Adhesins of Uropathogenic *Escherichia coli* (UPEC)

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Abstract

Uropathogenic *Escherichia coli* (UPEC) is the main pathogen associated with urinary tract infections namely cystitis, pyleonephritis and infectious complications. As a commensal, *E.coli* is mostly harmless in the gut. Some strains diverge and become more pathogenic. They express multiple virulence factors and invade the urinary tract (UT). The important ones are the 'adhesins' or specialized proteins with sticky ends, which help to break the inertia of urinary bladder mucosa and help to attach to them.

Host immune response trigger inflammatory reactions, resulting in symptoms of urinary tract infections (UTI). Recent studies help to get updated information about the molecular mechanisms behind the adhesins. This knowledge is helpful for better understanding of the pathogenesis of UTI which can then be applied to epidemiological research. It also helps to understand the revolutionary trends, to help with better prognosis and to devise new methods in lab diagnosis & vaccine development. This review is intended to unravel the molecular components that makeup the adhesions of UPEC.

Key words: Adhesins, AFA, Curli, CUP, Pap, SFA, UPEC

Introduction

Escherichia coli (*E.coli*) are highly versatile organisms. The commensal *E.coli* peacefully exists in mammalian gut niche. It is a successful competitor at this crowded site. There are other highly evolved strains among *E.coli* that cause broad spectrum of infections with the help of virulence factors. Expression of these factors are through the genetic elements that can be mobilized into different groups to form new combinations. Only the successful combinations persist long to become a Pathotype.(A group of strain of a single species that can cause common disease using a common set of virulence factors)(1).

E.coli is categorized into Diarrhoeagenic *E.coli*, the pathotype associated with enteric/diarrhoeal diseases, the Uropathogenic *E.coli* (UPEC), causing UTIs and *E.coli* causing Sepsis/meningitis, Meningitis associated *E.coli* (MNEC). The *E.coli* causing infections outside intestine is also termed as Extra Intestinal Pathogenic *E.coli* (ExPEC). The six categories among diarrhoegic *E.coli* include enteropathogenic *E.coli* (EPEC), enterohaemorrahagic *E.coli* (EHEC), entero invasive *E.coli* (EIEC), enterotoxigenic *E.coli* (ETEC) and diffusely adherent *E.coli* (DAEC). Pathotypes of Diarrhoeagenic *E.coli* give rise to gastroenteritis but not any infection outside intestine. The ExPEC will exist in gut without consequence but will disseminate and colonize other niches causing disease (2). UTI is defined as the presence of significant number of pathogenic organisms in urinary tract, along with symptoms, while recurrent UTI can be defined as two or more episodes within six months or three or more episodes in one year(3).

UPEC are responsible for more than 90% of UTI, in both sexes. *E.coli* is the primary cause for community acquired UTI (70-90%) and to a large part of nosocomial UTIs(50%), accounting for substantial medical costs and morbidity worldwide(4). The women & children are more prone. The incidence of UTI in women increases with age and has a peak in the twenties(5). Sexually active women aged 20-40years and postmenopausal women older than 60 years are the two populations at highest risk of UTI. The life time risk of symptomatic UTI among women has been found to be 60%(6). Factors such as shortness of urethra, sexual activity, contraceptives, estrogen deficiency, diabetes, obstructing lesions and genetic factors such as blood group secretor status increase a woman's likely-hood of contracting UTI(7).

The prevalence of asymptomatic bacteriuria (ABU) in healthy women has shown to increase with age by about 1% in age group 5 to 14 while about 20% in elderly living in community. In ABU carrier state *E.coli* strains exist without symptoms. For many groups ABU screening is not beneficial, while for other groups like pregnant women and people undergoing traumatic genitourinary procedures ABU screening is useful for better outcome(8).

It is believed that the primary reservoir for UPEC is the intestine and that *E.coli* get introduced into the urethra (ascending hypothesis). *E.coli* strains colonize the bladder after travelling from gut to reach vaginal and periurethral area will cause cystitis, the common form of UTI. It is marked with dysuria, frequency, burning sensation& pain. UTI can proceed from bladder, via ureters to the kidney to cause pyleonephritis. It can damage the kidneys and result in kidney failure. This is associated with flank pain, fever, nausea and vomiting and may even progress to septicemia. Pyleonephritis is less common type of symptomatic UTI than cystitis(3).

In chronic & complicated UTIs bacterial strains are often mixed(9). It has also been found UPEC strains isolated from sexually active patients match with their fecal isolates from their partners(10)(11). There are instances where spread of a specific closed group occurred through contaminated consumables & food (12). *E.coli* have been phylogenetically grouped into six groups designated as **A, B1, B2, C, D** & **E** .It has been shown **D** & **B2** harbor UPEC(13).

Stages of pathogenesis leading to development of UTIs include bacterial adherence to the host tissues, colonization within the host, avoidance of host defense mechanism and causing damage to host tissue(14). After successful attachment and colonization escaping the host defense mechanism will be the next priority. Pili and fimbriae help in attachment while toxins secreted by the pathogen will damage the host tissue and help to establish themselves.

Ascending route is the most common route in UTI. Upon entry into lower UT, UPEC face obstruction to colonize. There is the flushing action of the urine, the mucosal barrier of the urothelium. Host inflammatory response leading to cytokine production, neutrophil influx and exfoliation of the cells. Exfoliation of epithelium helps to clear many bacteria from urinary tract along with the flow of urine (15). At the same time the immature epithelial cells get exposed due to exfoliation, making them susceptible to infection. Infection which began with attachment and colonization of epithelial cells proceeds by invasion, dissemination and will spread to the underlying immature bladder cells.

UPEC binding to host tissues is a paramount step in UTI. The bacteria will break into the host cytosol and will multiply rapidly, forming large biofilm like intracellular communities (IBCs) by binding with actin (16). Though *E.coli* has been regarded as extracellular pathogen, it has been proposed that UPEC can form *quiescent intracellular reservoirs* (QIRs), where they persist for long periods (17). Host immune system will fail to detect them at these sites making them less immunogenic(18). As replication of these actin bound bacteria is limited antimicrobials will be less susceptible. These QIRs act as source for recurrent UTIs(2).

Virulence factors of UPEC

Virulence factors(VFs) are specific properties that enable organisms to overcome host defense and cause disease(11). Pathogens causing UTI unlike commensal bacteria possess many different virulence factors which influence the outcome of UTI. There are different virulence genes (VGs) expressed through pathogenicity associated island (PAI) in *E.coli*. Genes that encode microbial proteins and organelles that specifically aid in pathogenesis are known as virulence genes(17). The high degree of genetic diversity of UPEC isolates is due to the presence of mobile genetic elements called PAIs. (PAIs are discrete genetic units flanked by direct repeats, insertion sequences or *tRNA* genes, which act as sites for recombination into the DNA). Experimental and epidemiological data have shown that no single VF of UPEC is sufficient to cause disease. It is the timely stepwise expression of multiple factors that leads to successful manifestation of disease(19).

Virulence genes (VGs) help to survive in hostile environment and to persist in the UT. The different VFs play role in different steps in UTI pathogenesis and their expression can be versatile depending on the environment and the host. These include adhesins (like p-pili, Type1 fimbriae), toxins (hemolysins *Hly*, cytotoxic necrotizing factor; CNF), polysaccharide capsules (K1,K5) and siderophores (aerobactin, catecholate siderophores)(20). Other traits include resistance to bactericidal effect of serum, colicinogenicity, production of IgA protease(21),(22). The formation of 'pod 'or biofilm like intracellular reservoir acts as a virulence mechanism for persistence and recurrent UTI(15). It has been seen that history of chronic cystitis is a significant risk for recurrence of severe UTI.

UPEC Genomics: Genomic data of UPEC strains suggest UPEC are genetically diverse pathotypes without a common virulence plasmid or pathogenicity associated island(PAI) required for infection(23). *E.coli* got a plastic genome capable of rapid alteration to suit different environments. The immune response and various factors of the host like its physiology, anatomy influences the colonization of *E.coli*(2). UPEC genome is larger than commensal K12 *E.coli* genome. This facilitates their existence outside the gut. Whole genome sequencing of multiple *E.coli* strains have been done, for strains like CFT073, UTI189, 536 (24)(25)(23)(26). UPEC genome got adhesins, iron acquisition systems which help in their survival in bladder. It does not contain have Type III secretion system expressed by intestinal pathotypes of *E.coli*. Genes encoding VFs have been shown to be located on chromosome or plasmids. *Pap* & *hly* genes are exclusively chromosomal while *Afa*/*dra* can occur in either location and *traT* gene coding for serum resistance trait in outer membrane is exclusively plasmid mediated(19).

Expression of PAIs varies. They help in horizontal gene transfer. It can be inserted or removed from the genome of *E.coli* as it contain transposons and integrese(27),(28). There is 'phase variation', a process in which cross talk between fimbrial operon result in a switch in expression of one fimbriae type to another(29). This antigenic variation is helpful for the pathogen to overcome host defense.

Adhesin Assembly and Interactions: To establish an infection *E.coli* should attach to host surface. Adherence is mediated by adhesins that help in recognizing and binding to host receptors. The 'adhesins' are present on bacterial surface which mediate specific bonding with molecules on host epithelium called 'receptors'. The adhesins attach to their receptors first by *Vander Waal's*

forces and hydrophobic attraction, which leads to low affinity binding. During second step the bonds are strengthened by stereo chemical interactions(30). Adhesins are special proteins expressed by many pathogens including UPEC. The important adhesin is the fimbriae which are long surface proteins, extending out from bacterial surface.

Adherence helps the pathogen to prevent from being swept away by normal flow of body fluids. There occur molecular interactions with host and the pathogen. Binding brings many changes in the host and pathogen. The pathogen might express new genes to enhance its virulence. binding to host receptors trigger signal transduction cascades and will activate the immune response .At times it might slow down cellular process and aids in bacterial colonization(31). Synthesis and assembly of the adhesins is a multistep process. The *chaperone/ usher pathway* (*CUP*) is the much studied one. *CUP* pill an extra cellular fibres of a vast family are encoded by this pathway. These are critical virulence markers expressed by *E.coli* and many gram negative bacteria. *CUP* pili help to adhere to different niche and also help in biofilm formation. On the outer membrane of the bacteria there is a gated channel-usher which promote the assembly and extension of the pilus fibre to the extra cellular suface of the bacteria with the help of different chaperones. The Type 1 pili, P pili & S pili systems in *E.coli* are assembled by *CUP* biogenesis. Various structural protein subunits are organized to form the organelle(32).

This review will focus on the various adhesins of UPEC that enable them to facilitate infection and persist in the urinary tract. The *E.coli* adhesins are either *fimB*riae associated or adhesins independent of *fimB*riae.

Fimbrial Adhesin

Type I *fimb*riae: It is known as mannose sensitive adhesin because of the receptors biochemical characteristic that is mannose sensitive (MS). The adhesion is inhibited by solution of *D*-mannose. Phenotypically they can agglutinate RBCs (haemaggl-utination). They produce mannose sensitive haemagg-lutination (MSHA). RBCs of guinea pig, humans, rabbit etc contain the α-D mannose receptor. Type I fimbriae are important virulence determinants expressed in *E.coli* and most members of *Enterobacteriaceae* family and mediate binding to mannose oligosaccharides(33). Receptors for Type 1 fimbriae are present in different cells of humans like RBCs, muscular layers of blood, ureters, Henle's & proximal tubules. Type 1 fimbrial receptors are not present in the epithelium of human bladder or on distal tubules, collecting duct, glomerular endothelium(34).

The Type 1 fimbriae are peritrichously arranged around the bacterial cell and there will be about 100-500 fimbriae. These filamentous organelles are encoded by "*fim*' gene cluster , with the structural components of the *fimB*riae composed of *fimA*, *fimF*, *fimG* & *fimH* and the pilus encoded by *fimC* & *fimD*(35). These are subunits of genes for structure, adhesion and accessory proteins involved in transport and assembly of fimbriae. *fimB* & *fimE* are the regulatory genes that control phase variation of Type 1 fimbriae (36).At the distal tip of heteropolymeric Type1 Pilus rod, is *fimH* the adhesin protein responsible for binding to mannose containing host glycoproteins. The *uroplakin1a* (an integral membrane glycoprotein) present in bladder surface is the main receptor for *fimH*(16). *FimH* are also bound by integrins which are expressed by many host cell types. Integrins are extracellular matrix proteins, providing signaling between 'intra' &'outer' cell environment. Pathogens gain entry into host cell by manipulating integrins and signaling reactions.(37)(38). *FimH* also binds to pattern recognition receptors *TLR4.*

Attachment by Type 1 through *fimH* triggers, mast cell activation of bladder epithelium. They release histamine(31)(39). This inflammatory response is presented as the symptoms of UTI, pain &dysuria. Tamm Horse fall protein(THP), are soluble factors found in urine which protect the bladder by offering binding site for Type 1 fimbriae and subsequent elimin-ation of the pathogen without damage. If UPEC manages to adhere to the urothelium using adhesins and especially Type1 fimbriae the acute pathogenesis of UTI starts. UPEC enter the cytoplasm and will form IBCs by multiplying in the intracellular niche. This will be a protective haven from host immune response and thus helps the bacteria to gain foothold(40).

Type1 pili are highly conserved and are common virulence genes among UPEC and commensal isolates. Various studies have shown knockout of *fimH* diminishes UPEC ability to colonize.(41) The expression of Type1 pili is phase variable. Expression of Type1 pili is phase variable. Expression of Type1 is co-regulated with expression of P fimbriae associated with pyleonephritis. Bacteria switch 'on' or 'off' the product of virulence genes depending on environ-mental condition. *Phase switch* ability may account for differential expression of Type1 in different body sites and in pyleonephritis & cystitis(40)(20). Research done on Type1 pili gave varied results, depending on lab conditions, age, sex and location.

The general opinion is that Type 1 fimbriae have no importance in UTI while their role in lower UTI patients are varied. In humans severity of UTI was increased and immunological response was greater in children with infection caused by Type1 pilated UPEC strains(42). A review article by Johnson(24) has analyzed the expression of Type1 fimbriae in urinary (64%) and fecal (60%) samples, to be in similar proportions. The 'switch off' of expression of Type 1 by phase variation after invasion of tissues maybe a survival mechanism. The presence of fimbriae may favour human leukocytes to recognize the pathogen and may favor clearance(34).

The tip adhesin *fimH* has undergone pathoadaptive mutation in clinical isolates(40). Several allelic variation

of *fimH* has been identified with Phenotypes having different binding ability(70,71). Receptor recognition profile can be affected by minor amino acid sequence alteration in *fimH*(43). This variation in *fimH* adhesin enhances its binding to target such as laminin, collagen and fibronectin as well to different mannose derivatives. As UPEC are genetically diverse pathotypes without a common virulence plasmid, Norinder *et al*; 2011 suggested that the adhesins like *fimH* of Type1 *fimB*riae are required to initiate uncomplicated cystitis(44). The antiType1 fimbrial antibodies (62) and D mannopyranoside (a receptor analogue) protect against experimental infection in animals (63). Thus Type 1 pili is an interesting candidate for antiinfective compounds and vaccine.

Type 1 pili are formed on bacterial outer membrane by *Chaperone Usher pathway* (*CUP).* The pilus rod of Type 1 is made of *FimA* subunit arranged helically and to that is attached fibrillar structure Fim G. FimF and distal tip *FimH*(2). *FimH* the adhesive tip recognize mannosylated glycoproteins & N-linked oligosaccharides on *α3 & β1* integrins. Type 1 pili which is anchored in outer bacterial membrane protrudes out through CUP biogenesis. *SurA* is a bacterial periplasmic isomerase that helps in insertion of the *FimD* to outer membrane usher(45).

The receptor for Type 1 pili appears to depend upon the differentiation state of urothelial cells. Mature superficial umbrella cells express uroplakins on their luminal surface and *FimH* bind to mannosylated UPIa(46). Binding to receptor initiates signal transduction cascade and internalization of the UPEC by a zippering mechanism, involving actin rearrangement. (47). In undifferentiated urothelial cells *FimH* receptor is α3β1 integrins. Experimental UPEC pathogenesis in undifferentiated urothelium shows, bacterial proliferation and IBC formation in limited manner compared to differentiated umbrella cells where UPEC can survive in cytoplasm by vesicular escape. This could be because of the thick actin network, present in immature urothelium preventing bacterial spread(48). Type 1 fimbriae/ Type1 pili play a pivotal role in bacterial adhesion, invasion and growth in biofilm communities(16).

P-Pili: Expression of P-pili is associated with pyleonephritis(49). It is the mannose resistant adhesin. It was originally identified by their ability to mediate binding to human O type erythrocyte without inhibition by mannose, distinguishing it from Type 1 *fimB*riae.(50)

Glycolipids containing the *gal-gal* moiety (α-D galacto pyranosyl-(1-4) β-D galactopyranoside) are the receptors for adhering *E.coli* strains. Common P blood group antigen contains this receptors (49). This receptor for P fimbriae is present on RBCs of humans pigs , fowls, goats , dogs but not on those from cows, guinea pigs or horses.(51) Gal –gal moiety on the receptor for P fimbriae is found abundantly on surface epithelial cells lining urinary tract(52). P-pili expression is less in asymptomatic bacteriuria than in cystitis and pyleonephritis. The association of P-pili with pyleonephritis could be because of large amount of gal moiety receptors present in renal glycolipids. Studies by Wold *et al* suggest that P-pili has evolved in *E.coli*(53). The persistence in gut in enabled by the binding to gal receptors. They bind more to loosely associated substance in the gut and not to colonic cells. These gut strains belong to phylogenetic group **B2** and to lesser extent Group **D.** These groups got superior ability to persist & spread and to cause disease(54). Other studies by Zhang and co-workers found P-pili among young women associated with **B2** and **D**.(55)

The genome of P-pili is coded by *Pap* gene cluster in the chromosome. They are composed of *PapA*, which is a polymer, that forms a rigid stalk, that is connected to a flexible tip containing subunits *PapE* & *PapF* in limited numbers.(56) *PapD* transports several pilus subunits from the cytoplasmic membrane to the outer membrane.(57) The outer membrane usher is *Pap* C which forms a pore through which pili anchors the stalk *PapA* to outer membrane.*PapG* is the adhesin. *PapE* tip is joined to *PapG* using adapter protein *PapF*(4).

Three types of *PapG* adhesins have been identified namely *PapG*I, II, III iso receptors. The isoreceptors bound *PapG* variant contain a *gal-gal* moiety linked to ceramide, which acts as an agonist of TLR4, activating immune cell response. This crosstalk, favors production of pro inflammatory cytokines, chemokines (interleukin-6 and CXCL8 respectively) and recruitment of neutrophils. This initial response IS beneficial in initiating bacterial clearance, but it will also cause damage to the surrounding tissue and is associated with renal complications.(58)

Since Pfimbriae are implicated in triggering inflammation, it can be concluded that they are associated with manifestation of acute pyleonephritis.

Pap DNA exhibit considerable heterogencity leading to antigenic diversity. The selective pressure from host immune system might account for the expression of hyper variable immune gene domain(44). *Pap* fimbriae are subjected to rapid random phase variation & environmental influence. Because of this probable characteristic use of *Pap* fimbriae as vaccine candidate is limited. The P fimbriae donot adhere to human PMNLs as they produce small amount of gal –gal receptors(59). *PapG* allele II is commonly associated with acute pyleonephritis. *PapG* allele III is found in cystitis. The role of *PapG* allele I is debated. The exact role of P pili during course of UTI has remained elusive(60).

Melican & co workers have defined previously unknown synergistic functions for Type I & *Pap fimB*riae, which facilitate bacterial colonization in dynamic invivo condition. P *fimB*riae enhance early colonization of tubular epithelium, while Type 1 mediate colonization of the centre of the proximal & distal tubule

via a mechanism that involve inter bacterial binding and biofilm formation. This subsequently leads to obstruction and affects renal filtration and contribute to patho physiology of pyelonephritis.(61)

There is a structurally related gene cluster *Prs* (*Pap* related cluster). They have different adhesion moieties. *Prs* G is the adhesin tip. Genomic studies have shown UPEC containing *PapG*I bind to globo triaosyl-ceramide or *GbO3* (present in human uroepithelial cells), *PapG*II bind to *GbO4* (abundant on human uroepithelialcells) and *PapG*III adhesins or *Prs*G that bind to forssman antigen or *Gb05* (present in canine uropepithelium)(62). UPEC Strains 536 and UTI189 contain one copy of *Pap* encoding operon while CFT073 has two copies with separate pathogenicity associated island(PAI)(2). The P fimbriae are not only associated with UPEC causing UTI but also related to new born menenigitis *E.coli* (NMEC) & Avian pathogenic *E.coli* (APEC)(63).

S fimbriae: S fimbriae coded by *sfa* genes are detected from UTI, meningitis in newborns and septicemia. The *sfa* adhesins bind to epithelial and endothelial cell lines and endothelial cell lines derived from bovine UTI and kidney(64). S fimbriae help in bacterial dissemination within host tissues. 22.5% strains isolated from UTI showed *sfa*. In 18% cases they were associated with *pap* operon(65). The adherence gene of S fimbriae is distinct and codes for protein located at the tip.

The S-fimbriae recognizes neuraminic acid (sialic acid) containing structures other than mannoside or P antigen on human erythrocytes.(66) The specifically binds to sialyl galactoside, from which it got the name S fimbriae. This fimbriae is morphologically similar to Type 1and P fimbriae. *sfa* gene cluster consists of *sfaA* as major subunit protein and three minor units of proteins namely *sfaS, sfaG* and *sfa H*.(67) Regulation of *sfa* determinants is mediated by two regulatory proteins *sfaB* & *sfa*C. The S fimbriae also undergo phase variation. Depending on temperature, osmolarity, presence of glucose and other environmental factors(26). The S fimbriae are shown to bind to epithelial celss of the proximal & distal tubules collecting ducts and glomerulus, renal interstitium and renal vascular endothelium are known to be binding site for S. fimbriae(68). They bind to sialo-glycoproteins on brain micro vascular endothelial cells which could be the reason why S fimbriae containing *E.coli*, causes mengitis. Various genotyping studies have reported prevalence of S fimbriae among ExPEC isolates.(66)

FIC fimbriae: These fimbriae do not mediate haemagglutination to erythrocytes from human, guinea pig, horse ox and chicken. But they contribute to the adhesive propertives of the UPEC(65). The FIC fimbriae are encoded by foc gene cluster. Biogenesis of FIC is by *focA* genes, which encodes an important product required for fimbrial formation. *focG* & *foc H* encode for minor subunits and *focF* encodes a protein similar to protein of *focA*. *focH* is the tip located adhesin(28). The receptor for FIC has been revealed. It include glucosyl ceramide and gal-gal sequence of asialoGM1(69). Foc genetic cluster is related to S fimbriae genetic cluster. Though *sfa* & FIC antigen differ in their receptors for attachment. It is seen that receptor specificity varies with ceramide compounds. (70) In response to FIC attachment to human epithelial cell, innate immune response is triggered to produce proinflammtory cytokines& interleukin8(71).

A*fimB*rial Adhesins

***Dr* Fimbriae:** Dr. fimbriae are implicated in UTI especially in those with gestational pyleonephritis & recurring cystitis(72)(73). This is a heterogeneous group consisting of different but related adhesins. (≥70% homology). *Dr* adhesins can result in MRHA. They recognize different portions of the *Dr* blood group antigen, a component of IFC (Inab-Friberger Cromer) blood group complex(72). Dr/*Afa* adhesins recognize decay-accelerating factor (DAF) as a receptor which is a complement regulatory protein present on the surface of many human epithelial cells (including epithelial cells of the urinary tract). Dr Blood group substance was found on tubular basement membrane and Bowman's capsule of the human kidney. Dr fimbriae binds to a lesser extent to the bladder epithelium.(74). The genetic organization of Dr adhesin operon (dra) consists of five genes *draA, draB, draC, draD,* and *draE*. Four genes, *draA, draC, draD,* and *draE,* promote the expression of full, mannose-resistant haemagglu-tination. *draE* of *dra* operon bind to DAF. The group include *Afa*I, *AfaIII*(*a*fimbrial adhesins) and O75X (also known as *AfaII*). During pregnancy *E.coli* bearing dr adhesins pose a threat to patients because of its invasive nature(75). Binding of Dr adhesins is accompanied by the activation of signal transduction cascades, including activation of PI-3 kinase(1). Dr fimbriae is found in a lower proportion than *Pap*, *Sfa*, and foc fimbriae. Strains of diarrhogenic *E.coli* also harbor Dr adhesin.

***Afa* Adhesin:** 'Afimbrial' adhesins are associated with UTI. Purification of *Afa*-1 protein showed that they exist on the bacterial surface. They are free as macro molecular aggregate in spent culture medium. *Afa* protein can agglutinate human erythrocytes in the presence of D- mannose(76). For *Afa* there is *Afa* gene cluster, which consists of *AfaA*, *AfaE*, *AfaD*, *AfaB*, and *AfaC*. *AfaE-I* is the adhesin tip. (77)The products of *DrA* and *AfaA* are similar to chaperone usher product of *PapD* chaperone.(2) There exist at least four different *Afa* operons *Afa-1, Afa-2, Afa3, Afa-4* which encode protein *AFA-1, AFA-II, AFA-III, AFA-IV*. *AFA1* & *AFAIII* belong to Dr family of adhesins.(78) *AfaC* & *AfaD* genes are highly conserved. *AfaE* codes for adhesin and it is heterogenous in nature. *Afa* adhesins were isolated more from patients with cystits (26-65%) than with pyelonephritis (6-26%) and with ABU (6%) (34).

***Curli* Fibres:** They may be involved in the colonization of fibronectin coated surfaces. *Curli* are the third type of organelles expressed by *E.coli*, made of protein *Curli*. They are expressed as curled surface structure and is encoded by *Curli*n subunit gene (*csg*) cluster. *Curli* have been studied to attach to the proteins of extracellular matrix such as plasminogen, fibronectin and laminin thereby promoting adhesion of the bacteria to different human cells(79)(80) studies suggest that most of the pathogenic E. coli strains do not express *Curli* at higher temperature of 37ºC but at temperatures below 30ºC and under low nutrients and low media osmolarity, during the stationary growth phase. Assembly of *Curli* includes self-assembly of subunits *csg*. A sub-unit, to *csg*B which is a specific nucleator protein. *csg*D is the transcriptional activator and *csg*G is involved with stabilization.(81)

Newer Adhesins: A trimeric autotransporter adhesin *UpaG* was recently identified, which mediate aggregation of *E.coli* as well as adhesion to abiotic surfaces, T24 bladder epithelial cells and extra cellular membrane proteins.(82) *Yqi* is another adhesin found in UPEC & in ExPEC(83).

Role of Upec Adhesins in the Intestine: In gut we do find resident strains and transient strains. The former are those present in gut for many years, while transient strains are passed only for a short period(84). Resident strains include the commensal *E.coli* while the UPEC (ExPEC), Diarrhogenic *E.coli* form transient strains. *E.coli* is a normal inhabitant of the gut microbiota. It colonises the Gastrointestinal tract of human infants within few hours after birth and is acquired either from environment or from mother during parturition.(85) Commensal *E.coli* have a beneficial role by supporting digestion and producing Vitamin K and competing with other microbes and hindering colonization of pathogenic agents(86). Commensal strains are derived from phylogenetic groups **A** & **B1** which don't have specialized virulence attributes.

Colonization of the large intestine is the preliminary step in development of urinary tract infection. Among UPEC adhesins P-pili and Type1 fimbriae are found to enhance colonization within the gut.(87) There is also studies where expression of adhesins among fecal flora were found to be low. As adherence ability shown by bacteria helps to manipulate normal flora and considering the ascending route of UTI possibility of manipulating the relative composition of fecal, vaginal flora offers interesting possibilities in the prophylaxis of UTI(88).

Conclusion

Therapeutic use of receptor analogs to competitively inhibit attachment by UPEC is one possibility, in vaccine development. Moieties of adhesins expressed by UPEC has shown promising results. The *Pap subunit* vaccine to protect against pyleonephritis is under trial(89). Protective studies with animal models using *E.coli* adhesins gave encouraging results. Antibodies against *Pap* & Type1 Fimbriae prevented UPEC from binding to uroepithelium. Pilicides, novel inhibitors for pili biogenesis are being developed. Also research is into developing *FimH* vaccine, which will trigger formation of Anti–*FimH* antibodies. Targeted development of therapeutic molecules, to block CUP pilus assembly is underway. By increasing the immunogenicity of the adhesins through technology, vaccines look like an important alternative to treatment with antibiotics.

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