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Uropathogenic *Escherichia coli*: from molecular characteristics to clinical relevance

The search for an alternative treatment method

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Table of contents

1. Introduction.....	3
2. Uropathogenic <i>Escherichia coli</i>	3
2.1 Definition of UPEC	3
2.2 Serotyping.....	4
2.3 Genomics.....	4
2.3.1 The UPEC genome	4
2.3.3 Pathogenicity islands.....	5
3. Virulence factors.....	5
3.1 Adhesins	5
3.1.1 Type 1 fimbriae.....	6
3.1.2 P fimbriae	6
3.1.3 Other adhesins	7
3.2 Siderophores	7
3.3 Toxins.....	8
3.3.1 α -Hemolysin	8
3.3.2 Cytotoxic necrotizing factor 1	9
3.3.3 Autotransporter proteins	10
3.4 Intracellular bacterial communities	10
4. The link to clinical relevance	11
4.1 Pathogenesis	11
4.2 Clinical relevance.....	11
4.2.1 The economic burden of UTIs	11
4.2.2 Antibiotic resistance.....	12
4.2.3 Designing an alternative treatment method.....	12
5. Conclusion	13
6. Acknowledgements	14
7. References.....	14

1. Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases worldwide and form a major public health problem, affecting 150 million people each year (1, 2). These infections mainly affect women, estimating that the chance that each woman will develop a UTI in her lifetime is above 40-60% (3). Despite proper antibiotic treatment, recurrence is common: 44% of UTI patients develop another infection within a year (1, 4). These infections can involve the lower and upper urinary tract (3). While most infections remain restricted to the lower urinary tract which causes bladder infection (cystitis), the pathogen sometimes can ascend to the kidneys resulting in kidney infection (pyelonephritis) (1). In a few cases, an uncontrolled form of pyelonephritis can lead to life-threatening and potentially fatal complications like bacteraemia and sepsis (5).

UTIs can be caused by several pathogenic bacteria, but the primary causative agent of UTIs is uropathogenic *Escherichia coli* (UPEC), a pathogenic serotype diverged from the gram-negative, rod-shaped bacterium *E. coli* that resides in the gastrointestinal (GI) tract. While *E. coli* is known to be a commensal intestinal bacterium forming a beneficial symbiotic relationship with its host, some strains can diverge from their commensal cohorts and take on a more pathogenic nature. These strains are referred to as extra-intestinal *E. coli* (ExPEC), of which UPEC is a subtype (6). It is believed that UPEC that cause the patient's UTI originate from their own GI tract, but translocate to the urinary tract by colonization of the periurethral area (1). Several virulence factors, special components or products of the bacteria, make this colonization of the urinary tract possible. These virulence factors are needed to battle the strong innate immune defense that is induced as a response to UPEC infection and to promote the bacteria's survival in the poor environment of the human urinary tract (1, 6).

Since UTIs are infections that occur very frequently, they account for substantial medical costs, morbidity and mortality worldwide (6). They are currently well treatable with antibiotics, but just like for many other bacterial species, the number of reported antibiotic- or multidrug-resistant UPEC strains is increasing (7). This leads to the need for an alternative treatment method, since the rise of these resistant strains makes treatment with antibiotics difficult. When resistance against antibiotics increases, UTIs could turn into much more uncomfortable infections that last longer, leading to an increase in the economic burden of UTIs, and maybe even to an increase in mortality. In this review, we will therefore take a look at the genome of UPEC bacteria and the virulence mechanisms they possess. We will eventually link these molecular characteristics to challenges we currently face in a clinical setting. The main research question can therefore be formulated as follows: "*Can we find a molecular characteristic of uropathogenic Escherichia coli to design an alternative treatment method to antibiotics, for treatment of urinary tract infections?*".

2. Uropathogenic *Escherichia coli*

2.1 Definition of UPEC

To assess our research question, it is important to determine the definition of a UPEC strain, and how these are distinguished from other *E. coli* strains such as commensal or diarrheogenic strains. Some pathotypes, disease-causing variants of a certain microorganism, can be distinguished by the virulence factors their genomes encode or their physiological traits. This is for example the case for enterotoxigenic *E. coli* (ETEC) and neonatal meningitis *E. coli* (NMEC) (8). For UPEC, this is currently not possible due to a widespread diversity in genotype and phenotype for different strains. The virulence factors by which other types of *E. coli* can be classified, are expressed in different combinations and percentages across the genomes of different pathotypes of UPEC, leading to the conclusion that there is currently no single UPEC phenotypic profile (8). Therefore, UPEC are now defined as any isolate from the urine of a patient suffering from a UTI (1). Because of this, identification prior to infection remains impossible. However, there are some serogroups (see paragraph 2.2) or virulence factors (see Chapter

3) that are associated with the majority of UPEC strains of which their genomes have been sequenced. These factors will therefore be discussed in the following sections.

2.2 Serotyping

O:H:K serotyping is a commonly used method to characterize different strains of *E. coli*, depending on the type of lipopolysaccharides (O), flagella (H) and capsular antigens (K) the specific bacterium expresses. The specific combination of certain antigens such as O, H and K is called a serotype, and several studies have already demonstrated that some serotypes are more associated with a certain disease than others (9). This method of characterization is also applied to UPEC. UPEC strains are mainly distinguished by their expression of O-lipopolysaccharide antigens on their bacterial surface, since they function directly in protecting the bacterium against complement-mediated bacterial killing (1, 10). Despite the enormous diversity in *E. coli* strains, there is a small number of specific O-type groups that is currently known to account for the majority of UPEC strains. Therefore, the mainly used grouping of UPEC strains is by eight UTI-associated O-type groups: type O1, O2, O4, O6, O7, O8, O16, O16/72, O18, O25, O50 and O75 (1, 10).

Several studies showed that the virulence caused by the type of lipopolysaccharide (O) might be regulated by other virulence factors like P fimbriae or α -hemolysin (see paragraph 3.1.2 and 3.3.1) (10). Several associations of O-types with other virulence factors have indeed been reported since (11-13). Therefore, strains can also be identified based on their H (flagella) and K (capsule) serotypes, and sometimes even by the type of fimbriae they express (F). Certain associations have emerged from the O:K:H serotype and clinical manifestations of a UTI (10). However, there are some remarks to be made to O:H:K serotyping of UPEC strains. Even though this method has been used for years, recent studies showed that the genetic make-up of different *E. coli* strains is more complex than expected (9). During some studies, different surface molecules were found on bacteria within one serotype, showing that there are some differences between bacteria and we cannot say there is full homogeneity within one serotype (14). So even though serotyping results in association with several forms of disease and therefore can be useful in characterization of UPEC strains, this must be handled with caution.

2.3 Genomics

Understanding UPEC genomics is critical for understanding the pathogenesis of the urinary tract infections they cause. When genomes of different UPEC strains are sequenced and compared to those of commensal *E. coli* strains and to each other, it is possible to get an insight in how these UPEC strains can survive in the poor environment of the urinary tract, in how they can evade the human immune response and where we can potentially interfere with medication. Whole genome sequencing, determining the complete DNA sequence of an organism's genome, is an extremely helpful tool to assess these research questions. This technology has therefore emerged as an efficient research tool and is even being introduced in clinical use (15).

2.3.1 The UPEC genome

Published sequences of different *E. coli* strains shed light on the differences between the strains on a molecular level. The size of the *E. coli* genome ranges from approximately 4.5 to 5.5 Mb (commensal *E. coli* K-12 isolate MG1655 = 4.64 Mb, UPEC isolate CFT073 = 5.23 Mb and UTI89 = 5.07 Mb) (16). The genomes of pathogenic strains showed to be larger than those of commensal strains, probably because of the need for additional genes for survival and colonization outside of the gut.

Several genome sequences have been published with the help of whole genome sequencing, including those of the well-studied strains CFT073 (17, 18), UTI89 (19) and strain 536 (20). Comparison of these sequences with each other and with those of commensal strains revealed that only 131 genes were present in all of the UPEC isolates and absent from commensal strains. 106 of the 131 genes were found within 22 gene clusters of 2 or more genes, which indicates that these genes are not randomly distributed across the bacterial chromosome (10, 16). These clusters are called pathogenicity islands

(PAIs) and they are further explained in paragraph 2.3.3. The role of individual genes in virulence has been investigated by mutating genes one by one, but this didn't result in the same phenotype as the deletion of a whole pathogenicity island. This observation indicated a complex interplay between different virulence factors encoded by the UPEC genome. Several studies have also shown that UPEC strains differ significantly in their expression of virulence factors, which suggests that there is no single UPEC pathotype. We can therefore conclude that the genome of uropathogenic *E. coli* strains can be seen as a mosaic, which consists of a backbone of housekeeping genes, interfered with horizontally-acquired gene clusters called PAIs, that promote survival and colonization in the human urinary tract (1, 10, 18).

2.3.3 Pathogenicity islands

PAIs are large genomic regions (≥ 10 -200 kb) that are present in the genomes of pathogenic bacterial strains, but absent from the genomes of non-pathogenic strains (8, 21). These regions are acquired through horizontal gene transfer and carry genes encoding different kinds of virulence factors (56, 57), thereby contributing to the development of new pathogenic derivatives from commensal bacterial species. PAIs integrated in UPEC genomes for example contain genes encoding different kinds of adhesins and toxins such as *hly* (coding for α -hemolysin), *pap* and *prs* gene clusters (coding for P fimbriae) or *flu* (coding for Ag43) (21, 22). PAIs are characterized by a lower GC content than the neighboring DNA and they contain direct repeat (DR) sequences and insertion sequences (23). They are often integrated next to tRNA genes, that can act as integration sites for foreign DNA sequences (24). Selection takes place for PAI-associated genes that contribute to the pathogenicity and fitness of its bacterial host: if certain genes significantly contribute to these features, mobility genes regarding transfer, deletion or excision will be inactivated or deleted to make sure these genes remain present and functional in the associated PAI (24). Most UPEC strains contain more than one PAI: strain 536 for example contains five PAIs (25). Since PAIs encode virulence genes and are flexible gene pools that can be acquired by horizontal gene transfer, they severely contribute to the pathogenicity of UPEC strains.

3. Virulence factors

Several virulence factors that are coded by so-called virulence genes in the UPEC genome have proven to improve the fitness of the bacteria when they reside in the environmental niche of the urinary tract. Different strains of UPEC express these factors in different combinations or percentages. Some of these factors, like fimbriae (also called pili), aid in the adhesion of the bacteria to urothelial cells, which has shown to be an essential process for survival of the bacteria and their entry into host cells. Besides that, they also obtain iron, which is an essential nutrient for UPEC, from host cells by their iron acquisition systems called siderophores. Lastly, certain adhesins and toxins also aid the bacteria in proceeding from the lower to the upper urinary tract, eventually causing pyelonephritis and maybe even bacteraemia or sepsis (25). Virulence genes are often located on pathogenicity-islands, as explained in paragraph 2.3.2 (1) The different virulence factors that are expressed by UPEC are further explained in more detail.

3.1 Adhesins

Pathogenic *E. coli* strains as UPEC express specific adherence factors (adhesins) so that the bacteria can colonize niches that commensal *E. coli* strains do not normally inhabit, such as the urinary tract (8). Adherence of bacteria to specific receptors on host cells in the urinary tract is critical in development of UTIs, since it promotes bacterial survival in the urinary tract and leads to internalization of the bacteria into host cells (6, 26, 27). Several adhesins have been associated with UPEC infection, including type 1 and Pap (P) fimbriae, the Dr family of adhesins and S/F1C fimbriae (6, 8, 28, 29). Of these adhesins, type 1 and P fimbriae are considered as important virulence factors of UPEC and are therefore most intensively studied (28, 30).

3.1.1 Type 1 fimbriae

The majority of both uropathogenic and commensal *E. coli* strains encodes for type 1 fimbriae (32). These are hair-like fibres that account for UPEC adhesion by a ligand-receptor mechanism that is sensitive to the presence of mannose (27, 30, 33). Type 1 fimbriae consist of repeating FimA subunits, converging to a distal tip fibrillum structure containing two other proteins (FimF and FimG) (31, 32). The tip contains the adhesin FimH that binds to α -D-mannose-containing receptors on the surface of urothelial cells (see Fig.1) (8). A number of receptors for FimH has been reported, but the most relevant receptor in pathogenesis of UTIs is uroplakin 1a, a receptor specifically expressed on superficial umbrella cells, cells of the urothelium (1, 6).

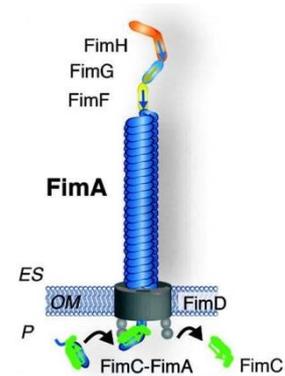


Figure 1. Structural image of the type 1 fimbria. The fimbria consists of the rod (FimA subunits) and the tip (FimF, FimG and adhesin FimH). FimD functions as an assembly platform, recruiting new FimA subunits from the periplasm, coordinating their assembly and secreting the fimbria through a pore to the ES. ES = extracellular space, OM = outer membrane, P = periplasm
Reference: (30, 31)

Binding of FimH to their receptors has several advantages for UPEC. It has been demonstrated that shear force enhances the type 1 fimbriae-mediated binding of the bacteria to host cells, which protects the bacteria from rinsing off by the frequent flow of urine. It also provides better resistance to host defenses (1, 34). Type 1 fimbriae-mediated binding to host cells can also lead to internalization of the bacteria into the cells (32). This results in the formation of intracellular bacterial communities (IBCs, see paragraph 3.4), bio-film-like structures of UPEC that reside inside the cell (8). Invasion requires reorganization of the host actin cytoskeleton, thus a number of integrin-associated signalling molecules and regulator proteins have been associated with this process (6, 34). Residing inside host cells benefits UPEC, since they can evade the host's defenses, facilitate dissemination within and across cellular barriers and gain access to a more nutrient-rich environment inside the cell (32). This invasion process is therefore critical in pathogenesis of UTIs.

The expression of type 1 fimbriae is under control of a process called phase variation. Expression can be turned 'on' or 'off' at a transcriptional level (33). This variation occurs at a high frequency, in the order of 10^{-3} per bacterium per generation (35). Phase variation is due to inversion of a 314-bp invertible DNA segment, the "phase switch", containing the promoter for the *fimA* gene, that encodes for the FimA subunit of type 1 fimbriae (26, 31, 35). Inversion of the phase switch alters the orientation of the promoter and leads to variation in the expression of type 1 fimbriae (35). Two recombinases have been reported to play a role in this mechanism, FimB and FimE. FimB promotes inversion in both directions and FimE stimulates on-to-off inversion of the phase switch (28).

It has been demonstrated that the expression of type 1 fimbriae is high during murine UTIs. Besides, molecular Koch's postulates have already been satisfied (36). Since successful colonization of the urinary tract is not possible without adherence of UPEC to urothelial cells, type 1 fimbriae have been considered as a major virulence factor during UTI pathogenesis.

3.1.2 P fimbriae

Pap (P) fimbriae also play a major role in pathogenesis of UTIs (1), but are particularly associated with UPEC strains causing pyelonephritis (6, 37). P fimbriae consist of a rod composed of PapA subunits, an adaptor subunit PapK, 5-10 copies of the PapE subunit, one copy of the adaptor subunit PapF and at the distal end the PapG subunit (see Fig. 2). The PapG subunit, which functions as the adhesin, is present at the distal end of the tip fibrillum (30). The PapG adhesin of P fimbriae binds to P blood group antigens, a family of oligosaccharides that contain Gal(α 1-4)Gal β sequences that are present on different cell types as erythrocytes and urothelial cells (36, 38). Three different classes of PapG have been identified. PapGI is very rare, PapGII is associated with pyelonephritis and PapGIII is associated with cystitis (1). Responses to P fimbriae-mediated binding of UPEC to urothelial cells include the

enhancement of cytokine responses to infection and the development of inflammation, probably mediated by Toll-like receptor 4 (TLR-4) (28, 37).

Expression of P fimbriae is subject to environmental cues and phase variation, but in another way than type 1 fimbriae (39). Phase variation of P fimbriae does not involve an invertible DNA element, but is regulated by the methylation of two GATC sites that are present in the *pap* regulatory region, the *papI-papB* intergenic region (1, 36). This intergenic region not only contains the two GATC sites, but also six leucine-responsive regulatory protein (Lrp) binding sites (39). The methylation status of these different sequences determines the binding of Lrp, which subsequently determines the phenotype of the bacterium. The phenotype is thus dependent on the competition between the binding of Lrp and methylation by Dam methyltransferase on the different sites (40).

Since the expression of P fimbriae is proven to be associated with certain stages of UTI and is needed for an ascending infection causing pyelonephritis (37), P fimbriae are defined as one of the most important virulence mechanisms of UPEC.

3.1.3 Other adhesins

Even though type 1 and P fimbriae are the most studied adhesins and are considered the most virulent ones, there are other adhesins that play a role in UTI pathogenesis. The other adhesins that have been characterized for UPEC strains, plus their receptors, functions and target tissues are summarized in Table 1 below.

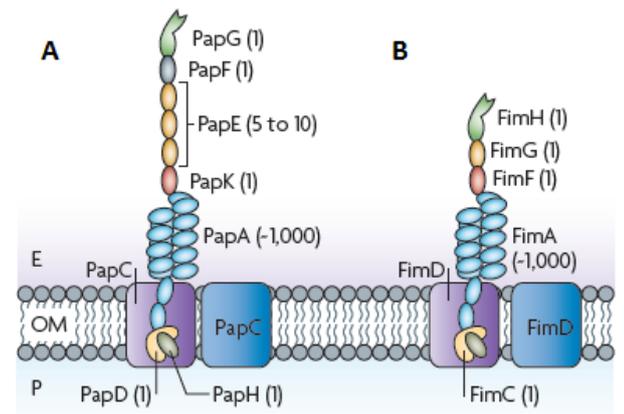


Figure 2. Schematic overview of P and type 1 fimbriae. Numbers indicate the amount of copies per subunit. A) P fimbriae, composed of different Pap subunits. B) Type 1 fimbriae, composed of different Fim subunits. The usher dimers of both types of fimbriae, that act as a recruiting and assembly platform, are shown in purple. E = extracellular space, OM = outer membrane, P = periplasmic space. Reference: (30)

Adhesin type	Target	Functions	Target tissues
Type 1 fimbriae	α -D-mannose-containing receptors, uroplakin 1a	Colonization	Proximal tubulus, vessel walls (bladder/kidney), bladder muscular layer
P fimbriae	Gal(α 1-4)Gal β sequences	Adherence, stimulation host responses, induces cytokine expression	All structures of the kidney*, bladder epithelium, vessel walls and muscular layer, urothelial cells
Dr adhesins	Dr blood group antigen (Dr ^a) on DAF	Facilitation of colonization	Uroepithelium lower/upper urinary tract, kidney, colonic glands
S fimbriae	α -sialyl-2,3-linked receptors, uroplakin 3a	Adherence, facilitation of colonization	All structures of the kidney*, bladder epithelium, vessel walls and muscular layer, connective tissue, urothelial cells
F1C fimbriae	β -GalNac-1,4 β -Gal residues on glycolipids	Adherence to host cells	Distal tubules, bladder, kidney, collecting ducts of the kidney

Table 1. Overview of the different UPEC adhesins. For each adhesin, their receptor, functions and target tissues are listed.

* = Bowman's capsule, glomerulus, proximal tubulus, distal tubulus, collecting duct and vessel walls of the kidney.

References: (6, 8, 10, 12, 26-33, 35-37, 40-44)

3.2 Siderophores

Iron is an essential nutrient for almost all micro-organisms, including UPEC. It is needed for a variety of functions, including the electron transport chain and several metabolic processes like reduction of oxygen for the synthesis of ATP (1, 45). When UPEC infect the urinary tract, they face an environment that is low on iron: iron is bound to human proteins like transferrin and lactoferrin, which lower the concentration of available iron (5). The concentration of iron in the urinary tract is only 10^{-24} M, while the bacteria require an intracellular iron concentration of 10^{-6} M (1). This results in a battle for iron on the host-pathogen interface.

As a result of iron scarcity, UPEC up-regulate genes that encode for iron-acquisition structures that scavenge iron from the environment and make it available to the bacterial cell: siderophores (45-47). Siderophores are low-molecular-weight compounds (500-1500 Da) with a high affinity and selectivity for iron (Fe^{3+}). These siderophores are secreted by the bacterial cell so that they can bind Fe^{3+} in the surroundings of the bacterium. Since they have a higher affinity for Fe^{3+} than transferrin and lactoferrin, they can outcompete these human proteins and thus can loot iron from the host. When siderophores bind Fe^{3+} , they form iron-siderophore complexes, which can then be internalized into the bacterial cell via outer membrane receptors that utilize the energy transduced by the TonB-ExbB-ExbD-complex (5, 48). The proteins ExbB and ExbD energize TonB with the proton-motive force that is generated at the inner membrane of the bacterial cell. TonB then transports this energy to the outer-membrane iron-receptor complexes, which results in translocation to their translocation to the periplasm via ABC transporters, using adenosine triphosphate (ATP) as an energy source (see Fig. 3) (46). Once inside, the iron is released via hydrolysis of the iron-siderophore complex, or via reduction of the iron from Fe^{3+} to Fe^{2+} (49).

The UPEC genome can code for four different siderophores: aerobactin, enterobactin, salmochelin and yersiniabactin (5, 46). As a response to UPEC infection, the host up-regulates the production of proteins that can bind to siderophores. This process is part of the so-called nutritional immunity, which protects the human body from UPEC colonization by limiting the source of nutrients. An example is the production of lipocalin-2 by the host's neutrophils and urothelial cells: lipocalin-2 recognizes and binds to enterobactin, preventing enterobactin-mediated uptake of iron by UPEC. However, UPEC has found a way around this protective mechanism by the host and uses the alternative siderophore salmochelin, a glycosylated derivative of enterobactin. Lipocalin-2 is not able to recognize salmochelin, which makes it a successful alternative to enterobactin (1, 5, 45, 46).

Since siderophores help the bacteria overcome the iron scarcity in the human urinary tract and therefore promote their survival, they are considered an important virulence factor.

3.3 Toxins

The UPEC genome codes for a wide variety of toxins that promote bacterial survival and enable the bacteria to exert cytopathic effects in their host (16). Due to the mosaic structure of the UPEC genome (see paragraph 2.3.1) the expression level of different toxins varies between different strains. The most thoroughly studied toxins that UPEC expresses are α -hemolysin, cytotoxic necrotizing factor 1 (CNF1) and autotransporter proteins (6).

3.3.1 α -Hemolysin

The toxin α -hemolysin, referred to as HlyA, is encoded by approximately 40-50% of UPEC isolates and belongs to the repeat-in-toxin (RTX) family of calcium-binding pore-forming toxins, secreted by type 1 secretion systems (ABC transporters). Although 40-50% of UPEC isolates encode for HlyA, this percentage increases up to 78% for more severe cases, for example of pyelonephritis. The effects of HlyA as a virulence factor during UPEC infection seems to vary between strains and between site of infection in the host (50). At high concentrations, HlyA mediates cell lysis by forming 2 nm-wide pores in host cells, releasing important substances and nutrients like iron (1, 6, 51).

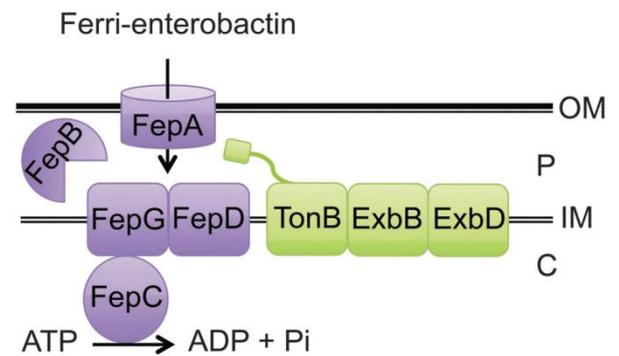


Figure 3. Enterobactin-mediated uptake of iron in UPEC. Iron-siderophore complexes are transported through FepA, using the energy transduced by the TonB-ExbB-ExbD-complex. FepB, a periplasmic-binding protein, transports the complex to the FepG/FepD-complex. FepC, an ATPase, then delivers the energy needed for translocation of the iron-siderophore complex across the inner membrane. Reference: (5)

Sub-lytic concentrations of HlyA proved to have more subtle effects than cell lysis, for example in the regulation of signalling pathways in host cells and exfoliation of urothelial cells (4, 50, 51).

Exfoliation of urothelial cells plays a central role in the development of UTIs. HlyA also has an influence on the exfoliation of these cells, both in lytic or sublytic doses. In lytic doses, HlyA induces exfoliation of the cells by the formation of 2 nm-wide pores which leads to cell lysis. They also activate several caspases (caspase-1, -3, -4 and -7), leading to the activation of apoptotic cell death pathways resulting in exfoliation of the cells (50). At lower, sub-lytic doses, HlyA activates host proteases such as metotrypsin. Activation of these kind of proteases leads to rapid degeneration of paxillin and other host proteins that hold cells together and function in cell-cell and cell-matrix interactions (4). When these proteins are broken down, cells will exfoliate from their substrate, gaining the bacteria access to deeper tissues (50).

HlyA can regulate several signalling pathways of the host, including the activation of MAP kinase signalling, the alteration of histone phosphorylation and acetylation patterns and the stimulation of the serine/threonine kinase Akt (6). Akt is a multifunctional signalling regulator that controls basic metabolic functions, but also inflammatory responses. The influence of HlyA on Akt may be used to downregulate the inflammatory response to UPEC infection. HlyA also appears to act directly on the host's leukocytes by impairing their membrane receptor function, reducing their immune responses to UPEC infection (50).

3.3.2 Cytotoxic necrotizing factor 1

A second toxin that is secreted by UPEC is cytotoxic necrotizing factor 1 (CNF1). CNF1 is a classical AB-toxin that contains two domains: the A domain functions as the receptor-binding domain, responsible for entry of the toxin into target cells via endocytosis, while the B domain is translocated across the endosomal membrane into the cytosol (1). When CNF1 binds its receptor laminin on the surface of urothelial cells, it enters the cell in endocytic vesicles via receptor-mediated endocytosis. The toxin then is transported to the endosomal compartment, and its catalytic domain (B) is translocated into the cytosol (53). CNF1 targets RhoA, Rac1 and Cdc42 that are present in the cytoplasm of the cells. These are small GTPases that are members of the larger Ras family and important regulators of actin and microtubule cytoskeleton dynamics (52, 54): RhoA is involved in the formation of bundles of filamentous actin and myosin (stress fibres), Rac1 stimulates the formation of lamellipodia and membrane ruffles and Cdc42 is involved in the formation of filopodia (54).

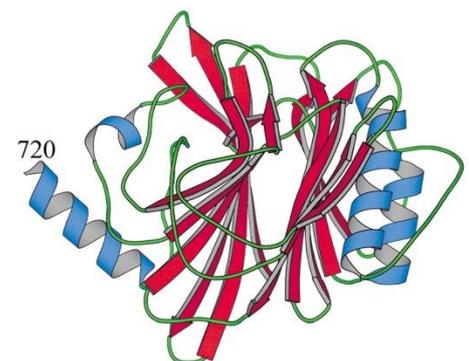


Figure 4. Three-dimensional structure of cytotoxic necrotizing factor-1 (CNF1). α -helices are blue, β -sheets are red and coils are green. Reference: (52)

The GTPases constantly oscillate between an inactive GDP-bound state and an active GTP-bound state, which is regulated by the regulatory protein GAP (52, 53). CNF1 deamidates glutamine residues on RhoA, Rac1 and Cdc42 (glutamine position 63 of RhoA, position 61 of Rac1 and Cdc42), which results in constitutive activation of the GTPases (55). Continuous activation of these GTPases leads to different effects on the affected cells and cells subsequently form actin stress fibres, membrane ruffles, lamellipodia and filopodia. Besides that, some cell types also become multinucleated (52, 55-59). CNF1 could even have effects on induction of apoptosis which could indicate a role in the exfoliation process of urothelial cells in response to UPEC infection (58). However, the final phenotype of the affected cells and the effects of CNF1 depend on the cell type and the GTPase that is affected (55). Because of their diverse effects in the host, CNF1 is also considered an important regulator of virulence during UPEC infection.

3.3.3 Autotransporter proteins

Another class of toxins that is secreted by UPEC is the group of autotransporter proteins, belonging to the type V secretion toxins (1, 6). All autotransporters are generally similar in structure (60), but despite this homology, their functions are very heterogeneous (61). Autotransporters consist of three domains: an N-terminal signal leader peptide, an α -domain (the passenger domain) and a C-terminal domain (60). After transport of the protein across the membrane, the α -domain is either released or remains bound to the bacterial cell (62). The autotransporter family consists of a lot of potential virulence factors in *E. coli*, such as cytotoxins and adhesins (63). Several autotransporters have been identified for UPEC, but a lot of them remain uninvestigated. The identified autotransporters include vacuolating autotransporter toxin (Vat), secreted autotransporter toxin (Sat) and Pic (6, 46, 60, 64, 65). The temperature-sensitive hemagglutinin (Tsh) has also been associated with UTI isolates, but this has not been confirmed yet. Next to these, additional autotransporters AIDA-1 and antigen 43 (Ag43) have been identified (60). The genes that encode these toxins and their functions during UTIs has been summarized in Table 2.

Autotransporter	Gene	Function and effects	Reference
Pic	<i>pic</i>	Mediate serum resistance, degradation one component of complement classical activation pathway, breaching mucus layer of the urinary tract, role in colonization	(1, 60, 62, 66)
Sat	<i>sat</i>	Cytotoxic effects (cell elongation, detachment from the monolayer), vacuolation of bladder and kidney cells, cellular damage	(62-67)
Vat	<i>vat</i>	Vacuolation of bladder and kidney cells, role in infection, proteolytic activity	(1, 6, 46, 60, 66)
Tsh	<i>tsh</i>	Expressed, but role not investigated	(66, 67)
Ag43	<i>flu/agn43</i>	Bacterial cell-cell aggregation, bio-film formation, adherence regulation	(51)
AIDA-1	<i>aidA-1</i>	Bacterial cell-cell aggregation, bio-film formation, adherence regulation	(51)

Table 2. Identified autotransporters in uropathogenic *E. coli* strains. Names, encoding genes and function and effects of different UPEC autotransporters are shown. References are listed in the table.

3.4 Intracellular bacterial communities

Another mechanism of virulence is the formation of intracellular bacterial communities of UPEC inside urothelial cells. During the acute phase of cystitis, type 1 fimbriae-mediated attachment to superficial umbrella cells, lining the lumen of the bladder, leads to internalization of the bacteria (69, 70). When UPEC enters the host cytosol, it faces a nutrient-rich environments that stimulates rapid bacterial multiplication (68, 71). This results in the formation of bio-film like structures called intracellular bacterial communities (IBCs).

After the formation of IBCs, the bacteria can re-emerge from the infected cells in a process called fluxing. The sensing of UPEC triggers apoptosis and subsequent exfoliation of the superficial umbrella cells as a mechanism to clear the infection, but the bacteria in the IBCs can escape from the exfoliating cells before this happens (72). These bacteria are often filamentous, so they can maintain contact with the urothelium as they leave the cell and can interact with the epithelial cells underlying the exfoliated cells. As they invade underlying cells, they establish a more stable bacterial community, called a quiescent intracellular reservoir (QIR) (69, 73). QIRs can exist for months, protecting the bacteria against antibiotics and cellular attacks (71). However, unknown cues may trigger intracellular bacterial proliferation and re-emergence of the QIR, leading to

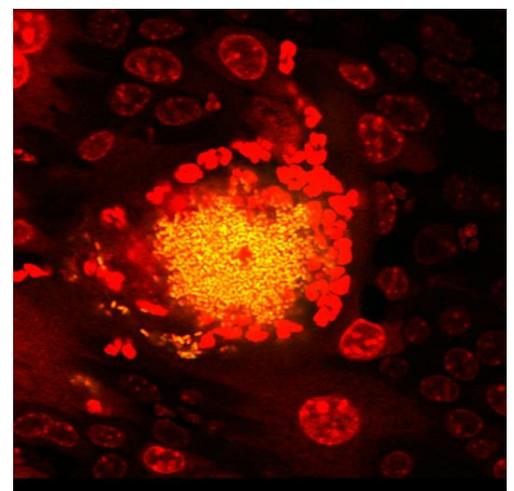


Figure 5. Intracellular bacterial community that is protected from neutrophil attack. Confocal microscopy image from a mouse bladder during acute cystitis (16h post infection) within a binucleate superficial umbrella cell. The cell is surrounded by neutrophils (red). The image shows a UPEC infection with strain UT189, expressing green fluorescent protein (GFP). Reference: (68)

a recurrent infection. IBCs and QIRs are therefore considered as one of the main reasons for the high recurrence rates of UTIs and considered a virulence factor (69).

4. The link to clinical relevance

To gain more insights into the molecular characteristics of UPEC and their role in the development of urinary tract infections, it is essential to understand the uropathogenesis and the exact role of their virulence factors. In the following paragraphs, a short description of uropathogenesis is given, followed by the current challenge of antibiotic resistance we now face in the clinic.

4.1 Pathogenesis

Even though the pathogenesis of UPEC-caused urinary tract infections is not fully understood, Kaper et al. (8) proposed a probable mechanism of infection. Since the UPEC strain isolated from the patient suffering from a UTI often matches rectal swabs from the same patient, it is believed that the UTI-causing strain originates from the patient's own gut (6, 46). Therefore, Kaper et al. propose that a UTI starts with colonization of the gut by a uropathogenic *E. coli* strain. The bacteria are then able to colonize the periurethral area and ascend the urethra to the bladder, where type 1 fimbriae enable the bacteria to bind mannose residues of uroplakin receptors on the urothelium. Attachment of the bacteria can result in two different outcomes: 1) the bacteria invade the urothelial cells and form IBCs (see paragraph 3.4) or 2) the bacteria remain bound and induce cell exfoliation and apoptosis, and sometimes even ascend to the kidneys, resulting in pyelonephritis (51).

As discussed in paragraph 2.2, some strains of UPEC are more associated with cystitis, while others are associated with pyelonephritis. Cystitis-associated strains only express type 1 fimbriae, which causes the infection to be limited to the bladder. If the UTI is caused by a pyelonephritis-associated UPEC strain, the invertible DNA element that controls the expression of type 1 fimbriae is switched to the 'off'-orientation, which releases the bacteria from the urothelial cells. As the bacteria ascend to the kidneys, subsequent expression of P fimbriae enables them to bind renal epithelial cells. At this stage, HlyA can exert different cytopathic effects on the kidney: it damages the epithelium, induces an acute inflammatory response and induces Ca^{2+} oscillations in renal epithelial cells. This results in the recruitment of phagocytes, particularly macrophages and neutrophils, and the secretion of cytokines IL-6 and IL-8 (68). Besides that, the secretion of autotransporter Sat has cytopathic effects on the glomeruli and renal epithelium (63, 67). In some patients, pyelonephritis can subsequently develop into bacteraemia due to damage of the proximal tubules of the kidney, which in healthy individuals functions as a barrier. This enables the bacteria to escape into the bloodstream and cause sepsis (8). This proposed mechanism of pathogenesis shows that the different virulence factors discussed in Chapter 3 enable the bacteria to colonize the whole urinary system, including the kidneys and even bloodstream.

4.2 Clinical relevance

Now that all the important molecular characteristics of UPEC have been summarized, it is important to discuss what impact this knowledge has for patients suffering from UTIs and how we can link it to their treatment. What do we know about the pathogenesis of UTIs caused by UPEC and how can we interfere? What can we do about the increasing resistance against certain antibiotics in the treatment of UPEC infections? These are some of the questions that emerge from the latest discoveries on UPEC, from their molecular characteristics to their clinical expression.

4.2.1 The economic burden of UTIs

Firstly, it is important to realize the economic burden of UTIs. On a global scale, it is estimated that 150 million UTIs occur, resulting in over \$6 billion spent every year (74). In the USA alone, UTIs account for nearly 7 million office visits and 1 million hospitalizations annually (3, 51). UTIs mostly occur among young adolescent women and the incidence was reported to be 0.5-0.7 per year. Recurrence is a major

problem in battling UTIs: 25-30% of patients suffer from another infection within a year. Even though most uncomplicated UTIs do not result in long-term effects, they do cause about 6-7 days of disability per infection. Altogether, this leads to significant morbidity because of their frequent occurrence and high chance of recurrence (3, 74, 75). UTIs are also among the most common hospital-acquired infections: catheter-associated UTIs account for 40% of all nosocomial infections. This not only leads to higher hospital costs (~\$400 per episode), but these infections also function as a source of multidrug-resistant *E. coli* bacteria in hospitalized patients (74). All these facts taken together, UTIs thus account for substantial medical costs worldwide. Therefore, the economic burden of UTIs is very high and research on the development and treatment of UTIs remains important.

4.2.2 Antibiotic resistance

Currently, UTIs are well treatable with antibiotics. The length, dose and type of antibiotic treatment depends on how severe the infection is, but most patients can be successfully treated with a short course of antibiotics (1 – 3 days) (76). However, the development of antibiotic resistance, which means that bacteria causing the infection can withstand the antibiotic treatment, is a common phenomenon observed for many bacterial species. Antibiotic resistance occurs naturally through mutation of normal genes and through horizontal gene transfer, but the widespread use and misuse of antibiotics accelerates these processes (77, 78). Since UPEC strains evolve by acquiring new genes from their environment through horizontal gene transfer, they can develop resistance to certain types of antibiotics by taking up antibiotic-resistance genes. Because UTIs are among the most common bacterial infections, their treatment plays a major role in the emergence of antibiotic resistance (7). Several studies have already documented antibiotic- and even multidrug-resistant UPEC strains (7, 79-82). As a result of that, UTIs are becoming less well treatable and thus more dangerous than they are now, becoming a real threat to public health. This leads to the need for an alternative treatment method (77).

4.2.3 Designing an alternative treatment method

As an alternative to antibiotics, several strategies are already known, of which cranberries are a popular and often-used method. The use of cranberries is known to be an easy way to treat a UTI, since it doesn't require a doctor's prescription and can be bought at the local supermarket. Cranberries were claimed to treat UTIs since they contain proanthocyanidins, tannins that are able to prevent the expression of P fimbriae, thus preventing the development of cystitis into pyelonephritis (3). However, these effects were shown to be dose-dependent and a lot of people ingesting cranberries or cranberry juice to treat their UTI never reach this dosages, thus limiting the beneficial effects of the tannins. However, the high intake of cranberry juice promotes frequent urination, which could help rinse the bacteria out of the urinary system, therefore promoting clearance of the infection. Cranberries as a way to treat UTIs are therefore not very effective, but form an easy way to treat the infection without having to go the doctor (1, 3, 83). Therefore, the search for an effective alternative treatment goes on.

An alternative treatment strategy should interfere with a characteristic that is present in UPEC strains, and absent from all other bacterial strains, in order to limit side-effects and only tackle UPEC strains. With the use of whole genome sequencing, it is possible to compare all known UPEC genomes to discover which molecular characteristic (or which combination of characteristics) is unique to UPEC strains. However, comparison of the published genomes of several UPEC strains so far has not resulted in any typical characteristics that could be used to separate a UPEC strain from any other *E. coli* strain. It is indeed true that some virulence factors as P fimbriae are associated with pyelonephritis (39), but they are not expressed by each known pyelonephritis-associated or general UPEC strain. On the other hand, type 1 fimbriae showed to be necessary for the acute phase of a developing UTI, but is also expressed by commensal strains and thus not unique to UPEC (8, 37). This leads to the conclusion that there is still a lot to unravel about the molecular characteristics of different UPEC strains and we cannot yet conclude there is no unique factor that distinguishes uropathogenic strains from any other *E. coli* strain. It is therefore important to continue doing research on different UPEC strains and their

genomes. Finding a UPEC-specific characteristic could be a major breakthrough, making it possible to design a UPEC-specific drug that targets this characteristic. The current knowledge on the genomes of several UPEC strains indicates that there may be a combination of molecular characteristics that could be associated with UPEC strains. With the genome sequences of more UPEC strains and the comparison between those sequences, we can gain more insights into the link between the expression of certain genes encoding the discussed virulence factors and the clinical expression resulting from an infection with that strain. If we continue to link expression levels of virulence factor-encoding genes in a certain UPEC strain with the clinical expression of their infection, we get to know more about the exact function of the virulence mechanisms in pathogenesis. Full understanding of the pathogenesis of UTIs that are caused by UPEC is essential for the development of an alternative treatment method.

5. Conclusion

Whole genome sequencing has taught us several things about uropathogenic *E. coli* and the infections they cause. We now know that there is a high level of heterogeneity between different strains and that there are no particular characteristics that distinguish uropathogenic strains from other strains of *E. coli*. We also discovered that most UPEC strains secrete toxins as α -hemolysin, cytotoxic necrotizing factor 1 and a wide range of autotransporter proteins to exert cytopathic effects in their host and to promote their own survival. While UTIs are currently relatively easily treatable with antibiotics, antibiotic resistance is an emerging phenomenon for UPEC strains as well as for a lot of other bacterial species. Antibiotic resistance can therefore be considered as a serious problem in public healthcare. UTIs are among the most common hospital-acquired infections, which means that their treatment plays a major role in the increased resistance to several types of antibiotics. The development of a new, alternative treatment method is therefore seriously needed, but the dynamic structure of the UPEC genome makes this very hard to realize. Comparison of the genome sequences of several UPEC strains has revealed that there are a lot of differences between different strains. Since there is no unique characteristic to UPEC strains, but there is a combination of virulence factors that these strains often express, it might be an idea to tackle this combination of factors to treat UTIs. However, our current knowledge on UPEC is not enough to design a universal alternative to antibiotics. Therefore, it is tremendously needed to continue with the ongoing research on UPEC strains and expand the knowledge on their genomics and virulence mechanisms. Luckily, the recent developments in biotechnology has led to the rise of whole genome sequencing as an efficient research tool, making it a lot easier and cheaper to sequence bacterial genomes for the sake of medical research. If we continue to sequence more UPEC strains, we will surely gain more insights into the relationship between certain virulence factors and the development and treatment of UTIs.

Unfortunately, it is therefore currently not possible to propose a possible alternative treatment method to antibiotics, even though the need for it is expanding. With UTIs being the most occurring bacterial infection and the rise of the number of antibiotic- or multidrug-resistant UPEC strains, it is necessary to still invest money into research projects focused on UPEC genomics. I believe that future research will lead to a suitable treatment method that can help us battle UTIs that have been caused by UPEC. Hopefully, this will eventually lead to less UPEC-caused urinary tract infections or at least less discomfort for patients, lower recurrence rates and a decreased economic burden of urinary tract infections caused by uropathogenic *E. coli*.

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