1   # Vaccination approaches for the prevention of urinary tract infection

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33

34 **Abstract**

35

36 Urinary tract infections (UTIs) are one of the most common infectious diseases of humans, with

37 approximately 150 million cases estimated to occur globally every year. UTIs usually start as a

38 bladder infection (cystitis), but can develop into acute kidney infection (pyelonephritis) and even

39 infection of the bloodstream (urosepsis). The high frequency of UTIs in community and nosocomial

40 settings places an enormous burden on healthcare systems worldwide. Multiple different pathogens

41 cause UTI, with uropathogenic *E. coli* (UPEC) the most common etiological agent. UTIs caused by

42 these pathogens are increasingly associated with antibiotic resistance, thus severely reducing

43 treatment options and significantly increasing UTI-associated morbidity and mortality. In this

44 review we present an overview of the recent advances in vaccine research targeted towards the

45 prevention of UPEC-mediated UTI. In the context of multidrug resistance, we conclude that

46 vaccination represents a viable approach for the prevention of chronic and recurrent UTI.

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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 $>10^5$ 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

122 significantly higher morbidity (up to 60.8%) than non-ESBL-producing *E. coli* strains (23.7%) [28].

123 In a recent retrospective study, human and animal *E. coli* isolates collected from 1950 and 2002

124 were assessed for historical changes in their resistance profile to 15 antibiotics. Multidrug resistance

125 (i.e. resistance to at least 3 drug classes) increased from 7.2% during the 1950s to 63.6% during the

126 2000s [29]. These alarming trends have led to an increase in the use of second- and third-line

127 therapies, further promoting the emergence of multidrug-resistant strains and highlighting the need

128 for alternative treatment and prevention approaches.

129

130 **Vaccination approaches to prevent UTI**

131

132 Infection of the urinary tract by UPEC is associated with the expression of multiple virulence

133 factors, including fimbrial adhesins, autotransporter proteins, toxins, siderophores, flagella and

134 polysaccharides that comprise specific capsule and O antigen surface structures [30] (Figure 1). It is

135 not surprising, therefore, that most vaccine efforts to-date have involved targeting these UPEC

136 surface or secreted factors, either as single or multi-component formulations.

137

138 *Preventing adhesion to the urinary tract*

139

140 Adherence represents a fundamental initial step in colonization of the urinary tract and is generally

141 mediated by fimbrial adhesins, which enable pathogens to bind to the uroepithelium, to avoid

142 clearance through the washing effect of urine and to mediate intimate interactions with host

143 epithelial cells. Adhesins are structurally variable and mediate specific attachment to different

144 receptors on epithelial cells in a lock-and-key fashion. Type 1 fimbriae are directly associated with

145 UPEC infection and persistence in the mouse urinary tract [31, 32] by virtue of their ability to

146 mediate binding to α-D-mannosylated proteins such as uroplakins Ia and Ib, two major

147 glycoproteins present on the apical surface of superficial bladder epithelial cells [33]. Type 1

148 fimbriae-facilitated adhesion is mediated specifically by FimH, a minor subunit localized at the tip

149 of the fimbrial structure [34, 35]. The use of FimH (truncated or in complex with the periplasmic

150 chaperone FimC) as a vaccine candidate has been shown to inhibit the binding of UPEC to human

151 epithelial bladder cells *in vitro* [36]. Moreover, systemic immunization with FimH (in combination

152 with the FimC chaperone protein) promoted a strong and long-lasting immune response that led to a

153 >99% reduction in bladder colonization in a murine model of cystitis, and prevention of kidney

154 infection over a 7-day period. Immunization with FimH also correlated with a high titer of anti-

155 FimH IgG antibodies in the urine of mice [36]. Further studies using a primate model of UTI

156 revealed that immunization with FimH (in complex with FimC) led to high vaginal anti-FimH titers

5

157 and resulted in significant protection from UTI following UPEC challenge [37]. A vaccine

158 containing the FimH protein fused to the FliC major flagellin subunit (FimH-FliC) has also been

159 recently described [38]. Mice immunized with the FimH-FliC protein induced a significant humoral

160 and cellular immune response, and were protected from UPEC infection of the bladder and kidney

161 in a mouse UTI model [38, 39].

162

163 P fimbriae, another adhesive component associated with UPEC infection of the urinary tract,

164 mediate adhesion to α-D-galactopyranosyl-(1-4)-β-D-galactopyranoside receptor epitopes in the

165 globoseries of glycolipids present on vaginal and kidney epithelial cells via the tip-located PapG

166 adhesin [40, 41, 42, 43]. Previous studies have demonstrated protection against renal colonization in

167 murine models of infection [44, 45]. PapG, in complex with the periplasmic chaperone PapD, was

168 used to vaccinate cynomolgus monkeys to evaluate protection against pyelonephritis. Vaccinated

169 monkeys possessed increased anti-PapDG IgG titers and were protected from pyelonephritis

170 following challenge. However, despite histological evidence for reduced inflammation, no

171 difference was observed in the number of bacteria recovered from the urine of vaccinated and

172 control groups [46]. It is possible these observations could be explained by the expression of other

173 adhesins, such as type 1 fimbriae, that also mediate binding to uroepithelial cells [46].

174

175 UPEC express a number of additional fimbrial adhesins that are associated with UTI, including

176 Afa/Dr fimbriae [47], F1C fimbriae [48], S fimbriae [49], and type 3 fimbriae [50]. Among these

177 adhesins, only Afa/Dr fimbriae, which mediate binding to decay-accelerating factor, type IV

178 collagen and carcinoembryonic antigen-related cell adhesion molecules in the upper urinary tract

179 [51], have been examined as a putative vaccine target. Vaccination with Afa/Dr fimbriae led to a

180 reduction in UPEC adherence to bladder cells in a UTI mouse infection model [52].

181

182 Several other UPEC virulence factors associated with adherence have been examined with respect

183 to their potential role as vaccine targets. The UPEC trimeric autotransporter UpaG was identified by

184 reverse vaccinology as a potentially protective antigen against extra-intestinal pathogenic *E. coli*

185 [53]. UpaG promotes cell aggregation, biofilm formation, and binding to human bladder epithelial

186 cells as well as to the extracellular matrix proteins fibronectin and laminin [54]. Using the prototype

187 pyelonephritis-associated UPEC strain CFT073 in a murine sepsis model, immunization with UpaG

188 (c4424) resulted in a 33% protection rate following active immunization and a 66% protection rate

189 following passive immunization [53]. Intranasal vaccination with FdeC (ECOK1_0290), an outer

190 membrane protein that mediates adhesion to human bladder and urethral epithelial cells, has also

191 been examined. Vaccination of mice with FdeC led to a 1.5-2.5 log reduction in UPEC kidney

192 colonization using a UTI model, thus demonstrating significant protection against pyelonephritis

193 [55]. FdeC has also been identified as a protective antigen in a murine model of sepsis [56].

194

195 *Preventing damage to uroepithelial cells*

196

197 An important feature of UPEC pathogenesis is the ability to scavenge nutrients from the urinary

198 tract. UPEC can secrete several toxins that damage uroepithelial cells and promote the release of

199 nutrients into the urine. Alpha hemolysin (HlyA) is a pore-forming cytotoxin secreted by some

200 UPEC strains [57]. Hemolysin has been purified from UPEC culture supernatants and tested as a

201 vaccine candidate in a murine model of pyelonephritis. Mice immunized with denatured hemolysin

202 showed less renal injury compared to control groups, however no significant effect was observed on

203 kidney colonization [58]. In a separate study, systemic immunization with recombinant HlyA led to

204 a protection rate of 86% in a murine model of sepsis [56]. Another UPEC toxin, the vacuolating

205 autotransporter cytotoxin (Vat), is a serine protease also secreted by avian pathogenic *E. coli*

206 (APEC) [59]. The *vat* gene is located on a pathogenicity island, which likely accounts for its high

207 prevalence in both UPEC and APEC strains. In a murine model of sepsis, Vat (c0393) led to a

208 protection rate of 32% and 78% by active and passive immunization, respectively [53].

209

210 *Preventing the scavenging of nutrients from urine*

211

212 The ability to capture nutrients in the resource-limited urinary tract is an essential feature of

213 pathogens that colonize this niche and cause UTI. Most UPEC strains possess a range of

214 mechanisms to effectively capture iron. This includes a number of siderophores, namely

215 enterobactin, salmochelin, aerobactin and yersiniabactin [60, 61, 62, 63]. IroN, a receptor for

216 salmochelin, is associated with UPEC colonization of the urinary tract [63, 64, 65]. Subcutaneous

217 immunization with denatured IroN led to a specific IgG response in serum and protection against

218 renal infection in a murine model of ascending UTI, however protection against bladder infection

219 and production of mucosal IgA was not observed [66]. IroN has also been tested as a vaccine

220 candidate in a murine model of sepsis, and led to a protection rate of 82% by active immunization

221 and 79% by passive immunization following UPEC challenge [53].

222

223 More recent work on UPEC iron receptors has demonstrated they hold significant promise as

224 potential vaccine candidates [53, 67]. Intranasal immunization with the iron receptor proteins IreA,

225 Hma and IutA, conjugated to cholerae toxin, was able to elicit a specific systemic and mucosal

226 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

296 chemotaxis as well as the respiratory burst [90], showed a protection efficacy of 33% in a murine

297 model of sepsis [56].

298

299 *Hypothetical proteins as vaccine targets*

300

301 Some UPEC proteins have been shown to elicit a strong immune response and provide protection in

302 an infection model despite their lack of an assigned function. This includes SslE (ECOK1_3385), a

303 secreted and surface-exposed *E. coli* lipoprotein [56, 91] potentially associated with the degradation

304 of mucosal glycoproteins such as mucin [92]. Active immunization of mice with SslE resulted in a

305 protection efficacy of 82% in a murine model of sepsis. SslE is secreted by a type 2 secretion

306 system (T2SS), and active immunization of mice with a surface-exposed component of this T2SS

307 (ECOK1_3374) also resulted in protection from infection, albeit at a significantly lower level.

308 Protection following passive immunization with rabbit polyclonal antibodies against SslE using the

309 same model reached 100%, and cross-protection against heterologous UPEC strains was also

310 demonstrated [56].

311

312 Several additional uncharacterized proteins have been shown to confer protection against UPEC in

313 a mouse sepsis infection model. This includes the OmpA-like protein c3389 (38% protection by

314 active immunization, 100% protection by passive immunization) [53], c1275 (45% protection by

315 active immunization) and c0975 (24% protection by active immunization) [56].

316

317 **Outlook and perspectives on vaccine development against UTI**

318

319 A vaccine aimed at the prevention of UTI would have a major impact on the quality of life for many

320 individuals who suffer from chronic and recurrent UTI. As UPEC represent the primary causative

321 agent of UTI, one could envisage that this pathogen would be the primary target of such a vaccine.

322 However, other common UTI pathogens should also be considered where feasible. The variation in

323 UPEC serotypes that cause UTI, together with the diversity of virulence factors that are expressed

324 by different UPEC strains, adds complexity to the design of an effective UTI vaccine. Ideally, one

325 would expect a UTI vaccine to be multi-component and have broad coverage that at a minimum

326 provides protection against all UPEC strain types. On the other hand, targeting a common

327 component of the commensal intestinal flora increases the challenge associated with such a vaccine,

328 as changes in the human microbiota may lead to other complications [93]. Considering the advances

329 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, enterohaemorragic, enteropathogenic, enteroinvasive, enteroaggregative and diffusely adherent *E. coli* pathotypes.

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357 **References**

358

359 [1] Foxman, B.; Ki, M.; Brown, P. Antibiotic resistance and pyelonephritis. *Clinical Infectious*
360 *Diseases,* **2007,** *45* (3), 281-283.

361 [2] Zilberberg, M.D.; Shorr, A.F. Secular trends in gram-negative resistance among urinary
362 tract infection hospitalizations in the United States, 2000-2009. *Infection Control and*
363 *Hospital Epidemiology,* **2013,** *34* (9), 940-946.

364 [3] Hooton, T.M. Clinical practice. Uncomplicated urinary tract infection. *The New England*
365 *Journal of Medicine,* **2012,** *366* (11), 1028-1037.

366 [4] Cohn, E.B.; Schaeffer, A.J. Urinary tract infections in adults. *The Scientific World Journal,*
367 **2004,** *4* (S1), 76-88.

368 [5] Foxman, B. Epidemiology of urinary tract infections: incidence, morbidity, and economic
369 costs. *The American Journal of Medicine,* **2002,** *113* (1A), 5S-13S.

370 [6] Little, P.; Merriman, R.; Turner, S.; Rumsby, K.; Warner, G.; Lowes, J.A.; Smith, H.;
371 Hawke, C.; Leydon, G.; Mullee, M.; Moore, M.V. Presentation, pattern, and natural course
372 of severe symptoms, and role of antibiotics and antibiotic resistance among patients
373 presenting with suspected uncomplicated urinary tract infection in primary care:
374 observational study. *BMJ,* **2010,** *340,* b5633.

375 [7] Foxman, B.; Barlow, R.; D'Arcy, H.; Gillespie, B.; Sobel, J.D. Urinary tract infection: self-
376 reported incidence and associated costs. *Annals of Epidemiology,* **2000,** *10* (8), 509-515.

377 [8] Schappert, S.M.; Rechtsteiner, E.A. Ambulatory medical care utilization estimates for 2007.
378 *Vital and Health Statistics,* **2011,** *13* (169), 1-38.

379 [9] Foxman, B. The epidemiology of urinary tract infection. *Nature Reviews. Urology,* **2010,** *7*
380 (12), 653-660.

381 [10] Hooton, T.M.; Besser, R.; Foxman, B.; Fritsche, T.R.; Nicolle, L.E. Acute uncomplicated
382 cystitis in an era of increasing antibiotic resistance: a proposed approach to empirical
383 therapy. *Clinical Infectious Diseases,* **2004,** *39* (1), 75-80.

384 [11] Bitsori, M.; Galanakis, E. Pediatric urinary tract infections: diagnosis and treatment. *Expert*
385 *Review of Anti-Infective Therapy,* **2012,** *10* (10), 1153-1164.

386 [12] Plowman, R.; Graves, N.; Esquivel, J.; Roberts, J.A. An economic model to assess the cost
387 and benefits of the routine use of silver alloy coated urinary catheters to reduce the risk of
388 urinary tract infections in catheterized patients. *The Journal of Hospital Infection,* **2001,** *48*
389 (1), 33-42.

390 [13] Saint, S. Clinical and economic consequences of nosocomial catheter-related bacteriuria.
391 *American Journal of Infection Control,* **2000,** *28* (1), 68-75.

392   [14]   Sastre, J.B.; Aparicio, A.R.; Cotallo, G.D.; Colomer, B.F.; Hernandez, M.C. Urinary tract
393   infection in the newborn: clinical and radio imaging studies. *Pediatric Nephrology,* **2007,** *22*
394   (10), 1735-1741.

395   [15]   Kanellopoulos, T.A.; Salakos, C.; Spiliopoulou, I.; Ellina, A.; Nikolakopoulou, N.M.;
396   Papanastasiou, D.A. First urinary tract infection in neonates, infants and young children: a
397   comparative study. *Pediatric Nephrology,* **2006,** *21* (8), 1131-1137.

398   [16]   Jacobsen, S.M.; Stickler, D.J.; Mobley, H.L.; Shirtliff, M.E. Complicated catheter-
399   associated urinary tract infections due to Escherichia coli and Proteus mirabilis. *Clinical*
400   *Microbiology Reviews,* **2008,** *21* (1), 26-59.

401   [17]   DeBusscher, J.; Zhang, L.; Buxton, M.; Foxman, B.; Barbosa-Cesnik, C. Persistent
402   extended-spectrum beta-lactamase urinary tract infection. *Emerging Infectious Diseases,*
403   **2009,** *15* (11), 1862-1864.

404   [18]   Foxman, B.; Buxton, M. Alternative approaches to conventional treatment of acute
405   uncomplicated urinary tract infection in women. *Current Infectious Disease Reports,* **2013,**
406   *15* (2), 124-129.

407   [19]   Nicolas-Chanoine, M.H.; Blanco, J.; Leflon-Guibout, V.; Demarty, R.; Alonso, M.P.;
408   Canica, M.M.; Park, Y.J.; Lavigne, J.P.; Pitout, J.; Johnson, J.R. Intercontinental emergence
409   of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. *Journal of Antimicrobial*
410   *Chemotherapy,* **2008,** *61* (2), 273-281.

411   [20]   Coque, T.M.; Baquero, F.; Canton, R. Increasing prevalence of ESBL-producing
412   Enterobacteriaceae in Europe. *Euro Surveillance,* **2008,** *13* (47), 1-11.

413   [21]   Lau, S.H.; Reddy, S.; Cheesbrough, J.; Bolton, F.J.; Willshaw, G.; Cheasty, T.; Fox, A.J.;
414   Upton, M. Major uropathogenic Escherichia coli strain isolated in the northwest of England
415   identified by multilocus sequence typing. *Journal of Clinical Microbiology,* **2008,** *46* (3),
416   1076-1080.

417   [22]   Totsika, M.; Beatson, S.A.; Sarkar, S.; Phan, M.D.; Petty, N.K.; Bachmann, N.; Szubert, M.;
418   Sidjabat, H.E.; Paterson, D.L.; Upton, M.; Schembri, M.A. Insights into a Multidrug
419   Resistant Escherichia coli Pathogen of the Globally Disseminated ST131 Lineage: Genome
420   Analysis and Virulence Mechanisms. *PLoS One,* **2011,** *6* (10), e26578.

421   [23]   Hannan, T.J.; Totsika, M.; Mansfield, K.J.; Moore, K.H.; Schembri, M.A.; Hultgren, S.J.
422   Host-pathogen checkpoints and population bottlenecks in persistent and intracellular
423   uropathogenic Escherichia coli bladder infection. *FEMS Microbiology Reviews,* **2012,** *36*
424   (3), 616-648.

425   [24]   Doi, Y.; Park, Y.S.; Rivera, J.I.; Adams-Haduch, J.M.; Hingwe, A.; Sordillo, E.M.; Lewis,
426   J.S., 2nd; Howard, W.J.; Johnson, L.E.; Polsky, B.; Jorgensen, J.H.; Richter, S.S.; Shutt,

K.A.; Paterson, D.L. Community-associated extended-spectrum beta-lactamase-producing Escherichia coli infection in the United States. *Clinical Infectious Diseases,* **2013,** *56* (5), 641-648.

[25]   Woodford, N.; Turton, J.F.; Livermore, D.M. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiology Reviews,* **2011,** *35* (5), 736-755.

[26]   Feglo, P.; Adu-Sarkodie, Y.; Ayisi, L.; Jain, R.; Spurbeck, R.R.; Springman, A.C.; Engleberg, N.C.; Newton, D.W.; Xi, C.; Walk, S.T. Emergence of a novel extended-spectrum-beta-lactamase (ESBL)-producing, fluoroquinolone-resistant clone of extraintestinal pathogenic Escherichia coli in Kumasi, Ghana. *Journal of Clinical Microbiology,* **2013,** *51* (2), 728-730.

[27]   Platell, J.L.; Trott, D.J.; Johnson, J.R.; Heisig, P.; Heisig, A.; Clabots, C.R.; Johnston, B.; Cobbold, R.N. Prominence of an O75 clonal group (clonal complex 14) among non-ST131 fluoroquinolone-resistant Escherichia coli causing extraintestinal infections in humans and dogs in Australia. *Antimicrobial Agents and Chemotherapy,* **2012,** *56* (7), 3898-3904.

[28]   Melzer, M.; Petersen, I. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E. coli compared to non-ESBL producing E. coli. *Journal of Infection,* **2007,** *55* (3), 254-259.

[29]   Tadesse, D.A.; Zhao, S.; Tong, E.; Ayers, S.; Singh, A.; Bartholomew, M.J.; McDermott, P.F. Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002. *Emerging Infectious Diseases,* **2012,** *18* (5), 741-749.

[30]   Totsika, M.; Moriel, D.G.; Idris, A.; Rogers, B.A.; Wurpel, D.J.; Phan, M.D.; Paterson, D.L.; Schembri, M.A. Uropathogenic Escherichia coli mediated urinary tract infection. *Current Drug Targets,* **2012,** *13* (11), 1386-1399.

[31]   Connell, I.; Agace, W.; Klemm, P.; Schembri, M.; Marild, 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* (18), 9827-9832.

[32]   Mulvey, M.A.; Lopez-Boado, Y.S.; Wilson, C.L.; Roth, R.; Parks, W.C.; Heuser, J.; Hultgren, S.J. Induction and evasion of host defenses by type 1-piliated uropathogenic Escherichia coli. *Science,* **1998,** *282* (5393), 1494-1497.

[33]   Wu, X.R.; Sun, T.T.; Medina, J.J. In vitro binding of type 1-fimbriated Escherichia coli to uroplakins Ia and Ib: relation to urinary tract infections. *Proceedings of the National Academy of Sciences of the United States of America,* **1996,** *93* (18), 9630-9635.

460 [34] Krogfelt, K.A.; Bergmans, H.; Klemm, P. Direct evidence that the FimH protein is the
461 mannose-specific adhesin of Escherichia coli type 1 fimbriae. *Infection and Immunity,* **1990,**
462 *58* (6), 1995-1998.

463 [35] Jones, C.H.; Pinkner, J.S.; Roth, R.; Heuser, J.; Nicholes, A.V.; Abraham, S.N.; Hultgren,
464 S.J. FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the
465 Enterobacteriaceae. *Proceedings of the National Academy of Sciences of the United States of*
466 *America,* **1995,** *92* (6), 2081-2085.

467 [36] Langermann, S.; Palaszynski, S.; Barnhart, M.; Auguste, G.; Pinkner, J.S.; Burlein, J.;
468 Barren, P.; Koenig, S.; Leath, S.; Jones, C.H.; Hultgren, S.J. Prevention of mucosal
469 Escherichia coli infection by FimH-adhesin-based systemic vaccination. *Science,* **1997,** *276*
470 (5312), 607-611.

471 [37] Langermann, S.; Mollby, R.; Burlein, J.E.; Palaszynski, S.R.; Auguste, C.G.; DeFusco, A.;
472 Strouse, R.; Schenerman, M.A.; Hultgren, S.J.; Pinkner, J.S.; Winberg, J.; Guldevall, L.;
473 Soderhall, M.; Ishikawa, K.; Normark, S.; Koenig, S. Vaccination with FimH adhesin
474 protects cynomolgus monkeys from colonization and infection by uropathogenic
475 Escherichia coli. *The Journal of Infectious Diseases,* **2000,** *181* (2), 774-778.

476 [38] Asadi Karam, M.R.; Oloomi, M.; Mahdavi, M.; Habibi, M.; Bouzari, S. Vaccination with
477 recombinant FimH fused with flagellin enhances cellular and humoral immunity against
478 urinary tract infection in mice. *Vaccine,* **2013,** *31* (8), 1210-1216.

479 [39] Karam, M.R.; Oloomi, M.; Mahdavi, M.; Habibi, M.; Bouzari, S. Assessment of immune
480 responses of the flagellin (FliC) fused to FimH adhesin of Uropathogenic Escherichia coli.
481 *Molecular Immunology,* **2012,** *54* (1), 32-39.

482 [40] Lund, B.; Lindberg, F.; Marklund, B.I.; Normark, S. The PapG protein is the alpha-D-
483 galactopyranosyl-(1----4)-beta-D-galactopyranose-binding adhesin of uropathogenic
484 Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of*
485 *America,* **1987,** *84* (16), 5898-5902.

486 [41] Roberts, J.A.; Marklund, B.I.; Ilver, D.; Haslam, D.; Kaack, M.B.; Baskin, G.; Louis, M.;
487 Mollby, R.; Winberg, J.; Normark, S. The Gal(alpha 1-4)Gal-specific tip adhesin of
488 Escherichia coli P-fimbriae is needed for pyelonephritis to occur in the normal urinary tract.
489 *Proceedings of the National Academy of Sciences of the United States of America,* **1994,** *91*
490 (25), 11889-11893.

491 [42] Hedges, S.R.; Agace, W.W.; Svanborg, C. Epithelial cytokine responses and mucosal
492 cytokine networks. *Trends in Microbiology,* **1995,** *3* (7), 266-270.

493   [43]   Bergsten, G.; Samuelsson, M.; Wullt, B.; Leijonhufvud, I.; Fischer, H.; Svanborg, C. PapG-dependent adherence breaks mucosal inertia and triggers the innate host response. *The Journal of Infectious Diseases,* **2004,** *189* (9), 1734-1742.

496   [44]   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* (6), 2102-2108.

500   [45]   Schmidt, M.A.; O'Hanley, P.; Lark, D.; Schoolnik, G.K. Synthetic peptides corresponding to protective epitopes of Escherichia coli digalactoside-binding pilin prevent infection in a murine pyelonephritis model. *Proceedings of the National Academy of Sciences of the United States of America,* **1988,** *85* (4), 1247-1251.

504   [46]   Roberts, J.A.; Kaack, M.B.; Baskin, G.; Chapman, M.R.; Hunstad, D.A.; Pinkner, J.S.; Hultgren, S.J. Antibody responses and protection from pyelonephritis following vaccination with purified Escherichia coli PapDG protein. *The Journal of Urology,* **2004,** *171* (4), 1682-1685.

508   [47]   Nowicki, B.; Labigne, A.; Moseley, S.; Hull, R.; Hull, S.; Moulds, J. The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated Escherichia coli belong to a family of hemagglutinins with Dr receptor recognition. *Infection and Immunity,* **1990,** *58* (1), 279-281.

512   [48]   Backhed, F.; Alsen, B.; Roche, N.; Angstrom, J.; von Euler, A.; Breimer, M.E.; Westerlund-Wikstrom, B.; Teneberg, S.; Richter-Dahlfors, A. Identification of target tissue glycosphingolipid receptors for uropathogenic, F1C-fimbriated Escherichia coli and its role in mucosal inflammation. *The Journal of Biological Chemistry,* **2002,** *277* (20), 18198-18205.

517   [49]   Korhonen, T.K.; Parkkinen, J.; Hacker, J.; Finne, J.; Pere, A.; Rhen, M.; Holthofer, H. Binding of Escherichia coli S fimbriae to human kidney epithelium. *Infection and Immunity,* **1986,** *54* (2), 322-327.

520   [50]   Ong, C.L.; Ulett, G.C.; Mabbett, A.N.; Beatson, S.A.; Webb, R.I.; Monaghan, W.; Nimmo, G.R.; Looke, D.F.; McEwan, A.G.; Schembri, M.A. Identification of Type 3 fimbriae in uropathogenic Escherichia coli reveals a role in biofilm formation. *Journal of Bacteriology,* **2007,** *190* (3), 1054-1063.

524   [51]   Selvarangan, R.; Goluszko, P.; Singhal, J.; Carnoy, C.; Moseley, S.; Hudson, B.; Nowicki, S.; Nowicki, B. Interaction of Dr adhesin with collagen type IV is a critical step in Escherichia coli renal persistence. *Infection and Immunity,* **2004,** *72* (8), 4827-4835.

527   [52]   Goluszko, P.; Goluszko, E.; Nowicki, B.; Nowicki, S.; Popov, V.; Wang, H.Q. Vaccination
528   with purified Dr Fimbriae reduces mortality associated with chronic urinary tract infection
529   due to Escherichia coli bearing Dr adhesin. *Infection and Immunity,* **2005,** *73* (1), 627-631.

530   [53]   Durant, L.; Metais, A.; Soulama-Mouze, C.; Genevard, J.M.; Nassif, X.; Escaich, S.
531   Identification of candidates for a subunit vaccine against extraintestinal pathogenic
532   Escherichia coli. *Infection and Immunity,* **2007,** *75* (4), 1916-1925.

533   [54]   Valle, J.; Mabbett, A.N.; Ulett, G.C.; Toledo-Arana, A.; Wecker, K.; Totsika, M.; Schembri,
534   M.A.; Ghigo, J.M.; Beloin, C. UpaG, a new member of the trimeric autotransporter family
535   of adhesins in uropathogenic Escherichia coli. *Journal of Bacteriology,* **2008,** *190* (12),
536   4147-4161.

537   [55]   Nesta, B.; Spraggon, G.; Alteri, C.; Gomes Moriel, D.; Rosini, R.; Veggi, D.; Smith, S.;
538   Bertoldi, I.; Pastorello, I.; Ferlenghi, I.; Fontana, M.R.; Frankel, G.; Mobley, H.L.;
539   Rappuoli, R.; Pizza, M.; Serino, L.; Soriani, M. FdeC, a Novel Broadly Conserved
540   Escherichia coli Adhesin Eliciting Protection against Urinary Tract Infections. *MBio,* **2012,**
541   *3* (2), e00010-00012.

542   [56]   Moriel, D.G.; Bertoldi, I.; Spagnuolo, A.; Marchi, S.; Rosini, R.; Nesta, B.; Pastorello, I.;
543   Corea, V.A.; Torricelli, G.; Cartocci, E.; Savino, S.; Scarselli, M.; Dobrindt, U.; Hacker, J.;
544   Tettelin, H.; Tallon, L.J.; Sullivan, S.; Wieler, L.H.; Ewers, C.; Pickard, D.; Dougan, G.;
545   Fontana, M.R.; Rappuoli, R.; Pizza, M.; Serino, L. Identification of protective and broadly
546   conserved vaccine antigens from the genome of extraintestinal pathogenic Escherichia coli.
547   *Proceedings of the National Academy of Sciences of the United States of America,* **2010,**
548   *107* (20), 9072-9077.

549   [57]   Mobley, H.L.; Green, D.M.; Trifillis, A.L.; Johnson, D.E.; Chippendale, G.R.; Lockatell,
550   C.V.; Jones, B.D.; Warren, J.W. Pyelonephritogenic Escherichia coli and killing of cultured
551   human renal proximal tubular epithelial cells: role of hemolysin in some strains. *Infection*
552   *and Immunity,* **1990,** *58* (5), 1281-1289.

553   [58]   O'Hanley, P.; Lalonde, G.; Ji, G. Alpha-hemolysin contributes to the pathogenicity of
554   piliated digalactoside-binding Escherichia coli in the kidney: efficacy of an alpha-hemolysin
555   vaccine in preventing renal injury in the BALB/c mouse model of pyelonephritis. *Infection*
556   *and Immunity,* **1991,** *59* (3), 1153-1161.

557   [59]   Parreira, V.R.; Gyles, C.L. A novel pathogenicity island integrated adjacent to the thrW
558   tRNA gene of avian pathogenic Escherichia coli encodes a vacuolating autotransporter
559   toxin. *Infection and Immunity,* **2003,** *71* (9), 5087-5096.

560 [60] Chu, B.C.; Garcia-Herrero, A.; Johanson, T.H.; Krewulak, K.D.; Lau, C.K.; Peacock, R.S.;
561 Slavinskaya, Z.; Vogel, H.J. Siderophore uptake in bacteria and the battle for iron with the
562 host; a bird's eye view. *Biometals,* **2010,** *23* (4), 601-611.

563 [61] Henderson, J.P.; Crowley, J.R.; Pinkner, J.S.; Walker, J.N.; Tsukayama, P.; Stamm, W.E.;
564 Hooton, T.M.; Hultgren, S.J. Quantitative metabolomics reveals an epigenetic blueprint for
565 iron acquisition in uropathogenic Escherichia coli. *PLoS Pathogens,* **2009,** *5* (2), e1000305.

566 [62] Miethke, M.; Marahiel, M.A. Siderophore-based iron acquisition and pathogen control.
567 *Microbiology and Molecular Biology Reviews* **2007,** *71* (3), 413-451.

568 [63] Watts, R.E.; Totsika, M.; Challinor, V.L.; Mabbett, A.N.; Ulett, G.C.; De Voss, J.J.;
569 Schembri, M.A. Contribution of siderophore systems to growth and urinary tract
570 colonization of asymptomatic bacteriuria Escherichia coli. *Infection and Immunity,* **2012,** *80*
571 (1), 333-344.

572 [64] Russo, T.A.; McFadden, C.D.; Carlino-MacDonald, U.B.; Beanan, J.M.; Barnard, T.J.;
573 Johnson, J.R. IroN functions as a siderophore receptor and is a urovirulence factor in an
574 extraintestinal pathogenic isolate of Escherichia coli. *Infection and Immunity,* **2002,** *70* (12),
575 7156-7160.

576 [65] Watts, R.E.; Tan, C.K.; Ulett, G.C.; Carey, A.J.; Totsika, M.; Idris, A.; Paton, A.W.;
577 Morona, R.; Paton, J.C.; Schembri, M.A. Escherichia coli 83972 expressing a P fimbriae
578 oligosaccharide receptor mimic impairs adhesion of uropathogenic E. coli. *The Journal of*
579 *Infectious Diseases,* **2012,** *206* (8), 1242-1249.

580 [66] Russo, T.A.; McFadden, C.D.; Carlino-MacDonald, U.B.; Beanan, J.M.; Olson, R.; Wilding,
581 G.E. The Siderophore receptor IroN of extraintestinal pathogenic Escherichia coli is a
582 potential vaccine candidate. *Infection and Immunity,* **2003,** *71* (12), 7164-7169.

583 [67] Alteri, C.J.; Hagan, E.C.; Sivick, K.E.; Smith, S.N.; Mobley, H.L. Mucosal immunization
584 with iron receptor antigens protects against urinary tract infection. *PLoS Pathogens,* **2009,** *5*
585 (9), e1000586.

586 [68] Hagan, E.C.; Mobley, H.L. Haem acquisition is facilitated by a novel receptor Hma and
587 required by uropathogenic Escherichia coli for kidney infection. *Molecular Microbiology,*
588 **2008,** *71* (1), 79-91.

589 [69] Russo, T.A.; Carlino, U.B.; Johnson, J.R. Identification of a new iron-regulated virulence
590 gene, ireA, in an extraintestinal pathogenic isolate of Escherichia coli. *Infection and*
591 *Immunity,* **2001,** *69* (10), 6209-6216.

592 [70] Brumbaugh, A.R.; Smith, S.N.; Mobley, H.L. Immunization with the Yersiniabactin
593 Receptor, FyuA, Protects Against Pyelonephritis in a Murine Model of Urinary Tract
594 Infection. *Infection and Immunity,* **2013,** *81* (9), 3309-3316.

595 [71] Ouyang, Z.; Isaacson, R. Identification and characterization of a novel ABC iron transport
596 system, fit, in Escherichia coli. *Infection and Immunity,* **2006,** *74* (12), 6949-6956.

597 [72] Wieser, A.; Romann, E.; Magistro, G.; Hoffmann, C.; Norenberg, D.; Weinert, K.; Schubert,
598 S. A multi-epitope subunit vaccine conveys protection against extraintestinal pathogenic
599 Escherichia coli in mice. *Infection and Immunity,* **2010,** *78* (8), 3432-3442.

600 [73] Svanborg, C. Urinary tract infections in children: microbial virulence versus host
601 susceptibility. *Advances in Experimental Medicine and Biology,* **2013,** *764,* 205-210.

602 [74] Whitworth, J.A.; Fairley, K.F.; O'Keefe, C.M.; Miller, T.E. Immunogenicity of Escherichia
603 coli O antigen in upper urinary tract infection. *Kidney International,* **1975,** *8* (5), 316-319.

604 [75] Kaijser, B.; Ahlstedt, S. Protective capacity of antibodies against Escherichia coli and K
605 antigens. *Infection and Immunity,* **1977,** *17* (2), 286-289.

606 [76] Kaijser, B.; Larsson, P.; Olling, S. Protection against ascending Escherichia coli
607 pyelonephritis in rats and significance of local immunity. *Infection and Immunity,* **1978,** *20*
608 (1), 78-81.

609 [77] Bauer, H.W.; Alloussi, S.; Egger, G.; Blumlein, H.M.; Cozma, G.; Schulman, C.C.;
610 Multicenter, U.T.I.S.G. A long-term, multicenter, double-blind study of an Escherichia coli
611 extract (OM-89) in female patients with recurrent urinary tract infections. *European*
612 *Urology,* **2005,** *47* (4), 542-548.

613 [78] Kruze, D.; Biro, K.; Holzbecher, K.; Andrial, M.; Bossart, W. Protection by a polyvalent
614 vaccine against challenge infection and pyelonephritis. *Urological Research,* **1992,** *20* (2),
615 177-181.

616 [79] Uehling, D.T.; Hopkins, W.J.; Beierle, L.M.; Kryger, J.V.; Heisey, D.M. Vaginal mucosal
617 immunization for recurrent urinary tract infection: extended phase II clinical trial. *The*
618 *Journal of Infectious Diseases,* **2001,** *183* (S1), S81-S83.

619 [80] Uehling, D.T.; Hopkins, W.J.; Elkahwaji, J.E.; Schmidt, D.M.; Leverson, G.E. Phase 2
620 clinical trial of a vaginal mucosal vaccine for urinary tract infections. *The Journal of*
621 *Urology,* **2003,** *170* (3), 867-869.

622 [81] Uehling, D.T.; Hopkins, W.J.; Balish, E.; Xing, Y.; Heisey, D.M. Vaginal mucosal
623 immunization for recurrent urinary tract infection: phase II clinical trial. *The Journal of*
624 *Urology,* **1997,** *157* (6), 2049-2052.

625 [82] Hopkins, W.J.; Elkahwaji, J.; Beierle, L.M.; Leverson, G.E.; Uehling, D.T. Vaginal mucosal
626 vaccine for recurrent urinary tract infections in women: results of a phase 2 clinical trial.
627 *The Journal of Urology,* **2007,** *177* (4), 1349-1353.

628   [83]   Billips, B.K.; Yaggie, R.E.; Cashy, J.P.; Schaeffer, A.J.; Klumpp, D.J. A live-attenuated
629   vaccine for the treatment of urinary tract infection by uropathogenic Escherichia coli. *The*
630   *Journal of Infectious Diseases,* **2009,** *200* (2), 263-272.

631   [84]   Sunden, F.; Hakansson, L.; Ljunggren, E.; Wullt, B. Escherichia coli 83972 bacteriuria
632   protects against recurrent lower urinary tract infections in patients with incomplete bladder
633   emptying. *The Journal of Urology,* **2010,** *184*, 179-185.

634   [85]   Klemm, P.; Hancock, V.; Schembri, M.A. Mellowing out: adaptation to commensalism by
635   Escherichia coli asymptomatic bacteriuria strain 83972. *Infection and Immunity,* **2007,** *75*
636   (8), 3688-3695.

637   [86]   Darouiche, R.O.; Thornby, J.I.; Cerra-Stewart, C.; Donovan, W.H.; Hull, R.A. Bacterial
638   interference for prevention of urinary tract infection: a prospective, randomized, placebo-
639   controlled, double-blind pilot trial. *Clinical Infectious Diseases,* **2005,** *41* (10), 1531-1534.

640   [87]   Wullt, B.; Connell, H.; Rollano, P.; Mansson, W.; Colleen, S.; Svanborg, C. Urodynamic
641   factors influence the duration of Escherichia coli bacteriuria in deliberately colonized cases.
642   *The Journal of Urology,* **1998,** *159* (6), 2057-2062.

643   [88]   Trautner, B.W.; Hull, R.A.; Darouiche, R.O. Escherichia coli 83972 inhibits catheter
644   adherence by a broad spectrum of uropathogens. *Urology,* **2003,** *61* (5), 1059-1062.

645   [89]   Russo, T.A.; Beanan, J.M.; Olson, R.; Genagon, S.A.; MacDonald, U.; Cope, J.J.; Davidson,
646   B.A.; Johnston, B.; Johnson, J.R. A killed, genetically engineered derivative of a wild-type
647   extraintestinal pathogenic E. coli strain is a vaccine candidate. *Vaccine,* **2007,** *25* (19), 3859-
648   3870.

649   [90]   Pastorello, I.; Rossi Paccani, S.; Rosini, R.; Mattera, R.; Ferrer Navarro, M.; Urosev, D.;
650   Nesta, B.; Lo Surdo, P.; Del Vecchio, M.; Rippa, V.; Bertoldi, I.; Gomes Moriel, D.;
651   Laarman, A.J.; van Strijp, J.A.; Daura, X.; Pizza, M.; Serino, L.; Soriani, M. EsiB, a novel
652   pathogenic Escherichia coli secretory immunoglobulin A-binding protein impairing
653   neutrophil activation. *MBio,* **2013,** *4* (4), e00206-00213.

654   [91]   Baldi, D.L.; Higginson, E.E.; Hocking, D.M.; Praszkier, J.; Cavaliere, R.; James, C.E.;
655   Bennett-Wood, V.; Azzopardi, K.I.; Turnbull, L.; Lithgow, T.; Robins-Browne, R.M.;
656   Whitchurch, C.B.; Tauschek, M. The type II secretion system and its ubiquitous lipoprotein
657   substrate, SslE, are required for biofilm formation and virulence of enteropathogenic
658   Escherichia coli. *Infection and Immunity,* **2012,** *80* (6), 2042-2052.

659   [92]   Nakjang, S.; Ndeh, D.A.; Wipat, A.; Bolam, D.N.; Hirt, R.P. A novel extracellular
660   metallopeptidase domain shared by animal host-associated mutualistic and pathogenic
661   microbes. *PLoS One,* **2012,** *7* (1), e30287.

662   [93]   Foxman, B.; Goldberg, D.; Murdock, C.; Xi, C.; Gilsdorf, J.R. Conceptualizing human
663   microbiota: from multicelled organ to ecological community. *Interdisciplinary Perspectives*
664   *on Infectious Diseases,* **2008,** *2008*, 613979.

665   [94]   Nagarajan, N.; Pop, M. Sequence assembly demystified. *Nature reviews. Genetics,* **2013,** *14*
666   (3), 157-167.

667   [95]   Nicholson, J.K.; Holmes, E.; Kinross, J.; Burcelin, R.; Gibson, G.; Jia, W.; Pettersson, S.
668   Host-gut microbiota metabolic interactions. *Science,* **2012,** *336* (6086), 1262-1267.

669   [96]   Human Microbiome Project, C. Structure, function and diversity of the healthy human
670   microbiome. *Nature,* **2012,** *486* (7402), 207-214.

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674 **Table 1 -** Analysis of the current pipeline (up to 5 years) for companies investing in *E. coli* vaccines
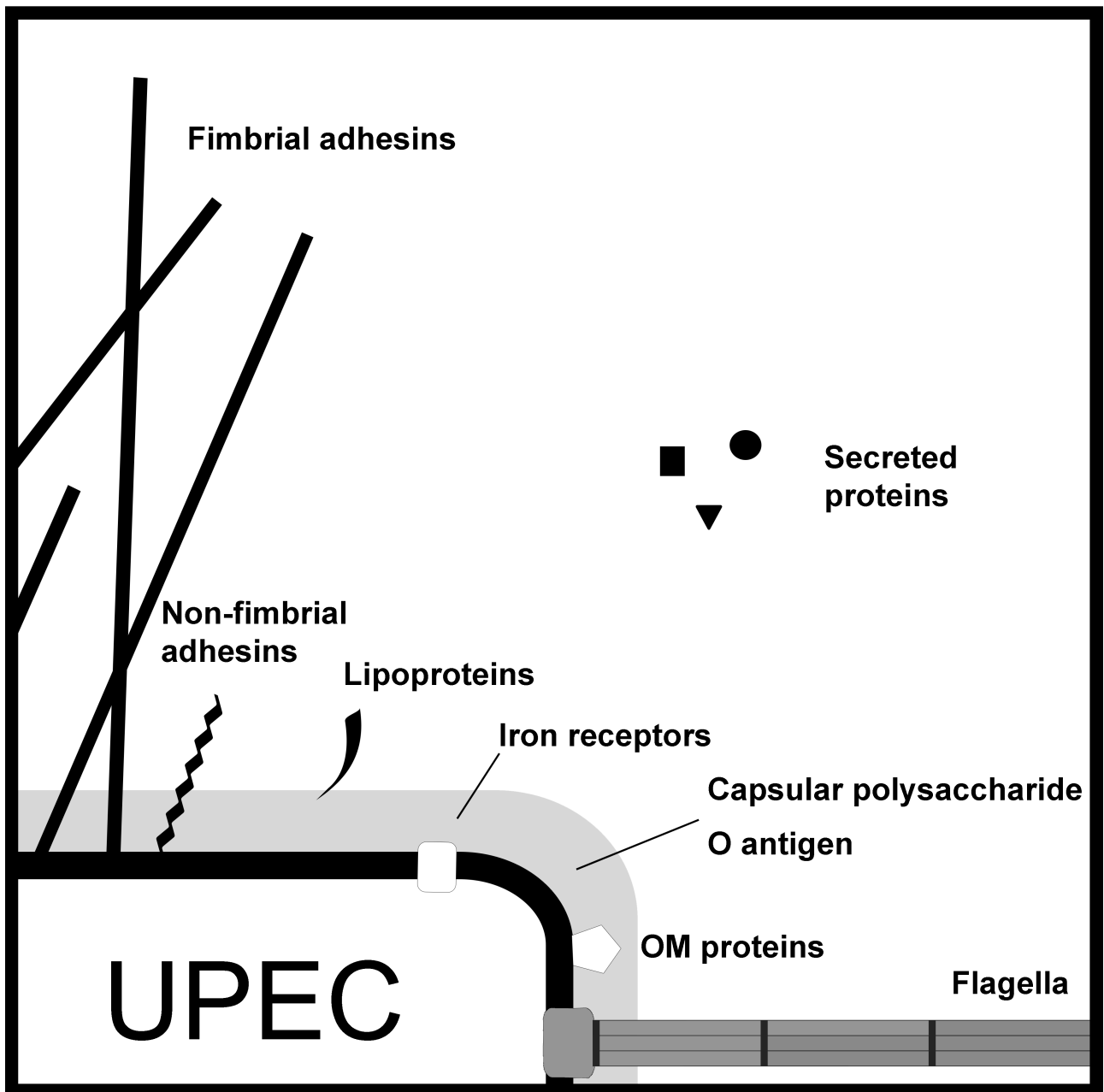675

Company	Stage	Description/Comments
Sequoia Sciences	PC	Vaccine consisting of *E. coli* 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.
GlycoVaxyn	PC	Conjugate vaccine against UPEC based on proprietary bioconjugate platform that enables the *in vivo* synthesis of polysaccharide-protein complexes.
NanoBio	PC	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.

676 PC - preclinical.

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678

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

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