Population structure of gut *Escherichia coli* and its role in development of extra-intestinal infections

Mohammad Katouli

*Faculty of Science, Health and Education, University of the Sunshine Coast, Queensland 4556, Australia.*

Received: February 2010, Accepted: May 2010.

**ABSTRACT**

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) strains are divided into uropathogenic *E. coli* (UPEC), strains causing neonatal meningitis and septicaemic *E. coli*. The most common pathotype of ExPEC is found among patients with urinary tract infection (UTI), defined as UPEC. These bacteria are responsible for >90% of cases of UTI and are often found amongst the faecal flora of the same host. *E.coli* strains are classified into four phylogenetic groups, A, B1, B2, and D. Groups A and B1 are commensal strains and carry few virulence-associated genes (VGs) while pathogenic group B2 and D usually possess VGs which enhance colonic persistence and adhesion in the urinary tract (UT). The gastrointestinal (GI) tract is widely accepted as a reservoir for UPEC and is believed that healthy humans have a reservoir of UPEC strains, belonging to phylogenetic group B2, and to a lesser extent, group D. These strains have superior ability to survive and persist in the gut of humans and can spread to cause extra-intestinal infections. ExPEC trains possess a range of VGs which are involved in their pathogenesis. These include adhesins, toxins, iron-acquisition systems (e.g. siderophores), and capsules. Evolutionary influences on the acquisition and main role of VGs amongst *E. coli* are widely debated, with some research holding that the prevalence of strains with VGs increases the likelihood of infections, whereas others believe that VGs provide a selective advantage for infection of extra-intestinal sites. This review is intended to present our existing knowledge and gaps in this area.

**Keywords:** *E.coli*, Urinary tract infection, Gut, Virulence factors.

| OVERVIEW OF GASTROINTESTINAL TRACT | 60 | P-pili | 64 |
| INTESTINAL MICROFLORA | 60 | Haemolysin | 66 |
| *E. coli* | 61 | Cytotoxic necrotising factor | 66 |
| Phylogenetic groups of *E. coli* | 61 | Siderophores | 66 |
| Diarrhoeagenic *E. coli* | 61 | Capsular polysaccharide | 67 |
| Urinary tract infection | 62 | **SURVIVAL OF UPEC IN THE GUT** | 67 |
| BACTERIAL PERSISTENCE | 62 | Gut as a source of UTI | 68 |
| ExPEC strains causing septicaemia | 62 | UPEC and septicaemia | 68 |
| ExPEC strains causing meningitis | 63 | **EVOLUTION OF UPEC AND CONCLUDING REMARKS** | 68 |
| VIRULENCE FACTORS OF EXPEC | 63 | REFERENCES | 68 |
| Type 1 fimbriae | 63 | | |

* Corresponding author: Dr. Mohammad Katouli
Address: Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore DC, 4556, Queensland, Australia.
Tel: + 61-754302845
Fax: + 61- 754302887
E-mail: mkatouli@usc.edu.au
OVERVIEW OF GASTROINTESTINAL TRACT

The body is protected from the external environment by the intact epithelial layer (1). The GI tract provides a barrier between the external environment and sterile, internal organs, whilst allowing absorption of essential nutrients. The GI system extends from the mouth and oesophagus to the rectum and can be divided into the upper (mouth and stomach) and lower tracts (small and large intestine) (2). Bacteria colonise the GI tract immediately after birth to form a complex milieu constituting hundreds of bacterial species, most of which are residing in the lower intestine. This flora develops through a process of ecological succession and has tremendous role in the state of health and disease. Among the important factors in this process is the influence of the intestinal physiology on the interaction between the microorganisms that contaminate the host, diet regime and food composition, immunological status of the host and the environment (3). The GI tract is composed of four layers: the mucosa, submucosa, muscularis externa and serosa (4, 5). The mucosa is the external layer of the epithelium, which is constantly exposed to bacteria (1, 4, 5).

The intestinal epithelium is more than just a physical barrier protecting internal body sites, with immune interactions to prevent infection and disease from constant exposure to bacteria (6). In the lower GI tract, bacteria must overcome host defences such as peristalsis, lysozyme secretions, intestinal mucus, and gut-associated lymphoid tissue (1, 7). Epithelial cells are protected by a layer of mucus and other nonspecific host defences, as well as a diverse range of mainly commensal bacteria (8). Mucous is secreted by goblet cells and acts as a lubricant for the smooth passage of food and faecal matter along the GI tract. It also helps to trap bacteria, preventing adherence to the GI epithelial cells (1, 6). The lower GI tract has a high rate of cell turnover, constantly shedding epithelial cells and many bacteria which have been able to adhere (6). These host defences help to prevent and decrease bacterial adhesion and colonisation in the lower GI tract.

Keywords: E.coli, Urinary tract infection, Gut, Virulence factors, Gastro-intestinal (GI).

INTESTINAL MICROFLORA

The large intestine supports the growth of commensal bacteria known as intestinal microflora. In the large intestine, these bacteria interact with, and colonise the epithelial cells. These bacteria survive within the GI tract, receiving nutrients from the host, whilst providing the host with essential nutrients and benefits (6). Intestinal microflora normally persists for long periods of time and their population size, depending on the species, can vary between $10^8$ and $10^{12}$ bacteria per gram of colon contents (9). Major bacterial species/genus that inhabit the large intestine are Bifidobacterium, Bacteroides, Lactobacillus, Clostridium, Fusobacterium and Enterobacteria (2). It is well established that a stable intestinal microflora can prevent the growth of pathogenic strains which have survived the gastric juice of the upper GI tract and have reached the large intestine (1, 7, 10). Intestinal microflora has an important role in preventing intestinal diseases by competitive inhibition of pathogenic strains, and due to a high number of bacteria and their functions it has been referred to as an organ of the body (3). Intestinal microflora has three main functions and effects on the mucosa: protective, structural, and metabolic (1, 5). It provides a protective function by competition with pathogens for nutrients and receptors within the GI tract. Some strains of this flora, such as lactic acid bacteria, produce bacteriocins and lactic acid which inhibit the growth of other bacterial species (1). The metabolic functions of this flora include the synthesis of essential vitamins such as biotin and folate, and fermentation of non-digestible dietary residues (3). Whilst the GI tract has many protective functions to prevent adherence of pathogenic strains, some functions encourage the growth of bacteria. The mucus layer contains mucins which produce saccharides that are utilised as a source of energy by commensal and pathogenic strains (8).

Many factors can impact homeostasis of intestinal microflora such as changes in diet, physical stressors, and degenerative and infectious diseases (10, 11). Food has been identified as a source for new bacterial species to enter the body and persist as microflora (12). Common foods such as yoghurt contain healthy bacteria known as probiotics, which have a protective role in the gut (13). Bifidobacteria are also common gut species with a protective role however, elderly males and females have shown to have lower populations of these protective bacteria than younger adults (14). Molecular based approaches have identified a shift in dominant microflora species amongst the elderly...
population towards a greater diversity of bacterial species than younger adults and children (15). Furthermore, fewer species isolated from elderly are able to be cultivated (15). Under certain conditions some of these bacteria are able to cause disease of extra-intestinal sites (3, 15). A common commensal GI tract bacterium responsible for many intestinal and extraintestinal infections is *Escherichia coli* (16).

**E. coli**

*E. coli* is commonly found in the large intestine of humans and other warm-blooded animals (2). These strains can be commensal, existing in a symbiotic state providing resistance against pathogenic organisms, or be pathogenic and cause diseases of intestinal and extra-intestinal sites (6). *E. coli* is found in relatively lower numbers than other major commensal bacteria; however, it is the most common cause of intestinal and extra-intestinal disease (10). The pathogenic strains of *E. coli* may carry several virulence factors directly involved in pathogenesis of these bacteria, although commensal strains may also cause disease in immunocompromised hosts (10, 17).

**Phylogenetic groups of E. coli**

Commensal and pathogenic *E. coli* can be collectively classified into four different phylogenetic groups; A, B1, B2, and D (18). Phylogenetic groups A and B1 mainly consist of commensal strains found in the large intestine of humans and animals, as well as in environmental samples (19). These strains do not normally carry any known virulence factors (20). In contrast, phylogenetic group B2, and to a lesser extent, group D, consist of pathogenic strains and normally carry virulence-associated genes (VGs) that are mostly associated with extra-intestinal diseases (18, 20). Some reports indicate that strains belonging to phylogenetic groups A and B1 can also cause disease of extra-intestinal sites (19), although this has not been consistently supported (21). A recent phylogenetic group E has also been identified, however it is uncommon, and is not widely used (22, 23). This group shares alleles with many *E. coli* strains and includes the enterohaemorrhagic strain O157:H7 (22). Pathogenic *E. coli* strains can cause three major types of infections: (i) enteric or diarrhoeagenic disease, (ii) UTI, and (iii) blood infection or sepsis and meningitis (24, 25).

**Diarrhoeagenic E. coli**

Diarrhoea is more common in developing countries and children under five due to poor sanitation and hygiene (26, 27). Intestinal pathogenic *E. coli* are responsible for majority of these cases worldwide (25). *E. coli* that cause disease of the intestinal tract are referred to as diarrhoeagenic *E. coli* (25). Diarrhoeagenic *E. coli* strains rarely translocate the GI epithelium and their pathogenic affect is mostly restricted to pathophysiological changes of the intestinal epithelial cells (6). The major pathotypes are enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EA-ggEC), and diffusely adherent *E. coli* (DAEC) (25). Since this review focuses on ExPEC strains, the mechanisms by which diarrhoeagenic strains of *E. coli* cause intestinal infection will be briefly discussed here.

Unlike commensal *E. coli*, diarrhoeagenic strains carry specific surface adhesins which enhance their ability to colonise the GI tract. Once established in the GI tract, the six pathotypes vary in their disease patterns and intensity (25). ETEC is known as traveller’s diarrhoea, and like many other diarrhoeagenic strains, it is associated with poor hygiene. ETEC strains produce two toxins, a cholera-like toxin called heat-labile toxin (LT), and a peptide hormone-like toxin called heat-stable toxin (ST) (6, 25). EPEC strains express bundle forming pili (BFP) which are involved in bacterium-to-bacterium adhesion and promotes the formation of bundles. These structural changes cause diarrhoea through the loss of absorptive capacity. EA-ggEC strains cause persistent diarrhoea in children which is believed to be caused by an unidentified diarrhoeal toxin, EA-ggEC heat-stable enterotoxin I (EAST1) (28). EHEC strains bind to mucosal cells through actin reorganisation as seen with EPEC strains. These strains produce Shiga-like toxin, with receptors found on intestinal cells and in the kidney, which can lead to kidney failure known as haemolytic uraemic syndrome (25).

EIEC is the only invasive pathotype, penetrating epithelial cells in an endocytic vesicle. These strains lyse vesicles to multiple within cells and then move through the cytoplasm to adjacent epithelial cells (29). Active invasion of colonic cells causes bloody diarrhoea. The most recent pathotype is DAEC which binds to the small bowel in a diffuse adhesion pattern, however there is limited research on the exact mechanism of action of this pathotype (30).
Other virulent strains of *E. coli* may cause a large range of diseases outside the GI tract (31). These pathotypes are referred to as ExPEC and are divided into UPEC which is the most common pathotype of ExPEC found among patients with UTI, strains causing neonatal meningitis and septicemia (32).

### Urinary tract infection

UTI is one of the most common diseases worldwide. Although it is one of the easiest treatable diseases, it may cause serious complications in healthy individuals if not optimally treated, with many women experiencing recurrent episodes (32). UTI has been defined as the presence of significant numbers of pathogenic bacteria or organisms in the urinary system, depending on the presence or absence of symptoms while recurrent UTI can be defined as two or more episodes within 6 months or three or more episodes within 12 months (33). UTIs are more common among females, with up to 60% of women having at least one episode in their lifetime (34). The disease is the most frequent hospital-acquired infection, affecting mainly women, children, and the elderly (35). 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 likelihood of contracting UTI (33, 36).

The lower GI tract is a common source of UTI causing bacteria, with some strains able to colonise the vagina (37-40). The UTI-causing strains have certain VGs that distinguish them from other commensal *E. coli* of the gut. These strains often originate from the stool where they enter the UT via colonisation of the vaginal introitus and the periurethral area (6). Bacteriuria is the presence of these bacteria in the UT when the patient has no physical symptoms normally associated with UTI such as pain, frequency and urgency (24). Under these conditions, *E. coli* strains exist in an asymptomatic carrier state without any obvious symptoms of UTI (41). *E. coli* strains that colonise the UT may ascend towards bladder to cause cystitis, which is usually associated with the classic symptoms of UTI, i.e. pain, frequency, and urgency. UTI can proceed from the bladder, via the ureters to the kidney, to cause pyelonephritis, with the possibility of causing irreversible kidney damage leading to kidney failure and death (42).

UPEC strains have VGs that distinguish them from other strains. Some of these VGs such as adhesins are essential for initiating infection in the host, whereas others (e.g. toxins) are responsible for damaging the host to benefit the bacterium. In UPEC strains adhesion is vitally important to overcome host defences in the UT and resist the flushing effect of the urine (6). UPEC can also carry VGs important for survival in body sites lacking essential metabolites, such as mechanisms for acquiring iron. Although *E. coli* is the most common cause of UTI, responsible for >90% of cases in young adults (37), and >60% of cases in elderly individuals (43), in chronic and complicated UTIs bacterial strains are often mixed (44).

### Bacterial Persistence

Women with UTIs that are not optimally treated, have up to 44% chance of recurrence of infection within 12 months (33). A recent study has found 67% of recurrent UTIs were caused by the same *E. coli* strain (37). This and many other results suggest a reservoir for these strains that enable them to cause recurrent infections (38, 39, 45). The former study has also found that the infecting strains for recurrent UTI to be present in the faecal flora of 78% of women, however the dominance of these clones was not reported (38). Moreno and co workers found 30 out of 42 patients carried a UTI-causing strain as the dominant faecal clone, however persistence of the strain in the GI tract was not measured (38). These findings reinforce the importance of faecal flora as a major reservoir for UTI-causing strains. In the case of recurrent UTI it is also shown that the responsible *E. coli* strains may protect themselves from the effect of antibiotics by hiding in the bladder cells. Using a mouse model of UTI it has been suggested that *E. coli* lie dormant within the bladder mucosa (46).

### ExPEC strains causing septicemia

Septicaemia is the presence of microorganisms in the blood (6). ExPEC strains are the most common group of microorganisms that cause septicemia. They may enter the blood through open wounds and burns, or may translocate from the kidney or GI tract, a process called bacterial translocation (47). Severe septicemia is known as sepsis and is associated with increased morbidity and mortality (48). Septicaemia which has originated from the UT is known as urosepsis (49, 50). From the blood, ExPEC strains can
infect distant organs, causing multiple organ failure and shock (51). A recent study investigating the virulence of septicemic E. coli strains has indicated these strains may carry a repertoire of VGs ranging from adhesins to iron acquisition system (52).

**ExPEC strains causing meningitis**

Meningitis is inflammation of the protective layers of the brain and spinal cord (53). It is a life threatening infection more commonly associated with neonates, and to a lesser extent, immunocompromised children and adults (54). The diseases now-a-days, is commonly caused by E. coli. Infection of the meninges differs from other body site attributable to the necessity for strains to cross endothelial cells which form the blood brain barrier. Meningitis-associated E. coli have acquired VGs to cross the blood brain barrier, most commonly K1 capsule polysaccharide and S-fimbriae, which enable E. coli to cross via transcytosis (5, 55). K1 capsule also protects the bacterium with serum resistance and antiphagocytic properties (54). E. coli strains that cause extra-intestinal infections such as UTI, meningitis and septicaemia have VGs which enable their survival and infection.

**VIRULENCE FACTORS OF EXPEC**

There are a number of VGs that help extra-intestinal E. coli to survive the hostile environment of the GI tract and persist in extra-intestinal sites to cause infection. These include adhesins (e.g. type 1 fimbiare and P-pili), toxins (e.g. haemolysin and cytotoxic necrotizing factor), polysaccharide capsules (e.g. K1 and K5), and siderophores (e.g. iron transport systems aerobactin and novel catecholate siderophore E. coli). Many of these VGs are controlled by phase-variable genetic switches that control their expression dependant on the environment conditions such as pH, osmolarity, temperature and amino acid concentration (24, 56). Two of the most studied adhesins amongst UPEC are type 1 fimbiare and P-pili.

**Type 1 fimbiare**

Type 1 fimbiare are filamentous organelles that cover the surface of the bacterium (57). These fimbiare are encoded by the fim gene cluster, with the structural components of the fimbiare composed of fimA, fimF, fimG, and fimH, and the pilus encoded by fimC and fimD (58). fimH is the tip of the pilus which mediates binding to glycoproteins (58, 59). These fimbiare bind to oligosaccharides which are located on the cell membrane of body cells, such as epithelial cells.

Type 1 fimbiare are the most common VG amongst UPEC, and are expressed by more than 80-90% of all E. coli, including commensal bacteria and pathogens (59). Due to the frequency of type 1 fimbiare amongst pathogenic and commensal strains, it has been concluded that this fimbiare has no correlation with UTI (24). On the other hand, several studies have shown that type 1 fimbiare are crucial during the first stages of infection by UPEC and mediate binding to urethral epithelial cells (57). Type 1 fimbiare, specifically fimH, have also been shown to have a critical role in activation of mast cells in the bladder epithelium, which are an inflammatory cell activated during UTI (57, 60). Activation of mast cells is associated with the release of histamine, which initiates the body’s inflammatory response to clear the infection, and is typically associated with the symptoms of UTI such as pain and frequency (60). Type 1 fimbiare lacking fimH are unable to invade bladder cells, emphasizing the importance of fimH for establishing infection of the UT (61). Tamm-Horsfall protein (THP) coats uroepithelial cells with the main role to bind type 1 fimbiare to prevent bacteria from adhering to epithelial cells (62). Distribution of THP varies throughout the UT which may influence results of different research methodologies (24).

UTI can proceed to the kidneys to cause pyelonephritis. THP is abundant within renal epithelial cells, which binds to UPEC strains expressing type 1 fimbiare, to prevent their adherence in the kidney (62). Although type 1 fimbiare are clearly important for adhesion to the uroepithelium, other VGs are needed for subsequent invasion of epithelial cells and infection. Expression of type 1 fimbiare is co-regulated with the expression of P-pili which are associated with with UTI and pyelonephritis. Bacteria are able to switch the production of VGs on or off, depending on the environment and the needs of the bacteria, to create many different phenotypes (56). Expression of the fim gene cluster is controlled by fimS (63, 64) and is co-regulated with expression of P-pili, with each bacterium expressing only one at a time (63, 64). Studies have shown papB (a regulatory protein of P-pili) inhibits the activity of fimB and increases fimE activity, both work together to inhibit the expression of type 1 fimbiare from being switched into the ‘on’ position, hence inhibiting type 1 fimbiare production (65, 66). Phase-switch ability
may account for differential expression of type 1 fimbriae in different body sites. A review article by Johnson (24) has analysed the expression of type 1 fimбриae and reported expression of similar portions in urinary (64%) and faecal strains (60%) with blood isolates having an increased expression (80%). These findings collectively suggest an important role of type 1 fimбриae among septicaemic strains of E. coli although their exact mechanism in this process is not known. Much research had been conducted on the prevalence of type 1 fimбриae with conflicting results. Several studies have found high expression of type 1 fimбриae amongst pyelonephritis, cystitis (67), or faecal isolates, and other studies have reported equal expression of this pilus among pyelonephritis and cystitis strains (60). These studies varied widely in laboratory culture conditions, age, gender, and geographical location of subjects which are likely factors involved in varied results. The switch-phase variation with P-pili could be responsible for the differential production of these fimбриae amongst cystitis and pyelonephritis strains, and may be responsible for variable expression amongst body sites and studies (67). The role of switch-phase of type 1 fimбриae in septicaemia is not well studied. Amongst E. coli strains carrying capsular antigen K1, type 1 fimбриae mutants with phase lock ‘on’ have been found to be more pathogenic during meningitis than those with phase lock ‘off’ (68).

Despite this, type 1 fimбриation has been shown to be associated with decreased bacteraemia amongst neonatal rats (69). Variable expression of adhesins and other virulence factors enables bacteria to adjust to different environments encountered during the infection process (56, 64).

Several allelic variations of the fimH gene have been identified, with at least three phenotypes having slightly different binding ability (70, 71). Pat least one study has suggested low-binding and high-binding variations of fimH to mannose residues (70). In this study approximately 80% of bacteria from the large intestine of healthy humans expressed low-binding to mannose, whereas 70% of bacteria isolated from UTI expressed high-binding to mannose (70). Although type 1 fimбриae are found amongst a majority of commensal and pathogenic E. coli strains, allelic variations may exist between these two groups.

Whilst type 1 fimбриae have an important role during colonisation of the UT, their role in colonisation of the GI tract is uncertain (31, 43, 72). These fimбриae are frequently found amongst faecal flora and are thought to be important for E. coli colonisation of the GI tract (45, 73).

fimH is also able to attach to different cell types such as erythrocytes and macrophages, however attachment is thought to occur through different mechanisms than epithelial cells (75). These fimбриae encourage adherence to phagocytes and subsequent phagocytosis (24). Once phagocytosed, type 1 fimбриated bacteria can survive within vacuoles as long as they are unopsonised (76). This remarkable ability helps bacteria to overcome host defences and persist to cause disease.

The GI tract has a range of protective defences, of which slg A is an important host defence against enteric bacteria (77). GI epithelial cells secrete slg A in mucus, which bind mannose-containing oligosaccharides and agglutinate bacteria in the gut to prevent epithelial cell adherence and penetration of the intestinal barrier. Mucin and slgA may contribute to this process by binding type 1 fimбриae and trapping bacteria in mucus (77). Production of a biofilm by commensal bacteria helps prevent pathogenic bacteria from adhering to and translocating the GI epithelial barrier (77). Type 1 fimбриae have also been shown to be important for the formation of intracellular bacterial communities, which have similar functions to biofilms (61).

P-pili

Another important adhesion for UPEC is pyelonephritis-associated pili, known as P-pili. P-pili are the second most common adhesion in UPEC strains, after type 1 fimбриae (24). These pili are associated with UTI and pyelonephritis, and have been isolated from more than 80% of pyelonephritis causing strains (78). P-pili are assembled by proteins from a pap gene cluster composed of subunits papE, papF, papG, papA, papH, papC. Other pap genes are important for fimбриae assembly but are not directly related to adhesion, such as papD which is responsible for stabilising translocation (6). papB is involved in regulating expression of type 1 fimбриae, as previously discussed (56, 64).

P-pili are common VGs of E. coli strains belonging to phylogenetic groups B2 and D, and are regularly found among strains that cause UTI (38, 79). While type 1 fimбриae are important for initial infection of the lower UT, P-pili are involved in colonisation of the upper UT (43, 64, 80). Furthermore, recent evidence
suggests that type 1 fimbriae and P-pili are inversely regulated, with individual bacteria expressing only one type of fimbriae sequentially (64). Regulation of genes appears to be related to pathogenesis by enabling sequential colonisation of different UT tissues (80, 81). Hence, both fimbriae play an important role in the survival and pathogenesis of UPEC, firstly in colonisation of the GI tract, and then invasion of the UT (38, 43, 73, 74).

P-pili were firstly recognised by the ability to agglutinate human type O erythrocytes without inhibition by mannose, distinguishing it from type 1 fimbriae (6). It has been shown that a common P blood group antigen glycosphingolipid with a lipid moiety and carbohydrate chain to be the receptor for P-pili, α-D-Gal-(1→4)-β-D-Gal (Gal-Gal moiety) (82). This antigen is present in glycophosphatidylinositol in humans and is found abundantly on the surface of epithelial cells lining the UT (82). The presence of this receptor on epithelial cells of the GI tract has not been fully investigated but it has been shown that resident E. coli strains of healthy adults contain a high rate of P-piliated E. coli strains (31). Attachment of P-pili to the receptor leads to the release of ceramide, acting as an agonist of Toll-like receptor 4, activating immune cell response (83). Epithelial cell activation leads to the production of cytokines and chemokines, such as interleukin (IL)-6, IL-8 and neutrophils (84). This in turn leads to the development of local inflammation and pain associated with UTI (84). P-pili expression of asymptomatic UTI has been found to be less than cystitis and pyelonephritis causing strains (85). Gal-Gal moiety receptors are found in larger amounts in renal glycolipids than those from shed uroepithelial cells, accounting for P-pili association with pyelonephritis. Receptor density (Gal-Gal moiety) on human uroepithelial cells are equal amongst men and women (24), suggesting other factors are important for increased UTI incidents among women.

Unlike type 1 fimbriae, P-pili do not adhere freely to human polymorphonuclear leukocytes (hPMNLs), given that these cells only produce small amounts of Gal-Gal receptors (83). In strains that also express type 1 fimbriae, P-pili may defend hPMNLs from adhering to and destroying the bacterium (6).

Healthy humans are believed to have a reservoir of ExPEC strains, belonging to phylogenetic group B2, and to a lesser extent, group D, which have superior ability to survive and persist in the gut of humans (86), and can spread to cause disease (87). Interestingly, it has been suggested that P-pili expression enhances colonisation in the GI tract (31, 38, 73, 88). Wold et al (31) studied the prevalence of E. coli strains carrying this VG among the resident strains of the gut in healthy individuals and found a majority carried P-pili. Based on these results, it has been suggested that P-pili have evolved in E. coli strains to promote their persistence in the gut by attaching to Galα1g4Galß-containing receptors on gut epithelial cells (73). Wold et al (74) also found type 1 fimbriae bound to colonic cells and to a substance loosely associated to the epithelium, however, P-pili only bound to the loosely associated substance and not the colonic cells (74). Continuous shedding of epithelial cells in the large intestine with Gal-Gal receptors may provide a niche for bacteria containing adhesins to establish colonisation within the gut (74). Gal-Gal binding strength to the UT epithelium is greater than that to colonic epithelial cells, and remains stronger after repeat washes, indicating that P-pili are well adapted for adherence in the UT. The dominance of these clones in the GI tract, and the presence of VGs are contributing factors of UTI (38). This suggests that colonic E. coli strains, which are persistent in the GI tract, have mechanisms associated with UTI that may be involved in early colonisation of the urethra (31, 73, 79, 86).

Resident strains of E. coli in infants, school girls, and young women commonly belong to the phylogenetic group B2 (79, 86, 90) and express P-pili and type 1 fimbriae more commonly than transient strains (86). For infants (aged 3 days to 12 months) half of these resident commensal B2 strains carried papC genes, which is believed to lead to persistence within the GI tract (79, 86). Zhang and co-workers found P-pili amongst young women (aged 18 – 39 years) was strongly associated with phylogenetic groups B2 and D (90). However group B2 had two distinct subgroups with differing levels of pathogenicity. Zhang’s findings suggest that healthy adults are capable of carrying B2 strains however with less virulent subclasses than UTI isolates (90). Contrary to these findings, Schlager et al (89) found in healthy young girls (aged 3 to 6 years), resident strains with P-pili were not associated with UTI, despite clones of non-dominant strains with P-pili present in the UT. This was based on the findings that dominant clones in the GI tract varied weekly and did not reflect those in the urine, hypothesising that UTI strains are in the gut for only a short period. These results differ from studies of women (15 – 65 years) with dominant faecal clones
representing the same urine clone however the study design only included one faecal sample at the time of UTI (38). Further studies have found dominant clones of the faecal flora are more likely to spread to the UT (38, 89, 90). Incidents and pathogenesis of male UTI are not as frequently studied as females (91).

P-pili mediate binding to epithelial layers containing Galα1g4Galβ through the use of the adhesion molecule papG located at the tip of the pilus (92, 93). There are three types of papG, each with slightly differing binding ability, these are papG allele I, papG allele II, and papG allele III (92, 93). The prevalence of papG allele I is debated, with some authors concluding it is uncommon and rarely found in humans (93), whilst others report its presence amongst uropathogenic isolates (94). PapG allele II binds to globoside, which is located in the human kidney (95) and is more commonly associated with acute pyelonephritis. Animal model studies have found that papG allele II enhances early colonisation of the kidney, however due to host immune defences, infection was not maintained (93). Cystitis is more commonly associated with papG allele III. The association of papG allele III with cystitis but not pyelonephritis or bacteraemia may be indication that this papG variant is not sufficient for invasion of the bloodstream in non-compromised hosts (93). A study by Otto et al indicates that whilst papG allele II was associated with healthy women of all ages, papG allele III was more common in men (96), though this study had a small sample size for male UTI. During bacteraemia, papG allele II has shown to be associated with E. coli urosepsis, and papG allele III associated typically with compromised hosts such as those with immunosuppression or UT abnormalities (93). Furthermore, papG allele II has been identified as the predominant variant in E. coli bacteraemia (93). Limited information is available on the role of papG variants for intestinal persistence, except that papG class I and II recognise the same receptors in the small intestine and presumably colonic proteins loosely associated with epithelial cells (97).

Haemolysin

Alpha haemolysin is a pore-forming toxin secreted by pathogenic E. coli to lyse erythrocytes and human renal epithelial cells (98). Lyses of erythrocytes releases iron which can be utilised through siderophore systems, hence the production of haemolysin is often regulated by iron availability. Haemolysin is toxic to many cells, leading to inflammation, tissue damage, and disruption of phagocyte function (24, 98). Alpha haemolysin is encoded chromosomally by the gene hlyA, compared to animals which are encoded in plasmids with differing nucleotide sequences (17). Unlike other toxins, hlyA gene is expressed without cleavage of peptides or cellular lysis (99). Haemolysin is most active during log phase of growth however activity declines when bacteria reach stationary phase despite continued cell production. This decrease is mainly due to toxic effects of excessive production to E. coli (99). Haemolysin production is increased during times of low iron concentration, and decreased in high iron situations (100).

Haemolysin is seen in E. coli strains associated with upper UTIs such as pyelonephritis, and is more common amongst invasive uropathogenic strains than healthy faecal isolates (24). It has been reported that an average 12% of faecal E. coli produce haemolysin, with similar results supported by other authors (101). Haemolysin producing strains are more prevalent amongst hosts without immune compromising conditions such as renal scarring or pregnancy (24).

Cytotoxic necrotising factor

Similar to α-haemolysin, cytotoxic necrotising factor 1 (CNF1) is also encoded chromosomally by cnf1, with both toxins often co-expressed in UPEC strains (24). CNF1 targets the Rho family of GTP-binding proteins and induces actin cytoskeleton reorganisation, leading to apoptosis, which facilitates bacterial invasion into deeper tissue layers of the UT (102, 103). This process enables bacteria to persist within the UT (104). CNF1 is not as well understood as other UPEC virulence factors. Some authors have reported CNF1 production to promote progressive infection (104-106), whereas others have suggested no impact within the UT (107). These differences may be attributable to rat models (106), cell lines (105), and human models (107). Haemolysin and CNF1 are strongly associated with phylogenetic group B2, with a stronger association amongst UTI strains than faecal isolates (90, 108, 109).

Siderophores

Iron is essential for normal bacteria metabolism (110). Free iron in human hosts is limited and not easily accessible to bacteria. Iron in the body is usually found as haemoglobin and heme (111). Bacterial infection can induce an acute-phase response known as
hypoferremia to further reduce iron availability (112). During infection, the body reduces the amount of iron available to bacteria, decreasing iron absorption from the gut and storing iron intracellularly (110). The release of transferrin tightly binds free iron, limiting the availability of iron for bacteria. In response to times of limited iron availability, some bacteria release iron chelators known as siderophores, which bind to iron with high affinity (110). There are many types of siderophores which function in different ways, some work to scavenge iron, whilst others compete with host defences to release iron from transferrin and lactoferrin (110). Iron is transported into the cell via outer membrane receptor proteins on the surface of the bacterium specific for each siderophore (110).

In *E. coli*, ferric aerobactin receptor is encoded by the gene *iutA*. Aerobactin has been strongly associated with pyelonephritis, cystitis, and bacteremia as opposed to asymptomatic bacteriuria or faecal strains (24). Aerobactin has been associated with 45 – 78% of UPEC strains (17, 113), with an increased association with bacteremia strains (113). Aerobactin is also associated with persistence in the GI tract, and is more common amongst resident than transient *E. coli* strains hence it may give a competitive advantage for survival and persistence in the GI tract (79, 88). As previously discussed, haemolysin also plays an important role for iron acquisition (114). It has been suggested as an alternative iron acquisition method, with clinical studies finding blood isolates lacking aerobactin were in fact positive for haemolysin production (114). Another identified iron siderophore is *iroN* <sub>E. coli</sub>. This catecholate siderophore gene is associated with pathogenesis of UPEC and is often coupled with other VGs (115).

**Capsular polysaccharide**

Capsules are mainly a polysaccharide structure covering bacteria which acts to protect the bacterium from the host immune system (6). Capsules enhance serum survival, and help to facilitate bacteria to avoid O antigen detection and hence bacterial phagocytosis by hPMNLs (116). The production of capsules, mainly K1 and K5, are more commonly seen amongst resident strains of the gut, with capsular antigen K5 recognised to enhance gut colonisation (21, 117). Similarly, capsular antigen K1 has been shown to efficiently colonise the large intestine (72), with greater frequency amongst adults than infants. Whilst capsule synthesis is common amongst faecal isolates, synthesis is significantly higher amongst UPEC strains (118). In the UT, capsules help to enhance survival by avoiding phagocytosis (116). *E. coli* strains expressing K1 capsule are the major cause of Gram-negative bacteraemia and meningitis in neonates and have been reported in *E.coli* strains causing cystitis (119).

**SURVIVAL OF UPEC IN THE GUT**

Bacterial colonisation of the large intestine is the first stage in the development of enteric, urinary and systemic infections (72). Resident strains are those which are present in the gut from months to years, whereas transient strains are present for a short period of time, usually days to weeks (73, 86). Resident strains are able to adhere and colonise the gut, and mostly constitute the dominant clones in the GI tract. Some studies have found resident strain commonly belong to phylogenetic group B2 and carry a number of VGs (79), whereas others found a majority belonged to commensal phylogenetic groups A and B1 (38, 120).

To survive the human GI tract, bacteria must evade host defences, acquire nutrients for survival, and competitively compete with other microflora (1, 10). Some VGs play a dual role to enhance colonisation of the GI tract and cause infection (38, 121). Bacteria with adhesin molecules specific to gut epithelium have therefore a better chance to colonise the GI tract. P-pili contribute to colonisation of the gut, with resident strains having higher expression than transient strains (31, 73, 74, 79). Studies of healthy adults have found up to 50% of *E. coli* strains carry P-pili (121, 122). However these results vary widely and may be attributable to geographical and lifestyle differences (122). Type 1 fimbriae are also believed to enhance colonisation of *E. coli* in the GI tract (74). Other VGs such as the aerobactin and capsule synthesis may also contribute to persistence in the large intestine (88). Contrary to the production of VGs for enhanced colonisation of the GI tract, Siitonen (101) found a low VGs profile amongst faecal *E. coli* isolates, leading to the conclusion that the gut is not a reservoir for infection. However, this has not been found by other researchers, with many supporting VGs enhancing colonisation of intestinal *E. coli* (73, 74, 79, 93, 121). This inconsistency in results suggests other factors influence the population structure of *E. coli* and their VGs in colonisation of GI tract.
**Gut as a source of UTI**

The gut is a common source of bacteria causing UTI. These bacteria can survive and persist in the gut from a few days to many years (10, 79). The finding that *E. coli* strains causing UTIs have been consistently isolated from the faecal flora of the same host (38, 45) strongly supports the gut-origin of UTIs. Intestinal *E. coli* strains causing UTI normally carry VGs necessary for colonisation of the UT and mainly belong to phylogenetic group B2 (90). Jantunen *et al* (50) found *E. coli* strains harbouring *papG* genes were the major facultative flora of the gut in children with urosepsis (aged 1 – 24 months). The way in which these strains come to inhabit the gut is not well established, with ingestion of contaminated poultry proposed as a source of UPEC strains (12, 123). Furthermore, birds have been suggested as reservoir of UPEC although additional reservoirs are likely to exist (124).

**UPEC and septicaemia**

In complicated cases of UTI or in compromised hosts, a simple UTI can lead to a serious infection known as septicaemia. From the kidneys, bacteria can enter the blood stream to cause sepsis (125). Urosepsis is the term given to patients with UTI associated sepsis, with the kidney believed to be the source of septicaemia. Studies into the incidents of urosepsis vary, with one study finding 5.9% of sepsis incidents originating from UTI (126), and another reporting a majority at 58.3% (127). Once bacteria have entered the blood they can infect other body organs to cause multiple organ failure, shock, and death (48, 49). Increased bacterial densities also increase the likelihood of a strain colonising the opening of the urethra to cause UTI. In urosepsis, *E. coli* is assumed to move from the UT and kidney to the blood. The gut has also been established as a reservoir of extraintestinal *E. coli*, with patients experiencing UTI, septicaemia, urosepsis or meningitis typically carrying strains with VGs amongst faecal flora (39, 128). Commensal and less virulent UPEC strains have been shown to cause disease in immunodeficient hosts as opposed to uncompromised hosts (17). Therefore, in elderly people, these bacteria have a better ability to translocate to the blood directly from GI tract rather than the UT.

**EVOLUTION OF UPEC AND CONCLUDING REMARKS**

It is widely accepted that UPEC strains originate from faecal flora, however whether their pathogenesis is due to higher prevalence within the faecal flora, or the acquisition of VGs, is widely debated (129). The “prevalence theory” holds that strains causing infection are predominant within the faeces, increasing the likelihood of these strains colonising the opening of the UT to cause UTI. This is opposed to the “special pathogenicity theory” based on the selective advantage of *E. coli* VGs specifically for infection of extra-intestinal sites (129). Irrespective of the evolutionary influences in the development of these VGs, UPEC are well developed for survival in extra-intestinal sites such as the UT. Furthermore, evidences suggest that VGs which contribute to fitness within the UT are likely to enhance GI tract colonisation (31, 85). Intestinal *E. coli* amongst infants, girls, and young women in health and during UTI has been widely studied, however; comparisons between genders and elderly individuals are limited. A review of the existing literature indicates that *E. coli* population structures between young adults and elderly, and genders, could be a predisposing factor to UTI with the GI tract being an established reservoir of infection. Furthermore, *E. coli* populations amongst elderly with weakening immune systems may be an important predisposing factor to disease, especially amongst hospitalised patients. Further researches are needed before these questions can be fully answered.

**REFERENCES**

1. Guarnier F, Malagelada J. Gut flora in health and disease. *Lancet* 2003; 360: 512-519.
2. Campbell NA, Reece JB. Biology, Pearson Education Inc, San Francisco, 2002.
3. O’Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO* reports 2006; 7: 688-693.
4. Yu Q, Yang Q. Diversity of tight junctions (TJs) between gastrointestinal epithelial cells and their function in maintaining the mucosal barrier. *Cell Biol Int* 2009; 33: 78-82.
5. Baumgart DC, Dignass AU. Intestinal barrier function. *Curr Opin Clin Nutr Metab Care* 2002; 5: 685-694.
6. Salyers AA, Whitt DD. Bacterial pathogenesis: a molecular approach, ASM Press, Washington DC, 2002.
7. Dogi CA, Galdeano CM, Perdigón G. Gut immune stimulation by non pathogenic gram (+) and gram (-) bacteria. Comparison with a probiotic strain. *Cytokine* 2008; 41: 223-231.
8. Moal VL, Servin AL. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clin Micro Rev* 2006; 19: 315-337.
9. MacFie J. Current status of bacterial translocation as a cause of surgical sepsis. *Brit Med Bull* 2004; 71: 1-11.
10. Stecher B, Hardt W. The role of microbioita in infectious disease. *Trends Microbiol* 2008; 16: 107-114.

11. Šmeňilová M, Vlková E, Nevorál J, Flajšmanová K, Killer J, Rada V. Comparison of intestinal microflora in healthy infants and infants with allergic colitis. *Folia Microbiol* 2008; 53: 255-258.

12. Rodríguez-Siek KE, Giddings CW, Doctcott C, Johnson TJ, Fakhri MK, Nolan LK. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology* 2005; 151: 2097-2110.

13. Guarné F, Perdigon G, Corthiér G, Salminen S, Koletzki B, Morelli L. Should yoghurt cultures be considered probiotic? *Brit J Nutr* 2005; 93: 783-786.

14. Gavini F, Cayuela C, Antoine J, Lecocq C, Lefebvre B, Membré J, et al. Differences in the distribution of bifidobacterial and enterobacterial species in human faecal microflora of three different (children, adults, elderly) age groups. *Microbial Ecol Health Dis* 2001; 13: 40-45.

15. Saunier K, Dore J. Gastrointestinal tract and the elderly: functional food, gut microflora and healthy ageing. *Digest Liver Dis* 2002; 34: S19-S24.

16. Bailey MT, Engler H, Sheridan JF. Stress induces the translocation of cutaneous and gastrointestinal microflora to secondary lymphoid organs of C57BL/6 mice. *J Neuroimmunol* 2006; 171: 29-37.

17. Johnson JR, Moseley SL, Roberts PL, Stamm WE. Aerobacterin and other virulence factor genes among strains of *Escherichia coli* causing urosepsis: association with patient characteristics. *Infect Immun* 1988; 56: 405-412.

18. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl Environ Microbiol* 2000; 66: 4555-4558.

19. Rijavec M, Müller-Premru M, Zakotnik B, Žagar-Bertok D. Virulence factors and biofilm production among *Escherichia coli* strains causing bacteremia of urinary tract origin. *J Med Microbiol* 2008; 57: 1329-1334.

20. Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. *J Infect Dis* 2001; 183: 78-88.

21. Bingen-Bidiois M, Clermont O, Bonacorsi S, Terki M, Brahimi N, Loukit C, et al. Phylogenetic analysis and prevalence of urosepsis strains of *Escherichia coli* bearing pathogenicity island domains. *Infect Immun* 2002; 70: 3216-3226.

22. Gordon DM, Clermont O, Tolley H, Denamur E. Assigning *Escherichia coli* strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. *Environ Microbiol* 2008; 10: 2484-2496.

23. Escobar-Páramo P, Clermont O, Blanc-Potard A, Bui H, Bouquénc CL, Denamur E. A specific genetic background is required for acquisition and expression of virulence factors in *Escherichia coli*. *Mol Biol Evol* 2004; 21: 1085-1094.

24. Johnson JR. Virulence factors in *Escherichia coli* urinary tract infection. *Clin Microbiol Rev*1991; 4: 80-128.

25. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998; 11: 142-201.

26. Qadri F, Svennerholm AM, Faruque ASG, Sack RB. Enterotoxigenic *Escherichia coli* in developing countries: Epidemiology, microbiology, clinical features, treatment, and prevention. *Clin Microbiol Rev* 2005; 18: 465-483.

27. Petri WA, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest* 2008; 118: 1277-1290.

28. Okeke IN, Nataro JP. Enterogaeggerative *Escherichia coli*. *Lancet Infect Dis* 2001; 1: 304-313.

29. Bekal S, Brousseau R, Masson L, Prefontaine G, Fairbrother J, Harel J. Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. *J Clin Microbiol* 2003; 41: 2113-2125.

30. Servin AL. Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clin Microbiol Rev* 2005; 18: 264-292.

31. Wolf AE, Caugant DA, Lidin-Janson G, deMan P, Svanborg C. Resident colonic *Escherichia coli* strains frequently display uropathogenic characteristics. *J Infect Dis* 1992; 165: 46-52.

32. Nicolle LE. Short-term therapy for urinary tract infection: success and failure. *Int J Antimicrob Agents* 2008; 31S: S40-S45.

33. Scholes D, Hooton TM, Roberts PL, Stapleton AE, Gupta K, Stamm WE. Risk factors for recurrent urinary tract infection in young women, *J Infect Dis* 2000; 182: 1177-1182.

34. Foxman B, Barlow R, D’Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. *Ann Epidemiol* 2000; 10: 509-515.

35. Tartof SY, Solberg OD, Riley LW. Genotypic analyses of uropathogenic *Escherichia coli* based on fimH single nucleotide polymorphism (SNPs). *J Med Microbiol* 2007; 56: 1363-1369.

36. Harrington RD, Hooton TM. Urinary tract infection risk factors and genders. *J Gender Specific Med* 2000; 3: 27-34.

37. Czaja C, Stamm E, Stapleton A, Roberts P, Hawn T, Scholes D, et al. Prospective cohort study of microbial and inflammatory events immediately preceding *Escherichia coli* recurrent urinary tract infection in women. *J Infect Dis* 2009; 200: 528-536.

38. Moreno E, Andreu A, Pigrau C, Kuskowski MA, Johnson JR, Prats G. Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal *E. coli* population of the host. *J Clin Microbiol* 2008; 46: 2529-2534.

39. Schlager TA, Hendley JO, Bell AL, Whittam TS. Clonal diversity of *Escherichia coli* colonizing stools and urinary tracts of young girls. *Infect Immun* 2002; 70: 1225-1229.

40. Obara-Yasuoka M, Ba-Thein W, Tsukamoto T, Yoshikawa H, Hayashi H. Vaginal *Escherichia coli* share common virulence factor profiles, serotypes and phylogeny with other extraintestinal *E. coli*. *Microbiology* 2002; 148: 2745-2752.

41. Mabbett AN, Ulett GC, Watts RE, Tree JJ, Totiska M, Ong CY, et al. Virulence properties of asymptomatic bacteriuria *Escherichia coli*. *Int J Med Microbio* 2009; 299: 53-63.

42. Scholes D, Hooton TM, Roberts PL, Gupta K, Stapleton
AE, Stamm WE. Risk factors associated with acute pyelonephritis in healthy women. Ann Intern Med 2005; 142: 20-27.

43. Snyder J, Haugen B, Lockatell CV, Maronec N, Hagan EC, Johnson DE, et al. Coordinate expression of fimbriae in uropathogenic Escherichia coli. Infect Immun 2005; 73: 7588-7596.

44. Nicolle LE. Urinary tract pathogens in complicated infection and in elderly individuals. J Infect Dis 2001; 183: S5-S8.

45. Moreno E, Andreu A, Perez T, Sabaté M, Johnson JR, Prats G. Relationship between Escherichia coli strains causing urinary tract infection in women and the dominant faecal flora of the same hosts. Epidemiol Infect 2006; 134: 1015-1023.

46. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent Escherichia coli reservoir during acute phase of a bladder infection. Infect Immun 2001; 69: 4572-4579.

47. Alexander JW, Gianotti L, Pyles T, Carey MA, Babcock GF. Distribution and survival of Escherichia coli translocating from the intestine after thermal injury. Ann Surg 1990; 213: 558-566.

48. Jaureguy F, Carbonnelle E, Bonacorsi S, Clec'h C, Jauréguy F, Carbonnelle E, Bonacorsi S, Clec'h C, Jantunen ME, Saxen H, Lukinmaa S, Ala-Houhala M, Alexander JW, Gianotti L, Pyles T, Carey MA, Babcock GF. Distribution and survival of Escherichia coli isolated from septicaemic and uroseptic patients. J Antimicrob Chemother 2007; 63: 630-631.

49. Johnson JR, Stell A. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 2000; 181: 261-272.

50. Jantunen ME, Saxen H, Lukinmaa S, Ala-Houhala M, Siitonen A. Genomic identity of pyelonephrogenic Escherichia coli isolated from blood, urine and faeces of children with urosepsis. J Med Microbiol 2001; 50: 650-652.

51. Reddy BS, Macfie J, Gatt M, Macfarlane-Smith L, Bitzopoulou K, Smelling AM. Commensal bacteria do translocate across the intestinal barrier in surgical patients. Clin Nutr 2007; 26: 208-215.

52. Ramos NL, Saayman ML, Chapman TA, Tucker JR, Smith HV, Faasgall J, et al. Genetic relatedness and virulence gene profiles of Escherichia coli strains isolated from sepsicemic and uroseptic patients. Eur J Clin Microbiol Infect Dis 2010; 29: 15-23.

53. Kaper JB, Nataro JP, Mobley HLT. Pathogenic Escherichia coli: Nat Rev Microbiol 2004; 2: 123-140.

54. Bingen E, Bonacorsi S, Brahim N, Denamur E, Elion J. Virulence patterns of Escherichia coli K1 strains associated with neonatal meningitis. J Clin Microbiol 1997; 35: 2981-2982.

55. Stins MF, Badger J, Kim KS. Bacterial invasion and transcytosis in transplanted human brain microvascular endothelial cells. Microbial Path 2001; 30: 19-28.

56. Holden NJ, Gally DL, Switches, cross-talk and memory in Escherichia coli adherence. J Med Microbiol 2004; 53: 585-593.

57. Martinez JJ, Mulvey MA, Schilling JD, Pinker JS, Hultgren SJ. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells EMBO J 2000; 19: 2803-2812.

58. Jones CH, Pinker JS, Roth R, Heuser J, Nicholes AV, Abraham SN, et al. FimH adhesion of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae. Proc Natl Acad Sci 1995; 92: 2081-2085.

59. Dhalak BK, Kulesus RR, Mulvey MA. Mechanisms and consequences of bladder cell invasion by uropathogenic Escherichia coli. Eur J Clin Invest 2008; 38: 2-11.

60. Abraham SN, Shin JS, Malaviya R. Type 1 fimbrinated Escherichia coli - Mast cell interactions in cystitis. J Infect Dis 2001; 183: S51-S54.

61. Wright KJ, Seed PC, Hultgren SJ. Development of intracellular bacterial communities of uropathogenic Escherichia coli depends on type 1 pil. Cell Microbiol 2007; 9: 2230-2241.

62. Pak J, Pu Y, Zhang Z, Hasty DL, Wu X. Tamm-Horsfall protein binds to type 1 fimbrinated Escherichia coli and prevents E. coli from binding to uropakin Ia and Ib receptors. J Biol Chem 2001; 276: 9924-9930.

63. Holden NJ, Blomfield IC, Uhlin BE, Totkiska M, Kulasekara DH, Gally DL. Comparative analysis of FimB and FimE recombine activity. Microbiology 2007; 153: 4138-4149.

64. Holden NJ, Totkiska M, Mahler E, Roe AJ, Catherwood K, Lindner K, et al. Demonstration of regulatory cross-talk between P fimbriae and type 1 fimbriae in uropathogenic Escherichia coli. Microbiology 2006; 152: 1143-1153.

65. Xia Y, Gally D, Forsmann-Semb K, Uhlin BE. Regulatory cross-talk between adhesin operons in Escherichia coli: inhibition of type 1 fimbriae expression by the papB protein. EMBO J 2000; 19: 1450-1457.

66. Holden NJ, Uhlin BE, Gally DL. PapB paralogues and their effect on the phase variation of type 1 fimbriae in Escherichia coli. Mol Microbiol 2001; 42: 319-330.

67. Gunther IV NW, Lockatell V, Johnson DE, Mobley HLT. In vivo dynamics of type 1 fimbria regulation in uropathogenic Escherichia coli during experimental urinary tract infection. Infect Immun 2001; 69: 2838-2846.

68. Teng C, Xie Y, Shin S, Cello FD, Paul-Satayaseela M, Cai M, et al. Effects of impA deletion on expression of type 1 fimbriae in Escherichia coli K1 strain RS218 and on the association of E. coli with human brain microvascular endothelial cells. Infect Immun 2006; 74: 5609-5616.

69. Xie Y, Yao O, Kolisnychenko V, Teng C, Kim KS. HbiF regulated type 1 fimbriation independently of FimB and FimE. Infect Immun 2006; 74: 4039-4047.

70. Sokurencov EV, Courtney HS, Maslow I, Siitonen A, Hasty DL. Quantitative differences in adhesiveness of type 1 fimbriated Escherichia coli due to structural differences in fimH genes. J Bacteriol 1995; 177: 3680-3686.

71. Ronald LS, Yakovenko O, Yazvenko N, Chattopadhyay S, Aprikian P, Thomas W E, et al. Adaptive mutations in the signal peptide of the type 1 fimbrial adhesin of FimE. J Infect Dis 2004; 190: 560-568.

72. Martindale J, Stroud D, Moxon ER, Tang CM. Genetic analysis of Escherichia coli K1 gastrointestinal colonization. Mol Microbiol 2000; 37: 293-1305.
73. Tullus K, Kühn I, Ørskov I, Ørskov F, Möllby R. The importance of P and type 1 fimbriae for the persistence of *Escherichia coli* in the human gut. *Epidemiol Infect* 1992; 108: 415-421.

74. Wold AE, Thorsén M, Hull S, Svanborg Edén C. Attachment of *Escherichia coli* via mannose- or Galα1-4Galβ-containing receptors to human colonic epithelial cells. *Infect Immun* 1988; 56: 2531-2537.

75. Hamrick TS, Harris SL, Spears PA, Havell EA, Horton JR, Russell PW, et al. Genetic characterization of *Escherichia coli* type 1 pilus adhesin mutants and identification of a novel binding phenotype. *J Bacteriol* 2000; 182: 4012-4021.

76. Goetz MB, Kuriyama SM, Silverblatt FJ. Phagolysoosome formation by polymorphonuclear neutrophilic leukocytes after ingestion of *Escherichia coli* that express type 1 pili. *J Infect Dis* 1987; 156: 229-233.

77. Bollinger RR, Everett ML, Palestrant D, Love SD, Lin SS, Parker W. Human secretory immunoglobulin A may contribute to biofilm formation in the gut. *Immunology* 2003; 109: 580-587.

78. Plos K, Connell H, Jodal U, Marklund B, Márild S, Wettergren B, et al. Intestinal carriage of P fimbriated *Escherichia coli* and the susceptibility to urinary tract infection in young children. *J Infect Dis* 1995; 171: 625-631.

79. Nowrouzian FL, Adlerberth I, Wold AE. Enhanced persistence in the colonic microbiota of *Escherichia coli* strains belonging to phylogenetic group B2: role of virulence factors and adherence to colorectal cells. *Microbes Infect* 2006; 8: 834-840.

80. Nowrouzian FL, Adlerberth I, Wold AE. P fimbriae capsule and aerobactin characterize colonic resident *Escherichia coli*. *Epidemiol Infect* 2001; 126: 11-18.

81. Snyder JA, Haugenn BJ, Buckles EL, Lockatell CV, Johnson DE, Donnenberg MS, et al. Transcriptome analysis of *Escherichia coli* J9680 during urinary tract infection. *Clin Infect Dis* 2004; 32: 637-648.

82. Leffler H, Svanborg Edén C. Chemical identification of a glycophasoglycoprotein receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. *JEMS Microbiol Lett* 1980; 8: 127-134.

83. Fischer H, Ellström P, Ekström K, Gustafsson L, Gustafsson M, Svanborg C. Ceramide as a TLR4 agonist; A putative signalling intermediate between sphingolipid receptors for microbial ligands and TLR4. *Cell Microbiol* 2007; 9: 1239-1251.

84. Bergsten G, Wüllt B, Svanborg C. *Escherichia coli*, fimbriae, bacterial persistence and host response induction in the human urinary tract. *Int J Med Microbiol* 2005; 296: 487-502.

85. Hull RA, Rudy DC, Donovan WH, Wieser IE, Stewart C, Darouiche RO. Virulence properties of *Escherichia coli* 83972, a prototype strain associated with asymptomatic bacteriuria. *Infect Immun* 1999; 67: 429-432.

86. Nowrouzian FL, Wold AE, Adlerberth I. *Escherichia coli* strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. *J Infect Dis* 2005; 191: 1078-1083.
S, Boquet P. *Escherichia coli* cytotoxic necrotizing factor 1: evidence for induction of actin assembly by constitutive activation of the p21 Rho GTPase. *Infect Immun* 1995; 63: 3936-3944.

103. Mills M, Meysick KC, O’Brien AD. Cytotoxic necrotizing factor type 1 of uropathogenic *Escherichia coli* kills cultured human urethelial 5637 cells by an apoptotic mechanism. *Infect Immun* 2000; 68: 5869-5880.

104. Rippere-Lampe KE, O’Brien AD, Conran R, Lockman HA. Mutation of the gene encoding cytotoxic necrotizing factor type 1 (cnf 1) attenuates the virulence of uropathogenic *Escherichia coli*. *Infect Immun* 2001; 69: 3954-3964.

105. Hertting O, chromek M, Slamová Z, Kádas L, Söderkvist M, Vaninumäe I, et al. Cytotoxic necrotizing factor 1 (CNF1) induces an inflammatory response in the urinary tract in *vivo* but not in *vivo*. *Toxicol* 2008; 51: 1544-1547.

106. Rippere-Lampe KE, Lang M, Ceri H, Olson M, Lockman HA, O’Brien AD. Cytotoxic necrotizing factor type 1-positive *Escherichia coli* causes increased inflammation and tissue damage to the prostate in a rat prostatitis model. *Infect Immun* 2001; 69: 6515-6519.

107. Johnson DE, Drachenberg C, Lockatell CV, Island MD, Warren JW, Donnenberg MS. The role of cytotoxic necrotizing factor-1 in colonization and tissue injury in a murine model of urinary tract infection. *FEMS Immunol Med Microbiol* 2000; 28: 37-41.

108. Siqueira AK, Ribeiro MG, Leite DS, Tiba MR, Mora C, Lopes MD, et al. Virulence factors in *Escherichia coli* strains isolated from urinary tract infection and pyometra cases and from feces of healthy dogs. *Res Vet Sci* 2009; 86: 206-210.

109. Brauner A, Katouli M, Tulluss K, Jacobson SH. Production of cytotoxic necrotizing factor, verocytotoxin and haemolysin by *Escherichia coli* strains isolated from urinary tract infection and pyometra. *Infect Immun* 2009; 77: 4032-4037.

110. Litwin CM, Calderwood SB. Role of iron in regulation of virulence genes. *Clin Microbiol Rev* 1993; 6: 137-149.

111. Kumar S, Bandoryadhyay U. Free heme toxicity and its detoxification systems in human. *Toxicol Lett* 2005; 157: 175-188.

112. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediated hyperferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004; 113:1271-1276.

113. de Brito BG, Leite DS, Linhares REC, Vidotto MC. Virulence-associated factors of uropathogenic *Escherichia coli* strains isolated from pigs. *Vet Microbiol* 1999; 65: 23-132.

114. Opal SM, Cross AS, Gemski P, Lyhte LW. Aerobactin and α-hemolysin as virulence determinants in *Escherichia coli* isolated from human blood, urine, and stool. *J Infect Dis* 1990; 161: 794-796.

115. Bauer RJ, Zhang L, Foxman B, Siitonen A, Jantunen ME, Saxen H, et al. Molecular epidemiology of 3 putative virulence genes for *Escherichia coli* urinary tract infection - *usp, iha*, and *iro*N* _{e, col i}*, *J Infect Dis* 2002; 185: 1521-1524.

116. Maruvada R, Blom AM, Prasadaro NV. Effects of complement regulators bound to *Escherichia coli* K1 and Group B Streptococcus on the interaction with host cells. *Immunology* 2008; 124: 265-276.

117. Herias MV, Midveldt T, Hanson LA, Wold AE. *Escherichia coli* K5 capsule expression enhances colonization of the large intestine in the gnotobiotic rat. *Infect Immun* 1997; 65: 531-536.

118. Marrs CF, Zhang L, Tallman P, Manning SD, Somsel P, Raz P, et al. Variations in 10 putative uropathogen virulence genes among urinary, faecal and peri-urethral *Escherichia coli*. *J Med Microbiol* 2002; 51: 138-142.

119. Katouli M, Brauner A, Kajiser B, Muratov V, Haghhighi LK, Möllby R. Virulence characteristics of *Escherichia coli* strains causing acute cystitis in young adults in Iran. *J Infect Dis* 2005; 50: 312-321.

120. Duriez P, Clermont O, Bonacorsi S, Bingen E, Chaventre A, Elion J, et al. Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. *Microbiology* 2001; 147: 1671-1676.

121. Moreno E, Johnson JR, Pérez T, Prats G, Kuskowski MA, Andrea A. Structure and urovirulence characteristics of the *Escherichia coli* population among healthy women. *Microbes Infect* 2009; 11: 274-280.

122. Kahar I, Grabnae M, Žgur-Bertok D. Virulence determinants of uropathogenic *Escherichia coli* in fecal strains from intestinal infections and healthy individuals. *FEMS Microbiol Lett* 1998; 164: 243-248.

123. Ron EZ. Host specificity of septicemic *Escherichia coli*: human and avian pathogens. *Curr Opin Microbio* 2006; 9: 28-32.

124. Skyberg JA, Johnson TJ, Johnson JR, Clabots C, Logue CM, Nolan NK. Acquisition of avian pathogenic *Escherichia coli* plasmids by a commensal *E. coli* isolate enhances its ability to kill chicken embryos, grow in human urine, and colonize the murine kidney. *Infect Immun* 2006; 74: 6287-6292.

125. MacFie J, O’Boyle C, Mitchell CJ, Buckley PM, Johnstone D, Sudworth P. Gut origin of sepsis: a prospective study investigating associations between bacterial translocation, gastric microflora, and septic morbidity. *Gut* 1999; 45: 223-228.

126. Finfer S, Bellomo R, Lipman J, French C, Dobbs G, Myburgh J. Adult-population incidence of severe sepsis in Australian and New Zealand intensive care units. *Intensive Care Med* 2004; 30: 589-596.

127. Bian A, Brauner A, Li Y, Normark S. Expression of and cytokine activation by *Escherichia coli* curli fibers in human sepsis. *J Infect Dis* 2000; 181: 602-612.

128. Johnson JR, Owens K, Gajewski A, Kuskowski MA. Bacterial characteristics in relation to clinical source of *Escherichia coli* isolates from women with acute cystitis or pyelonephritis and uninfected women. *J Clin Microbiol* 2005; 43: 6064-6072.

129. Blanco M, Bianco E, Alonso M P, Blanco J. Virulence factors and O groups of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *Eur J Epidemiol* 1996; 12: 191-198.