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Chapter 2

Virulence Factors of Uropathogenic *E. coli*

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Abstract

In order for a successful infection and creating a satisfactory environment inside the host, strains of uropathogenic *Escherichia coli* (UPEC) need some special features that are achieved by expressing particular genes, called virulence factors. Two of the most important surface virulence factors of UPEC are type 1 fimbriae and *P* fimbriae that are crucial for the colonization process inside the urinary tract. Expression of these virulence factors converts a commensal strain into an uropathogen. Beside these factors, outer membrane proteins also contribute to virulence being involved in the secretory machinery; an example of such type is TolC protein that transfers *α*-hemolysin across the outer membrane of *E. coli*. However, *α*-hemolysin along with many other toxins serves various pathogenic roles during UTIs including adhesion, colonization, cytotoxic activity, etc. Moreover, virulence factors located on bacterial surface including capsule and lipopolysaccharides may also have the contribution to UTIs providing antiphagocytosis and antibactericidal complement activity.

Keywords: virulent factors, *E. coli*, UPEC, urinary tract infection, type 1 fimbriae, *P* fimbriae, *α*-hemolysin, cytotoxic necrotizing factor 1 (CNF1), siderophores, hemin uptake system, flagellar motility

1. Introduction

The most commonly living microorganism of the human gastrointestinal tract and also the most common causative agent of bacterial urinary tract infection is *E. coli* [1]. Though they remain in a good relationship with their hosts, they might appear as a subject of consideration in immunocompromised hosts. This common inhabitant of the gastrointestinal tract usually remains in a symbiotic relationship with the host and plays a role in maintaining the homeostasis of the intestinal tract. Though most of the strains of *E. coli* are harmless, some serotypes can cause food poisoning. *E. coli* present in the normal human microbiota produces...
vitamin K2. Strains of E. coli, however, obtaining ability to colonize inside the urinary tract and to make themselves safe from the host immune system, become uropathogenic E. coli. UPEC causes >80% of UTI [2]. Urinary tract infections are very common, and approximately 10% of people [3] and half of all women (at least one time) become infected throughout their life. According to a study, more than 100,000 patients in the United States are hospitalized annually due to urinary tract infections [4], and in the year 2011, 400,000 patients were hospitalized, and the estimated cost was about 2.8 billion USD [5]. Infections can occur in both upper and lower urinary tracts. Lower urinary tract infection is known as cystitis, and in the case of upper urinary tract infection, it is called pyelonephritis. Without distinction of site, in order to cause infection, the causative agent must at first dodge the host’s immune system and colonize in the urinary tract [6]. Several different virulent factors are needed for the bacterial population to cause infections [7]; for instance, pathogenic strains of E. coli express adherence factors which form pili or fimbriae of different types for their attachment in the sites where they usually do not live [7]; these are structural virulence factors and predominantly include P fimbriae and type 1 fimbriae [1]. Fimbrial adhesins such as PapG and CsgA are virulence factors that facilitate the attachment of E. coli [8]. In animal models, type 1 fimbriae aggrandize the chance survival of E. coli [9]. Beside these, UPEC can impair host immune system by a variety of ways [10], such as toxins and iron acquisition systems, and these are called secreted virulence factors. The production of these virulence factors by UPEC may cause an inflammatory response which makes a possible pathway for UTI symptoms [1]. However, both the host and the uropathogenic E. coli strain have different roles in the establishment and colonization process in the urinary tract [11]. Here in this chapter, different types of important virulence factors of uropathogenic E. coli will be discussed. In Gram-negative and some Gram-positive bacteria, virulence genes are allocated in particular segments (about 10–200 kilo bases in size) of their genome which have different G + C content than the other parts of the genome that are termed as pathogenicity islands. They are present in the virulent strains but present rarely in the nonpathogenic strains of the same species. These sequences can be transferred horizontally from species to species [12]. Pathogenicity islands encode virulence factors such as adherence factors, toxins, and iron acquisition systems which are important virulence factors of UPEC.
surface of bacteria that recognize specific compounds usually carbohydrates of the target host cells [11. Pili are the short form of fimbriae and might be used interchangeably with fimbriae. Fimbriae consist of oligomeric pilin proteins. These proteins are arranged in such a manner that they form a helical cylindrical structure and are both thinner and shorter than flagellum. These proteinaceous structures are expressed in uropathogenic strains of \textit{E. coli} and are considered as virulence factors [11. Most of the receptors for these fimbriae are carbohydrates. They include type 1 fimbriae, P fimbriae, and thin aggregative fimbriae [16. Many bacterial pathogens can produce an array of these adhesins, and often inhibition of a single adhesin may cost enough to a bacterium to lose its virulence. Functions of pili or fimbriae are not limited only to adhesion and can help in many other crucial pathways for the microbe to survive and evade the immune system of the host. Evolution of different types of adhesins plays a role in tissue tropism. In gram-negative bacteria like UPEC, adhesins are unveiled by chaperone-usher-assisted pathway. This pathway involves two proteins, one is a periplasmic chaperone, and the other is a protein called usher. Usher act as the base of the structure, and the function of chaperone is folding and recruitment of the subunits [17, 18. In absence of the chaperone, pilin proteins are degraded and misfolded and thus cannot be assembled in the form of a mature pilus. On the other hand, usher helps to mature the fimbriae and its transportation through the outer membrane guaranteeing integrity of the outer membrane. The constituents of usher proteins are an N-terminal domain (NTD), 24-stranded beta-barrel channel, a plug domain, and two C-terminal domains (CTD). In uropathogenic \textit{E. coli} strains, chaperone-usher family fimbriae are more abundant. 2.1. Type 1 fimbriae In 99% of \textit{E. coli} strains, genes to encode type 1 fimbriae are present [19, and during urinary tract infections, they damage urinary tract cells by mediating an increased inflammation [20. In order to enter into the host cells of the urinary tract, type 1 fimbriae play a great role. Type 1 fimbriae are remarkably versatile virulence factors of UPEC that can stabilize the attachment of the bacteria to different type of cells throughout the urinary tract. Though in Bowman's capsules and glomerulus their binding sites could not be identified, a strong affinity of type 1 fimbriae was found in proximal tubules and vessel walls. In the bladder, they bind strongly to muscular layers and moderately to vessel walls. Receptors for type 1 fimbriae were also found in the distal tubules and collecting ducts. They can also induce their binding to the surface of macrophages [9. These fimbriae recognize uroplakin from bladder epithelial cells and mannoside-containing host proteins. Unlike many other important types of adhesins, these are encoded by the bacterial backbone DNA [21 and are mainly composed of FimA proteins along with FimF, FimG, and FimH [17. FimA proteins are most in number but are not pivotal for virulence. Among other subunits of type 1 fimbriae, allelic variations of FimH determine the sugar specificity and deletion of \textit{fimH} results in less amount of colonization in mouse models of ascending UTI, and colonization could be restored by expression of plasmid with \textit{fimH} gene [20. FimH alone or in association with LPS can stimulate toll-like receptor 4 (TLR4) to initiate particular signaling cascade that may activate the humoral immune response. Many studies revealed that expression of type 1 fimbriae results in virulence and loss of expression results in loss of expression but their presence cannot be correlated with UTI as normal fecal strains also have equally expressed type 1 fimbriae [22. However, type 1 fimbriae-mediated attachment is a crucial stage for cystitis. Adhesins of these fimbriae are mannose sensitive.
2.2. P fimbriae

P-fimbriated *E. coli* are pyelonephritogenic and attach to the carbohydrate structure alpha-D-Galp-(1-4)-beta-D-Galp. In the kidney, they bind strongly to Bowman’s capsule, glomerulus, and endothelial cells of vessel walls. This highly organized composite structure is composed of six subunits at least. Once P fimbriae expressed *E. coli* enter the urinary tract, they establish bacteriuria and help to cross the epithelial barrier to enter the bloodstream and can cause hemagglutination of erythrocytes [14]. This type of fimbriae is encoded by *pap* gene cluster (also known as *fso* and *fst*), and *pap +* strains remain longer in the intestinal flora than *pap -* strains [23]. P antigens are expressed in the cell surface of red blood cells and in various cells lining in the urinary tract. P1 (present in glycoproteins in human), P, P*, P K*, and LKE antigens act as the receptors for P-fimbriated UPEC. P-fimbriated *E. coli* cannot agglutinate red blood cells that lack P antigen. Isolated P fimbriae can bind to a synthetic analogue of its receptor, and experimental application of that analogue impedes infection process.

There are at least nine genes in the *pap* gene cluster with two restriction sites at two ends. The regulatory part starts the following Eco R1 consisting of *papI* and *papB*. Then *papA*, *papH*, *papC*, *papD*, *papE*, *papF*, and *papG* are situated, and after these, Bam HI is present. Approximately 1000 of subunits form a P fimbria, being united in a helical manner. Among them the major constituent is the protein subunit PapA (19.5 KD), and minor subunits are PapE (16.5 KD), PapF (15 KD), and PapG (35 KD). In the periplasmic space, PapD (27.5 KD) may be present and can also be incorporated in the structure. Another protein PapC, which is the largest one with 80 KD of mass, assists the process by transporting the subunits outside the part of the cell. Though PapA is the major constituent, it is not mandatory for attachment, and among many serotypes, PapA molecules show high homology with the amino acids of N and C termini. PapA also has an average level of similarity with structural subunits of other *E. coli* fimbriae including type 1 fimbriae. The minor subunits at the tip of fimbria determine the specificity to the receptor. Many mutational analyses revealed that mutation in PapA does not affect the adherence, while mutation in other genes (i.e. *papEFG*) does not hamper fimbrial structural appearance. In the fine structure of P fimbriae, a PapF-PapG complex is formed which is attached to PapA (bulk potion of the structure) subunits through PapE subunits. Finally, PapH terminates the assembly of the fimbriae and attaches thereby [16]. An important thing is that the amino acid sequence of PapG is approximately similar to that of Shiga toxin. Shiga toxin is found in some serotypes of *E. coli*. Another role of PapG was found in some variants of P fimbriae which is they can initiate subunit polymerization [14]. Many experiments show that expression of these fimbriae is not relevant to urinary tract infection, while more sophisticated other experiments have concluded about their role in pathogenesis. However, during infection in immunocompromised patients, less expression of P fimbriae is observed, which indicated that P fimbriae are needed to overcome certain types of host immune attacks. Although P fimbriae can initiate inflammatory responses by activating TLR4 [24], it protects UPEC from human polymorphonuclear leukocytes (hPMNLs). In the rapidly changing environment through the urinary tract, environmental influences affect the expression of P fimbriae. Expression of P fimbriae is favored at 37°C and inhibited at a range of 18–22 °C, but there are some variations in this phenomenon. The temperature-dependent expression is controlled by a region close to *papB* of the *pap* gene cluster.
2.3. Dr/Afa adhesins

Dr blood group antigen is a membrane protein of red blood cells and located on the decay accelerating factor (DAF) that protects red blood cells from being degraded or lysed by autologous complements. Another important function of DAF is to regulate complement cascade [25]. These antigens are recognized by Dr and Afa adhesin family of uropathogenic E. coli.

There are both fimbrial (F1845 and O75X) and non-fimbrial (AFA I and AFA II) types of adhesins. Immuno-invasion of UPEC by hiding from the host humoral immune response is somehow mediated by Dr family of adhesins [26]. These microscopically invisible fimbriae are present in the cell wall, and their structural and organization properties are quite different from other types of fimbriae [13]. Chloramphenicol can inhibit O75X binding to a specific part of the Dr antigen, but it cannot inhibit other adhesins of this family which indicates that Dr family adhesins can recognize specific sites at the Dr [25]. For years, several studies were conducted to identify specific sites for binding of Dr family hemagglutinins. For instance, a strong affinity of O75X was found to Bowman's capsule, proximal and distal tubules, and the collecting duct basement membranes. In the bladder, they strongly bind to connective tissues.

2.4. Other fimbriae as virulence factors

F1C is a virulence factor responsible for urinary tract infections, which is encoded by an operon of seven genes, i.e., focAICDFGH, where FocA is the major subunit and FocH is the tip adhesin [26]. F1C receptors are present in bladder endothelium and muscular layer. They cannot bind to the epithelium. They bind to glomeruli, distal tubules, collecting ducts, and vascular endothelial cells. Studies show that F1C fimbriae and pyelonephritis are correlated though there is a little difference in the prevalence of type 1 fimbriae in UTI strains and nor-normal fecal isolates. Prevalence of F1C fimbriae in normal fecal isolates is 10% which is 16% in UTI strains [26]. S fimbriae are genetically identical to F1C fimbriae and differ only by the tip adhesin SfaS. Criteria that are needed to be recognized as a virulence factor were determined by different studies regarding S fimbriae. There are some other adhesins that are not crucial for the survival of UPEC strains such as F9 fimbriae.

3. Toxins

Several toxic substances or proteins secreted by uropathogenic strains of E. coli play a consequential role as virulence factors in UTIs. However, toxins have the ability to alter the host cell signaling cascade and modulate inflammatory responses. Several in vitro and in vivo studies showed that toxins also contribute to the stimulation of the host cell death and releasing of necessary nutrients, which provide the ability to access deeper tissues within the urinary tract [27]. In 1987, CDT toxin (cyclomodulins) was first reported as virulent toxin in UPEC [28] which opened a new door in the study of the pathogenesis of UTIs, and then many other toxins in UPEC were reported including α-hemolysin (HlyA), cytotoxic necrotizing factor 1 (CNF1), secreted autotransporter toxin (SAT), cytolysin A, plasmid-encoded toxin (PET), vacuolating autotransporter toxin (VAT), Shigella enterotoxin-1 (ShET-1), arginine succinyl-transferase (AST), etc.
3.1. α-hemolysin

Among all the toxins, α-hemolysin (HlyA) is very important which is a lipoprotein and belongs to the RTX (repeats in toxin) toxins family [13, 29, 30]. HlyA is a pore-forming toxin and causes inducible nitric-oxide-synthase (iNOS)-mediated cell membrane injury and apoptosis [31]. However, HlyA can lyse erythrocytes and nucleated host cells at high concentration by a process enabling UPEC which may damage the host immune effector cells for gaining enhanced access to the host nutrients and iron stores. But when the concentration is low, HlyA can induce the apoptosis of target host cells and promote the exfoliation of bladder epithelial cells [13, 32, 33]. Besides, HlyA can also contribute to nephropathogenicity, which was proved by infecting mice transurethrally or intravesically with toxin producer and nonproducer isogenic clone pairs of E. coli [34]. A recent study showed that HlyA regulates the dephosphorylation of Akt, which is a multifunctional signaling regulator and responsible for controlling inflammatory responses in the host, as well as the cell cycle control [35]. Moreover, HlyA has the role in the increased production of IL-6 and IL-8 by inducing Ca\textsuperscript{2+} oscillations in renal epithelial cells [36].

3.2. Cytotoxic necrotizing factor 1 (CNF1)

Another virulence factor secreted by E. coli named cytotoxic necrotizing factor 1 (CNF1) is also involved in UTIs and stimulates actin stress fiber formation and membrane ruffle formation in a Rho GTPase-dependent manner that results in the entry of E. coli into the cells [37]. The toxin has a remarkable effect on the actin skeletal of HEp-2 cells and produces large vacuoles in HEp-2 cells [28]. However, several in vitro and in vivo studies showed that this protein interferes with polymorphonuclear phagocytosis and evokes apoptotic death of bladder epithelial cells and may lead to bladder cell exfoliation and to enhanced bacterial access to underlying tissue [38, 39]. In addition, there is also a possibility of the association of CNF1 with the hemolysin in the virulence mechanism, which is beneficial for the bacteria [28].

3.3. Secreted autotransporter toxin (SAT)

Secreted autotransporter toxin (SAT) may also be important as a virulence factor for the pathogenesis of UTIs being had a toxin activity against cell lines of bladder or kidney origin. SAT is a serine protease autotransporter which falls within one subgroup of autotransporters recently classified as the SPATE (serine protease autotransporters of Enterobacteriaceae) family and associated with pyelonephritic E. coli strains [40, 41]. SAT may have the cytopathic activity that results in the damage of the host tissue and may increase the propagation ability of the UPEC. However, this toxin may facilitate entry of pyelonephritogenic strains into the bloodstream resulting from specific damage to the glomeruli and proximal tubules [40].

3.4. Cytolethal distending toxin (CDT)

Cytolethal distending toxin, having a unique property of damaging the DNA of the target cell, was first reported in pathogenic E. coli in 1987 [28, 42]. This toxin has the ability to arrest the cell cycle and contributes to the pathogenesis of UTIs [43, 44]. However, CDT is an operon product encoding three proteins including CdtA, CdtB, and CdtC proteins which are encoded
by cdtA, cdtB, and cdtC genes, respectively [28]. CDT has DNase I-like enzymatic activity and attacks DNA, while the other bacterial toxins attack the cell membrane or different targets within the cytoplasm [45]. This unique property of attacking DNA damages the target cell DNA which results in progressive cell distending leading to cell death [27].

3.5. Other toxins

Some others including cytolysin A and toll/interleukin (IL-1) receptor (TIR) domain-containing protein (Tcp) are also considered as virulence factors in UTIs [46, 47]. The former causes apoptosis of the host cells [47], while the other has the ability to subvert TLR signaling that gives a survival advantage during UTIs [46]. However, Tcp is associated with pyelonephritis but rare in environmental E. coli, in fecal flora of healthy children and in less severe forms of UTI [27]. Besides these, Tcp has also the role in the human avoidance system and cytopathic effect on the kidney [48].

In addition to these toxins, vacuolating autotransporter toxin (VAT), Shigella enterotoxin-1 (ShET-1) and arginine succinyltransferase (AST) may also contribute to UTIs. VAT has the cytotoxic effect on the bladder and kidney, while the two others are involved in the invasion of the infections [48]. However, VAT is a highly protected immunogenic protein that belongs to the protease family of SPATE [28].

4. Siderophores

Iron is a very important molecule for all living beings, and E. coli uses iron for transporting and storing oxygen, DNA synthesis, electron transport, and metabolism of peroxides. But the amount of iron availability is reduced in the host urinary tract during UTIs [49]. In response to this, E. coli possesses some multiple functionally redundant systems that mediate iron uptake by secreting low-molecular-weight Fe$^{3+}$-chelating molecules which are known as siderophores [50]. Iron utilization, mediated by these siderophores, is critical for colonization of the urinary tract by UPEC [51]. There are four distinct siderophore systems found in E. coli such as yersiniabactin, aerobactin, enterobactin, and salmochelin [52]. These systems also include some genes such as ent genes encoding enterobactin, iuc genes encoding aerobactin, and iro genes encoding an ent-like system. However, all these systems are expressed under low-iron conditions and are negatively regulated by ferrous iron and the ferric uptake regulator Fur [53].

4.1. Aerobactin

Aerobactin is a low-weight molecule and a hydroxamate siderophore with a higher Fe$^{3+}$-binding stability in acidic environments and is maximally produced at low pH [44, 53]. This siderophore extracts Fe$^{3+}$ from host iron-binding proteins and is taken up through an outer membrane receptor protein [44]. However, aerobactin has many advantages over other siderophores and is formed from the condensation of two lysine molecules and one citrate catalyzed by an enzyme named aerobactin synthase [13, 25, 30].
4.2. Enterobactin

Enterobactin is another specialized highly prevalent catecholate siderophore which is less soluble and less stable than aerobactin [53–55]. But this siderophore has a higher iron affinity and can deferrate transferrin more rapidly than aerobactin in aqueous solution [13, 54]. However, iron is released from enterobactin through the hydrolysis of this siderophore [13]. Besides these, enterobactin may afford UPEC the ability to colonize within an iron-limiting environment such as the urinary tract [56]. But this siderophore has a limitation that it can be inactivated by host proteins such as serum albumin and siderocalin [25].

4.3. Yersiniabactin

Yersiniabactin, a mixed-type siderophore, is widespread in Enterobacteriaceae including E. coli and encoded on the high-pathogenicity island [53]. Yersiniabactin has a high iron affinity and produced yersiniabactin-Fe$^{3+}$ complex binding to the iron molecule which recognizes the specific bacterial outer membrane TonB-dependent receptor and Fyu (Psn). The iron molecule is released from yersiniabactin in the cytosol with the help of membrane-embedded proteins [57]. In addition, this siderophore increases resistance to copper stress by chelating Cu$^{2+}$[10].

4.4. Salmochelin

Salmochelin is a glucosylated derivative of enterobactin which is not recognized by siderocalin and thus escapes from the host immune response [53]. However, siderocalin, neutrophil gelatinase-associated lipocalin is also known as lipocalin 2 that binds enterobactin and prevents its uptake [53, 56]. To overcome this, enterobactin is modified to salmochelin by glucosylation via the action of glucosyltransferase and is not recognized by lipocalin 2 [56]. However, a recent study found that salmochelin siderophore receptor IroN is involved in the invasion of urothelial cells, and thus IroN may play both an iron uptake receptor and an internalization factor in the establishment of urinary tract infections [26].

4.5. Hemin uptake system

There is another iron acquisition system called hemin uptake system including ChuA and Hma, which involves direct upregulation of haem receptors. This system uptakes free iron during UTIs, and several studies found its role in bacterial growth and biofilm formation [48, 58, 59]. ChuA expression is regulated by other regulatory proteins, for instance, in uropathogenic E. coli strain 536, increase in RfaH level induces the expression of ChuA [60]. But the other receptor Hma functions independently of ChuA, and a residue, Tyr-126, is necessary for its function. However, both ChuA and Hma contribute to haem utilization which is required for the maximum kidney colonization [51].

5. Capsule

The main role of a capsule is to cover and protect the bacterium from various unfavorable conditions as well as the host immune system, which is mainly constituted of polysaccharide [1].
Phosphorylation of CREB results in the expression of IL-6 and IL-8 [24]. Mutation in TLR4 in murine models revealed its role on bacterial pathogenesis. There are other receptors related to UTI pathogenesis. One of such is CXCR1, but there are both types of evidences that demonstrate the positive and “no correlation” of CXCR1 with recurrent UTIs. Polymorphisms in IL-8 genes were found to have a correlation with pyelonephritis in the case of no correlation with CXCR1 mutation [19, 64]. TLR4 can be activated by the presence of type 1 fimbriae and P fimbriae. As there are enough studies to evidence the activation of immune response against UPEC strains, there must be some ways that are used by these bacteria to overcome unfavorable situations early in the infection. Incubation of human urothelial cells with type 1-fimbriated UPEC strains resulted in increased apoptosis. In the case of a nonpathogenic type 1-fimbriated strain (HB101) of *E. coli*, rate of apoptosis was approximately 50% of that of pathogenic strains of UPEC [65]. UPEC blocks NF-ĸB, and this results in apoptosis and a decreased cytokine secretion. Another indispensable way is the expression of toll/IL-1 receptor domain-containing protein (TcpC), which was discovered in UPEC strain CFT073. TcpC interacts with myeloid differentiation primary response 88 (MyD88), a protein that, in human, is encoded by *MYD88* gene. Interaction of TcpC and MyD88 subsequently stops downstream signaling pathways mediated by TLRs. Modification of capsular lipopolysaccharides specific to the pathogenic strain can cause the failure of TLR4 to recognize the pathogen. However, LPS biosynthetic genes encoded by *rfa*, *rfb* operons, and *surA* are the factors responsible for the suppression of TLR-initiated signaling cascades. Biosynthesis of a number of outer membrane proteins and fimbriae is facilitated by the protein encoded by *surA*, which is a periplasmic cis-trans prolyl isomerase [66, 67].

9. Conclusions

Several epidemiological, serological, and bacteriological studies revealed that uropathogenic *E. coli* is the pathogen most frequently associated with UTIs. In recent years, our understanding of virulence factors and behavior of this pathogen is increased remarkably. Several studies showed that *E. coli* colonizes the urinary tract and may ascend toward the bladder to cause cystitis. If it is left untreated, UPEC may ascend the ureters to the kidney and establish a secondary infection. Our increased understanding of its virulence factors can uncover novel approaches to control UPEC-mediated UTIs. However, accumulation of theoretical knowledge through virulence studies allows practical applications and may facilitate the application of more precise approaches in phenotypic or molecular diagnosis and epidemiology.

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