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Investigating Differences Between Virulence Factors of Escherichia coli and Pseudomonas aeruginosa in Causing Urinary Tract Infections

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Abstract

Although the urinary tract (UT) of healthy individuals was traditionally considered sterile, we now know that bacteria persist in the UT of healthy humans. Moreover, bacteriophages are abundant in healthy human TU and probably play a role in modulating the diversity and relative abundance of bacteria within the community. Understanding the urinary microbiota of healthy people has helped us understand the symptoms and diseases of UT in humans. Pseudomonas aeroginosa and Escherichia coli are ubiquitous microorganisms, which is present in diverse environmental. P. aeruginosa and E.coli an increasingly problematic drug-resistant bacterium in today's world. Uropathogenic Escherichia coli (UPEC) and Pseudomonas aeroginosa express a multitude of virulence factors, which enable the bacteria to establish UTI.

Introduction

Urinary tract infections (UTIs) are one of the most frequent ambulatory bacterial infections with a lifetime incidence of 50 - 60% among adult females. Lower UTIs affect the urethra and bladder. On the other hand, upper urinary tract infections affect kidney function and can be life-threatening when bacteria invade the blood stream from infected kidneys, a condition called urosepsia. Risk factors associated with the host include immune deficiency of the host, urinary tract abnormality, bladder dysfunction in type 2 diabetes, behavioural factors, and estrogen deficiency (Lin et al., 2022).

Bacterial factors are also reported to be associated with UTI pathogenesis and progression (Wang et al., 2002). *Pseudomonas aeruginosa* is one of the most important nosocomial pathogens causing a variety of infections with limited treatment options due to its antibiotic resistance (Sabharwal et al., 2014). Catheter associated urinary tract infections (CAUTIs) are responsible for 40% of nosocomial infections. P. aeruginosa within the catheter frequently develops as biofilm by directly attaching to its surface (Pearson et al., 1995).

Quorum sensing (QS) via acyl-homoserine lactone (HSL), controls the expression of an array of virulence genes in *P. aeruginosa*. The autoinducer synthase, LasI, synthesises N-(3-oxododecanoyl) homoserine lactone (3OC12-HSL), which regulates the production of elastase, exotoxin A and alkaline protease, while RhlI synthesizes the autoinducer N-butyryl homoserine lactone (C4-HSL), which regulates the production of rhamnolipid, alkaline protease, elastase, cyanide and pyocyanin (Winson et al., 1995). Because of the regulatory control of production of virulence factors, QS mechanisms are being proposed as a novel target for development of innovative strategies to control infections. Moreover, importance of QS in the establishment of a successful infection has been shown in different types of animal model studies such as acute pulmonary infection, burn wound infection, microbial keratitis, chronic lung infection and urinary tract infections (Kumar et al., 2011).

Uropathogenic *Escherichia coli* (UPEC) is the predominant infectious agent in both uncomplicated and complicated UTIs. Furthermore, UPEC strains show great diversity in genetic content, virulence factors, genomic islands, and pathogenicity islands (1). Previous studies have shown that isolated E. coli strains have more *neu*A, *pap*GII, *afa*, and *hly*A virulence genes associated with bacterial traits in diabetic patients with E. coli that cause urinary tract infections. In patients with upper UTIs, the *pap*G class II gene plays a critical role in the development of E. coli bacteremia (Wang et al., 2002; Wang et al., 2013). Moreover, *fim*H adhesin plays a role not only in lower UTI, but also in kidney infection by acting synergistically with papGII adhesin (Tseng et al., 2022). Carriage of putative urovirulence factors is thought to enhance E. coli uropathogenicity and is used to measure and categorize clinical UPEC strains isolated from different patient populations (Johnson et al., 2015).

Virulence factors identification

E.coli Virulence factors

K1 capsule antigen and 15 virulence factor genes of UPEC were detected by PCR. Primer pairs specific for K1 capsule gene (*neu*A), 3 PapG adhesion genes of P-fimbriae (papG class I to III), type 1 fimbrial adhesins (fimH), S-/F1Cfimbriae (sfa/foc), afimbrial adhesins (afa), iron regulated gene A homologue adhesins (iha), hemolysin (hlyA), cytotoxic necrotizing factor 1 (cnf1), catecholate siderophore receptor (iroN), aerobactin receptor (iutA), outer membrane protease T (ompT), and uropathogenic specific protein (usp), were described in our previous studies (Naziri et al., 2020; Lin et al., 2018).

In Table 1, the virulence factors of E. coli are placed in four general groups of immunosuppression, Toxins, Siderophores and Adhesins.

Authors	Virulence factor	Role in UTIs
Bunduki et al. Su et al.	Siderophores (aer, chuA, fyuA, iuD, iutA, yfcv)	Production of siderophores by E. coli which takes up iron from the host and helps in colonization and survival of pathogen. They contribute to the process of nutritional passivation of metal ions, in which UPEC access these vital nutrients while simultaneously protecting themselves from their toxic potential.
Bunduki et al. Kaper et al. Felores-Mireles et al.	Adhesins (afa, CSH, fimH, fimP, kpsmtII, pap, sfa, traT)	UPEC adhesins can contribute to virulence in different ways: • directly triggering host and bacterial cell signalling pathways, • facilitating the delivery of other bacterial products to host tissues, and • promoting bacterial invasion. Adhesins help in the adhesion of organism to epithelial cell surface, thereby it escapes from flushing action during micturition. Fimbriae is responsible for adhesion, colonization, invasion of host epithelium and makes UPEC to escape from the innate immune system by internalization process within urothelial cells which is mediated by the transduction cascades.
Mariano et al. Lloyd et al.	Immune suppressors (PAI, shiA, sisA, sisB, sivH, Eco274)	UPEC induces a non-sterilizing adaptive immune response in the bladder. Its causes long-lasting changes in the bladder urothelium, conferring resistance or increased susceptibility to subsequent infections depending on the outcomes of the first infection. The invasins play a key role in suppressing the host immune response during the initial stages of infection.
Terlizzi et al. Bunduki et al.	Toxins (Cnf1, hlyA, saT, vaT)	Toxins like haemolysin and Cytotoxic Necrotising Factor (CNF) act by their cytotoxicity and invasiveness. Haemolysin production could inhibit the cytokine production of host cells and promote the cytotoxicity. It causes lysis of the erythrocytes which release nutrients and other vitamins available for the bacteria. At the same time it releases inflammatory mediators and enzymes which are cytotoxic to renal proximal tubular epithelial cells, erythrocytes and leukocytes, thereby causing renal epithelial damage. CNF interferes with the phagocytosis of E. coli by the WBCs and thus it leads to exfoliation and apoptosis of bladder epithelial cells. It further enhances the easy access of bacteria into the underlying tissue. These toxins can alter signalling pathways, provoke the inflammatory response and prevent the apoptosis thereby they cause the UPEC population to expand.

Table 1: Role of E.coli virulence factors in UTIs.

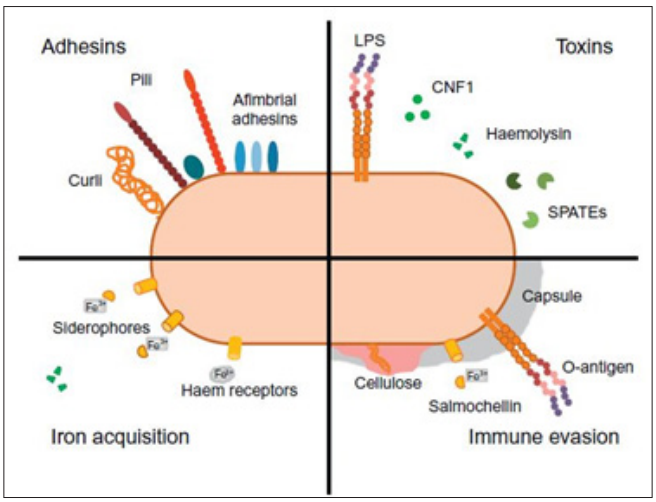

Figure 1: Virulence and fitness factors of uropathogenic E. coli. *E. coli* employs different strategies to infect the urinary tract, to resist immune defences of the host and to persist.

P. *aeruginosa* Virulence factors

P. *aeruginosa* has a large number of virulence factors that can play important roles in the pathogenicity of this bacterium. The main virulence factors are toxin A (toxA), alkaline protease (aprA), elastase, and exoenzymes (S, U, and T exoS, exoU, exoT). Two phospholipases C encoded by plcH and plcN may also play important roles in the hydrolysis of phospholipids in pulmonary surfactants (Borchaloee & Haghkhah, 2019; Badamchi et al., 2017).

Authors	Virulence factor	Role in UTIs
Engel and Balachandran	ExoS	Levels increase over time in UTIs, could aid persistence and immune evasion
Chiu et al	ExoA	Could aid immune evasion; stimulation of inflammation in the kidney could aid persistence
Wood et al.	ExoT	Could aid immune evasion
Tielen et al.	ExoU	Some UTI isolates found with ExoU and low cytotoxicity; ExoU may serve other function or be a hindrance
Beckert et al	ExoY	Unclear
Stapper et al.	Alginate	Although alginate plays a role in biofilm formation, the contribution of alginate in the urinary tract is thought to be minimal
Spencer et al.	LasA	Aid breakdown of host tissues (including elastin in the urinary tract) which could facilitate invasion and/or amino acid metabolism
van der Plas et al.	LasB	Biofilm formation; immunomodulation; Aid breakdown of host tissues (including elastin) and which could facilitate invasion and/or amino acid metabolism
Cornelis and Dingemans	Pyoverdine and pyochelin	Urine, particularly in the bladder, is a low iron environment and siderophores would facilitate bacterial growth
Hall et al.	Pyocyanin	May impair ability of urothelial cells to repair and cause pain and urinary urgency in infection; induce inflammation
Tielen et al.	Phospholipase A	Could be implicated in apoptosis of host cells; possible generation of ROS
Ostroff, Vasil and Vasil	Phospholipase C	Haemolytic activity could aid iron availability in the iron scarce urinary tract
Jiang et al	Phospholipase D	Could aid persistence and/or invasion in the urinary tract

Table 2 : Role of P. *aeruginosa* virulence factors in UTIs.

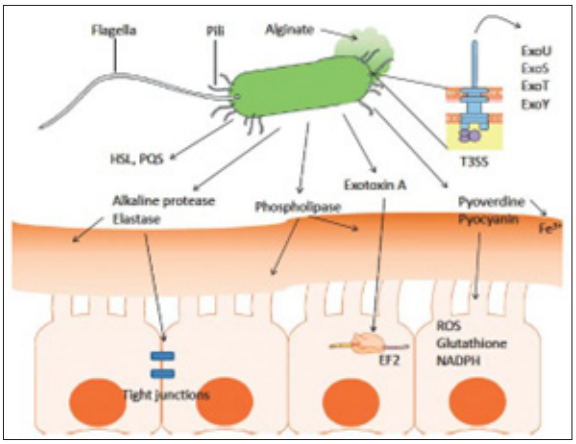

Figure 2: A multitude of virulence factors are produced by Pseudomonas aeruginosa. Flagella and type 4 pili are the main adhesins, capable of binding to host epithelial gangliosides, asialoGM1 and asialoGM2. Along with lipopolysaccharide, these surface appendages are also highly inflammatory. Once contact with host epithelia has occurred, the T3SS can be activated, which is able to inject cytotoxins directly into the host cell. Several virulence factors are secreted by P. *aeruginosa* and have varying effects on the host. Several proteases are produced, which can degrade host complement factors, mucins, and disrupt tight junctions between epithelial leading to dissemination of the bacteria. Lipases and phospholipases can target lipids in the surfactant as well as host cell membranes. Pyocyanin, a blue-green pigment, can interfere with host cell electron transport pathways and redox cycling. Pyoverdine captures Fe3+ to allow for a competitive edge in an environment in which free iron is scarce.

Adhesions

Adherence to host cells can be mediated by fimbriae but also afimbrial adhesins. Fimbriae, or pili, are complex structures and thus encoded by gene clusters coding for fimbrial subunits, assembly and secretion machinery. Pathogenic but also commensal E. coli harbor numerous different operons coding for fimbriae in the genome, most of them belonging to the usher chaperon family (Wurpel et al., 2013). A large proportion of non-fimbrial adhesins belong to the group of autotransporter proteins. An autotransporter protein is composed of different domains, allowing its own transport across the bacterial membranes (Henderson et al., 1998). In addition, UPEC expresses other types of adhesins, such as the amyloid fibres curli and the afimbrial adhesins of the Dr adhesin family. Moreover, structures which are primarily associated to other functions may also promote adhesion and invasion. For example, flagella might, partly independent from their role for mobility, mediate entry into cells of the renal collecting duct (Pichon et al., 2009).

Siderophore

Siderophores are secreted iron-chelating molecules which are then, loaded with iron, taken up by the bacterial cell via specific receptors at the outer membrane (Garenaux, et al., 2011). Four siderophore systems have been investigated in UPEC in the context of infection; enterobactin and its receptor FebA, salmochelin and Iron, aerobactin and IutA, and yersiniabactin and FyuA. The redundancy of siderophore systems in UPEC makes it however complicated to identify certain systems as virulence factors while others might not confer that property (Garcia et al., 2011).

The enterobactin system is expressed by virtually all E. coli strains and consists of the siderophore enterobactin and its receptor FebA. Binding of enterobactin to its receptors is outcompeted by the mammalian protein lipocalin-2, which is upregulated in response to infection and thus counteracts the bacterial attempt of iron acquisition (Fischbach et al., 2006).

Iron chelation is a vital part of establishing infections and the progression to a chronic infection, as the host environment has little free iron due to its own sequestration molecules such as lactoferrin and transferrin. The siderophore, pyoverdine, is both able to sequester iron from host depots and to act as a signaling molecule. Iron-bound pyoverdine interacts with the Pseudomonas cell receptor FPVA, and this complex in turn interacts with the anti-sigma factor FPVR, causing the upregulation of exotoxin A, endoprotease, and of pyoverdine itself (Jimenez et al., 2012). Several other iron siderophore transport systems exist, enabling uptake of iron complexed with endogenous siderophores (e.g. pyochelin), host heme, or the siderophores of other microorganisms (e.g. enterobactin) (Gellatly & Hancock, 2013).

Toxins

P. *aeruginosa* can produce a variety of toxins, including four type III toxins: Exoenzyme (Exo) S, Exo U, Exo T and Exo Y (Fig. 2). In vitro expression of these toxins from isolates has been identified in P. *aeruginosa* isolates from various infection settings, particularly from acute infections. Exo S is an effector protein of the type III secretion system and functions as an ADP-ribosylating enzyme (Newman et al., 2017). Levels of Exo S were significantly higher in vitro in P. aeruginosa isolates from wound and UTIs when compared to tracheal isolates and increased with persistent infection. Infection isolates isolated longitudinally produced higher levels of Exo S, regardless of the site of infection suggesting a role for this enzyme in persistence. Exo U is a cytotoxin secreted by the type III secretion system. Exo U has phospholipase A2 activity and also impairs the recruitment of phagocytes (Diaz et al., 2008). The presence of Exo U has been identified in isolates from the urinary tract (Pobiega et al., 2016).

Toxins are important virulence factors in a variety of E. coli-mediated diseases. Production of toxins by colonizing E. coli may cause an inflammatory response, a possible pathway for UTIs symptoms. The most important secreted virulence factor of uropathogenic E. coli is a lipoprotein called α-haemolysin (HlyA), which is associated with upper UTIs such as pyelonephritis) (Johnson, 1991). The HlyA is a pore-forming toxin, which belongs to the family of RTX (repeats in toxin) toxins that are widespread among the Gram-negative pathogens. This toxin has been shown to exert dual concentration-dependent activities on primary epithelial cells

originating from renal proximal tubules (Bien et al., 2012).

Biofilms

Biofilms are multicellular communities, composed of bacterial cells embedded in an extracellular matrix. Within these biofilms, bacteria are protected from adverse environmental conditions, including antimicrobial treatment and endogenous host defense mechanisms. The ability to form biofilm could therefore be considered the sum of adhesion, production of extracellular matrix and growth characteristics (Sharma et al., 2019; Fux et al., 2005).Also uropathogenic E. coli form more biofilm in vitro compared to commensal faecal isolates, demonstrating the contribution of biofilm to E. coli urovirulence, Moreover, increased biofilm formation as well as the production of exopolysaccharides is associated to more severe and persisting infections (Tapiainen et al., 2014; Kai-Larsen et al., 2010). The ability to form biofilm could therefore be considered the sum of adhesion, production of extracellular matrix and growth characteristic. Type 1 fimbriae are implicated as virulence factors in animal models of urinary tract infection, but their function in human pathology remains unclear. Role of the type 1 fimbriae in human disease is difficult to reconcile because they are expressed in both pathogenic and commensal strains. Specifically, there is no significant difference in the fim gene frequency between more or less virulent strains in the urinary tract. In the murine UTIs model, the type 1 fimbriae have been shown to enhance bacterial survival, to stimulate mucosal inflammation, and to promote invasion and growth as a biofilm (Bien et al., 2012). Recently, Melican and co-workers have defined previously unknown synergistic functions of the both types of fimbriae, which facilitate bacterial colonization under dynamic in vivo conditions. P fimbria have been shown to enhance early colonization of the tubular epithelium, while the type 1 fimbriae mediate colonization of the center of the tubule via a mechanism that involves inter bacterial binding and biofilm formation (Melican et al., 2011). P. aeruginosa can produce several exopolysaccharides, including alginate, Psl, Pel, and lipopolysaccharide. In this review, we highlight the roles of each exopolysaccharide in P. aeruginosa biofilm development and how bacteria coordinate the biosynthesis of multiple exopolysaccharides and bacterial motility. In addition, we present advances in antibiofilm strategies targeting matrix exopolysaccharides, with a focus on glycoside hydrolases (Ma et al., 2022).

Discussion

Pseudomonas aeruginosa and of *Escherichia* coli are opportunistic pathogens that is able to infect humans with a normal immune system. These bacteria are the most important pathogens associated with hospital infections.

The infection process involves several steps in which E. coli interacts with the host cell, each promoted by different virulence factors. While type 1 fimbriae are a pre-requisite for an infection of the urinary tract, several other factors might be dispensable but nevertheless confer an advantage during a particular stage of infection. Therefore together with factors of the host, the combination of bacterial virulence and fitness factors expressed by one particular strain might predict the fate of infection.

Pseudomonas aeruginosa has various pathogenic factors that it uses to intervene in the host's immune system. Pathogenesis in *Pseudomonas aeruginosa* targets the facilitation of binding to the host cell and extracellular matrix. The tendency of *Pseudomonas aeruginosa* to produce biofilm protects the bacterium against antibiotics and the host's immune system. *Pseudomonas aeruginosa* is inherently resistant to a large number of antibiotics and can become resistant to many other antibiotics and make treatment difficult. P*seudomonas aeruginosa* induces a strong inflammatory response during the infectious process.

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