

Urinary Tract Infections of *Escherichia coli* Strains of Chaperone-Usher System

BEATA M. ZALEWSKA-PIĄTEK*

Department of Microbiology, Gdańsk University of Technology, Gdańsk, Poland

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Abstract

Urinary tract infections are a very serious health and economic problem affecting millions of people each year worldwide. The most common etiologic agent of this type of bacterial infections, involving the upper and lower urinary tract, are *E. coli* strains representing approximately 80% of cases. Uropathogenic *E. coli* strains produce several urovirulence factors which can be divided into two main types, surface virulence factors and exported virulence factors. Surface-exposed structures include mainly extracellular adhesive organelles such as fimbriae/pili necessary in adhesion, invasion, biofilm formation and cytokine induction. Among the surface-exposed polymeric adhesive structures there are three most invasive groups, type 1 pili, type P pili and Dr family of adhesins which are bioassembled *via* the conserved, among Gram-negative bacteria, chaperone-usher secretion system. Type 1 and P-piliated *E. coli* cause cystitis and pyelonephritis. The Dr family of adhesins recognizing DAF receptor is responsible for cystitis, pyelonephritis (especially in pregnant women) and diarrhoea (in infants). In addition, Dr-positive *E. coli* strains carry the risk of recurrent urinary tract infections. Pyelonephritis in pregnant women leads to a series of complications such as bacteremia, urosepsis, acute respiratory distress syndrome and even death. In the era of increasing drug resistance of bacteria, the development of vaccines, drugs termed pilicides and inhibitors of adhesion may be a promising tool in the fight against urogenital infections.

Key words: chaperone-usher, uropathogenic *E. coli*, cystitis, pyelonephritis

Urinary tract infections caused by *Escherichia coli*

Urinary tract infections (UTIs) involving the bladder (cystitis) or the kidneys (pyelonephritis) are the most common bacterial infections. *Escherichia coli* strains are the major infecting agent (identified in 75–80% of analyzed cases) colonizing the urinary tract. UTIs affect more than 60% of women during their lifetime, about 25% of them have a second episode of urologic disease within 6 months because of recurrence (Milar and Cox, 1997; Hooton and Levy, 2001; Foxman, 2002; 2003a; Ronald, 2002; Foxman and Brown, 2003b).

UTIs can have severe, recurrent or chronic course. The classification of UTIs in most cases depends on the part of urinary tract which is actually colonized by the pathogenic bacteria. Lower UTIs including cystitis are the primary sites of infections in about 95%. Cystitis is connected with pelvic discomfort, frequent voiding and suprapubic pain. Acute pyelonephritis, a complication that involves the kidneys (upper UTIs) is identified by the characteristic clinical symptoms, such as fever, nausea, flank pain and, sometimes vomiting. In pregnant women *E. coli* strains as a primary cause of

pyelonephritis account for 65–80% of cases (Milar and Cox, 1997). Pyelonephritis in these kinds of patients can lead to serious consequences like urosepsis, bacteremia, acute respiratory syndrome and even death (Ventura *et al.*, 1997). The stages of pregnancy development can be also disordered and result in intrauterine growth retardation and preterm labor (Cunningham and Lucas, 1994).

Urinary disease can be characterized by stormy clinical symptoms or especially in recurrent infection the illness can take its asymptomatic course. Elder children complain of acute stomachache, pollakiuria, painful vesical tenesmus, nausea, vomiting, constipation or diarrhea and fever. In newborn babies and infants the clinical symptoms of UTIs are non-specific and localized outside the urinary tract. The symptoms include fever or hypothermia, aversion towards sucking, flatulence of stomach, prolonged jaundice, anxiety, cyanosis or pallor skin. The illness can also have a form of septicemia (Watson, 1994; Wyszowska, 1994; Lewczyk and Jankowski, 2003). In adults the symptoms are very similar to elder children and include pollakiuria, fever, nausea, headache, stomachache, difficulty

* Corresponding author: B. Zalewska-Piątek, Department of Microbiology, Gdańsk University of Technology; G. Narutowicza 11/12, 80-952 Gdańsk, Poland; phone/fax (+48) 58 3471822; e-mail: beazalew@pg.gda.pl

or pain on urination (dysuria), low back and pubic pain (Sobotová, 2011).

The development of urinary tract infection and its course depends on general defensive mechanisms of organism, the property of pathogenic factors, diagnosis and applied treatment (Wyszyńska, 1994; Sobotová, 2011).

Virulence factors of uropathogenic *Escherichia coli*

Uropathogenic strains of *E. coli* (UPEC) harbour different virulence factors which enable them colonization and persistence in the urinary tract. The virulence factors are encoded by genes located at the selected regions of chromosomal DNA, plasmids and transposons. The DNA fragments forming a complex of virulence determinants (adhesins, toxins, secretion mechanisms, capsules and iron uptake systems) are called pathogenicity islands (PAIs). PAIs are flexible genetic elements, holding the mobility sequences, which are transferred horizontally between the bacterial cells (the mechanism of gene transfer important in the evolution of UPEC virulence) (Blum *et al.*, 1994; Picard *et al.*, 1999; Hacker and Kaper, 2000; Johnson and Kuskowski, 2000; Oelschlaeger *et al.*, 2002; Johnson, 2003).

Virulence determinants associated with UPEC include the family of adhesive fimbrial organelles, fimbrial polyadhesins and adhesive pili (P and type 1 pili, Dr family of adhesins, S and F1C fimbriae), polysaccharide coatings (group II capsules), toxins (hemolysin, secreted autotransporter toxin, Sat and cytotoxic necrotizing factor), and siderophores (aerobactin system) (Oelschlaeger *et al.*, 2002; Emödy *et al.*, 2003; Arisoy *et al.*, 2006; Yamamoto, 2007).

Pili and fimbriae are necessary for attachment to specific uroepithelial cells which can activate mechanisms facilitating bacterial multiplications, invasion and sometimes growth as a biofilm. The presence of pili (especially type 1 pili) can also promote the development of local inflammation (Mulvey *et al.*, 1998). Among binding targets, host cell receptors, of adhesive fimbrial structures are manno-oligosaccharides (Connell *et al.*, 1996; Thankavel *et al.*, 1997; Bahrani-Mougeot *et al.*, 2002), digalactoside component of glycosphingolipids (Roberts *et al.*, 1994), glycoproteins and proteins (CD55/decay accelerating factor, DAF and carcinoembryonic antigen-related molecules, CEACAMs) (Nowicki *et al.*, 1988; 2001; Berger *et al.*, 2004; Servin 2005; LeBougenec and Servin, 2006; Korotkova *et al.*, 2008a; 2008b; Guignot *et al.*, 2009).

Among the most frequent urovirulence factors with filamentous morphology are type 1 pili, P pili and Dr family of adhesins. Type 1 pili causes cystitis associated

with the ability of bacteria to recognize the monomannosyl residues, localized on the bladder epithelium (Sokurenko *et al.*, 1997). However, P pili by recognition of P antigen in the renal tubular epithelium leads to acute pyelonephritis (Källénus *et al.*, 1981). In pregnant patients with pyelonephritis, P- and Dr-positive *E. coli* strains (representing gestational age-dependent profiles) predominate during the second and third trimester, respectively (Nowicki *et al.*, 1994).

The Dr adhesins are the third most common group of colonization factors of UPEC, behind type 1 and P pili (Nowicki *et al.*, 2001). They are responsible for 25–50% cases of cystitis and 30% of pyelonephritis in pregnant woman (Labigne-Roussel and Falkow, 1988). Dr adhesins can also increase the risk of recurrent UTIs (Foxman *et al.*, 1995).

The growing Dr superfamily of adhesins includes Dr hemagglutinin (Dr/DraE adhesin), Dr-II, F1845, Afa-I, -II, -III, -IV, -V, -VII and -VIII, Nfa-I, Aaf-I and Aaf-II (of human or animal origin). All the adhesins are encoded by gene clusters of similar organization. In most cases, they bind to the common epithelial cell receptor, DAF (Nowicki *et al.*, 2001; Servin 2005; Le Bougenec and Servin, 2006). Interaction of the Dr adhesin with DAF glycoprotein leads to the internalization of bacterial cells to the epithelial cells. Dr⁺ *E. coli* strains are able to survive long-term in human epithelial cells (Goluszko *et al.*, 1997; 1999). In the kidneys of experimental animals the above *E. coli* strains can also persist for several months (Selvarangan *et al.*, 2004).

Dr hemagglutinin among the whole family of Dr adhesin is the only member which can bind to type IV collagen (Westerlund *et al.*, 1989; Carnoy and Moseley, 1997). DrII13T mutant totally lost its ability to bind type IV collagen (Carnoy and Moseley, 1997; Selvarangan *et al.*, 2004). Besides type IV collagen and DAF, the DraE adhesin can interact with members of the carcinoembryonic antigen family, CEA-related cellular adhesion molecules, CEACAM1, CEACAM5 and CEACAM6 (Berger *et al.*, 2004; Korotkova *et al.*, 2008a; 2008b; Guignot *et al.*, 2009). Dr haemagglutinin also triggers the bacterial mobilization of $\alpha_3\beta_1$ integrin (Guignot *et al.*, 2009).

The capsule material on the bacterial surface provides protection against complement mediated bactericidal effect in the host organism and phagocytic engulfment. LPS molecules induce the cytokine synthesis (IL-1, TNF α) and stimulate the inflammatory response (Morrison and Ryan, 1987; Rietschel, *et al.*, 1996). Other surface located molecules can form a part of a secretory machinery of virulence factors exported outside the bacterial cells like the outer membrane haemin receptor protein termed ChuA (required to gain access to the source of iron ions) (Torres and Payne, 1997).

Exported virulence factor, α -hemolysin, is responsible for a destruction of cytoplasmic membrane of erythrocytes, endothelial and renal epithelial cells which causes uncontrolled outflow of ions from the cells (Smith, 1963; Keane *et al.*, 1987). The serine auto-transporter protease, Sat, also belonging to exported determinants, reveals a toxic activity against bladder or kidney cell lines (Guyer *et al.*, 2002). The toxin has an ability to induce vacuolization within the cytoplasm of human cells of urinary tract origin contributing to the pathogenicity of UPEC by damage of host tissues and increase of bacterial propagation. Another secreted factor, the cytotoxic necrotising factor 1, CNF1 evokes apoptosis of bladder epithelial cells (Caprioli *et al.*, 1987; Fiorentini *et al.*, 1997). Siderophores, like aerobactin, found in 30–60% of uropathogenic *E. coli* strains are low molecular weight compounds exported from the bacterial cell to gain Fe^{3+} cations from the host components chelating the iron. The process of iron utilization is controlled by ferric-siderophore receptors located in the bacterial outer membrane (Guerinot, 1994; Braun *et al.*, 1998; Schubert *et al.*, 2002).

Chaperone-usher pathway as the secretion mechanism of adhesive organelles of UPEC

A multitude of Gram-negative pathogens utilize the highly conserved secretion pathway termed chaperone-usher for the bioassembly of at least 30 diverse adhesive organelles that are required in host-pathogen interactions critical for the initial step of infection (Hung *et al.*, 1996; Thanassi *et al.*, 1998; Sauer *et al.*, 2004). Among those fibers, P and type 1 pili, expressed by uropathogenic *E. coli* strains, are mainly used as the model systems to understand their structure and function. The chaperone-usher secretion system is also studied on a basis of *E. coli* Dr family of adhesins and polymeric F1 capsular antigen of *Yersinia pestis* (Zavialov *et al.*, 2003; Anderson *et al.*, 2004; Pettigrew *et al.*, 2004; Sauer *et al.*, 2004; Piątek *et al.*, 2005a; 2005b).

The chaperone-usher secretion system is based on two components, chaperone located in periplasmic space and usher inserted in the outer-membrane. Including the sequence analysis two immunoglobulin-like periplasmic chaperone families have been identified, FGL (F1-G1 long) named Caf1M-like with a long and flexible loop (21–29 residues) between F1 and G1 β -strands and FGS (F1-G1 short) named PapD-like with a short loop (10–20 residues) connecting F1 and G1 β -strands (Zav'yalov *et al.*, 1995; Hung *et al.*, 1996; 1998; Saulino *et al.*, 2000; Sauer *et al.*, 2000; Zavialov *et al.*, 2007).

The division of chaperones is strictly connected with morphological properties of adhesive structures.

The FGL chaperone-assembled organelles, called fimbrial polyadhesins (diameter of 2 nm), consist of linear polymers of one or two types of protein subunits (each subunit possesses adhesive properties). The adhesive organelles have nonpilus, amorphous or capsule like-morphology. The FGS assembled organelles, termed adhesive pili (pilus rod of 10 nm diameter), are composite structures built from multiple different pilus subunits with single adhesive subunit at the tip (Hung *et al.*, 1996; Nishiyama *et al.*, 2005; Remaut *et al.*, 2006; Zavialov *et al.*, 2003; 2005; 2007).

The outer membrane molecular ushers form a conserved family of proteins (Fimbrial Usher Proteins, FUP) without any specific differences in sequences involved in the assembly of fimbrial polyadhesins or adhesive pili (Thanassi *et al.*, 2002; Capitani *et al.*, 2006a; 2006b; Zavialov *et al.*, 2007).

The chaperones of both families play many functions in biogenesis of pili/fimbriae. They stabilize subunits in the periplasm (after secretion *via* the Sec pathway) by formation of soluble chaperone-subunit complexes, cap their interactive surfaces which prevents premature interactions of the subunits, aggregation and degradation by periplasmic proteases. They also facilitate the folding of subunits after their emergence from the inner membrane in semi-unfolded conformations and transport the subunits to an outer membrane molecular usher. All functions are determined by a donor strand complementation mechanism in which G1 β -strand of the chaperone and the portion of the F1-G1 loop complete the immunoglobulin fold of the subunit (Zav'yalov *et al.*, 1995; Hung *et al.*, 1996; Thanassi *et al.*, 1998; Knight *et al.*, 2000; Sauer *et al.*, 1999; 2000; 2004; Zavialov *et al.*, 2001; 2003; 2005; 2007; Remaut *et al.*, 2006).

The ushers release the subunit from the complex with the chaperone in the periplasm, form an assembly polymerization platform of subunits in linear fibers/pilus structures and secrete adhesive organelles *via* the oligomeric usher pores to the bacterial cell (Nishiyama *et al.*, 2005; Remaut *et al.*, 2006). The pilus/fiber assembly is carried out by a donor strand exchange mechanism in which the amino terminal strand of a neighboring subunit replaces the G1 β -strand of the chaperone. Thus, in the mature adhesive organelle every subunit is required for the complementation of the immunoglobulin fold of its neighbor (Barnhart *et al.*, 2000).

Present and future treatment of UTIs

Modern therapy of the urinary tract infections is based on their treatment with antibiotics. The choice of anti-bacterial treatment and the time of application is dependent on a clinical character of urinary tract

infection and patient condition. One should take into consideration the course of drug action, the concentration of the medicine in urine, excretion in unaltered form, undesirable symptoms after its application and the cost of treatment. The drugs with a long-term effect and capacity of sequential treatment (initial parenteral administration of a drug and a continuation of treatment by the oral form of preparation) are recommended (Gutierrez, 1996).

Increasing resistance of bacteria to antibiotics and understanding of pathogenesis of UTIs create the need for designing chemotherapeutics that target new molecular targets (Stamm and Hooton, 1993; Hooton and Levy, 2001; White and McDermott, 2001; Gupta, 2003). There are several potential strategies which can be used in future to treat UTIs. One strategy blocking the colonization of urinary tract by uropathogens includes inhibition of pili/fimbriae biogenesis. Another strategy concerns the process of blocking of intracellular bacterial communities (IBC) which are cellular structures similar to biofilm preventing the bacteria from immune response and antibiotic therapy. The next strategy is connected with abolishing of binding of bacterial adhesins to host cellular receptors. The most promising approaches are related to inhibition of pili/fimbriae biogenesis and blocking of bacterial adhesion (Wright and Hultgren, 2006).

The adhesion of bacterial cells can be blocked by using small molecules, so called adhesin inhibitors which strictly interact with the target adhesins (Firon *et al.*, 1987; Ohlsson *et al.*, 2002; Ofek *et al.*, 2003). Aromatic alpha-glycosides of mannose for a long time are considered to be excellent inhibitors of adhesion of type 1 piliated *E. coli* (mediated by FimH adhesin) to target tissues (Firon *et al.*, 1987). Fruit juices, especially cranberry juice, are widely used in the treatment of UTIs. Positive effects of cranberry juice have also been observed in clinical trials (Avorn *et al.*, 1994; Kontiokari *et al.*, 2001; Di Martino *et al.*, 2006). The active inhibition ingredient in cranberry juice is fructose (Zafriri *et al.*, 1989). Fructose, as well as aryl mannosides, binds to FimH adhesin of type 1 pili ~ 15 times weaker than mannose (Bouckaert *et al.*, 2005) (mannose-containing receptors of the bladder epithelium are critical for establishment of cystitis) (Connell *et al.*, 1996; Thankavel *et al.*, 1997; Bahrani-Mougeot *et al.*, 2002), and tightly than the natural globoside, (Gala1-4Gal) (globoside-containing receptors present on the kidney epithelium are necessary for pyelonephritis) (Lund *et al.*, 1987; Roberts *et al.*, 1994) to PapG-II adhesin of type P pili. Among several variants of PapG adhesins, PapG-II and PapG-III are predominant for colonization of the upper urinary tract (Strömberg *et al.*, 1990; 1991; Striker *et al.*, 1995). Galabiose derivatives appear to be potent inhibitors of adhe-

sion mediated by PapG adhesin of *pap⁺ E. coli* strains (Ohlsson *et al.*, 2002).

The surface adhesive organelles (pili/fimbriae) of uropathogenic *E. coli* strains as virulence factors target for the development of a new class of anti-infective agents called pilicides. The pilicides function by interfering with the chaperone and usher activity, thus preventing the biogenesis of pili/fimbriae (Pinkner *et al.*, 2001; Svensson *et al.*, 2001). Effective activity of pilicides has been demonstrated for type 1 and type P pili. In this case, the reduction of biogenesis of the studied adhesive structures, was observed (Hendenström *et al.*, 2005; Pinkner *et al.*, 2006; Chorell *et al.*, 2010). In both cases, inhibitors of adhesion and pilicides, further *in vivo* studies are required.

Among the traditional methods used in combating infectious diseases is vaccination. There are high expectations about the vaccine based on the FimCH binary complex composed of chaperone and adhesin (encoded by type 1 piliated *E. coli*), which was tested in mice and cynomolgus monkeys. Vaccination of the studied animals with FimCH resulted in greater than 99% reduction of mucosal colonization (Langermann and Ballou WR Jr, 2001) and 75% reduction in both colonization and inflammation, respectively (Langerman *et al.*, 2000). Until now, there are no clinical trials performed on the basis of PapDG chaperone-adhesin complex (of P-piliated *E. coli*).

Finally, the best treatment should apply to the combination of bacterial adhesins and be directed to different sites of colonization by bacterial strains. The development of vaccines and anti-adhesive drugs can prevent anticipated and recurrent UTIs.

Summary

Urinary tract infections are very common health problem world wide. Most of these infections are caused by the uropathogenic *E. coli* strains (about 80%). Virulence of the bacterial strains is mainly determined by the surface-exposed adhesive polymeric structures (fimbriae/pili) responsible for recognition of the selected cellular receptors on the target cells. Interaction of the adhesive subunits with the specific receptors on the surface of host cells is the crucial step in the pathogenesis. Strong immunogenic properties of the adhesive polymeric structures are often used to design vaccines based on the conserved antigenic domains of the adhesins (so called adhesin-based vaccines) which can be useful for the prevention of a number of different diseases. An alternative to adhesin-based vaccines is an inhibition of adhesion by using adhesin inhibitors interacting with target adhesin in many cases more tightly than the physiological ligands. Because of the

crucial role of the adhesive organelles in the colonization of the invaded environment, their biogenesis is highly conserved among the multitude of Gram-negative bacteria. Detailed characteristics of the chaperones, a component of the chaperone-usher pathway, showed their usefulness as an ideal target for the developing of the potential drugs, termed pilicides. Such drugs, by interactions with chaperone and usher proteins, have the potential to block pili formation in a broad range of pathogenic bacteria parallel with the inhibition of adhesion and invasion of bacterial cells into the eucariotic cells.

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