Chapter

New Strategies for the Prevention of Urinary Tract Infections by Uropathogenic *Escherichia coli*

Juan Xicohtencatl-Cortes, Sara A. Ochoa, Ariadnna Cruz-Córdova, Marco A. Flores-Oropeza and Rigoberto Hernández-Castro

Abstract

Uropathogenic *Escherichia coli* (UPEC) is the leading causal agent of urinary tract infections (UTIs), which present high morbidity and limitations in antibiotic treatments. UTIs can also manifest as recurrent (RUTIs) in children and adults and represent a severe public health problem, mainly because there are no treatment and control alternatives that are 100% effective. Patients with RUTIs have a decreased quality of life and are prone to significant complications of UTIs, such as pyelonephritis and urosepsis. Recently, we described UPEC clinical strains related to UTI that have a high profile of antibiotic resistance [multidrug-resistant (MDR) and extensively drug-resistant (XDR)] and genes encoding several fimbrial adhesins, such as FimH of type 1 fimbriae, PapG of fimbriae P, and CsgA of Curli fimbriae. Recently, the expression of fimbrial adhesins (FimH, CsgA, and PapG) was shown to be involved in the release of the interleukins (IL) 6 and IL-8 *in vitro*. This work aims to present a broad overview and description of the pathogenic attributes of UPEC, including the infection processes, pathogenicity mechanisms, and host immune responses, as well as an integral perspective to generate new studies that would contribute to the implementation of preventive strategies against UTI.

Keywords: uropathogenic *Escherichia coli*, resistance, adherence, vaccine, prevention

1. Introduction

Urinary tract infections (UTIs) represent a severe public health problem, and 150 million cases occur annually worldwide. In addition, approximately 40% of women and 12% of men experience at least one UTI event with symptoms in their lives. Different epidemiological data have indicated that a quarter of women who have developed a UTI may present a recurrent infection within six to 12 months [1].

Despite the regular flow of urine, the physiological barriers, and the different defense mechanisms of the host, the urinary tract constitutes one of the most common sites affected by bacterial infection. UTIs originate from the presence of microorganisms in the urinary tract in sufficient quantity to cause clinical symptoms. Depending on the characteristics of the infectious process and the affected site, UTIs can be defined as lower UTIs (cystitis) and uncomplicated pyelonephritis; complicated UTIs with or without pyelonephritis, urinary sepsis or urethritis; and UTIs considered to be special disease types (prostatitis, epididymitis, and orchitis). Depending on the evolution time, they are considered acute and chronic due to symptomatic and asymptomatic clinical manifestations (**Figure 1**) [2].

From a microbiological perspective, UTIs exist when pathogenic microorganisms are detected in the urine, urethra, bladder, kidney, or prostate. Regarding the pathogenesis of UTIs, most begin as a bladder infection (cystitis) caused by bacteria that colonize the perineum, later reach the urethra, and finally colonize the bladder. In cases where cystitis is not properly treated, the bacteria that colonize the bladder can ascend through the ureters and cause pyelonephritis or acute kidney infection, which can even lead to permanent kidney damage (**Figure 1**). In severe cases of pyelonephritis, the bacteria infect the epithelium and endothelium in the kidney and thereby pass into the bloodstream (bacteremia) and cause systemic infection and sepsis, which can result in fatal consequences for the affected individual [3, 4]. The UTI diagnosis, in general, is attained using a general urine test, looking for the presence of leukocyte esterase, the reduction of nitrates to nitrites, the inflammatory cell count (more than 10 cells), and the presence of bacteria. This test has a sensitivity of 75–90% and a specificity of 70–82%. However, urine culture has the limitation of the ability to have an adequate sample for the process; if the urine is obtained from a collection bag, the sensitivity and specificity are very low, since the samples may be contaminated. Additionally, if urine is obtained by catheter, the sensitivity and specificity are greater than 70%, while with collection by suprapubic puncture, the presence of any number of bacterial colonies allows us to ensure the diagnosis. The number of colony-forming units (CFU) necessary to establish the diagnosis of a UTI depends on the type of sample obtained, although it has been considered that this number should be equal to or greater than 10^5 CFU/mL. In the diagnosis, procedures such as excretory urography with a voiding histogram and ultrasound can be used, and this approach has gradually displaced the previous procedure [5].

UTIs are caused by gram-negative and gram-positive uropathogens, in order of importance: uropathogenic *E. coli* (UPEC), *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B β-hemolytic *Streptococcus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida spp*. [6]. UPEC is the causal agent of more than 80% of community-acquired cystitis, more than 70% of unreported acute pyelonephritis, and 3.6–12% of UTIs complicated by urosepsis [7].

E. coli is a commensal bacterium of the intestinal tract of humans and animals and is widely distributed in hospital units. Several clinical features of intestinal infections are caused by extraintestinal *E. coli* (ExPEC) and diarrheagenic *E. coli* pathotypes [8–10]. Meanwhile, the diarrheagenic pathotypes of *E. coli* tend to produce self-limited infections. ExPEC causes infections in various nonintestinal anatomical sites, rapidly progressing to complicated infections of bacteremia, sepsis, and meningitis, which requires immediate antibiotic treatment. In addition, ExPECs have independent virulence factors that confer their ability to survive diverse ecological niches and cause damage [10, 11]. Various lineages of ExPEC have been responsible for infections in

DOI: http://dx.doi.org/10.5772/intechopen.108911

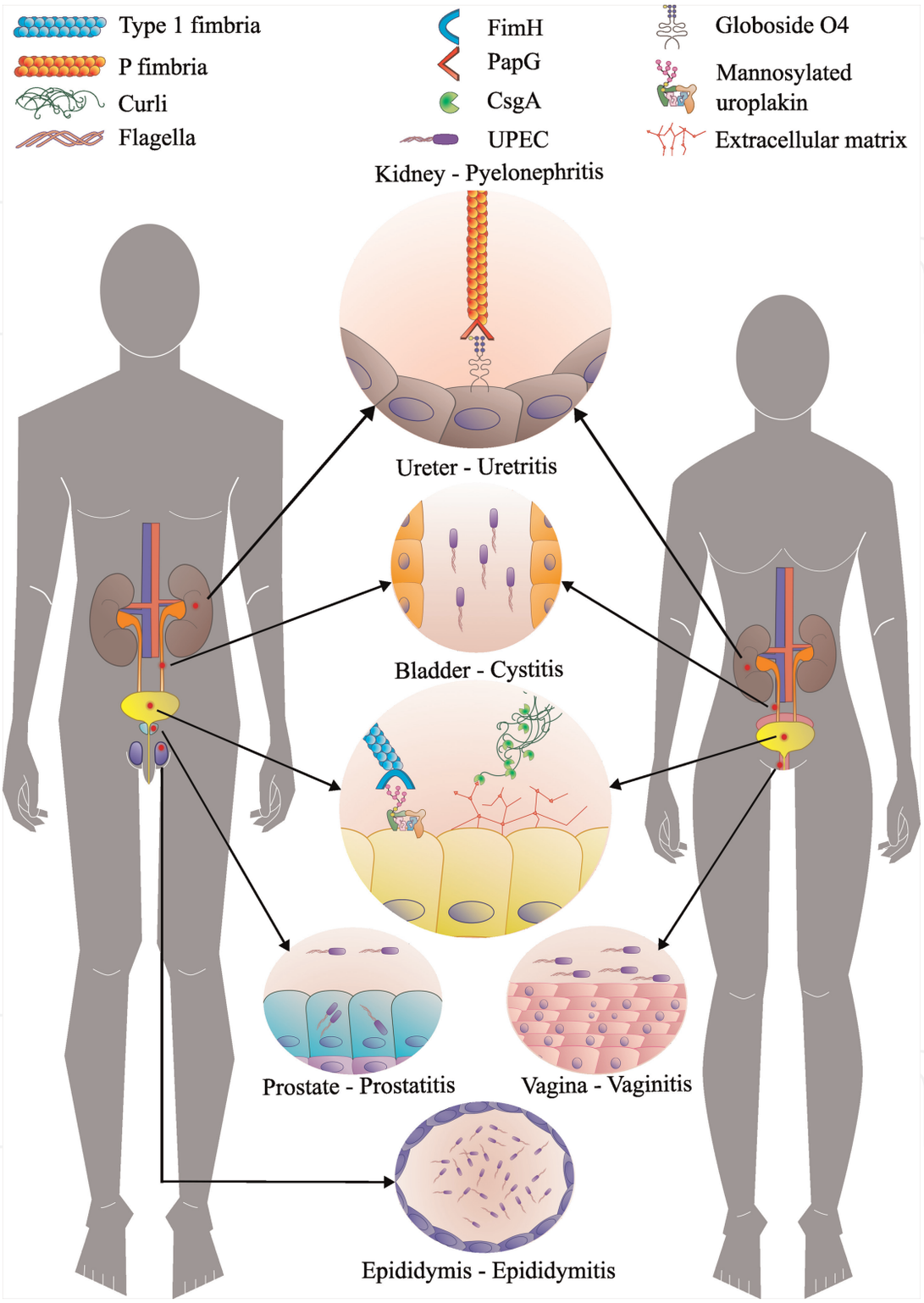

Figure 1.
Urinary tract infection localizations. Men and women have different localizations and usual reservoirs for complicated UTI. Infection establishment starts in the bladder by FimH adhesin from fimbria type 1 binding to mannosylated uroplakins, which are strongly associated with invasion processes, and by CsgA adhesin from Curli, which binds to the extracellular matrix. Some flagellated UPEC organisms are capable of ascending to the ureters and to the kidney; next, P fimbriae bind to globoside receptors by the recognition of PapG. In male patients, the forms of infection and probable reservoirs involve the prostate and epididymis. In the prostate, invasion of the epithelia has also been observed, while in the epididymis, metabolically active UPEC bacteria have been localized in the ducts. In female patients, the vagina is a reported reservoir. In the prostate, vagina, and epididymis, the adhesins involved in the adherence and invasive process are not known.

humans and animals worldwide [12]. The ExPEC pathotypes recognized so far are neonatal meningitis *E. coli* (NMEC), sepsis-associated *E. coli* (SEPEC), avian pathogenic *E. coli* (APEC), and mammary pathogenic *E. coli* (MPEC) [10]. Diarrheagenic *E. coli* strains and ExPEC have been recovered from animal infections, such as pneumonia in pigs, bovine and porcine mastitis, pyometra, and UTI in dogs [13, 14].

2. Virulence of UPEC to the urinary tract

UPEC is the leading etiological agent and has been related to more than 80% of UTIs [15]. The plasticity of this bacterium has allowed it to acquire different pathogenic attributes as tools to colonize the epithelium of the urinary tract. Analysis of various UPEC genomes has shown that the acquisition of virulence factors occurs in pathogenicity islands, plasmids, and phages through horizontal gene transfer [16]. Some virulence factors are secreted, while others are anchored in the outer membrane as molecules that promote bacterial colonization of the urinary tract and, therefore, the development of a clinical pathology [17]. Among the main colonization and virulence factors involved in UPEC pathogenicity are toxins, fimbriae, iron acquisition systems, autotransporter proteins, flagellum, lipopolysaccharides (LPS), and capsules [7, 18]. Briefly, the pathogenicity mechanism of UPEC mainly begins with adherence through the participation of three fimbrial adhesins (FimH, PapG, and CsgA), which are assembled in the distal part (type 1, P, and Curli fimbriae) as shown in **Figure 1** [19, 20]. These adhesins interact with cell receptors (α-D-mannosylated proteins, glycosphingolipids, neuraminic acid, factors that accelerate decay, and extracellular matrix proteins) in the urinary tract and favor bacterial colonization through different signaling pathways, generating apoptosis [21, 22]. The expression of α-hemolysin (HlyA), secreted autotransporter toxin (Sat), and cytotoxic necrotizing factor (CNF-1) has been related to an increase in the cytotoxicity of UPEC to the urinary tract (**Table 1**) [17]. Iron uptake *via* the yersiniabactin (*fyuA*) and aerobactin (*iutD*) systems are necessary elements for UPEC to colonize and persist in anatomical areas with low iron levels (**Table 1**) [17].

Category	Proteins	Name	Function	Evaluated in vaccines	Reference
Adhesin	FimH	Type 1 fimbriae	Adherence to the uroepithelium; binding to mannosylated residues	Yes	[7, 23–30]
	CsgA	Fimbriae P	Adherence to kidney cells; binding to sphingolipids	Yes	[7, 23]
	PapG	Curli	Adherence to the uroepithelium; binding to the extracellular matrix	Yes	[7, 23, 24, 27]
Hemolysin	HlyA	Alpha hemolysin	Increase in cytotoxic capabilities	Yes	[17, 23]
Toxin	Sat	Secreted autotransporter toxin	Increase in cytotoxic capabilities	No	[17]
	CNF-1	Cytotoxic necrotizing Factor	Increase in cytotoxic capabilities	No	[17]

DOI: http://dx.doi.org/10.5772/intechopen.108911

Category	Proteins	Name	Function	Evaluated in vaccines	Reference
	Vat	Vacuolating autotransporter toxin	Increase in cytotoxic capabilities	No	[23]
Siderophore	FyuA	Yersiniabactin	Iron metabolism; iron acquisition	No	[17]
	IutA	Aerobactin	Iron metabolism; iron acquisition	Yes	[17, 29, 30]
	Ent	Enterobactin	Iron metabolism; iron acquisition	No	[23]
	IroN	Salmochelin receptor	Iron metabolism; iron acquisition	Yes	[23, 29, 30]
	ChuA	Heme receptor	Iron metabolism; iron acquisition	Yes	[29, 30]
	Hma	Haem acquisition protein	Iron metabolism; iron acquisition	Yes	[29, 30]
	Iha	Iron regulated-gene-homolog adhesin	Iron metabolism; iron acquisition	Yes	[29, 30]
	IreA	Catecholate siderophore (iron-regulating element)	Iron metabolism; iron acquisition	Yes	[29, 30]
Protectins	Iss	Increased Survival Serum factor	Bloodstream survival; critical for urosepsis development	No	[23]
	ProP	Proline permease	Osmoprotection; survival in urine	No	[31]
Motility	FliC	Flagella	Motility; related to pyelonephritis develop	Yes	[32]

Table 1.
UPEC virulence factors and their vaccine-evaluation status.

UPEC adhesion to the urinary tract cells is an initial process prior to the invasion, and a mechanism to withstand the flow of urine, the activity of antibodies and proteins with bactericidal properties, and the action of antibiotics (**Figure 2**) [33]. Invasion occurs through a "zipper" type mechanism, a process that involves the host cell membrane enveloping the bacteria *via* the activation of several proteins (tyrosine kinases, phosphoinositol-3 (PI-3) kinase, and cell division control proteins), which promote complexes between components of the cytoskeleton (actin, microtubules, and vinculin) [34]. Each of these steps allows UPEC to survive within macrophages as a mechanism of dissemination in the urinary tract [35]. In the cytoplasm, the bacterium initiates the formation of intracellular bacterial communities (IBCs) in three stages [early stage (IBC formation), intermediate stage (IBC maturation), and late stage (IBC efflux and release)], which are encapsulated in RAB27b spindle-shaped vesicles to associate with intermediate filaments of urinary tract cells (**Figure 2**) [36, 37]. The interaction of LPS with the "Toll"-like receptor (TLR) 4 favors an increase in cyclic adenosine monophosphate (cAMP) and the expulsion of UPEC wrapped in RAB27b^{+} vesicles [36, 38, 39]. TLR4 activation by UPEC *via* LPS, FimH, and/or PapG generates an intracellular oxidative state that causes filamentation by

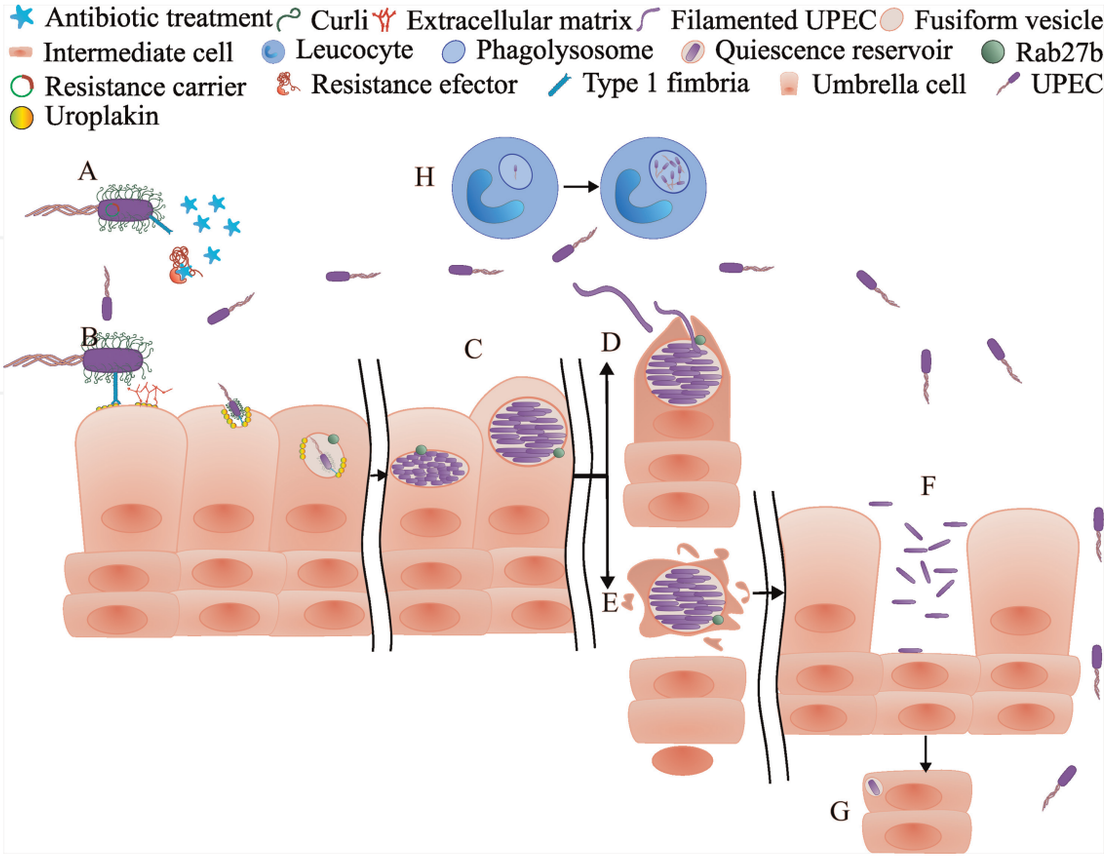

Figure 2.
Events associated with recurrence of urinary tract infections. A) Antibiotic resistance: UPEC strains can harbor resistance genes that allow them to evade the antibiotic function, with eventual failure of the antibiotic treatment. B) Adherence and invasion of UPEC: Fimbria type 1 binding facilitates invasion of the superficial cells from the uroepithelium, by the realigning of actin in Rab26b fusiform vesicles. Some of these vesicles can be released. C) Maturation of the intracellular bacterial community: After the invasion of cells by metabolically active UPEC strains, the bacteria multiply inside fusiform vesicles in a biofilm-like matrix. The increased stress environment makes division difficult, and the UPEC organisms appear as filiform nonsegmented bacteria. The maturation of this structure can lead to different paths. D) Efflux: Filiform bacteria can be released by the fusion of fusiform vesicles with the cellular membrane. E) Apoptosis: The host cell begins programmed cell death, and apoptosis results in the release of filiform bacteria. F) Adherence to intermediate cells: The apoptosis of superficial cells endangers the intermediate cells, and the UPEC bacteria bind to and invade this new layer. G) Quiescence reservoirs: In the intermediate cell layers, the UPEC organisms become quiescent, and these low-metabolic and nonmultiplying bacteria can lie dormant until a better environment is encountered, at which time the bacteria can readily reactivate. H) Strong resistance to phagocytosis: Some strains of UPEC are capable of resisting phagocytosis by neutrophils. By avoiding reactive oxygen species and resisting bactericidal mechanisms, UPEC organisms can be released from the phagolysosome.

inhibiting bacterial division [40]. The growth of UPEC filaments promotes host cell lysis, bacterial efflux, and the initiation of a new cycle of infection [41, 42]. Moreover, UPEC can enter a quiescent state for prolonged periods in exosomes, a mechanism that favors the bacteria to go unnoticed by the immune system (**Figure 2**) [43]. The TRP mucolipin-type channel 3 is expressed on the surface of exosomes activated by UPEC, promoting the neutralization and exocytosis of exosomes with bacteria in a quiescent state [44]. The release of UPEC wrapped in spindle-shaped vesicles is probably promoted by its fusion in the cell membrane of the uroepithelium, where the increase in the cell surface and distention of the bladder is favored (**Figure 2**) [45]. The exit of the bacteria from the quiescent state promotes the reinfection of the urinary tract by the same bacteria, defined as a recurrent UTI (RUTI), and its complications lead to the presence of pyelonephritis and urosepsis [45, 46]. UPEC

DOI: http://dx.doi.org/10.5772/intechopen.108911

pathogenicity, through different mechanisms, promotes colonization, persistence, and recurrence of infection, although the host also mounts an immune response against UTIs.

3. UPEC is mainly responsible for recurrent urinary tract infections (RUTIs)

UTIs are an inflammatory response to the colonization and multiplication of a microorganism, such as UPEC, mainly in any organ of the urinary system, which are accompanied by symptoms, such as dysuria, hematuria, urinary frequency and urgency, and occasionally suprapubic pain [47, 48]. UTIs occur more frequently in women, and the incidence rate of cystitis has been reported to be 12.6% in women and 3.0% in men in the USA [49]. Additionally, it has been estimated that 25% of UTI cases will present a new infectious episode after 3 months [50].

RUTIs occur when a patient has more than two episodes of UTIs within 6 months or more than three episodes within 1 year [51]. RUTIs represent a global health problem and have been related to poor clinical outcomes and a negative impact on patient's quality of life [52]. Recurrent cystitis in women and children is one of the most frequent infections; such episodes are usually disabling and present a more significant number of symptoms, and more than 80.3% are treated with various antibiotics. Recurrence prophylaxis is a clinical option; however, 73% of antibiotic prophylaxis patients have persistent infections and a high percentage have mental stress [53]. The incidence and prevalence of UTIs vary and depend on age, sex, and comorbidities. In Mexico, the epidemiological journal of the Ministry of Health recently reported 4,348,079 cases of UTIs, considering patients from < 2 years to >60 years. UTIs are the third cause of morbidity in the Mexican population, with a higher-frequency age group between 25 and 44 years; however, in pediatric patients, UTIs are the most frequent infections considered healthcare-associated infections (HAIs). The rate of recurrence of UTIs in pediatrics is 15–20%, especially in the first year of life; after an initial case, the risk increases with the number of previous occurrences, ranging from 60 to 75% of cases with three or more occurrences [54, 55]. Timely diagnosis of RUTIs in pediatric patients is complicated since the signs and symptoms reported by the patient, or in some cases by family members, may be nonspecific. The damage that a RUTI can cause in this age group can be reflected in poor growth and anatomical function of the urinary tract, in addition to the generation of antimicrobial resistance in pediatric strains [55].

One of the essential characteristics of RUTIs is the number of previous infectious episodes, and it has been identified that when there is a history of previous episodes, the recurrence rate is 25% with an increase of up to 75% when there are more than two episodes [56]. Complications of UTIs can lead to changes in kidney function, even where the first episode of acute pyelonephritis can be the cause of kidney damage in 57% of cases [3, 57]. In children, one of the most critical risk factors for RUTI is urinary reflux, alone or in combination with dysfunctional urination [58, 59]. A second important risk factor is antimicrobial therapy since, in recent years, it has become more common to observe an increase in resistance to different antimicrobials in different parts of the world [60, 61]. This situation is complicated in children with vesical-urinary reflux because they require prophylactic treatment for prolonged periods, which invariably contributes to the selection of resistant strains.

Recently, RUTIs have been considered reinfections in the urinary tract; in these cases, it is considered that the etiological agent is usually different from the one that caused the initial symptoms. Among the associated microorganisms are *Klebsiella* spp., *Proteus* spp., and *Enterobacter* spp., and the participation of new and different strains of *E. coli*, a microorganism that in the RUTI is also considered the main agent responsible for this disease. Under this proposal, it has been proposed that the selective pressure exerted by antimicrobials during therapy is one of the factors responsible for the emergence of new strains of *E. coli* resistant to antimicrobials, considering the intestine as the main reservoir of the bacteria [62].

Generally, RUTIs are caused by different strains than those that caused the original condition; therefore, a RUTI is considered a reinfection with a new clinical strain [63]. Other studies have reported that in 50–78% of the cases, the collected strains contain the same characteristics as the identified initial strains; thus, the infection is considered a persistence of the original strain [64, 65]. The persistence of UPEC strains in the bladder could be related to their presence as part of the fecal biota for long periods, leading to recurrent ascending infections [4]. Local treatment of the perineum with antibiotics does not prevent the recurrence of UTIs, which indicates that the migration of the pathogen from the anus to the urinary tract is not an event associated with RUTIs (**Figure 2**) [66]. There are reservoirs of intracellular bacteria that persist for weeks and months in the urethral epithelium. Likewise, bacterial communities, as structures similar to biofilms, have been identified in the cells of women with cystitis. Other data have confirmed that UPEC strains invade the epithelium and form reservoirs of bacteria to produce RUTIs (**Figure 2**) [67–69]. In a study carried out from 2007 to 2011, the authors described 75 cases of RUTI whose etiological agent was UPEC strains; 52% (39/75) of them were persistent infections, and 48% (36/75) were associated with reinfections [63]. The average persistence fluctuated between 15 days and 42 months regarding reinfections. In addition, some patients presented reinfection by different UPEC strains for more than 36 months. This information reflects the importance of both clinical events (persistence and reinfection), with a high possibility of kidney damage due to the duration of the infectious process.

4. UPEC resistance impacts the course of UTI

Multidrug resistance (MDR) is a critical factor in UPEC strains that cause RUTIs worldwide due to the high costs of antimicrobial treatments [70]. One of the main resistance mechanisms is the participation of genes that code for extended-spectrum β-lactamases (ESBLs) and resistance genes that code for quinolones and sulfonamides [71]. Additionally, three mechanisms by which UPECr may cause RUTIs have been suggested: (1) The presence of resistance genes, which can cause treatment failure, as well as the selection of MDR strains that persist in the urinary tract. UPEC MDR strains can reactivate once treatment is completed. (2) The activation of invasins, toxins, siderophores, and metabolic fitness factors allows the invasion of uroepithelial cells, forming IBCs. In this process, the UPECr strains remain inside the cells of the transitional urinary epithelium, isolated and protected from antimicrobial treatment and the host's immune response. (3) The formation of quiescent reservoirs (QRs) by UPECr strains is maintained in the deepest layers of the urinary epithelium, where they can remain for long periods and be reactivated by various signaling systems that have not been described in detail (**Figure 2**). These mechanisms can be independent or act jointly in a single process. For the prevention of RUTIs, specifically in women,

DOI: http://dx.doi.org/10.5772/intechopen.108911

various treatment options, including continuous antibiotic prophylaxis, accompanied by behavioral therapy, probiotics, estrogens, and intravesical instillations of hyaluronate, have been proposed. However, the results have shown variable efficacy in the short and long term [72].

5. Use of antimicrobials in UTIs

The World Health Organization (WHO) has considered that the excessive use of antimicrobials is one of the main causes related to the increase in bacterial resistance, generating an important public health problem of inadequate medical prescription, excessive use, and increasingly (and sometimes unnecessarily) prolonged times in the treatment schemes. In addition, the administration of nonoptimal doses and failures in medication consumption has contributed significantly to the increase in antimicrobial resistance rates [73, 74]. The purpose of antimicrobial treatments against UTIs is to use antibiotics that guarantee the eradication of the responsible microorganisms, such as UPEC; however, it is crucial to consider the exact timing of antibiotic use to avoid adverse side effects. The selection of antimicrobials will depend on the causal agent, the sensitivity patterns in the community and/or hospital environment, and patient's characteristics (age, sex, pregnancy, anatomical location of the infection, and comorbid conditions). Factors related to the antimicrobial to be used include its pharmacodynamics, adverse effect profile, and ease of administration [75].

In general terms, antimicrobial therapy resolves most symptomatic cases of UTIs. Among the most frequently used antibiotics of choice are trimethoprim-sulfamethoxazole (TMP-SMX), fluoroquinolones, nitrofurantoin, amoxicillin with or without clavulanic acid, second- and third-generation cephalosporins, and aminoglycosides. In recent years, issues related to inadequate drug management, such as incomplete dosages, intolerance, and side effects, have been host factors that, together with bacterial resistance to antibiotics, lead to failures in the management and treatment of infections. In cases of complicated UTIs, the duration of antibiotic treatment should not be less than 7 days, and between 10 and 15 days is recommended. Severe infections often require treatment with broad-spectrum cephalosporins, such as cefotaxime or ceftriaxone, or with other beta-lactams that have excellent activity against microorganisms and good tissue penetration. A clinical study conducted in the United States of America (USA) showed that of 10,161 urine culture samples, 17% of *E. coli* and *P. mirabilis* isolates, 11% of *K. pneumoniae* isolates, and 3% of *Streptococcus saprophyticus* isolates were resistant to TMP-SMX. However, the frequencies and antimicrobials vary between hospital-acquired and community-acquired infections; an example is resistance to ampicillin (63%) in *E. coli* isolates from samples of nonhospitalized children in Israel.

Recently, in the HIMFG, we described some specific characteristics of 105 UPEC strains isolated from urine samples of children diagnosed with acute UTIs, observing a resistance rate of 86% for ampicillin, 69% for amoxicillin-clavulanic acid, and 55% for nalidixic acid. Regarding the presence of multidrug resistance, 19% of the isolates were resistant to 3 or more groups of antibiotics. In 2010, a prospective study of UTIs, including 289 *E. coli,* isolates showed that 78% were resistant to ampicillin; 60%, to trimethoprim-sulfamethoxazole; 40%, to ciprofloxacin; 50%, to ceftriaxone; and 32%, to gentamicin. These data show how resistance to the different antimicrobials used for treating UTIs is maintained with a high frequency and, in some cases, higher than that reported in other parts of the world.

6. Biofilms formed by UPEC favor the development of UTIs

The formation of biofilms by UPEC is a process that is made up of a series of sequential events, where virulence and aptitude factors are activated and repressed; in addition, it is regulated by environmental signals that allow the change in the condition of planktonic cells until the establishment of biofilms, with the relevant changes in the gene expression of bacterial strains [76, 77]. Bacteria express various virulent factors through the use of nutrients to form biofilms, which change their spatial organization and alter the expression of surface molecules as a mechanism of resistance to environmental stress. Changes in environmental conditions within the bacterial biofilm can lead to the emergence of bacterial subpopulations that express different genes in response to the availability of nutrients and oxygen [78–80]. Biofilms, as highly organized structures, confer an advantage to bacterial pathogenesis and make eradicating such organisms more complex, resulting in chronic infection. Biofilm formation during UTIs is an event that can lead to severe infections in hospitalized and community patients [81, 82]. UPEC is the uropathogen most closely related to RUTIs, employing a complex pathogenic cascade to colonize extra- and intracellular niches during the infectious process [83]. UPEC strains activate numerous virulence factors during colonization of the urinary tract, such as adhesins, toxins, iron acquisition systems, capsular structures, flagellum, and islands of pathogenicity [82, 84, 85]. The processes of adhesion, invasion, and formation of CBI as mechanisms that contribute to the persistence of UPEC are related to the expression of external appendages, known as fimbriae or pili (**Figure 2**). Several fimbriae that participate in the adherence to various epithelia (such as the urothelium) or processes of invasion and CBI formation, thereby facilitating the persistence of UPEC, have been described (**Figure 2**).

Bacterial biofilms are a significant cause of antibiotic resistance and infection persistence; in this context, biofilm control is essential in reducing MDR bacterial infections. Recently, the reuse of drugs with anti-biofilm activity has been implemented in treating UTIs due to UPEC. Auranofin is a drug approved by the FDA in 1985 for treating rheumatoid arthritis and has shown effectiveness as an antibacterial agent [86]. Interestingly, auranofin significantly inhibits the biofilm formation of UPEC strains [87]. Likewise, trans-cinnamaldehyde (CNMA) is a natural molecule derived from *Cinnamomum zeylanicum*, which has shown its usefulness in treating high blood pressure and low blood glucose in diabetic patients. CNMA and its derivatives maintain an antimicrobial spectrum and anti-biofilm activity against UPEC at a minimum inhibitory concentration of 50−100 μg/mL. Additionally, they inhibit flagellar mobility and reduce the production of extracellular polymeric substances in UPEC [88]. Trans-resveratrol and oxyresveratrol are compounds with antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer, neuroprotective, and anti-biofilm activities against several bacterial pathogens [89–93].

Trans-resveratrol and oxyresveratrol significantly reduce biofilm formation by UPEC at sub-inhibitory concentrations of 10−50 μg/mL, and they also present an antivirulence strategy against the persistence of bacterial infections through a reduction in the production of fimbriae and swarming mobility. Furthermore, t-resveratrol and oxiresveratrol markedly decrease the hemagglutinating capacity of UPEC and increase the killing of bacteria by the human blood complex [94]. Finally, many plant-derived compounds can act in conjunction with existing antibiotics. A synergistic effect has been demonstrated between Type A procyanidin and the antibiotic nitrofurantoin at pH 5.8 due to a deregulation process of the expression of UPEC

DOI: http://dx.doi.org/10.5772/intechopen.108911

fimbrial adhesins [95]. These studies support the need to carry out synergism tests between various agents used in treating chronic diseases and derived from plants in conjunction with antibiotics as a strategy to identify anti-biofilm agents and with antimicrobial effects that support the treatment of infections by UPEC-MDR [87].

7. Fimbria type 1, PapG, and Curli in the CBI process by UPEC

UPEC is the main uropathogen recovered in more than 80% of RUTIs [96]. The pathophysiology of RUTIs due to UPEC is a complex process that has not yet been fully characterized. Several studies have described the participation of various virulence factors associated with UPEC pathogenicity, such as adhesion fimbriae: *fim* (fimbria type 1), *csg* (Curli), *pap* (fimbria P); siderophores: *ent* (enterobactin), *iut* (aerobactin), *fyu* (yersiniabactin); toxins: *hly* (hemolysin), sat (autotransporter secreted toxin), vat (autotransporter vacuolating toxin); and capsule production and variations in lipopolysaccharides. The presence of metabolic regulators and protectins, such as *iss* (factor for increasing serum survival) and *pro* (proline permease), also have an essential role in the persistence of UPEC (**Table 1**) [23].

8. Nonantibiotic alternatives for the treatment of UTIs

The vaccines available for treating of UTIs are products focused on stimulating the systemic immune response due to the difficulty of stimulating mucosal immunity [97]. Different bacterial antigens, such as O antigen (major compound of LPS), FimCH (bound of the FimC chaperone and FimH adhesin), and PapDG (the bound to the chaperone PapD and PapG adhesin), HlyA and IroN, have generated a systemic specific response but not generated a response at the mucosa level (**Table 1**) [24–27]. Indeed, bacterial antigens efficiently induce mucosal immunogenicity *via* intranasal (IN), intravaginal, and oral administration. Meanwhile, parenteral administration induces an inefficient response [98].

The SolcoUrovac® vaccine approach (rebranded StroVac®) is a formulation from a suspension of 10 *E. coli*, which were inactivated from different serotypes and including other uropathogens. Administration through the vaginal mucosa significantly reduces recurrent UTIs, according to phase II clinical studies; however, they have generated adverse reactions such as pain and irritation of the vaginal epithelium (**Table 2**) [99, 100]. In a randomized, double-binding, placebo-controlled, parallel-group study, intramuscular administration of three injections 2 weeks apart showed a nonstatistical reduction in clinically relevant UTI episodes (86/188 patients, 46.0%) when compared with the placebo group (97/188 patients, 51.6%). However, the same data showed a statistical reduction of UTIs in patients with more than seven UTIs in the previous year [101].

The oral administration of the immunomodulator Urostim®, another uropathogens lysate, in phase clinical II the one tablet dose for 3 months, stimulates the cellular phagocytosis and secretory IgA humoral response, without generating protection against UTIs [31]. Oral administration of OM-89/Uro-Vaxom®, a lyophilizate biological extract from 18 *E. coli* strains, reduces RUTIs; however, it produces adverse effects related to immunological tolerance and clinical manifestations at the gastrointestinal level (**Table 2**) [102].

Name	Formulation	Administration	Doses and treatment duration	Adverse responses and protection effect
SolcoUrovac® vaccine (rebranded StroVac®)	Heat lysate of 10 uropathogenic strains, *Escherichia coli* (6), *Morganella morganii, Proteus mirabilis, Enterococcus faecalis* and *Klebsiella pneumoniae*.	Intramuscular injection	Three injections of 0.5 mL, at intervals from 1 to 2 weeks.	Significant reduction in RUTI. There are no long-term clinical studies. Localized adverse reactions such as pain and irritation. Systemic reactions such as fever, headache, dizziness, and nausea.
Immunomodulator Urostim®	Uropathogenic bacterial lysates from *E. coli, P. mirabilis, E. faecalis,* and *K. pneumoniae*	Oral	One tablet per day for two to three consecutive months.	Stimulation of the cellular and humoral response. It does not generate protection.
OM-89/Uro-Vaxom® vaccine	Lyophilized biological extract of *E. coli* (18c).	Oral	One tablet per day for three consecutive months.	Reduction in recurring UTIs. It produces immunological tolerance and clinical manifestations at the gastrointestinal level, diarrhea, nausea, and abdominal pain. At the cutaneous level, it can lead to pruritus and rash.
ExPEC4V vaccine	Conjugated with the exotoxin A of *Pseudomonas aeruginosa* bound to four polysaccharide antigens of the serotypes of *E. coli* O1A, O2, O6A, and O25B.	Intramuscular	One intramuscular injection of 0.5 mL.	Low reduction in RUTIs; mild adverse effects.
UROMUNE® (MV140) vaccine	Whole bacterial cells from inactive uropathogens: *E. coli, Proteus vulgaris, E. faecalis,* and *K. pneumoniae*	Sublingual	Two sprays daily for 3 to 6 months.	Reduction of 55.7% and 58% in the frequency of RUTIs in 3 and 6 months of treatment, respectively. The 1.7% of patients manifest mild adverse effects.

Table 2.
Available commercial vaccines for the treatment and prevention of RUTI.

The ExPEC4V conjugate vaccine is a long-term development for a bioconjugate of exotoxin A from *P. aeruginosa* with four polysaccharide antigens of *E. coli* strains. The parental vaccination with the tetravalent o-conjugate was well tolerated with mild adverse events but safe. A preliminary study showed a low UTI reduction; the functional immune response was shown for a bacterial count reduction (**Table 2**) [103, 104]. The efficacy and safety of the UROMUNE® (MV140) vaccine showed only 1.7% of mild adverse responses. This vaccine was generated with glycerinated

DOI: http://dx.doi.org/10.5772/intechopen.108911

suspensions of heat-inactivated whole bacteria of four uropathogens and administrated daily by a sublingual spray for 3 months. While reported evidence suggests that this vaccine can be effective, reports concluded that some patients with RUTI can achieve a non-UTI status (**Table 2**) [105].

Finally, the transurethral immunization of mice with attenuated UPEC strains is not persistent in the urinary tract and favors nonspecific protection [106]. Intranasal immunization (IN) with different UPEC antigens (ChuA, Hma, Iha, IreA, IroN, IutA, and FimH) induces the activation of high concentrations of IgA in saliva, vagina, and urine (**Table 1**) [29, 30]. According to this information, IN vaccination of fimbrial adhesins may be a potential strategy to generate a humoral immune response with IgA antibodies in the mucosa of the urinary tract as a mechanism of host protection against UTIs by UPEC.

9. Fimbrial adhesins and immune response

Various studies have described the development of effective strategies for the prevention, treatment, and/or management of UTIs caused by UPEC. However, there are no effective vaccines generated from fimbrial adhesins, autotransporters, toxins, siderophores, flagella, and outer membrane proteins of UPEC. These proteins, located on the bacterial surface, are expressed during infectious processes and can stimulate an immune response from the host [107]. UPEC mainly expresses three types of fimbrial adhesins during the colonization of urinary tract cells: FimH located in the upper part of the type 1 fimbria, PapG in the P fimbria, and CsgA in the Curli fimbriae [19].

Data generated by our working group have revealed that 90% of clinical strains of UPEC isolated from pediatric patients express fimbria type 1. This fimbrial adhesin has been related to adherence, invasion, and formation of bacterial colonies in the urinary tract (**Figure 2**) [108]. In addition, we have reported that more than 95% of clinical strains of UPEC contain csgA, a gene that codes for the CsgA protein (a structural adhesin of the Curli fimbriae) and has been associated with urosepsis processes [109, 110]. More than 35% of UPEC clinical strains express the P fimbria, a homopolymeric structure that participates in the colonization of the kidney *via* interaction with globoceramides located in renal cells. The diversity of globoceramides in the kidney has favored the appearance of three allelic variants in the papG gene [papGJ96 (variant I), papGAD/IA2 (variant II), and prsGJ96 (variant III)] [111]. It is important to note that the FimH, CsgA, and PapGII adhesins are three essential protein structures in the pathogenesis of UPEC and may be viable biomolecules for the generation of an effective vaccine that stimulates an immune response.

10. FimH of type 1 fimbria is immunogenic

The FimH adhesin (fimbria type 1) is a protein used in preliminary studies in animal models to evaluate its potential as a viable vaccine. Sera from C3H/HeJ mice immunized with the FimH adhesin and type 1 fimbria inhibit adherence to human bladder cells [28]. Other studies have shown that the mannose-binding domain of FimH, plus the complete protein and in association with FimC (flagellin and chaperone), significantly reduces adherence in the bladder and kidney in mice and cynomolgus monkeys; in addition, specific anti-FimH antibodies inhibit the

colonization of UPEC [26, 28, 112–114]. The recombinant FimH protein fused with the FliC protein induces a significant increase in the cellular and humoral immune response against UTIs in a murine model [101]. Subcutaneous immunization induced high levels of immunoglobulins (IgG1 and IgG2a) and cytokines [(INFγ: interferon gamma) and IL4 (interleukin 4)] [32]. IN immunization with FimH (UPEC) and MrpH (*P. mirabilis*) protein as a fused molecule induces the release of IgG and IgA antibodies in mouse samples (serum, urine, nasal wash, and vaginal). Th1- and Th2-type cellular immunity generated by FimH/MrpH proteins with or without MPL adjuvant suggests that one of these proteins functions as an adjuvant molecule [115]. The FimH protein interacts with TLR4 through the α-mannosylated coreceptor, favoring the activation of CD4- epithelial cells *via* Tirap-MyD88 to recruit neutrophils in the mucosa [116, 117].

11. The PapG adhesin is an immunogenic molecule

The expression of the adhesin PapG contributes to the UPEC colonization in the kidney during the pyelonephritis process in humans [118]. The interaction of PapG with TLR4 activates the secretion of IL-6 and IL-8 [118, 119]. Intraperitoneal immunization of the P fimbria and a protein complex of PapDG (chaperone) generates protection against kidney inflammation and production induces specific antibodies in mice and cynomolgus monkey sera [24, 120]. In these studies, no significant differences showed between the number of bacteria recovered in urine and the control group, probably due to the expression of other accessory fimbriae involved in bacterial colonization [81].

12. CsgA is an immunogenic protein

Preliminary data obtained by our working group showed that CsgA from the Curli fimbriae is an adhesin that contributes as an accessory molecule in the adherence of UPEC to bladder cells; however, more studies are still required to determine the specific role in the pathogenesis of UPEC [110]. High levels of anti-CsgA antibodies recognize the CsgA protein in sera from patients convalescing from sepsis. These data suggest the expression of the protein *in vivo*; however, more studies are necessary to evaluate the immunogenicity of this protein [110]. In other *E. coli*, the Curli fimbriae induce the release of proinflammatory cytokines (TNFα, IL6, and IL8) in macrophage cells [121]. The recombinant protein CsgA from *Salmonella* and Curli from *E. coli* MC4100 participate in the release of IL8 in human THP-1 macrophage cells through the cooperative interaction of TLR1 and TLR2 [122]. The CsgA protein has also been considered a pathogen-associated molecular pattern (PAMP), responsible for generating an IL6 and IL1β response *via* the inflammasome (NLRP3) [123, 124].

13. Conclusions

Several studies have reported that UPEC is responsible for 90% of community UTIs and approximately 60% of hospital-acquired UTIs; however, more studies are required to elucidate the specific characteristics of UPEC, such as its serotypes, virulence factors, and antimicrobial susceptibility. The innate plasticity of the genome of

DOI: http://dx.doi.org/10.5772/intechopen.108911

this bacterium allows it to acquire various virulence factors, resistance mechanisms, and adaptative advantages to colonize the urinary tract. The presence of clinical strains of MDR and XDR UPEC is a highly worrisome phenomenon that alerts health specialists who are left without therapeutic options to treat acute and recurrent UTIs effectively. The FimH, CsgA, and PapG adhesins are the main virulence factors of UPEC. These adhesins can be considered new targets in the elaboration of biomolecules with immunogenic potential for protection against UTIs.

The search for new alternative antibiotics has led to the emergence of potential biomolecules that can generate an efficient vaccine that protects against UTIs, significantly reducing or eliminating disease cases. These biomolecules with the capacity to generate protection can act alone or in association with antibiotics of a lesser spectrum for treating UTIs. Recently, we reported that the best way to generate protection is by intranasal inoculation of mixtures of these protein adhesins with the ability to generate antibodies in mucous membranes; however, more studies are still necessary to solidify this premise. Finally, frontier science may lead us to the paradigm of improving our understanding of mucosal immunity and the role of these adhesin-based biomolecules as an alternative to the overuse of antimicrobials to treat UTIs.

Acknowledgements

The data generated by my workgroup and published in other journals have been approached in this book chapter with an integral focus on addressing their relevance to UTIs. The different experiments conducted were supported by Public Federal Funds HIM-2017-107 SSA. 1421, HIM-2018-045 SSA. 1503, HIM-2019-038 SSA. 1592, and HIM-2021-030 FF SSA. 1730 from the Hospital Infantil de México Federico Gómez. These grants were approved by the Research Committee (Dr. Juan Garduño Espinosa), Ethics Committee (Dr. Luis Jasso Gutiérrez), and Biosecurity Committee (Dr. Marcela Salazar García) of Hospital Infantil de México Federico Gómez.

Conflict of interest

The authors declare no conflict of interest.

Abbreviations

UPEC	uropathogenic *E. coli*
UTI	urinary tract infection
RUTI	recurrent urinary tract infections
MDR	multidrug-resistant
XDR	extensively drug-resistant
CFU	colony-forming units
IBCs	intracellular bacterial communities
LPS	lipopolysaccharides
TLR	"Toll"-like receptor
cAMP	cyclic adenosine monophosphate
TRP	transient receptor potential
HAIs	healthcare-associated infections

ESBL	extended-spectrum β-lactamases
UPECr	UPEC recurrent strains
QR	quiescent reservoirs
WHO	World Health Organization
IN	intranasal immunization
MPL®	monophosphoryl lipid A
IL	interleukin
PAMP	pathogen-associated molecular patterns
TNFα	tumor necrosis factor-alpha
NLRP3	NOD-, LRR- and pyrin domain-containing protein

Author details

Juan Xicohtencatl-Cortes[1*], Sara A. Ochoa[1], Ariadnna Cruz-Córdova[1], Marco A. Flores-Oropeza[1] and Rigoberto Hernández-Castro[2]

1 Laboratorio de Investigación en Bacteriología Intestinal, Hospital Infantil de México Federico Gómez, Ciudad de México, Mexico

2 Departamento de Ecología de Agentes Patógenos, Hospital General "Dr. Manuel Gea González", Ciudad de México, Mexico

*Address all correspondence to: juanxico@yahoo.com

IntechOpen

References

[1] Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clinical Microbiology Reviews. 2008;**21**: 26-59. DOI: 10.1128/cmr.00019-07

[2] Beetz R, Mannhardt W, Fisch M, Stein R, Thüroff JW. Long-term follow-up of 158 young adults surgically treated for vesicoureteral reflux in childhood: The ongoing risk of urinary tract infections. The Journal of Urology. 2002; **168**:704-707; discussion 707. DOI: 10.1016/s0022-5347(05)64729-5

[3] Lin KY, Chiu NT, Chen MJ, Lai CH, Huang JJ, Wang YT, et al. Acute pyelonephritis and sequelae of renal scar in pediatric first febrile urinary tract infection. Pediatric Nephrology. 2003; **18**:362-365. DOI: 10.1007/s00467-003-1109-1

[4] Hooton TM. Clinical practice. Uncomplicated urinary tract infection. The New England Journal of Medicine. 2012;**366**:1028-1037. DOI: 10.1056/NEJMcp1104429

[5] Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clinical Infectious Diseases. 2004;**38**:1150-1158. DOI: 10.1086/383029

[6] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nature Reviews. Microbiology. 2015;**13**(5): 269-284. DOI: 10.1038/nrmicro3432

[7] Luna-Pineda VM, Ochoa S, Cruz-Córdova A, Cázares-Domínguez V, Vélez-González F, Hernández-Castro R, et al. Urinary tract infections, immunity, and vaccination. Boletin Medico del Hospital Infantil de Mexico. 2018;**75**: 67-78. DOI: 10.24875/bmhim.m18000011

[8] Levine MM. *Escherichia coli* that cause diarrhea: Enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. The Journal of Infectious Diseases. 1987; **155**(3):377-389. DOI: 10.1093/infdis/155.3.377

[9] Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, et al. Diarrheagenic *Escherichia coli*. Brazilian Journal of Microbiology. 2016;**47**(Suppl 1):3-30. DOI: 10.1016/j.bjm.2016.10.015

[10] Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A. Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. Pathogens. 2021;**10**(11):1355. DOI: 10.3390/pathogens10111355

[11] Foxman B, Brown P. Epidemiology of urinary tract infections: Transmission and risk factors, incidence, and costs. Infectious Disease Clinics of North America. 2003;**17**:227-241. DOI: 10.1016/S0891-5520(03)00005-9

[12] Riley LW. Pandemic lineages of Extraintestinal pathogenic *Escherichia coli*. Clinical Microbiology and Infection. 2014;**20**:380-390. DOI: 10.1111/1469-0691.12646

[13] Müştak HK, Günaydin E, Kaya İB, Salar MÖ, Babacan O, Önat K, et al. Phylo-typing of clinical *Escherichia coli* isolates originating from bovine mastitis and canine pyometra and urinary tract infection by means of quadruplex PCR. Veterinary Quarterly. 2015;**35**(4): 194-199. DOI: 10.1080/01652176.2015.1068963

[14] Kong L-C, Guo X, Wang Z, Gao Y-H, Jia B-Y, Liu S-M, et al. Whole genome sequencing of an ExPEC that caused fatal pneumonia at a pig farm in Changchun, China. BMC Veterinary Research. 2017;**13**:169. DOI: 10.1186/s12917-017-1093-5

[15] Kline KA, Lewis AL. Gram-positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. In: Mulvey MA, Klumpp DJ, Stapleton AE, editors. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management. Chichester: Wiley; 2017. pp. 459-502

[16] Lloyd AL, Rasko DA, Mobley HL. Defining genomic islands and uropathogen-specific genes in uropathogenic *Escherichia coli*. Journal of Bacteriology. 2007;**189**:3532-3546. DOI: 10.1128/jb.01744-06

[17] Subashchandrabose S, Mobley HL. Virulence and fitness determinants of uropathogenic *Escherichia coli*. In: Mulvey MA, Klumpp DJ, Stapleton AE, editors. Urinary Tract Infections: Molecular Pathogenesis and Clinical Management. 2nd edition. Wiley; 2017. pp. 235-261. DOI: 10.1128/9781555817404.ch12

[18] Nielubowicz GR, Mobley HLT. Host–pathogen interactions in urinary tract infection. Nature Reviews Urology. 2010;**7**:430-441. DOI: 10.1038/nrurol.2010.101

[19] Antão E-M, Wieler LH, Ewers C. Adhesive threads of extraintestinal pathogenic *Escherichia coli*. Gut Pathogens. 2009;**1**:22. DOI: 10.1186/1757-4749-1-22

[20] Spaulding CN, Hultgren SJ. Adhesive pili in UTI pathogenesis and drug development. Pathogens. 2016;**5**:30. DOI: 10.3390/pathogens5010030

[21] Lüthje P, Brauner A. Virulence factors of uropathogenic *E. coli* and their interaction with the host. Advances in Microbial Physiology. 2014;**65**:337-372. DOI: 10.1016/bs.ampbs.2014.08.006

[22] Thumbikat P, Berry RE, Zhou G, Billips BK, Yaggie RE, Zaichuk T, et al. Bacteria-induced uroplakin signaling mediates bladder response to infection. PLoS Pathogens. 2009;**5**:e1000415. DOI: 10.1371/journal.ppat.1000415

[23] Johnson JR, Russo TA. Acute pyelonephritis in adults. The New England Journal of Medicine. 2018;**378**:48-59. DOI: 10.1056/nejmcp1702758

[24] Roberts JA, Kaack MB, Baskin G, Chapman MR, Hunstad DA, Pinkner JS, et al. Antibody responses and protection from pyelonephritis following vaccination with purified *Escherichia coli* PAPDG protein. The Journal of Urology. 2004;**171**:1682-1685. DOI: 10.1097/01.ju.0000116123.05160.43

[25] Russo TA, McFadden CD, Carlino-MacDonald UB, Beanan JM, Olson R, Wilding GE. The Siderophore receptor IroN of extraintestinal pathogenic *Escherichia coli* is a potential vaccine candidate. Infection and Immunity. 2003;**71**:7164-7169. DOI: 10.1128/iai.71.12.7164-7169.2003

[26] Langermann S, Möllby R, Burlein JE, Palaszynski SR, Auguste CG, DeFusco A, et al. Vaccination with FimH adhesin protects cynomolgus monkeys from colonization and infection by uropathogenic *Escherichia coli*. The Journal of Infectious Diseases. 2000;**181**:774-778. DOI: 10.1086/315258

[27] O'Hanley P, Lalonde G, Ji G. Alpha-hemolysin contributes to the pathogenicity of piliated digalactoside-binding *Escherichia coli* in the kidney: Efficacy of an alpha-hemolysin vaccine

in preventing renal injury in the BALB/c mouse model of pyelonephritis. Infection and Immunity. 1991;**59**: 1153-1161. DOI: 10.1128/iai.59.3.1153-1161.1991

[28] Langermann S, Palaszynski S, Barnhart M, Auguste G, Pinkner JS, Burlein J, et al. Prevention of mucosal *Escherichia coli* infection by FimH-adhesin-based systemic vaccination. Science. 1997;**276**:607-611. DOI: 10.1126/science.276.5312.607

[29] Mike LA, Smith SN, Sumner CA, Eaton KA, Mobley HL. Siderophore vaccine conjugates protect against uropathogenic *Escherichia coli* urinary tract infection. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**: 13468-13473. DOI: 10.1073/pnas.1606324113

[30] Alteri CJ, Hagan EC, Sivick KE, Smith SN, Mobley HL. Mucosal immunization with iron receptor antigens protects against urinary tract infection. PLoS Pathogens. 2009;**5**: e1000586. DOI: 10.1371/journal.ppat.1000586

[31] Markova R, Nikolaeva S, Kostadinova R, Mitov I, et al. Cellular and humoral systemic and mucosal immune responses stimulated by an oral polybacterial immunomodulator in patients with chronic urinary tract infections. International Journal of Immunopathology and Pharmacology. 2005;**18**:457-473. DOI: 10.1177/039463200501800306

[32] Karam MRA, Oloomi M, Mahdavi M, Habibi M, Bouzari S. Vaccination with recombinant FimH fused with flagellin enhances cellular and humoral immunity against urinary tract infection in mice. Vaccine. 2013;**31**:1210-1216. DOI: 10.1016/j.vaccine.2012.12.059

[33] Dhakal BK, Kulesus RR, Mulvey MA. Mechanisms and consequences of bladder cell invasion by uropathogenic *Escherichia coli*. European Journal of Clinical Investigation. 2008;**38**(Suppl 2): 2-11. DOI: 10.1111/j.1365-2362.2008.01986.x

[34] Dhakal BK, Mulvey MA. Uropathogenic *Escherichia coli* invades host cells via an HDAC6-modulated microtubule-dependent pathway. The Journal of Biological Chemistry. 2009; **284**:446-454. DOI: 10.1074/jbc.M805010200

[35] Bokil NJ, Totsika M, Carey AJ, Stacey KJ, Hancock V, Saunders BM, et al. Intramacrophage survival of uropathogenic *Escherichia coli*: Differences between diverse clinical isolates and between mouse and human macrophages. Immunobiology. 2011;**216**:1164-1171. DOI: 10.1016/j.imbio.2011.05.011

[36] Bishop BL, Duncan MJ, Song J, Li G, Zaas D, Abraham SN. Cyclic AMP–regulated exocytosis of *Escherichia coli* from infected bladder epithelial cells. Nature Medicine. 2007;**13**:625-630. DOI: 10.1038/nm1572

[37] Scott VC, Haake DA, Churchill BM, Justice SS, Kim JH. Intracellular bacterial communities: A potential etiology for chronic lower urinary tract symptoms. Urology. 2015;**86**:425-431. DOI: 10.1016/j.urology.2015.04.002

[38] Song J, Bishop BL, Li G, Grady R, Stapleton A, Abraham SN. TLR4-mediated expulsion of bacteria from infected bladder epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:14966-14971. DOI: 10.1073/pnas.0900527106

[39] Song J, Bishop BL, Li G, Duncan MJ, Abraham SN. TLR4-initiated and cAMP-

mediated abrogation of bacterial invasion of the bladder. Cell Host & Microbe. 2007;**1**:287-298. DOI: 10.1016/j.chom.2007.05.007

[40] Justice SS, Lauer SR, Hultgren SJ, Hunstad DA. Maturation of intracellular *Escherichia coli* communities requires SurA. Infection and Immunity. 2006;**74**: 4793-4800. DOI: 10.1128/iai.00355-06

[41] Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ. Bad bugs and beleaguered bladders: Interplay between uropathogenic *Escherichia coli* and innate host defenses. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**: 8829-8835. DOI: 10.1073/pnas.97.16.8829

[42] Klein K, Palarasah Y, Kolmos HJ, Møller-Jensen J, Andersen TE. Quantification of filamentation by uropathogenic *Escherichia coli* during experimental bladder cell infection by using semi-automated image analysis. Journal of Microbiological Methods. 2015;**109**:110-116. DOI: 10.1016/j.mimet.2014.12.017

[43] Leatham-Jensen MP, Mokszycki ME, Rowley DC, Deering R, Camberg JL, Sokurenko EV, et al. Uropathogenic *Escherichia coli* metabolite-dependent quiescence and persistence may explain antibiotic tolerance during urinary tract infection. mSphere. 2016;**1**:e00055-e00015. DOI: 10.1128/mSphere.00055-15

[44] Miao Y, Li G, Zhang X, Xu H, Abraham SN. A TRP channel senses lysosome neutralization by pathogens to trigger their expulsion. Cell. 2015;**161**: 1306-1319. DOI: 10.1016/j.cell.2015.05.009

[45] Truschel ST, Wang E, Ruiz WG, Leung SM, Rojas R, Lavelle J, et al. Stretch-regulated exocytosis/endocytosis in bladder umbrella cells. Molecular Biology of the Cell. 2002;**13**:830-846. DOI: 10.1091/mbc.01-09-0435

[46] Kodner CM, Thomas Gupton EK. Recurrent urinary tract infections in women: Diagnosis and management. American Family Physician. 2010;**82**: 638-643

[47] Anger J, Lee U, Ackerman AL, Chou R, Chughtai B, Clemens JQ, et al. Recurrent uncomplicated urinary tract infections in women: AUA/CUA/SUFU guideline. Journal of Urology. 2019;**202**: 282-289. DOI: 10.1097/JU.0000000000000296

[48] Burnett LA, Hochstedler BR, Weldon K, Wolfe AJ, Brubaker L. Recurrent urinary tract infection: Association of clinical profiles with urobiome composition in women. Neurourology and Urodynamics. 2021; **40**:1479-1489. DOI: 10.1002/nau.24707

[49] Wagenlehner FME, Bjorklund Johansen TE, Cai T, Koves B, Kranz J, Pilatz A, et al. Epidemiology, definition and treatment of complicated urinary tract infections. Nature Reviews Urology. 2020;**17**:586-600. DOI: 10.1038/s41585-020-0362-4

[50] Foxman B. The epidemiology of urinary tract infection. Nature Reviews Urology. 2010;**7**:653-660. DOI: 10.1038/nrurol.2010.190

[51] Haddad JM, Ubertazzi E, Cabrera OS, Medina M, Garcia J, Rodriguez-Colorado S, et al. Latin American consensus on uncomplicated recurrent urinary tract infection—2018. International Urogynecology Journal. 2020;**31**:35-44. DOI: 10.1007/s00192-019-04079-5

[52] Wagenlehner FM, Tandogdu Z, Johansen TEB. An update on classification and management of

urosepsis. Current Opinion in Urology. 2017;**27**:133-137. DOI: 10.1097/mou.0000000000000364

[53] Wagenlehner FME, Naber KG. A new way to prevent urinary tract infections? The Lancet Infectious Diseases. 2017;**17**:467-468. DOI: 10.1016/s1473-3099(17)30107-x

[54] Garrido D, Garrido S, Gutiérrez M, Calvopiña L, Harrison AS, Fuseau M, et al. Clinical characterization and antimicrobial resistance of *Escherichia coli* in pediatric patients with urinary tract infection at a third level hospital of Quito, Ecuador. Boletín Médico del Hospital Infantil de México. 2017;**74**: 265-271. DOI: 10.1016/j.bmhimx.2017.02.004

[55] Jayaweera J, Reyes M. Antimicrobial misuse in pediatric urinary tract infections: Recurrences and renal scarring. Annals of Clinical Microbiology and Antimicrobials. 2018;**17**:27. DOI: 10.1186/s12941-018-0279-4

[56] Conway PH, Cnaan A, Zaoutis T, Henry BV, Grundmeier RW, Keren R. Recurrent urinary tract infections in children: Risk factors and association with prophylactic antimicrobials. Journal of the American Medical Association. 2007;**298**:179-186. DOI: 10.1001/jama.298.2.179

[57] Hoberman A, Charron M, Hickey RW, Baskin M, Kearney DH, Wald ER. Imaging studies after a first febrile urinary tract infection in young children. The New England Journal of Medicine. 2003;**348**:195-202. DOI: 10.1056/NEJMoa021698

[58] Dias CS, Silva JM, Diniz JS, Lima EM, Marciano RC, Lana LG, et al. Risk factors for recurrent urinary tract infections in a cohort of patients with primary vesicoureteral reflux. The Pediatric Infectious Disease Journal. 2010;**29**:139-144. DOI: 10.1097/inf.0b013e3181b8e85f

[59] Zorc JJ, Kiddoo DA, Shaw KN. Diagnosis and management of pediatric urinary tract infections. Clinical Microbiology Reviews. 2005;**18**: 417-422. DOI: 10.1128/cmr.18.2.417-422.2005

[60] Huovinen P. Bacteriotherapy: The time has come. BMJ. 2001;**323**:353-354. DOI: 10.1136/bmj.323.7309.353

[61] Karki T, Truusalu K, Vainumäe I, Mikelsaar M. Antibiotic susceptibility patterns of community- and hospital-acquired *Staphylococcus aureus* and *Escherichia coli* in Estonia. Scandinavian Journal of Infectious Diseases. 2001;**33**: 333-338. DOI: 10.1080/003655401750173904

[62] Worby CJ, Olson BS, Dodson KW, Earl AM, Hultgren SJ. Establishing the role of the gut microbiota in susceptibility to recurrent urinary tract infections. The Journal of Clinical Investigation. 2022;**132**:e158497. DOI: 10.1172/jci158497

[63] Luo Y, Ma Y, Zhao Q, Wang L, Guo L, Ye L, et al. Similarity and divergence of phylogenies, antimicrobial susceptibilities, and virulence factor profiles of *Escherichia coli* isolates causing recurrent urinary tract infections that persist or result from reinfection. Journal of Clinical Microbiology. 2012;**50**:4002-4007. DOI: 10.1128/jcm.02086-12

[64] Jantunen ME, Saxén H, Salo E, Siitonen A. Recurrent urinary tract infections in infancy: Relapses or reinfections? The Journal of Infectious Diseases. 2002;**185**:375-379. DOI: 10.1086/338771

[65] Skjøt-Rasmussen L, Hammerum AM, Jakobsen L, Lester CH, Larsen P, Frimodt-Møller N. Persisting clones of *Escherichia coli* isolates from recurrent urinary tract infection in men and women. Journal of Medical Microbiology. 2011;**60**:550-554. DOI: 10.1099/jmm.0.026963-0

[66] Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. Annual Review of Microbiology. 2010;**64**: 203-221. DOI: 10.1146/annurev.micro.112408.134258

[67] Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infections. Science. 2003;**301**:105-107. DOI: 10.1126/science.1084550

[68] Kerrn MB, Struve C, Blom J, Frimodt-Møller N, Krogfelt KA. Intracellular persistence of *Escherichia coli* in urinary bladders from mecillinam-treated mice. Journal of Antimicrobial Chemotherapy. 2005;**55**:383-386. DOI: 10.1093/jac/dki002

[69] Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ. Detection of intracellular bacterial communities in human urinary tract infection. PLoS Medicine. 2007;**4**:e329. DOI: 10.1371/journal.pmed.0040329

[70] Brown ED, Wright GD. Antibacterial drug discovery in the resistance era. Nature. 2016;**529**:336-343. DOI: 10.1038/nature17042

[71] Khoshnood S, Heidary M, Mirnejad R, Bahramian A, Sedighi M, Mirzaei H. Drug-resistant gram-negative uropathogens: A review. Biomedicine & Pharmacotherapy. 2017;**94**:982-994. DOI: 10.1016/j.biopha.2017.08.006

[72] Pigrau C, Escolà-Vergé L. Infecciones urinarias recurrentes: desde la patogenia a las estrategias de prevención. Medicina Clínica. 2020;**155**: 171-177. DOI: 10.1016/j.medcli.2020.04.026

[73] Carson C, Naber KG. Role of fluoroquinolones in the treatment of serious bacterial urinary tract infections. Drugs. 2004;**64**:1359-1373. DOI: 10.2165/00003495-200464120-00007

[74] Matthew GB, Matthew AM. Persistence of uropathogenic *Escherichia coli* in the face of multiple antibiotics. Antimicrobial Agents and Chemotherapy. 2010;**54**:1855-1863. DOI: 10.1128/AAC.00014-10

[75] Chardavoyne PC, Kasmire KE. Appropriateness of antibiotic prescriptions for urinary tract infections. The Western Journal of Emergency Medicine. 2020;**21**:633-639. DOI: 10.5811/westjem.2020.1.45944

[76] Pratt LA, Kolter R. Genetic analysis of *Escherichia coli* biofilm formation: Roles of flagella, motility, chemotaxis and type I pili. Molecular Microbiology. 1998;**30**:285-293. DOI: 10.1046/j.1365-2958.1998.01061.x

[77] Prigent-Combaret C, Brombacher E, Vidal O, Ambert A, Lejeune P, Landini P, et al. Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the csgD gene. Journal of Bacteriology. 2001;**183**:7213-7223. DOI: 10.1128/jb.183.24.7213-7223.2001

[78] Lewis K. Persister cells and the riddle of biofilm survival. Biochemistry (Moscow). 2005;**70**:267-274. DOI: 10.1007/s10541-005-0111-6

[79] Domka J, Lee J, Bansal T, Wood TK. Temporal gene-expression in *Escherichia*

coli K-12 biofilms. Environmental Microbiology. 2007;**9**:332-346. DOI: 10.1111/j.1462-2920.2006.01143.x

[80] Monds RD, O'Toole GA. The developmental model of microbial biofilms: Ten years of a paradigm up for review. Trends in Microbiology. 2009;**17**: 73-87. DOI: 10.1016/j.tim.2008.11.001

[81] Ferrières L, Aslam SN, Cooper RM, Clarke DJ. The yjbEFGH locus in *Escherichia coli* K-12 is an operon encoding proteins involved in exopolysaccharide production. Microbiology. 2007;**153**:1070-1080. DOI: 10.1099/mic.0.2006/002907-0

[82] Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. Experimental and Molecular Pathology. 2008;**85**:11-19. DOI: 10.1016/j.yexmp.2008.03.007

[83] Schwartz DJ, Chen SL, Hultgren SJ, Seed PC. Population dynamics and niche distribution of uropathogenic *Escherichia coli* during acute and chronic urinary tract infection. Infection and Immunity. 2011;**79**(10):4250-4259

[84] Wright KJ, Seed PC, Hultgren SJ. Uropathogenic *Escherichia coli* flagella aid in efficient urinary tract colonization. Infection and Immunity. 2005;**73**: 7657-7668. DOI: 10.1128/iai.73.11.7657-7668.2005

[85] Anderson GG, Goller CC, Justice S, Hultgren SJ, Seed PC. Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. Infection and Immunity. 2010;**78**:963-975. DOI: 10.1128/iai.00925-09

[86] AbdelKhalek A, Abutaleb NS, Elmagarmid KA, Seleem MN. Repurposing auranofin as an intestinal decolonizing agent for vancomycin-resistant enterococci. Scientific Reports. 2018;**8**:8353

[87] Jang HI, Eom YB. Repurposing auranofin to combat uropathogenic *Escherichia coli* biofilms. Journal of Applied Microbiology. 2019;**127**(2): 459-471. DOI: 10.1111/jam.14312

[88] Kim Y, Kim S, Cho KH, Lee JH, Lee J. Antibiofilm activities of Cinnamaldehyde analogs against Uropathogenic *Escherichia coli* and *Staphylococcus aureus*. International Journal of Molecular Sciences. 2022; **23**(13):7225. DOI: 10.3390/ijms23137225

[89] Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science. 1997;**275**:218-220. DOI: 10.1126/science.275.5297.218

[90] Cottart CH, Nivet-Antoine V, Laguillier-Morizot C, Beaudeux JL. Resveratrol bioavailability and toxicity in humans. Molecular Nutrition & Food Research. 2010;**54**:7-16. DOI: 10.1002/mnfr.200900437

[91] Oh M, Lee J-H, Bae SY, Seok JH, Kim S, Chung YB, et al. Protective effects of red wine and resveratrol for foodborne virus surrogates. Food Control. 2015;**47**:502-509. DOI: 10.1016/j.foodcont.2014.07.056

[92] Ma DSL, Tan LT, Chan KG, Yap WH, Pusparajah P, Chuah LH, et al. Resveratrol-potential antibacterial agent against foodborne pathogens. Frontiers in Pharmacology. 2018;**9**:102. DOI: 10.3389/fphar.2018.00102

[93] Vestergaard M, Ingmer H. Antibacterial and antifungal properties of resveratrol. International Journal of

Antimicrobial Agents. 2019;**53**:716-723. DOI: 10.1016/j.ijantimicag.2019.02.015

[94] Lee JH, Kim YG, Raorane CJ, Ryu SY, Shim JJ, Lee J. The anti-biofilm and anti-virulence activities of *trans*-resveratrol and oxyresveratrol against uropathogenic *Escherichia coli*. Biofouling. 2019;**35**(7):758-767. DOI: 10.1080/08927014.2019.1657418

[95] Vasudevan S, Thamil Selvan G, Bhaskaran S, Hari N, Solomon AP. Reciprocal cooperation of type a Procyanidin and nitrofurantoin against multi-drug resistant (MDR) UPEC: A pH-dependent study. Frontiers in Cellular and Infection Microbiology. 2020;**10**:421. DOI: 10.3389/fcimb.2020.00421

[96] Thänert R, Reske KA, Hink T, Wallace MA, Wang B, Schwartz DJ, et al. Comparative genomics of antibiotic-resistant uropathogens implicates three routes for recurrence of urinary tract infections. MBio. 2019;**10**:e01977-e01919. DOI: 10.1128/mBio.01977-19

[97] Lamm ME. Interaction of antigens and antibodies at mucosal surfaces. Annual Review of Microbiology. 1997;**51**: 311-340. DOI: 10.1146/annurev.micro.51.1.311

[98] Levine MM. Immunization against bacterial diseases of the intestine. Journal of Pediatric Gastroenterology and Nutrition. 2000;**31**:336-355. DOI: 10.1097/00005176-200010000-00003

[99] Kochiashvili D, Khuskivadze A, Kochiashvili G, Koberidze G, Kvakhajelidze V. Role of the bacterial vaccine Solco-Urovac® in treatment and prevention of recurrent urinary tract infections of bacterial origin. Georgian Medical News. 2014;**231**:11-16

[100] Hopkins WJ, Elkahwaji J, Beierle LM, Leverson GE, Uehling DT. Vaginal mucosal vaccine for recurrent urinary tract infections in women: Results of a phase 2 clinical trial. The Journal of Urology. 2007;**177**:1349-1353; quiz 1591. DOI: 10.1016/j.juro.2006.11.093

[101] Nestler S, Peschel C, Horstmann AH, Vahlensieck W, Fabry W, Neisius A. Prospective multicentre randomized double-blind placebo-controlled parallel group study on the efficacy and tolerability of StroVac® in patients with recurrent symptomatic uncomplicated bacterial urinary tract infections. International Urology and Nephrology. 2022;**54**:1-8

[102] Bauer HW, Alloussi S, Egger G, Blümlein HM, Cozma G, Schulman CC. A long-term, multicenter, double-blind study of an *Escherichia coli* extract (OM-89) in female patients with recurrent urinary tract infections. European Urology. 2005;**47**:542-548; discussion 548. DOI: 10.1016/j.eururo.2004.12.009

[103] Huttner A, Gambillara V. The development and early clinical testing of the ExPEC4V conjugate vaccine against uropathogenic *Escherichia coli*. Clinical Microbiology and Infection. 2018; **24**(10):1046-1050

[104] Huttner A, Hatz C, van den Dobbelsteen G, Abbanat D, Hornacek A, Frölich R, et al. Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: A randomised, single-blind, placebo-controlled phase 1b trial. The Lancet Infectious Diseases. 2017;**17**(5): 528-537

[105] Nickel JC, Saz-Leal P, Doiron RC. Could sublingual vaccination be a viable

option for the prevention of recurrent urinary tract infection in Canada? A systematic review of the current literature and plans for the future. Canadian Urological Association Journal. 2020;**14**(8):281

[106] Billips BK, Yaggie RE, Cashy JP, Schaeffer AJ, Klumpp DJ. A live-attenuated vaccine for the treatment of urinary tract infection by uropathogenic *Escherichia coli*. The Journal of Infectious Diseases. 2009;**200**:263-272. DOI: 10.1086/599839

[107] Brumbaugh AR, Mobley HL. Preventing urinary tract infection: Progress toward an effective *Escherichia coli* vaccine. Expert Review of Vaccines. 2012;**11**:663-676. DOI: 10.1586/erv.12.36

[108] Connell I, Agace W, Klemm P, Schembri M, Mărild S, Svanborg C. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**:9827-9832. DOI: 10.1073/pnas.93.18.9827

[109] Norinder BS, Köves B, Yadav M, Brauner A, Svanborg C. Do *Escherichia coli* strains causing acute cystitis have a distinct virulence repertoire? Microbial Pathogenesis. 2012;**52**:10-16. DOI: 10.1016/j.micpath.2011.08.005

[110] Cordeiro MA, Werle CH, Milanez GP, Yano T. Curli fimbria: An *Escherichia coli* adhesin associated with human cystitis. Brazilian Journal of Microbiology. 2016;**47**:414-416. DOI: 10.1016/j.bjm.2016.01.024

[111] Manning SD, Zhang L, Foxman B, Spindler A, Tallman P, Marrs CF. Prevalence of known P-fimbrial G alleles in *Escherichia coli* and identification of a new adhesin class. Clinical and Diagnostic Laboratory Immunology. 2001;**8**:637-640. DOI: 10.1128/cdli.8.3.637-640.2001

[112] Thankavel K, Madison B, Ikeda T, Malaviya R, Shah AH, Arumugam PM, et al. Localization of a domain in the FimH adhesin of *Escherichia coli* type 1 fimbriae capable of receptor recognition and use of a domain-specific antibody to confer protection against experimental urinary tract infection. The Journal of Clinical Investigation. 1997;**100**: 1123-1136. DOI: 10.1172/jci119623

[113] Langermann S, Ballou WR Jr. Vaccination utilizing the FimCH complex as a strategy to prevent *Escherichia coli* urinary tract infections. The Journal of Infectious Diseases. 2001; **183**:S84-S86. DOI: 10.1086/318857

[114] Langermann S, Ballou WR. Development of a recombinant FIMCH vaccine for urinary tract infections. In: Atala A, Slade D, editors. Bladder Disease, Part a: Research Concepts and Clinical Applications. Boston, MA: Springer US; 2003. pp. 635-653

[115] Habibi M, Asadi Karam MR, Shokrgozar MA, Oloomi M, Jafari A, Bouzari S. Intranasal immunization with fusion protein MrpH·FimH and MPL adjuvant confers protection against urinary tract infections caused by uropathogenic *Escherichia coli* and *Proteus mirabilis*. Molecular Immunology. 2015;**64**:285-294. DOI: 10.1016/j.molimm.2014.12.008

[116] Fischer H, Yamamoto M, Akira S, Beutler B, Svanborg C. Mechanism of pathogen-specific TLR4 activation in the mucosa: Fimbriae, recognition receptors and adaptor protein selection. European Journal of Immunology. 2006;**36**: 267-277. DOI: 10.1002/eji.200535149

[117] Samuelsson P, Hang L, Wullt B, Irjala H, Svanborg C. Toll-like receptor 4

expression and cytokine responses in the human urinary tract mucosa. Infection and Immunity. 2004;**72**:3179-3186. DOI: 10.1128/iai.72.6.3179-3186.2004

[118] Lane MC, Mobley HLT. Role of P-fimbrial-mediated adherence in pyelonephritis and persistence of uropathogenic *Escherichia coli* (UPEC) in the mammalian kidney. Kidney International. 2007;**72**:19-25. DOI: 10.1038/sj.ki.5002230

[119] Frendéus B, Wachtler C, Hedlund M, Fischer H, Samuelsson P, Svensson M, et al. *Escherichia coli P. fimbriae* utilize the toll-like receptor 4 pathway for cell activation. Molecular Microbiology. 2001;**40**:37-51. DOI: 10.1046/j.1365-2958.2001.02361.x

[120] Pecha B, Low D, O'Hanley P. Gal-gal pili vaccines prevent pyelonephritis by piliated *Escherichia coli* in a murine model. Single-component gal-gal pili vaccines prevent pyelonephritis by homologous and heterologous piliated E. coli strains. The Journal of Clinical Investigation. 1989;**83**:2102-2108. DOI: 10.1172/jci114123

[121] Bian Z, Brauner A, Li Y, Normark S. Expression of and cytokine activation by *Escherichia coli* curli fibers in human sepsis. The Journal of Infectious Diseases. 2000;**181**:602-612. DOI: 10.1086/315233

[122] Tükel C, Nishimori JH, Wilson RP, Winter MG, Keestra AM, van Putten JP, et al. Toll-like receptors 1 and 2 cooperatively mediate immune responses to curli, a common amyloid from enterobacterial biofilms. Cellular Microbiology. 2010;**12**:1495-1505. DOI: 10.1111/j.1462-5822.2010.01485.x

[123] Bian Z, Yan Z-Q, Hansson GK, Thorén P, Normark S. Activation of inducible nitric oxide synthase/nitric oxide by curli fibers leads to a fall in blood pressure during systemic *Escherichia coli* infection in mice. The Journal of Infectious Diseases. 2001;**183**: 612-619. DOI: 10.1086/318528

[124] Rapsinski GJ, Wynosky-Dolfi MA, Oppong GO, Tursi SA, Wilson RP, Brodsky IE, et al. Toll-like receptor 2 and NLRP3 cooperate to recognize a functional bacterial amyloid, curli. Infection and Immunity. 2015;**83**: 693-701. DOI: 10.1128/iai.02370-14