

Host–pathogen interactions in urinary tract infection

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Abstract | The urinary tract is a common site of bacterial infections; nearly half of all women experience at least one urinary tract infection (UTI) during their lifetime. These infections are classified based on the condition of the host. Uncomplicated infections affect otherwise healthy individuals and are most commonly caused by uropathogenic *Escherichia coli*, whereas complicated infections affect patients with underlying difficulties, such as a urinary tract abnormality or catheterization, and are commonly caused by species such as *Proteus mirabilis*. Virulence and fitness factors produced by both pathogens include fimbriae, toxins, flagella, iron acquisition systems, and proteins that function in immune evasion. Additional factors that contribute to infection include the formation of intracellular bacterial communities by *E. coli* and the production of urease by *P. mirabilis*, which can result in urinary stone formation. Innate immune responses are induced or mediated by pattern recognition receptors, antimicrobial peptides, and neutrophils. The adaptive immune response to UTI is less well understood. Host factors TLR4 and CXCR1 are implicated in disease outcome and susceptibility, respectively. Low levels of TLR4 are associated with asymptomatic bacteriuria while low levels of CXCR1 are associated with increased incidence of acute pyelonephritis. Current research is focused on the identification of additional virulence factors and therapeutic or prophylactic targets that might be used in the generation of vaccines against both uropathogens.

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Introduction

The urinary tract is one of the most common sites of bacterial infection in humans.¹ Lower urinary tract infections (UTIs), such as cystitis, are typically characterized by symptoms including frequency, urgency, and dysuria.² If left untreated, these infections can progress to an upper UTI, known as acute pyelonephritis or kidney infection, which can be associated with additional symptoms such as fever, nausea, vomiting, and flank pain. These infections also carry the risk of possible progression to bacteremia. An estimated 40% of women and 12% of men will experience a symptomatic UTI during their lifetime, and approximately a quarter of affected women will suffer recurrent UTI within 6–12 months.³ Infants and children are also susceptible to UTI. Febrile UTIs in children tend to be associated with vesicoureteral reflux and the potential for renal scarring. Pediatric UTI might predispose patients to adult disease.⁴ In 2006, UTIs were the cause of more than 11 million physician visits, 1.7 million emergency room visits, and almost half a million hospitalizations; the societal cost of these infections is 3.5 billion dollars annually in the US alone.^{5,6}

UTIs can be classified as uncomplicated or complicated. Uncomplicated infections occur in patients who are otherwise considered healthy. Complicated UTIs, on the other hand, occur in patients who are compromised in some way, for example, if they have anatomical or

functional abnormalities in their urinary tract, are suffering from another illness, are immunocompromised, or are undergoing long-term catheterization. The vast majority of uncomplicated UTIs are caused by *Escherichia coli*. By contrast, complicated UTIs, especially those associated with long-term catheterization, might be polymicrobial.^{7,8} These infections are typically caused by *Proteus mirabilis*, *Providencia stuartii*, *Morganella morganii*, *Klebsiella pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa*, among others.

This Review will focus on uropathogenic *E. coli* and *P. mirabilis* as representative pathogens causing uncomplicated and complicated UTI, respectively. Here, we summarize the common mechanisms of uropathogenesis utilized by uropathogenic *E. coli* and *P. mirabilis*, including adherence, toxin production, motility, metal acquisition, and evasion of host immune defenses. In addition, we will highlight differences between the two pathogens, such as the formation of intracellular bacterial communities by uropathogenic *E. coli* and the production of urease by *P. mirabilis*. The innate immune response to UTI will be outlined, including the pattern recognition receptors Toll-like receptor (TLR) 4, 5, and 11. Host factors that impact human disease outcomes, namely TLR4 and CXC chemokine receptor type 1 (CXCR1), will also be explored. Finally, current prophylactic and treatment strategies will be briefly outlined and the need for vaccine development, as well as recent advances in the field, will be addressed.

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Competing interests

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Uropathogenesis and virulence factors

The vast majority of UTIs are ascending infections; both *E. coli* and *P. mirabilis* can be found in the intestinal tract, which is likely to be the source of organisms infecting the urinary tract. Uropathogenic *E. coli* are a specific subset of extraintestinal pathogenic *E. coli*; that is, not all strains of *E. coli* are capable of causing UTI.⁹ By contrast, it appears that all strains of *P. mirabilis*, regardless of isolate origin, are capable of infecting the urinary tract.¹⁰ Prototypical strains of each pathogen have been sequenced: *E. coli* strains CFT073,¹¹ 536,¹² UTI89,¹³ IAI 39,¹⁴ and UMN026,¹⁴ and *P. mirabilis* strain HI4320.¹⁵ Horizontally acquired genetic elements have been identified in both species: uropathogenic *E. coli* strains have up to 13 pathogenicity islands^{16–19} and an integrative and conjugative element was recently identified in *P. mirabilis*.²⁰ Horizontal gene transfer is relevant to bacterial pathogenesis because these horizontally transferred genes often encode traits that contribute to virulence, niche specificity, or antibiotic resistance.²¹

Common strategies of pathogenesis employed by both uropathogenic *E. coli* and *P. mirabilis* include adherence, motility, acquisition of metals, toxin production, and immune evasion (Figure 1). For the purpose of this article, we define a virulence factor as a protein (such as a toxin) or macromolecular structure (such as a fimbria or flagellum) that contributes to the ability of the pathogen to cause disease and a fitness factor as a protein or macromolecular structure that, while not required for virulence, offers a competitive advantage during infection. Additionally, each pathogen displays unique characteristics not shared by the other, namely, uropathogenic *E. coli* can form intracellular bacterial communities and *P. mirabilis* synthesizes urease. Elucidation of these pathogenic mechanisms resulted from years of work carried out by many investigators. Much of this work was made possible by the availability of sequenced genomes, development of genetic screens, and use of animal models of UTI. The outcome of infection by these bacteria is dictated by the immune response to UTI and host factors that influence susceptibility to disease.

Uropathogenic *E. coli*

Adherence via fimbriae

Fimbriae are complex surface structures that, in general, mediate adherence of bacteria to host epithelial receptors. The recognized uropathogenic *E. coli* reference strain CFT073 encodes 13 distinct fimbrial gene clusters, including type 1, P, F1C, Dr, Auf, S, and M fimbriae.^{9,11} These gene clusters, or operons, encode all of the genes necessary for assembly of the fimbriae, including structural subunits as well as chaperone and usher proteins that aid in their secretion.

Type 1 fimbria, a definitive virulence factor in the murine model of ascending UTI,²² facilitates adherence to the bladder cell surface glycoprotein uroplakin and other host proteins that contain mannositides.²³ Expression of type 1 fimbriae is phase variable (Box 1). Although type 1 fimbriae are almost always present in uropathogenic strains of *E. coli* isolated from human patients, the

Key points

- The urinary tract is a common site of bacterial infection
- Uropathogenic *Escherichia coli* and *Proteus mirabilis* are representative pathogens of uncomplicated and complicated urinary tract infection (UTI), respectively
- Virulence and fitness factors synthesized by both pathogens include fimbriae, toxins, flagella, iron acquisition systems, and proteins that function in immune evasion
- Additional factors that contribute to infection include the formation of intracellular bacterial communities by *E. coli* and the production of urease by *P. mirabilis*, which can result in urinary stone formation
- Innate immune responses are induced or mediated by pattern recognition receptors, antimicrobial peptides, and neutrophils; the adaptive immune response to UTI is less well understood
- Host factors TLR4 and CXCR1 are implicated in disease outcome and susceptibility, respectively; low levels of TLR4 are associated with asymptomatic bacteriuria; low levels of CXCR1 are associated with increased incidence of acute pyelonephritis
- Reverse vaccinology approaches are currently underway to identify potential vaccine candidates for both pathogens

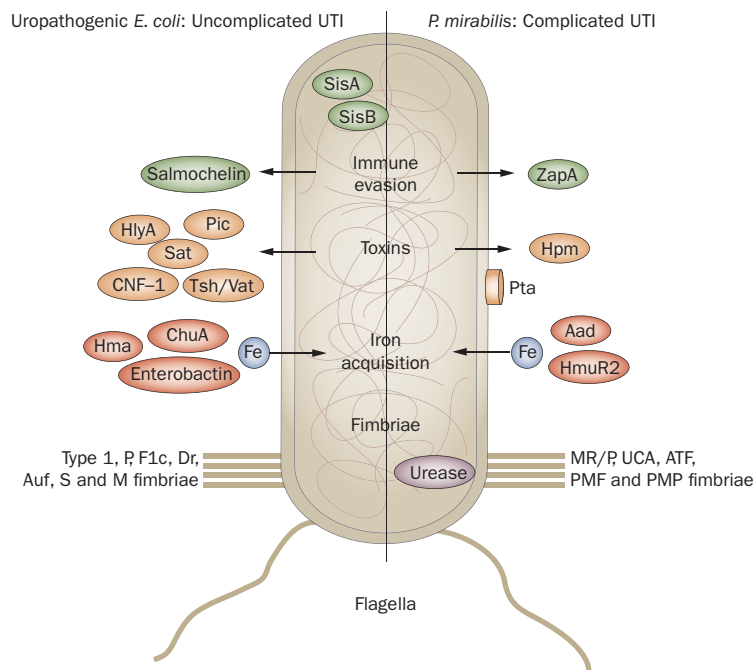


Figure 1 | Overview of uropathogen virulence factors. The left hand side of this schematic bacterial cell represents uropathogenic *Escherichia coli*, and the right hand side represents *Proteus mirabilis*. Proteins that contribute to each mechanism of pathogenesis—immune evasion, toxin production, iron acquisition, adherence, and motility—are highlighted for each pathogen. Some strains of uropathogenic *E. coli* encode the proteins SisA and SisB, which suppress the host inflammatory response during early stages of infection. *P. mirabilis* produces urease, which plays a role in the formation of urinary stones.

presence of type 1 fimbriae does not correlate with virulence because these fimbriae are found equally in UTI and fecal strains.⁹ P fimbriae are associated with *E. coli* strains that cause acute pyelonephritis in humans, are produced and displayed during infection, and trigger a mucosal response to infection in humans, although they play only a subtle role in pathogenesis in the murine model.²⁴ The Dr family of adhesins is comprised of

Box 1 | Phase variation of fimbriae by uropathogens

Type 1 fimbriae in uropathogenic *Escherichia coli* and MR/P fimbriae in *Proteus mirabilis* undergo phase variation.^{109,185} In each species, expression of these fimbrial operons is under the control of a promoter located on an invertible element upstream of the genes of interest. When the promoter is in the correct orientation, referred to as 'on', fimbrial genes are transcribed, and fimbriae are synthesized. In the opposite orientation ('off') expression and production of fimbriae does not occur.

In uropathogenic *E. coli*, the orientation of the promoter is controlled by multiple recombinase enzymes. Two of these enzymes, FimB and FimE, are encoded upstream of the fimbrial operon.¹⁸⁶ Two additional recombinases, IpuA and IpbA, mediate switching of the invertible element independent of FimB and FimE.¹⁸⁷ FimB and IpuA can mediate bidirectional switching of the promoter, while FimE and IpbA can only turn expression off and on, respectively. The regulation of this process is complex, affected by a multitude of environmental factors (including pH, osmolarity, temperature, and oxygen levels) and at least three regulatory proteins (Lrp, IHF, and H-NS).⁹ By contrast, there is only one recombinase, MrpI, in *P. mirabilis* that acts on the invertible element containing the promoter of the MR/P fimbrial operon.⁸⁰ Like expression of type 1 fimbriae in uropathogenic *E. coli*, expression of MR/P fimbriae is increased under oxygen limitation.¹⁸⁸

This type of regulation of virulence factors could be advantageous in the host, especially in the presence of antibodies directed against a protein constituent of MR/P or type 1 fimbriae. Bacteria with their promoters in the 'on' position that are expressing fimbrial genes could be targeted, whereas bacteria with their promoters in the 'off' position would not be recognized by these antibodies, essentially escaping this mechanism of the host defense.

Box 2 | Coordinated regulation of motility and adherence

In general, fimbriae mediate adherence and flagella mediate motility. Both adherence and motility are important pathogenic traits, but they have apparently opposing functions. It has been proposed that one bacterium should not be simultaneously adherent and motile. Thus, it is hypothesized that regulation of these traits must be coordinated; indeed, proteins have been identified in both uropathogenic *Escherichia coli* and *Proteus mirabilis* that seem to function in this way.

Sequencing of the MR/P operon in *P. mirabilis* revealed the presence of a transcriptional regulator, MrpJ, at the 3' end of the fimbrial operon. Overexpression of MrpJ led to a reduction in the expression of flagellin protein (FlaA), production of flagella, and motility.^{189,190} MrpJ appears to bind to the promoter of the *flhDC* operon, which encodes the so-called 'master regulator' of flagellar motility, FlhDC. Interestingly, the genome sequence of *P. mirabilis* HI4320 revealed the presence of 14 MrpJ paralogs, most of which repress motility when overexpressed.^{15,190} At least one of these proteins binds to the *flhDC* promoter, suggesting this mechanism might be shared by at least some of these MrpJ paralogs.¹⁹⁰ Based on the sequences of these proteins, a conserved sequence was identified, and most residues in this conserved sequence seem to be necessary for the function of MrpJ.¹⁹⁰

The 3' end of the P fimbrial operon of uropathogenic *E. coli* encodes a protein with similar function to MrpJ, PapX, which represses production of flagella and motility when overexpressed.¹⁹¹ PapX also functions at the transcriptional level, binding as a repressor to the promoter sequence of *flhDC* in uropathogenic *E. coli*.¹⁹¹

surface structures (both fimbrial and afimbrial) that bind to the bladder epithelium and type IV collagen on basement membranes.²⁵ Dr adhesins are associated with cell invasion *in vitro* and contribute to pathogenesis in animal models of UTI.^{26,27} FIC fimbriae are expressed *in vivo* during human UTI, but their role in pathogenesis is unknown.²⁸ Auf fimbriae are expressed by uropathogenic *E. coli* found in the urine of infected mice and are antigenic, but they play no apparent role in colonization of the murine urinary tract.²⁹ In addition to these fimbrial adhesins, the nonfimbrial adhesin UpaG is an

autotransporter that promotes cell aggregation and the formation of biofilms and mediates adhesion to human bladder epithelial cells. However, UpaG is not required for colonization.³⁰ These various fimbriae encoded by uropathogenic *E. coli* bind to different receptors and are therefore speculated to aid in colonization of different areas in the host urinary tract.

Toxins

Three main types of toxin are produced by uropathogenic *E. coli*: hemolysin, cytotoxic necrotizing factor 1 (CNF1), and secreted autotransporter toxins. Hemolysin, encoded by the *hlyCABD* operon, is a prototypical calcium-dependent repeats-in-toxin secreted protein that is found more commonly in uropathogenic strains of *E. coli* than fecal strains.³¹ This toxin inserts into host cell membranes and is active against many cell types, including uroepithelial cells.^{32,33} Hemolysin, which is also implicated in invasion, stimulates cytokine production and leads to an inflammatory response.^{34,35} Although hemolysin is not required for colonization in experimental UTI, it does contribute to virulence because it is responsible for epithelial damage and bladder hemorrhage *in vivo* and has been implicated in causing renal damage and increasing risk for septicemia.^{36,37}

CNF1 activity leads to constitutive activation of members of the Rho family of GTP-binding proteins, resulting in cytoskeleton rearrangements in host cells.^{38,39} CNF1 has been implicated in adherence to and invasion of host cells.^{40,41} This toxin also causes apoptosis of bladder cells, which might stimulate their exfoliation *in vivo*, thereby exposing underlying tissue.⁴² CNF1 is not required for infection, but might be a fitness factor.^{43,44} In addition, CNF1-positive strains cause more inflammation than strains lacking this toxin.⁴⁴

Three autotransporter toxins (Sat, Pic, and Tsh) have been characterized in uropathogenic *E. coli* and six additional autotransporters (not including UpaG, described above) have been predicted but remain uncharacterized.^{11,45} Sat (secreted autotransporter toxin) is a serine protease that mediates cytopathic effects on bladder and kidney cell lines *in vitro* and elicits tissue damage and an immune response in infected mice.⁴⁶ Pic, another autotransporter with serine protease activity, is expressed during infection but is neither required nor advantageous for colonization.^{45,47} The autotransporter Tsh (also referred to as Vat [vacuolating autotransporter toxin] in CFT073), which lacks detectable serine protease activity, is expressed during infection in mice⁴⁷ but is not required for colonization (E. C. Hagan and H. L. T. Mobley, unpublished work).

Flagella-mediated motility

Much like adherence, bacterial motility is another trait often associated with virulence of bacterial pathogens (Box 2). Motility is mediated by complex surface structures called flagella. Like many pathogens, uropathogenic *E. coli* is capable of flagella-mediated motility and flagella contribute to the fitness of uropathogenic *E. coli* during UTI.^{48,49} Flagellar genes are poorly expressed

during chronic uropathogenic *E. coli* infection in mice, where they are generally downregulated *in vivo* compared with their expression during culture in rich laboratory medium.⁵⁰ However, the synthesis of flagella appears to be tightly regulated and coincides with ascension of bacteria from the bladder to the kidneys in mice. Thus, flagella contribute to ascending *E. coli* infection.⁵¹

Metal acquisition

Iron, an essential nutrient for all living things, is sequestered by the host, and successful bacterial pathogens must have the means to acquire it. Uropathogenic *E. coli* is no exception and the CFT073 genome contains at least 10 characterized iron uptake systems and several putative transporters.¹¹ Uptake of heme, enterobactin, and other siderophores contribute to the fitness of uropathogenic *E. coli* during infection, but because of the functional redundancy of iron acquisition systems, no single system is absolutely required for virulence.^{52–56}

Much like iron, zinc is an essential nutrient, and the importance of zinc acquisition has recently been demonstrated for a number of human pathogens. Indeed, the high-affinity zinc transport system ZnuACB was shown to contribute to the fitness of uropathogenic *E. coli* during experimental UTI.⁵⁷

Strategies for evading host defenses

In addition to colonizing the host and damaging surrounding tissue, a hallmark of pathogens is the ability to avoid the host immune response. For example, as noted above, iron is a critical nutrient that must be acquired by uropathogenic *E. coli* during infection, and the siderophore enterobactin represents one mechanism of iron acquisition. However, the host protein lipocalin-2, which is expressed and released by neutrophils, specifically binds to and sequesters enterobactin, thereby rendering it unable to supply uropathogenic *E. coli* with iron.⁵⁸ Interestingly, uropathogenic *E. coli* is capable of modifying enterobactin by glycosylation, and this modified enterobactin, known as salmochelin, is not recognized and sequestered by lipocalin-2 (Figure 2). Thus, uropathogenic *E. coli* that synthesize salmochelin escape the host defense mechanism mediated by lipocalin-2.^{59,60}

Uropathogenic *E. coli* also synthesize factors that inhibit cytokine responses^{61–63} and enhance tolerance to reactive oxygen and nitrogen species.^{64–67} Extracellular polysaccharides have also been implicated as contributing factors to evading host defenses, but definitive roles for these various surface molecules during UTI are not yet clear.⁹ Additionally, some uropathogenic *E. coli* strains encode the proteins SisA and SisB, which suppress the host inflammatory response during early stages of infection. A strain of uropathogenic *E. coli* in which functional copies of these genes were deleted caused significantly more inflammation during experimental infection and was more easily cleared from the host than the wild-type strain.⁶⁸ In addition, the complement component C3 can be bound by uropathogenic *E. coli*, which can then enter epithelial cells via C3 receptors.⁶⁹ Finally, phase variation of type 1 fimbrial genes might contribute to the ability of

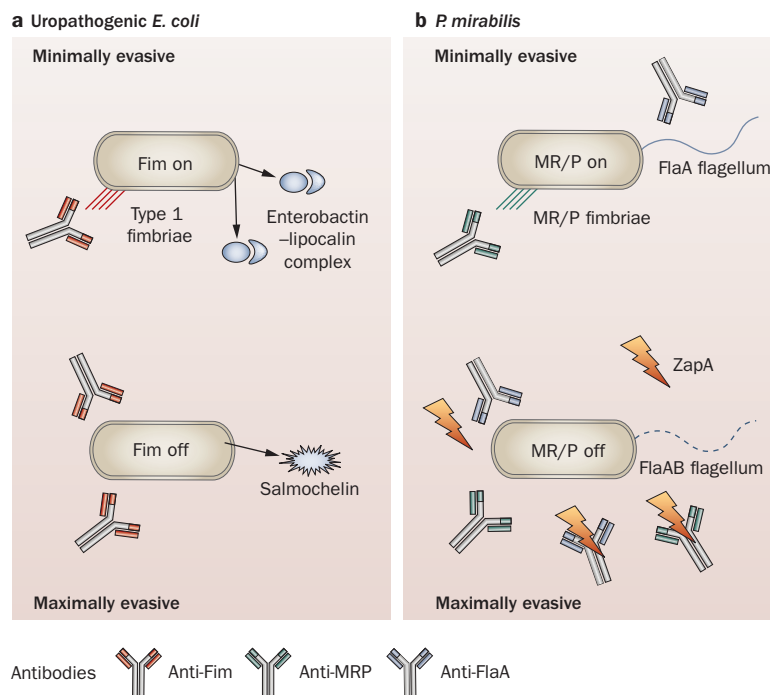


Figure 2 | Known mechanisms of immune evasion. **a** | Uropathogenic *Escherichia coli* strategies for evading the host immune response include phase variation of type 1 fimbriae and the production of salmochelin. In its minimally evasive state, uropathogenic *E. coli* expresses type 1 fimbriae, which can be bound by antibodies, and produces enterobactin, which can be bound and sequestered by the host protein lipocalin-2. In its maximally evasive state, uropathogenic *E. coli* does not produce type 1 fimbriae and is thus resistant to type 1 fimbria-specific antibodies. Furthermore, salmochelin, which is resistant to lipocalin-2, is synthesized rather than enterobactin. **b** | *Proteus mirabilis* MR/P fimbriae are also phase variable and can be targeted by host antibodies. Thus, *P. mirabilis* can evade host defenses when it does not produce these fimbriae. Additionally, *P. mirabilis* can avoid the immune response by producing recombinant FlaAB flagellum that is not recognized by anti-FlaA antibodies. *P. mirabilis* can also synthesize the protease ZapA, which can degrade a broad range of host proteins, including antibodies.

uropathogenic *E. coli* to avoid detection by the immune system (Box 1).

Intracellular bacterial communities

Uropathogenic *E. coli* are capable of forming intracellular bacterial communities (IBCs) and quiescent intracellular reservoirs (QIRs).⁷⁰ IBCs have been identified in experimentally infected mouse bladders and in voided uroepithelial cells collected from human patients.^{71,72} IBCs appear to be the result of clonal invasion of bladder epithelial cells. Because they remain intracellular in what are proposed to be biofilm-like structures, IBCs are protected from components of the host immune response, including neutrophils and antibiotics. Thus, this mechanism might contribute to the recalcitrance of uropathogenic *E. coli* UTIs to antibiotic therapy.⁷³ IBCs and QIRs might also contribute to the recurrence of UTIs owing to bacteria fluxing out of infected host cells and seeding new invasive events.⁷⁴ However, it is currently difficult to pinpoint whether the source of recurrent infections is intestinal colonization, vaginal colonization, or intracellular bacteria within the urinary tract.

P. mirabilis

Adherence via fimbriae

The sequencing and annotation of *P. mirabilis* HI4320 revealed that this strain can potentially express a diverse array of fimbriae. The genome predicts 17 distinct fimbrial operons and 13 additional orphan fimbrial genes not associated with complete operons.¹⁵ Of these, only a handful have been studied: mannose-resistant *Proteus*-like (MR/P), uroepithelial cell adhesin (UCA; also called NAF for nonagglutinating fimbriae), ambient temperature fimbriae (ATF), *Proteus mirabilis* fimbriae (PMF), and *Proteus mirabilis* P-like fimbriae (PMP).⁷⁵

Perhaps the best understood are MR/P fimbriae, which are synthesized *in vivo*, elicit a strong immune response during infection, and have been implicated in autoaggregation and biofilm formation.^{76–78} Although not absolutely required, MR/P fimbriae contribute to virulence.^{79,80} UCA fimbriae facilitate binding to uroepithelial cells and bind to host factors such as asialo-GM₁, asialo-GM₂, lactosyl ceramide, and galectin-3.^{81–83} However, the role of UCA fimbriae in the virulence of *P. mirabilis* *in vivo* has not been reported. PMF fimbriae represent a fitness factor during experimental UTI, but their exact role in adherence to host cells is unclear.^{84,85} ATF are optimally expressed at ambient temperature, as opposed to host body temperature,⁸⁶ so perhaps it is not surprising that these fimbriae do not play a role in colonization of the urinary tract.⁸⁷ PMP fimbriae were identified from a canine UTI isolate, but the genes encoding this fimbria have also been found in at least one human clinical isolate.^{15,88} However, their role during UTI has not been reported. Given the important role of many fimbriae on the pathogenic processes, it will be interesting to see what roles, if any, the other potential fimbriae encoded in the genome of *P. mirabilis* HI4320 have during UTI.

Toxins

Two toxins encoded by *P. mirabilis* have been characterized: hemolysin (HpmA) and *Proteus* toxic agglutinin (Pta). HpmA is secreted from the bacterium (in a process mediated by HpmB) and has calcium-independent hemolytic and cytotoxic activities.^{89,90} Pta, an autotransporter with subtilisin-like serine protease activity, remains anchored at the bacterial surface and mediates autoagglutination of bacteria as well as toxicity against cultured human bladder and kidney cells *in vitro*.⁹¹ HpmA and Pta both contribute to tissue damage observed during infection.⁹² Pta contributes to the ability of *P. mirabilis* to colonize the urinary tract; HpmA does not.^{91,92}

Flagella-mediated motility

Perhaps the most defining characteristic of *P. mirabilis* is swarming motility, a specialized form of flagella-mediated movement that requires the differentiation of cells into an elongated morphotype teeming with flagella.⁹³ These elongated cells seem to be in the minority during infection.⁹⁴ However, *P. mirabilis* is capable of swarming across the surface of urinary catheters.⁹⁵ Perhaps this phenomenon represents the mechanism by

which bacteria gain access to the host, which suggests that this morphotype might be encountered, at the very least, during early stages of human infection.

The roles of swarming and swimming motility during infection are difficult to distinguish because both types of motility are mediated by flagella. Strains lacking flagella are weakened in the murine model, implying a role for flagella during ascending UTI.⁹⁶ However, a strain of *P. mirabilis* lacking flagella has been isolated from a human patient, suggesting that flagella are not strictly required for virulence of this uropathogen.⁹⁷

Metal acquisition

Historically, it was thought that *P. mirabilis* did not produce any of the siderophores known to be produced by other members of the *Enterobacteriaceae* family.⁹⁸ However, sequencing and annotation of HI4320 revealed that this strain of *P. mirabilis* encodes two seemingly complete siderophore synthesis operons.¹⁵ Careful re-examination of siderophore synthesis has revealed that *P. mirabilis* does, in fact, produce siderophores, and the structure of these siderophores is currently under investigation (S. Himpf and H. L. T. Mobley, unpublished work). Additionally, *P. mirabilis* can utilize heme and hemin via the receptor HmuR2, which contributes to the fitness of *P. mirabilis* during experimental UTI.⁹⁹

P. mirabilis produces α -hydroxyisovaleric acid, which has been suggested to function in iron acquisition.⁹⁸ α -keto acids, formed by the deamination of amino acids by the enzyme amino acid deaminase (Aad), can also be utilized as lower affinity siderophores by *P. mirabilis*.^{100,101} Surprisingly, the *aad* gene is not affected by iron concentrations, leading to speculation that perhaps iron acquisition is not the sole function of Aad.¹⁰¹ Interestingly, it has been speculated that Aad might be essential because multiple attempts to generate an isogenic mutant lacking *aad* were unsuccessful.¹⁰¹ Thus, the role of Aad during infection, or even its unequivocal role in iron uptake, has not been confirmed.

Similarly to uropathogenic *E. coli*, the ZnuACB high-affinity zinc transport system has recently been shown to be a fitness factor for *P. mirabilis*. This system contributes to, but is not required for, experimental UTI.¹⁰²

Strategies for evading host defenses

At least three known mechanisms utilized by *P. mirabilis* contribute to immune evasion during infection (Figure 2). One is the production of ZapA, a zinc metalloprotease that is capable of cleaving a broad range of host proteins, including serum and secretory IgA1, IgA2, and IgG, complement proteins, and antimicrobial peptides.¹⁰³ ZapA activity has been detected in the urine of infected patients and contributes to the ability of *P. mirabilis* to colonize the urinary tract.^{104,105}

Another mechanism requires recombination of two independent copies of flagellin genes, *flaA* and *flaB*.¹⁰⁶ FlaA is the predominate version of flagellin expressed by *P. mirabilis*; the *flaB* locus is normally silent.¹⁰⁷ However, upon recombination of these genes, *P. mirabilis* produces flagella that are antigenically distinct

and therefore potentially capable of subverting the immune response.^{107,108}

Lastly, MR/P fimbriae are capable of undergoing phase variation, which might facilitate immune response avoidance (Box 1).¹⁰⁹

Urease production

One of the hallmarks of UTI caused by *P. mirabilis* is urolithiasis, the production of stones in the urinary tract. The formation of these stones is the result of activity of the bacterial enzyme urease, which hydrolyzes urea into ammonia and carbonate (Figure 3). The production of ammonia significantly raises the local pH, resulting in the precipitation of ions that are normally soluble in urine. These stones, such as magnesium ammonium phosphate (struvite), can block the flow of urine through catheters and result in renal scarring owing to calculus formation within the renal pelvis. Additionally, bacteria can be found within these urinary stones, where they are thought to be protected from host defenses and antibiotic treatment.¹¹⁰ Indeed, following antibiotic therapy, bacteria from the stones can reseed the urine.

Overview of host defenses

The vast majority of work characterizing host defenses against UTI has focused on uropathogenic *E. coli* infections. Less is known about the response to *P. mirabilis*, but because both organisms affect the same organ system, aspects of the host defense might be similar in response to both pathogens.

Urine flow and cell exfoliation

Organisms infecting the urinary tract must overcome the mechanical force of the flow of urine. Paradoxically, the shear stress of urine flow enhances the binding interactions of FimH (the tip adhesin of type 1 fimbria), and therefore adherence of uropathogenic *E. coli*.¹¹¹ Superficial umbrella cells in the bladder are covered with membrane proteins known as uroplakins.¹¹² Type 1 fimbriae of uropathogenic *E. coli* bind to uroplakin-1a,¹¹³ triggering exfoliation of the bladder epithelium. The process of exfoliation serves to remove adherent bacteria but also allows access to underlying tissue that is normally not exposed, despite rapid differentiation of these underlying cells into superficial facet cells in response to exfoliation.^{114,115} During uropathogenic *E. coli* infection, this exfoliation is the result of an apoptosis-like mechanism promoted by FimH.^{114,116}

Innate and adaptive immune responses to UTI

These topics have been covered extensively in other review articles.^{117,118} A limited overview will be presented here (Figure 4).

Innate immune response to UTI

Antimicrobial peptides are short, positively charged peptides that bind to and disrupt bacterial membranes. β -defensin 1 and the cathelicidin LL-37, for example, have both been implicated in the response to UTI.^{119–121} As discussed above, iron is an essential nutrient that

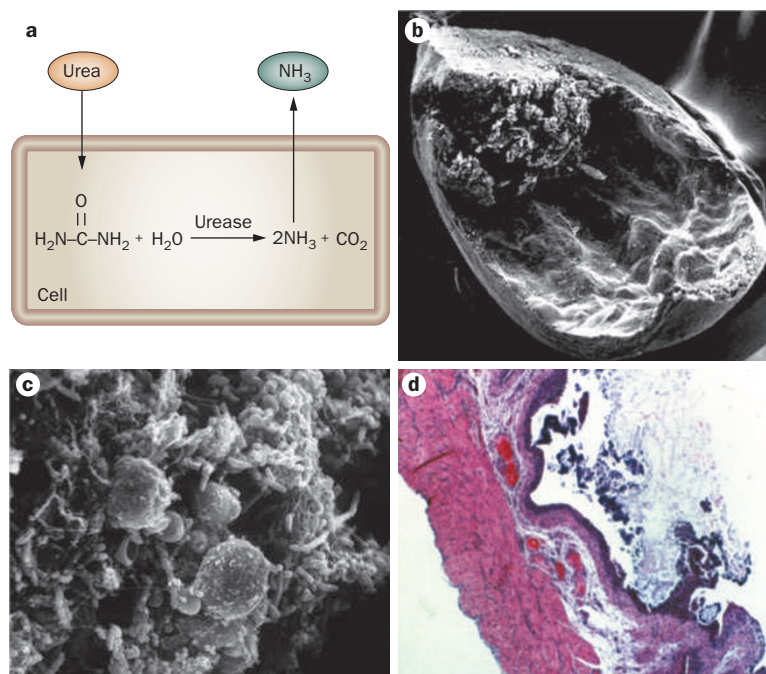


Figure 3 | Formation of urinary stones is the result of bacterial urease. **a** | Urease catalyzes the hydrolysis of urea into ammonia and carbonate. The production of ammonia significantly raises the local pH, resulting in the precipitation of ions that are normally soluble in urine. **b** | Scanning electron micrograph of a segment of mouse bladder after experimental *Proteus mirabilis* infection. A stone can be seen at the inferior end of the bladder. **c** | 100-fold larger magnification of the stone shown in panel B, in which bacterial cells, erythrocytes, neutrophils, and host cell fragments are visible. **d** | Hematoxylin and eosin-stained section of a *P. mirabilis*-infected mouse bladder carrying a luminal stone. Permission obtained from American Society for Microbiology © Li, X. et al. *Infect. Immun.* **70**, 389–394 (2002).

the host sequesters. This iron sequestration is mediated by factors such as lactoferrin, transferrin, and lipocalin-2.¹²² Tamm-Horsfall protein, also known as uromodulin, is a high-molecular-weight glycoprotein present in human urine that binds to type 1 fimbriae, thereby limiting interaction of uropathogenic *E. coli* with host receptors.¹²³ Tamm-Horsfall protein is also effective against *P. mirabilis*.¹²⁴

A large panel of cytokines and chemokines are upregulated in response to uropathogenic *E. coli* infection in mice.¹²⁵ Most of these peak by 1 day postinfection and return to levels near baseline by 2 weeks postinfection, except for IL-17A, which was recently shown to contribute to innate clearance of uropathogenic *E. coli*.¹²⁶ IL-8, the main neutrophil attractant in humans, is secreted by both bladder and kidney cell lines in response to uropathogenic *E. coli*.^{127–129} Indeed, neutrophils are the most rapid and robust responders to UTI and their migration to the site of uropathogenic *E. coli* infection in the urinary tract is dependent on IL-8.^{130–133}

Pathogen recognition receptors, specifically TLR4 and TLR5 in humans, as well as TLR11 in mice, have been implicated in the response to UTI. By contrast, TLR2 does not appear to be required for a normal response to UTI.¹³⁴ TLR4 knockout mice infected with uropathogenic *E. coli* have higher bacterial burdens because TLR4 is important for clearance and the inflammatory response

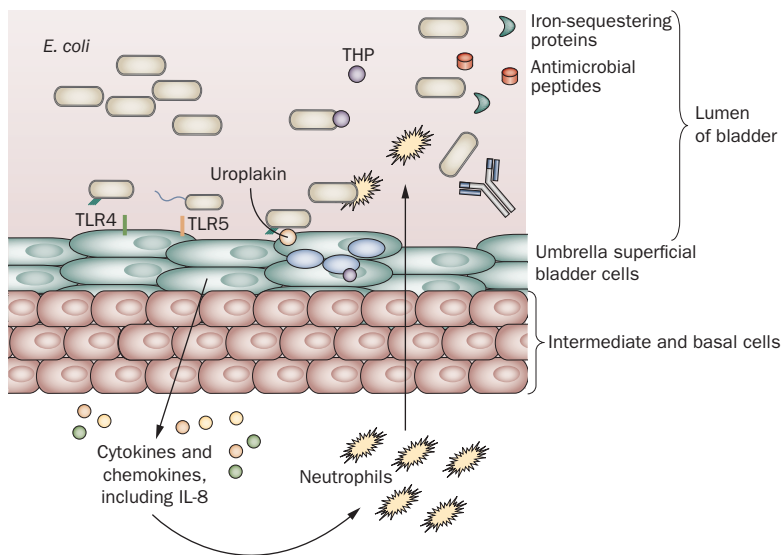


Figure 4 | Uropathogenic *Escherichia coli* interaction with components of the host immune system. In the lumen of the bladder, uropathogenic *E. coli* must face such challenges as binding by THP, attack by antimicrobial peptides (such as β -defensin 1 and the cathelicidin LL-37), and competition with host iron-sequestering proteins (such as lipocalin-2, transferrin, and lactoferrin). Uropathogenic *E. coli* can be detected by the bladder epithelium via binding to TLR4 (mediated by either type 1 or P fimbriae), TLR5 (mediated by flagella) and uroplakin-1a (via type 1 fimbriae). These interactions with the uroepithelium result in secretion of cytokines and chemokines, including IL-8, which results in the recruitment of neutrophils to the bladder. Although not as much is known about the adaptive immune response to UTI, antibodies have been implicated in this role. Abbreviations: THP, Tamm-Horsfall protein; TLR, Toll-like receptor.

in the bladder and kidneys.^{135–137} TLR4 signaling can be induced by multiple mechanisms, including a P fimbriae-dependent and lipopolysaccharide-independent pathway^{138–140} and a lipopolysaccharide-dependent and type 1 fimbriae-dependent pathway.^{128,141} In addition, FimH can directly interact with TLR4.^{135,142} Regardless of the source of stimulation, TLR4-mediated signaling results in the activation of NF κ B and expression of proinflammatory genes, such as IL-6 and IL-8.¹⁴³ TLR5 recognizes flagella, is involved in the response to uropathogenic *E. coli* infection, and results in the production of proinflammatory factors.^{144,145} Mice lacking TLR11 are more susceptible to uropathogenic infection; however, it appears that humans lack functional TLR11 owing to an early stop codon.¹⁴⁶

Adaptive immune response to UTI

In general, data regarding the adaptive immune response to UTI are limited. Results from one seminal study suggest that the protection derived from infection is antibody-mediated.¹⁴⁷ In addition, there is evidence that uropathogenic *E. coli* clearance is also antibody-mediated.^{148–151} At this point, it is unclear if the immune response is skewed toward a T_H1-mediated or T_H2-mediated response, and the role of T_{REG} cells has not been elucidated.

Genetic variations that influence UTI

This topic has been reviewed thoroughly elsewhere,^{134,152} but a brief overview will be presented here, focusing

on two host factors that can impact susceptibility and outcome of disease: TLR4 and CXC chemokine receptor type 1 (CXCR1). Susceptibility is also affected by the density of epithelial cell receptors—patients who are prone to UTI seem to have higher numbers of receptors for bacterial fimbriae.¹⁵³

As described above, TLR4 signaling is important for the host response to UTI. Interestingly, infection of mice with mutations in TLR4 resulted in an asymptomatic carrier state.^{143,154} However, it should be noted that these mice were not spared from death following these infections.¹⁵⁵ TLR4 might also be associated with asymptomatic infections in humans, as children with asymptomatic infections have lower expression of TLR4 mRNA and protein in neutrophils than age-matched controls.¹⁵⁶

CXCR1, the receptor for IL-8, is essential for the migration and activation of neutrophils. In mice lacking the IL-8 receptor, infection with uropathogenic *E. coli* resulted in higher bacterial titers and progression to bacteremia and renal scarring than in infected wild-type mice, suggesting that functional CXCR1 plays a role in protecting the host from severe infection.^{157–159} In human patients, CXCR1 mRNA and protein levels are significantly lower in neutrophils isolated from the blood of children who are prone to pyelonephritis.¹⁵⁷ Further work revealed that these UTI-prone children had single nucleotide polymorphisms in their *CXCR1* genes.¹⁶⁰ In addition, CXCR1 expression was also lower in relatives of these children, suggesting that low expression, and perhaps also the resultant increased susceptibility to acute pyelonephritis, is inheritable.¹⁶¹

Treatment and prevention of UTI

Current prophylaxis and treatment strategies

Beyond continuous or intermittent (postcoital) antibiotic usage, current prophylactic strategies are limited to consumption of cranberry juice and, for postmenopausal women, estrogen treatment.¹⁶² The utilization of different compounds for the prevention of UTI in animal models has been reported, including the glucose analog N-butyldeoxyojirimycin,¹⁶³ forskolin,¹⁶⁴ small molecule inhibitors,¹⁶⁵ and anti-FimH antibodies.¹⁶⁶ None of these compounds have been tested in human UTI patients, to our knowledge. Treatment with protamine sulfate is associated with increased exfoliation of umbrella cells and, in mice, reduced bacterial burden in the bladder.⁷⁰ However, this treatment caused significant discomfort in human volunteers.¹⁶⁷ The standard treatment for UTI remains antibiotic therapy, which unfortunately can also disrupt the microbiota and result in the selection of resistant strains.

Catheter-associated complicated UTIs

More than 5 million patients are catheterized each year, and each one is at significant risk for UTI.¹⁶⁸ The first steps to reducing the risk of these infections are to limit the use of urinary catheters, which are generally over-used, and to make sure they are removed promptly when no longer medically necessary.^{168,169} There has been much interest in developing catheter coatings to reduce bac-

terial colonization, but, to date, no single surface has been demonstrated to effectively prevent long-term colonization.¹⁶⁸ Early studies employing the catheter balloon, rather than the surface of the catheter, as a reservoir for antimicrobial compounds have been encouraging.¹⁷⁰ In addition, the probiotic use of nonpathogenic *E. coli* strains might prevent catheter colonization by pathogenic strains.¹⁷¹ Infection with urease-positive strains, such as *P. mirabilis*, is a further complication because urinary stones potentially serve as a reservoir for bacteria, which can rapidly colonize replacement catheters.¹⁷²

Vaccines to prevent UTI

Given the large impact of these infections (on both individual patients and the health-care system) and the current lack of effective, nonantibiotic prophylactic strategies, there is much interest in the development of vaccines to prevent both uncomplicated and complicated UTIs. Different patient populations are likely to benefit from these respective vaccines. We anticipate that a uropathogenic *E. coli* vaccine could be administered to individuals who suffer from, or are at increased risk for, recurrent infections, whereas a *P. mirabilis* vaccine would be most beneficial to patients at an increased risk of developing complicated infections, including individuals with known functional or anatomical urinary tract abnormalities and patients at the onset of long-term catheterization.

Uropathogenic E. coli vaccines

Vaccines targeting uropathogenic *E. coli* have recently been reviewed.¹¹⁷ Perhaps the two most successful vaccine formulations to date are Uro-Vaxom (OM Pharma, Geneva, Switzerland), a daily oral capsule containing membrane proteins from 18 *E. coli* strains, and SolcoUrovac (Solco Basel AG, Birsfelden, Switzerland), a vaginal suppository containing ten heat-killed uropathogenic strains.^{173,174} However, there is currently no licensed uropathogenic *E. coli* vaccine available in the US and active research toward this goal is ongoing.

Historically, vaccines against uropathogenic *E. coli* have been directed against known virulence factors, specifically components of P and type 1 fimbriae. Recently, additional vaccine candidates were identified via multiple 'omics' approaches, including genomic, proteomic, and metabolomic screens.¹⁷⁵ These approaches identified a number of protein vaccine candidates that are pathogen-specific, antigenic, surface-exposed, and expressed *in vivo*. Interestingly, the top candidates (those identified from multiple screens that fit all of the above criteria) were outer membrane iron receptors. Intranasal vaccination with three outer membrane iron receptors protected mice from uropathogenic *E. coli*-mediated UTI.¹⁷⁶ Protection correlated with production of antigen-specific IgA in the urine and class switching from IgM to antigen-specific IgG.¹⁷⁶ One goal of future research should be to determine if these findings will translate to human UTI patients.

P. mirabilis vaccines

Early studies showed that initial infection with *P. mirabilis* does not prevent re-infection, and heat-killed

preparations do not prevent initial infection.^{177,178} Immunization with outer membrane preparations protected mice from renal colonization, tissue damage, and death.¹⁷⁹ Varying degrees of protection were offered by immunization with fimbrial structural proteins MrpA, UcaA, and PmfA.^{180,181} One of these recent studies employed *Lactococcus lactis* as an antigen delivery vehicle, a novel approach in *Proteus* vaccinology that, in general, is a promising idea for the development of new vaccines against mucosal pathogens, since these vaccines seem to have fewer side effects than systemic vaccines.¹⁸⁰ One of the most promising vaccines to date has been intranasal immunization with the N-terminal domain of MrpH, the tip adhesin of MR/P fimbriae.¹⁸² Recently, an immunoproteomic screen was performed to identify outer membrane proteins of *P. mirabilis* that can elicit an immune response in infected mice.¹⁸³ Since these proteins are expressed *in vivo* and are exposed to the surface (where they could potentially interact with the host), they represent exciting new potential vaccine candidates. Indeed, immunization with one of these proteins, Pta, protected mice from subsequent transurethral challenge.⁹²

Conclusions

Uropathogenic *E. coli* and *P. mirabilis* are pathogens of the urinary tract, a common site of bacterial infection in humans. Proteins that are required for, or contribute to, the virulence of each pathogen have been identified, and these discoveries have contributed to our understanding of the mechanisms of pathogenesis. Such virulence or fitness factors include fimbriae (such as type 1 and P fimbriae in uropathogenic *E. coli* and MR/P and UCA fimbriae in *P. mirabilis*) that mediate attachment to host tissues, toxins (such as hemolysins and autotransporter toxins in both species), flagella, iron acquisition systems, and proteins that function to dampen or evade the host immune response (such as SisA and SisB in uropathogenic *E. coli* and ZapA in *P. mirabilis*). In addition, each pathogen has some traits not shared with the other: uropathogenic *E. coli* is capable of forming intracellular bacterial communities, and *P. mirabilis* produces the enzyme urease, the action of which can lead to the formation of urinary stones and block the flow of urine through catheters.

To successfully colonize and persist in the host, these uropathogens must overcome host defenses mediated by the innate and adaptive immune systems. Innate responses to UTI are more well-defined than adaptive responses. Tamm-Horsfall protein, antimicrobial peptides, and iron sequestration proteins such as transferrin and lactoferrin are active in the urinary tract. TLRs can be engaged by multiple uropathogenic *E. coli* components, including flagella and both P and type 1 fimbriae. Numerous cytokines and chemokines are upregulated in response to infection; the chemoattractant IL-8 is required for the migration of neutrophils to the infected urinary tract. Not surprisingly, deviations in components of the host immune system can impact susceptibility to, or outcome of, infection. Reduced levels of TLR4 are associated with

the development of asymptomatic bacteriuria, while reduced levels of CXCR1 are associated with increased susceptibility to acute pyelonephritis.

UTIs cause significant morbidity and are typically treated with antibiotics; there is currently not a licensed vaccine available in the US against uropathogenic *E. coli* or *P. mirabilis*. Thus, there has been interest in the generation of vaccines against both uropathogens. Much of current research is focused on pathogen gene and protein expression *in vivo* during infection of the host, with the hope of identifying additional virulence factors and therapeutic or prophylactic targets. Recently, an ‘omics’ approach, consisting of multiple screens, led to the identification of several uropathogenic *E. coli* vaccine candidates that were able to protect mice from subsequent infection. Similar screens are currently underway in *P. mirabilis*. These types of ‘reverse vaccinology’ approaches have broad appeal and have led to the

successful identification of potential vaccine candidates in other human pathogens, such as *Neisseria meningitidis* serogroup B, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*.¹⁸⁴ In addition, further study will be required to elucidate adaptive immune responses in the urinary tract. These findings will aid understanding of the disease process and might also provide insight toward the generation of lasting immunity against uropathogens.

Review criteria

We searched for original articles on PubMed using the search terms “urinary tract infection”, “uropathogenic *E. coli*”, and “*Proteus mirabilis*”. All papers identified were English-language full-text papers. We also searched the reference lists of identified articles for further papers. Most cited articles were published between 1994 and 2010, but some earlier seminal studies were also included.

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