Vaccination approaches for the prevention of urinary tract infection

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Abstract

Urinary tract infections (UTIs) are one of the most common infectious diseases of humans, with approximately 150 million cases estimated to occur globally every year. UTIs usually start as a bladder infection (cystitis), but can develop into acute kidney infection (pyelonephritis) and even infection of the bloodstream (urosepsis). The high frequency of UTIs in community and nosocomial settings places an enormous burden on healthcare systems worldwide. Multiple different pathogens cause UTI, with uropathogenic *E. coli* (UPEC) the most common etiological agent. UTIs caused by these pathogens are increasingly associated with antibiotic resistance, thus severely reducing treatment options and significantly increasing UTI-associated morbidity and mortality. In this review we present an overview of the recent advances in vaccine research targeted towards the prevention of UPEC-mediated UTI. In the context of multidrug resistance, we conclude that vaccination represents a viable approach for the prevention of chronic and recurrent UTI.

Key words: Vaccine; urinary tract infection; Escherichia coli
Clinical importance and impact of urinary tract infections (UTIs)

UTIs are one of the most common infections of humans. They affect approximately 12% of women and 3% of men in the United States every year [1] and also represent a major cause of hospitalization [2]. UTIs present as uncomplicated or complicated infections of the bladder (cystitis) or kidney (pyelonephritis) and are frequently observed in both nosocomial and community settings. Acute cystitis and pyelonephritis episodes in healthy premenopausal, non-pregnant women with no evidence of an abnormal urinary tract are usually classified and treated as uncomplicated. Complicated UTIs generally affect patients with structural or functional abnormalities that may compromise therapy and lead to urosepsis. UTIs are also categorized as isolated, unresolved or recurrent (due to reinfection or relapse) and, altogether, these classifications inform the selection and duration of antibiotics used in treatment [3, 4]. Another form of UTI, termed asymptomatic bacteriuria (ABU), represents an asymptomatic carrier state in which patients may carry >10⁵ CFU/ml of a single organism for years without provoking a host response. ABU is generally left untreated unless there are additional risk factors, such as during pregnancy [5].

Cystitis, the most common infection of the urinary tract, generally resolves quickly in response to antibiotic treatment (3.32 ± 2.54 days). The mean duration of symptoms is increased when incorrect antibiotics are administered due to infection with drug-resistant strains (4.73 ± 2.91 days) or where treatment is delayed (4.94 ± 3.82 days) [6]. Overall, the global burden of UTI is responsible for huge health care costs throughout the world. Indeed, in 1995, it was estimated that 11.3 million women received treatment for a UTI in the United States, leading to an estimated direct cost of 1.6 billion dollars [7]. Community-acquired UTIs also represent approximately 0.7% of ambulatory care visits, which in 2007 alone corresponded to 8.6 billion patient episodes in the USA [8].

The total burden of UTI is significantly higher in women than men. This is strongly linked to anatomical differences in the urinary tract; women have a shorter distance between the bladder and the urethra, and the urethral opening of women is proximate to vaginal cavity and rectum, thus increasing the opportunity for infection [9]. It is estimated that one in three women experience a UTI by the age of 24 years and at least 40-50% of women experience a UTI in their lifetime [5]. Uncomplicated UTIs in women are often associated with sexual activity, with the peak incidence of disease occurring between 18-39 years of age [10]. UTIs are also a common infection in childhood, affecting approximately 7-8% of girls and 2% of boys. It is estimated that one out of ten girls and one out of thirty boys will present with a UTI by the age of 16 years [11].
Nosocomial UTIs also contribute a significant economic burden to hospitals and health care facilities. The incidence of nosocomial UTI in catheterized inpatients is estimated to be 7.3% [12]. In the UK, it is estimated that acquisition of a UTI following surgery results in a mean of 3.6 additional hospital days per infected patient. This equates to an approximate cost of one thousand British pounds per patient, and constitutes a major economic impact given that approximately 1.6% of inpatients are estimated to acquire a UTI [12].

Urosepsis is a severe complication of UTI that results when bacteria cross into the bloodstream and can be life-threatening. Urosepsis is estimated to develop in 3.6-12.6% of UTI cases [13, 14], with a mortality rate of up to 12.7% [13].

**Causative agents and antibiotic resistance**

Uropathogenic *Escherichia coli* (UPEC) is the most common causative agent of UTI, and is responsible for 75-95% of all cases of uncomplicated cystitis and pyelonephritis [3]. Other common Gram-negative and Gram-positive bacterial pathogens that cause UTI include *Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, Enterobacter cloacae, Enterobacter aerogenes, Morganella morganii, Acinetobacter baumannii, Staphylococcus saprophyticus* and *Enterococcus* species [15]. The range of pathogens that cause nosocomial UTI is generally more diverse [16].

UTIs are the second most common reason for antibiotic prescription, preceded only by otitis media [9]. Community-acquired uncomplicated UTIs are generally treated empirically, since UPEC causes the majority of infections and short-course therapies are usually completed before laboratory analysis is available. This strategy has likely contributed to the increased incidence of extended-spectrum β-lactamase (ESBL)-producing strains worldwide, as well as to episodes of subclinical persistence and recurrence following treatment [17, 18]. The emergence and dissemination of multidrug-resistant clones such as *E. coli* sequence type 131 (*E. coli* ST131), as well as other sequence types including ST69, ST73 and ST95, has significantly reduced treatment options and threatens to make UTI a major threat to public health worldwide [19, 20, 21, 22, 23, 24, 25, 26, 27]. Indeed, several large surveillance studies performed over the last two decades have demonstrated that in some regions across the globe, 20-50% of all UPEC strains are resistant to commonly prescribed antibiotics such as trimethoprim-sulfamethoxazole, fluoroquinolones and β-lactams [9]. Recent studies also indicate that ESBL-producing strains are associated with 6.6% of community-acquired and 26.8% of nosocomial bacteremia caused by *E. coli*, and are associated with
significantly higher morbidity (up to 60.8%) than non-ESBL-producing *E. coli* strains (23.7%) [28]. In a recent retrospective study, human and animal *E. coli* isolates collected from 1950 and 2002 were assessed for historical changes in their resistance profile to 15 antibiotics. Multidrug resistance (i.e. resistance to at least 3 drug classes) increased from 7.2% during the 1950s to 63.6% during the 2000s [29]. These alarming trends have led to an increase in the use of second- and third-line therapies, further promoting the emergence of multidrug-resistant strains and highlighting the need for alternative treatment and prevention approaches.

**Vaccination approaches to prevent UTI**

Infection of the urinary tract by UPEC is associated with the expression of multiple virulence factors, including fimbrial adhesins, autotransporter proteins, toxins, siderophores, flagella and polysaccharides that comprise specific capsule and O antigen surface structures [30] (Figure 1). It is not surprising, therefore, that most vaccine efforts to-date have involved targeting these UPEC surface or secreted factors, either as single or multi-component formulations.

**Preventing adhesion to the urinary tract**

Adherence represents a fundamental initial step in colonization of the urinary tract and is generally mediated by fimbrial adhesins, which enable pathogens to bind to the uroepithelium, to avoid clearance through the washing effect of urine and to mediate intimate interactions with host epithelial cells. Adhesins are structurally variable and mediate specific attachment to different receptors on epithelial cells in a lock-and-key fashion. Type 1 fimbriae are directly associated with UPEC infection and persistence in the mouse urinary tract [31, 32] by virtue of their ability to mediate binding to α-D-mannosylated proteins such as uroplakins Ia and Ib, two major glycoproteins present on the apical surface of superficial bladder epithelial cells [33]. Type 1 fimbriae-facilitated adhesion is mediated specifically by FimH, a minor subunit localized at the tip of the fimbrial structure [34, 35]. The use of FimH (truncated or in complex with the periplasmic chaperone FimC) as a vaccine candidate has been shown to inhibit the binding of UPEC to human epithelial bladder cells *in vitro* [36]. Moreover, systemic immunization with FimH (in combination with the FimC chaperone protein) promoted a strong and long-lasting immune response that led to a >99% reduction in bladder colonization in a murine model of cystitis, and prevention of kidney infection over a 7-day period. Immunization with FimH also correlated with a high titer of anti-FimH IgG antibodies in the urine of mice [36]. Further studies using a primate model of UTI revealed that immunization with FimH (in complex with FimC) led to high vaginal anti-FimH titers.
and resulted in significant protection from UTI following UPEC challenge [37]. A vaccine containing the FimH protein fused to the FliC major flagellin subunit (FimH-FliC) has also been recently described [38]. Mice immunized with the FimH-FliC protein induced a significant humoral and cellular immune response, and were protected from UPEC infection of the bladder and kidney in a mouse UTI model [38, 39].

P fimbriae, another adhesive component associated with UPEC infection of the urinary tract, mediate adhesion to α-D-galactopyranosyl-(1-4)β-D-galactopyranoside receptor epitopes in the globoseries of glycolipids present on vaginal and kidney epithelial cells via the tip-located PapG adhesin [40, 41, 42, 43]. Previous studies have demonstrated protection against renal colonization in murine models of infection [44, 45]. PapG, in complex with the periplasmic chaperone PapD, was used to vaccinate cynomolgus monkeys to evaluate protection against pyelonephritis. Vaccinated monkeys possessed increased anti-PapDG IgG titers and were protected from pyelonephritis following challenge. However, despite histological evidence for reduced inflammation, no difference was observed in the number of bacteria recovered from the urine of vaccinated and control groups [46]. It is possible these observations could be explained by the expression of other adhesins, such as type 1 fimbriae, that also mediate binding to uroepithelial cells [46].

UPEC express a number of additional fimbrial adhesins that are associated with UTI, including Afa/Dr fimbriae [47], F1C fimbriae [48], S fimbriae [49], and type 3 fimbriae [50]. Among these adhesins, only Afa/Dr fimbriae, which mediate binding to decay-accelerating factor, type IV collagen and carcinoembryonic antigen-related cell adhesion molecules in the upper urinary tract [51], have been examined as a putative vaccine target. Vaccination with Afa/Dr fimbriae led to a reduction in UPEC adherence to bladder cells in a UTI mouse infection model [52].

Several other UPEC virulence factors associated with adherence have been examined with respect to their potential role as vaccine targets. The UPEC trimeric autotransporter UpaG was identified by reverse vaccinology as a potentially protective antigen against extra-intestinal pathogenic E. coli [53]. UpaG promotes cell aggregation, biofilm formation, and binding to human bladder epithelial cells as well as to the extracellular matrix proteins fibronectin and laminin [54]. Using the prototype pyelonephritis-associated UPEC strain CFT073 in a murine sepsis model, immunization with UpaG (c4424) resulted in a 33% protection rate following active immunization and a 66% protection rate following passive immunization [53]. Intranasal vaccination with FdeC (ECOK1_0290), an outer membrane protein that mediates adhesion to human bladder and urethral epithelial cells, has also been examined. Vaccination of mice with FdeC led to a 1.5-2.5 log reduction in UPEC kidney
colonization using a UTI model, thus demonstrating significant protection against pyelonephritis [55]. FdeC has also been identified as a protective antigen in a murine model of sepsis [56].

Preventing damage to uroepithelial cells

An important feature of UPEC pathogenesis is the ability to scavenge nutrients from the urinary tract. UPEC can secrete several toxins that damage uroepithelial cells and promote the release of nutrients into the urine. Alpha hemolysin (HlyA) is a pore-forming cytotoxin secreted by some UPEC strains [57]. Hemolysin has been purified from UPEC culture supernatants and tested as a vaccine candidate in a murine model of pyelonephritis. Mice immunized with denatured hemolysin showed less renal injury compared to control groups, however no significant effect was observed on kidney colonization [58]. In a separate study, systemic immunization with recombinant HlyA led to a protection rate of 86% in a murine model of sepsis [56]. Another UPEC toxin, the vacuolating autotransporter cytotoxin (Vat), is a serine protease also secreted by avian pathogenic *E. coli* (APEC) [59]. The vat gene is located on a pathogenicity island, which likely accounts for its high prevalence in both UPEC and APEC strains. In a murine model of sepsis, Vat (c0393) led to a protection rate of 32% and 78% by active and passive immunization, respectively [53].

Preventing the scavenging of nutrients from urine

The ability to capture nutrients in the resource-limited urinary tract is an essential feature of pathogens that colonize this niche and cause UTI. Most UPEC strains possess a range of mechanisms to effectively capture iron. This includes a number of siderophores, namely enterobactin, salmochelin, aerobactin and yersiniabactin [60, 61, 62, 63]. IroN, a receptor for salmochelin, is associated with UPEC colonization of the urinary tract [63, 64, 65]. Subcutaneous immunization with denatured IroN led to a specific IgG response in serum and protection against renal infection in a murine model of ascending UTI, however protection against bladder infection and production of mucosal IgA was not observed [66]. IroN has also been tested as a vaccine candidate in a murine model of sepsis, and led to a protection rate of 82% by active immunization and 79% by passive immunization following UPEC challenge [53].

More recent work on UPEC iron receptors has demonstrated they hold significant promise as potential vaccine candidates [53, 67]. Intranasal immunization with the iron receptor proteins IreA, Hma and LutA, conjugated to cholerae toxin, was able to elicit a specific systemic and mucosal immune response capable of conferring protection in a murine model of UTI. Vaccination with
Hma, a haem receptor required for kidney colonization [68], protected mice against colonization of the kidney, while vaccination with IreA, a less-well characterised iron receptor required for bladder colonization [69], showed significant protection against bladder infection, even against heterologous UPEC strains [67]. IutA, a receptor for aerobactin, was also able to confer significant protection against UPEC infection of the mouse bladder and kidney [67]. In a murine model of sepsis, FyuA, the yersiniabactin receptor, led to a protection rate of 53% by active immunization and 72% by passive immunization [53]. Intranasal immunization with FyuA also resulted in a 29-fold decrease in kidney colonization in a murine model of UTI compared to control groups [70]. Finally, vaccination of mice with FitA (ECOK1_3457), a putative iron receptor [71], resulted in a protection efficacy of 25% in a sepsis model [56].

Iron receptors are integral outer membrane proteins that contain surface loops exposed to the external milieu and interact with siderophore-iron complexes. The external loops of IroN and IutA have been synthesized as synthetic linear peptides and tested for their ability to protect mice from UTI. In these studies, a 2-log reduction in colonization of the kidney was observed after UPEC challenge [67]. Furthermore, when administered as a multi-epitope vaccine, synthetic vaccine proteins with concatenated epitopes Vol1 (combination of IutA, IhaA, FyuA and Usp epitopes) and Vol2 (IreA, ChuA and IroN epitopes) resulted in a significant 2-log reduction in bacterial load in the spleen and liver using a murine model of peritonitis infection [72].

 Preventing escape from immune cells

Protection against innate host defenses is strongly associated with UPEC pathogenesis of the urinary tract. Several specific O antigen and capsular polysaccharide types are highly prevalent among UPEC strains, and confer resistance against antibacterial peptides, phagocytosis and complement mediated killing [73]. The use of chemically inactivated UPEC strains to generate specific antibodies against O and K antigens was one of the first strategies employed to prevent UTI [74, 75]. Targeting of UPEC surface polysaccharides has been attempted using both active (intraperitoneal and intravesical) and passive (urine transfer) immunization in a rat ascending UTI model, showing increased protection against homologous strains but a reduced impact on heterologous strains [76].

Uro-Vaxom, a lyophilized lysate prepared from 18 UPEC strains and licensed for human application (Galenica Group), has been tested in a double-blind randomized study of patients with recurrent UTI from 52 centers and nine different countries [77]. Female patients aged 18-65 years
with at least three documented episodes of UTI in the previous year were included in the investigation. Capsules containing the treated UPEC lysates were administered daily during the first three months, followed by no treatment during months 4-6, and then one capsule daily during the first ten days of months seven to nine. In this trial, the treated group achieved a 14.7% reduction in UTI episodes compared to control group. Interestingly, after month seven, the reduction increased to 43%, possibly associated with the administration of booster doses. The vaccine significantly impacted on the number of recurrent UTI episodes, which were reduced by 49% in the treated group compared to placebo [77].

Urovac, another lyophilized vaccine formulation, contains heat-killed bacteria from ten uropathogenic strains (six UPEC strains, *K. pneumoniae*, *P. mirabilis*, *M. morganii* and *E. faecalis*) [78]. After several Phase II clinical studies using different schedules of immunization to determine vaccine efficacy [79, 80, 81], Urovac was tested in a randomized, double-blind clinical trial using women who had suffered at least three recurrent UTIs in the previous year. Patients were initially immunized with three vaginal suppositories at weekly intervals followed by three additional suppositories at monthly intervals. Overall, vaccination with Urovac significantly reduced recurrent UTI over a six-month period (46% infection-free) compared to a placebo control group (16.7% infection-free). Moreover, a major impact of vaccination with Urovac was observed in sexually active women [82].

A live-attenuated vaccine against UTI has also been proposed using a rough mutant of the UPEC strain NU14. This mutant strain stimulated a strong urothelial cytokine response and protection in mice for up to 8 weeks against challenge by direct inoculation into the bladder. The same pattern of protection was demonstrated for several other UPEC strains, demonstrating a reduction in bladder colonization and cross-protection against different UPEC serotypes [83]. While used prophylactically rather than directly as a vaccine, the ABU *E. coli* strain 83972 has also been shown to reduce UPEC colonization in animal and human infection studies [84, 85, 86, 87, 88]. The potential of killed whole-cell vaccines has also been examined through the generation of a genetically engineered UPEC strain deficient in capsule and O-antigen synthesis. A formalin-killed preparation of this strain administered intranasally into mice led to an increased humoral immune response and increased opsonophagocytosis of UPEC following challenge [89].

Other antigens may also contribute to the ability of UPEC to avoid immune responses in the urinary tract. For example, EsiB (c5321), a protein that interacts with secretory IgA and inhibits neutrophil
chemotaxis as well as the respiratory burst [90], showed a protection efficacy of 33% in a murine model of sepsis [56].

_Hypothetical proteins as vaccine targets_

Some UPEC proteins have been shown to elicit a strong immune response and provide protection in an infection model despite their lack of an assigned function. This includes SslE (ECOK1_3385), a secreted and surface-exposed _E. coli_ lipoprotein [56, 91] potentially associated with the degradation of mucosal glycoproteins such as mucin [92]. Active immunization of mice with SslE resulted in a protection efficacy of 82% in a murine model of sepsis. SslE is secreted by a type 2 secretion system (T2SS), and active immunization of mice with a surface-exposed component of this T2SS (ECOK1_3374) also resulted in protection from infection, albeit at a significantly lower level. Protection following passive immunization with rabbit polyclonal antibodies against SslE using the same model reached 100%, and cross-protection against heterologous UPEC strains was also demonstrated [56].

Several additional uncharacterized proteins have been shown to confer protection against UPEC in a mouse sepsis infection model. This includes the OmpA-like protein c3389 (38% protection by active immunization, 100% protection by passive immunization) [53], c1275 (45% protection by active immunization) and c0975 (24% protection by active immunization) [56].

_Outlook and perspectives on vaccine development against UTI_

A vaccine aimed at the prevention of UTI would have a major impact on the quality of life for many individuals who suffer from chronic and recurrent UTI. As UPEC represent the primary causative agent of UTI, one could envisage that this pathogen would be the primary target of such a vaccine. However, other common UTI pathogens should also be considered where feasible. The variation in UPEC serotypes that cause UTI, together with the diversity of virulence factors that are expressed by different UPEC strains, adds complexity to the design of an effective UTI vaccine. Ideally, one would expect a UTI vaccine to be multi-component and have broad coverage that at a minimum provides protection against all UPEC strain types. On the other hand, targeting a common component of the commensal intestinal flora increases the challenge associated with such a vaccine, as changes in the human microbiota may lead to other complications [93]. Considering the advances in metagenomics [94] and the recent outcomes of the Human Microbiome Project [95, 96], a
comparison of the gut microbiota between pre- and post-vaccinated individuals would finally address the direct and indirect impact of a UTI vaccine on intestinal homeostasis.

The use of multiple animal infection models in combination with a range of UPEC targets has demonstrated the feasibility of a vaccine to prevent UTI in humans. Systemic and mucosal immunization has also been shown to confer protection in the urinary tract, even though the generation of long-lasting responses still needs to be demonstrated. So why is a broadly protective UTI vaccine still not available? Our analysis of publicly available pipelines only identified three vaccine companies that are directly investing in a vaccine against UTI (Table 1). The main reasons for this may be a combination of marketing strategies and the target population for such a vaccine.

Despite the huge increase in multidrug resistant strains that cause UTI over the last decade and the consequent economic burden associated with treatment failure (including extended therapy and increased recovery time), UTIs can in most cases still be treated by an appropriate antibiotic regimen. Therefore, despite the impact of a UTI vaccine on specific patient groups, overall this represents a small target population and a limited market. A UTI vaccine would not be inserted into national immunization schedules and thus would most likely only attract a private market, far away from the revenues of a blockbuster vaccine. One possible way to circumvent this roadblock would be the design of a universal *E. coli* vaccine that targets common surface proteins from both extraintestinal and intestinal *E. coli* pathotypes. Such a vaccine would impact on multiple human diseases, including extraintestinal infections such as urinary tract infection, meningitis and sepsis, as well as severe intestinal diseases caused by enterotoxigenic, enterohaemorrhagic, enteropathogenic, enteroinvasive, enteroaggregative and diffusely adherent *E. coli* pathotypes.

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Table 1 - Analysis of the current pipeline (up to 5 years) for companies investing in *E. coli* vaccines

<table>
<thead>
<tr>
<th>Company</th>
<th>Stage</th>
<th>Description/Comments</th>
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<tbody>
<tr>
<td>Sequoia Sciences</td>
<td>PC</td>
<td>Vaccine consisting of <em>E. coli</em> adhesins in development for the prevention of chronic UTI. Objective to enter clinical trials in 2013 after the optimization of adjuvants and vaccination protocols. Exclusive license to US Patent 6,500,434 obtained from Washington University in Saint Louis.</td>
</tr>
<tr>
<td>GlycoVaxyn</td>
<td>PC</td>
<td>Conjugate vaccine against UPEC based on proprietary bioconjugate platform that enables the <em>in vivo</em> synthesis of polysaccharide-protein complexes.</td>
</tr>
<tr>
<td>NanoBio</td>
<td>PC</td>
<td>Vaccine targeting UPEC surface antigens. Objective to develop an intranasally delivered vaccine using proprietary nanoemulsion adjuvant technology. Licencing agreement announced in 2011 in partnership with the University of Michigan.</td>
</tr>
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</table>

PC - preclinical.
**Figure 1** - Schematic diagram of the major cell-surface associated or secreted virulence factors of UPEC that contribute to colonization of the urinary tract. Fimbrial and non-fimbrial adhesins mediate attachment to host epithelial cells. Flagella mediate motility and chemotaxis and contribute to UPEC dissemination. Iron receptors facilitate the uptake of iron by UPEC. Secreted toxins induce host cell lysis and disrupt host inflammatory signaling cascades. Outer membrane (OM) proteins and lipoproteins are integral components of the UPEC outer membrane. Capsule and O-antigen promote host evasion and contribute to survival in the bloodstream.