]. UpaG is a member of the trimeric autotransporter family of adhesins in UPEC and is strongly associated with other *E. coli* strains. UpaG proteins encoded by *upaG* genes mediates adhesion to human bladder epithelial cells[47]. UpaH is a identified autotransporter protein that contributes to biofilm formation and bladder colonization by UPEC. UpaH encodes a large cell surface-located AT protein that contributes to biofilm formation[48]. As we mentioned before, *ompA*, *upaB*, *upaC*, *upaG*, and *upaH* genes encode various proteins, such as ag43, *upaB*, *upaC*, *upaG* and *upaH* proteins, with biofilm formation, adhesion and chronic infection tasks, causing chronic UTIs.

Effective antibiotics on UPEC

For the treatment of urinary tract infections, the first and second lines of the treatment can be used in a variety of antibiotics, for example: fosfomycin, nitrofurantonin, pivmecillinam, trimethoprim, sulfamethoxazole, ciprofloxacin, levofloxacin, and prulifloxacin[49].

It is noteworthy that ciprofloxacin, levofloxacin and prulifloxacin should not be used as the first line of treatment due to their high side effects[49].

Conclusions

Urinary tract infections are demonstrated to be one of the most controversial infections all over the world. The commonest cause of UTI is uropathogenic *Escherichia coli*, with different virulence factors encoding by virulence genes, such as *mrk* (encoding type 3 fimbriae), *kpsM* (encoding type 3 fimbriae), *foc* (encoding F1C), *auf* (Auf fimbriae), *C* (F9 fimbriae), *Kps* (encoding K antigen group), *chuA*, *hma*, *ireA*, *iha*, *iutA* (encoding Hemin uptake system), *fliC* (encoding H antigen), and *ompA*, *upaB*, *upaC*, *upaG* and *upaH*. Among these virulence factors, genes by encoding make the course of treatment harder.

More studies are also need to find the most appropriate treatment for this issue, but we searched and found some antibiotics group for the treatment, such as: fosfomycin, nitrofurantonin, pivmecillinam, trimethoprim, sulfamethoxazole, ciprofloxacin, levofloxacin and prulifloxacin. ■

Conflict of interests: The authors declare no conflict of interests.

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