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Urinary tract infections: microbial pathogenesis, host-pathogen interactions and new treatment strategies

Roger D. Klein[a,b], **Scott J. Hultgren**[a,b,#]

[a]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA

[b]Center for Women's Infectious Disease Research (CWIDR), Washington University School of Medicine, St. Louis, Missouri, USA

Abstract

Urinary tract infections (UTIs) are common, recurrent infections that can be mild to life-threatening. The continued emergence of antibiotic resistance, together with our increasing understanding of the detrimental effects conferred by broad-spectrum antibiotic use on the health of the beneficial microbiota of the host, has underscored the weaknesses in our current treatment paradigm for UTIs. In this Review, we discuss how recent microbiological, structural, genetic and immunological studies have expanded our understanding of host-pathogen interactions during urinary tract pathogenesis. These basic scientific findings have the potential to shift the strategy for UTI treatment away from broad-spectrum antibiotics targeting conserved aspects of bacterial replication towards pathogen-specific antibiotic-sparing therapeutics that target core determinants of bacterial virulence at the host-pathogen interface.

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In this Review, Klein and Hultgren discuss recent advances in our understanding of the interplay between pathogens and host during urinary tract infections, and how the insights into host-pathogen interactions and pathogenesis are guiding the development of antibiotic-sparing therapeutics.

hultgren@wustl.edu.

Author contributions

R.D.K and S.J.H. researched data for the article, contributed to discussion of content, wrote the article, and reviewed and edited the manuscript before submission.

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S.J.H. has an ownership interest in Fimbrion Therapeutics, and may benefit if the company is successful in marketing mannosides. S.J.H. is also the chief scientific officer of QureTech Bio. R.D.K. declares no competing interests.

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Introduction

Urinary tract infections (UTIs) are common infections caused predominantly by uropathogenic *Escherichia coli* (UPEC), which cause approximately 80% of UTIs. The annual incidence of physician-diagnosed UTIs in the United States is greater than 10% for females and 3% for males, and more than 60% of females will be diagnosed with a UTI in their lifetime[1,2]. Individual risk for infection depends on various factors, including age, sexual activity, family history, medical comorbidities and an individual history of UTI (Box 1) [3]. Recurrent UTIs (rUTIs; defined as three or more UTIs during a 12 month period [4]) contribute greatly to the incidence and morbidity rates of UTI, as more than 30% of women will suffer from a subsequent infection within 12 months of resolution of initial symptoms despite appropriate antibiotic therapy [5]. UTIs are becoming increasingly difficulty to treat owing to the rapid spread of drug resistance among Gram-negative organisms, including UPEC [6].

Most UTIs are initiated when UPEC enter the urinary tract through the urinary meatus before ascending up the urethra and into the bladder lumen. Isolated infections of the bladder and lower urinary tract without signs or symptoms of upper tract or systemic infection are referred to as ‘uncomplicated’ cystitis or ‘simple’ cystitis (Table 1). Acute cystitis in non-pregnant, premenopausal patients without functional urinary tract abnormalities is classified as uncomplicated[7]. Cystitis is classified as ‘complicated’ cystitis in pregnant or immunocompromised patients and patients with functional urinary tract abnormalities, an indwelling catheter or a history of renal transplantation [8]. In approximately 0.34% of cases, pathogens causing cystitis ascend further through the ureters into the kidney, where they cause an infection of the renal pelvis, calices and cortex that leads to the clinical signs and symptoms of pyelonephritis [9]. If left untreated, pathogens may spread from the kidney into the bloodstream (bacteremia) and, if a concurrent systemic inflammatory response is present this may lead to septicemia [10]. In patients presenting to the emergency department with sepsis, 27% of all cases can be attributed to a previous urinary isolate, and are thus termed urosepsis [11]. In hospitalized patients, the proportion of sepsis cases attributed to UTI increases to 42% [12]. There is an urgent need to develop antibiotic-sparing therapeutics that can break the vicious cycle of rUTI, antibiotic resistance and the interconnection to sepsis.

The risk of UTI progression to pyelonephritis and urosepsis is dependent, in part, on the setting in which the UTI was acquired. For example, the pathogen population that causes healthcare-associated UTIs due to indwelling urethral catheters (catheter-associated UTIs (CAUTIs)) comprises a larger group of bacteria from those responsible for community-acquired UTIs. Insertion of a urinary catheter into the urethra and bladder results in the release of host factors such as fibrinogen, which coat the catheter. These factors enable diverse pathogens, including members of the genera *Enterococcus* and *Staphylococcus,* to bind and assemble biofilms on the catheter surface [13]. The genomes of these pathogens encode fibrinogen-binding adhesins that are crucial to biofilm formation on the catheter, and confer increased mortality if the infections progress to pyelonephritis and urosepsis [14,15].

Traditionally, broad-spectrum antibiotics have been the drug of choice to combat both community- and hospital-associated UTIs. However, the continued emergence of antibiotic resistance, coupled with our increasing appreciation for the role of commensal members of the host microbiota, have underscored the urgent need for antibiotic-sparing therapeutics that can selectively treat UTIs without altering the structure of the gut and vaginal microbiota. In the gastrointestinal tract, antibiotic consumption increases inflammation, undercuts the host immune environment and promotes the proliferation of *E. coli* by increasing nitrate availability [16]. As the gastrointestinal tract is frequently the ultimate reservoir for UPEC, expansion of UPEC within the gastrointestinal habitat is also associated with increased risk of subsequent UTI recurrence [17,18]. Antibiotic treatment can also disrupt the protective vaginal microbiota by diminishing colonization with peroxide-producing Lactobacillus species that suppress vaginal UPEC colonization and subsequent bacterial ascension [19,20]. Thus, paradoxically, the antibiotics used to treat UTIs are also risk factors for rUTIs, probably because of their effect on the gut and vaginal microbiotas [16, 21, 22].

In this Review, we discuss recent advances in our understanding of the dynamic and diverse interplay between pathogens and the host during infection, and how these molecular insights into host-pathogen interactions are guiding the design and development of antibiotic-sparing therapeutics. We first outline the pathogenic cascade of uncomplicated acute UTI and critical virulence factors involved in disease progression, followed by a discussion of CAUTI pathogenesis and virulence factors involved in establishing colonization and infection. We discuss how new insights into host-pathogen interactions are leading to the development of promising antibiotic-sparing therapeutics including small-molecular inhibitors of adhesion, inhibitors of nutrient uptake, immunomodulatory therapy that alters the host response to infection and vaccination against microbial targets. Such promising antibiotic-sparing therapeutics have the potential to curb recurrence and circumvent existing antimicrobial resistance.

UPEC pathogenesis during UTI

Recent metagenomic and metatranscriptomic studies on a panel of clinical isolates, combined with *in vitro*, *in vivo*, animal and human studies have provided tremendous molecular insights into the wide range of virulence factors used by UPEC to overcome the innate and adaptive immune response of the host to facilitate bladder colonization during UTI [23]. These studies have also extended to virulence factors promoting the colonization of extraurinary tissues, including the vaginal, peri-urethral and gastrointestinal tracts en route to ascending UTI. In this section, we provide an overview of host-pathogen interactions critical in uncomplicated UTI. A discussion of novel insights into the interplay between virulence factor carriage and bacterial virulence is found in Box 2.

The UTI cycle and host immune response.

Over the past 20 years, various mouse models have been developed to better dissect the molecular mechanisms of UTI pathogenesis (Supplementary Figure S1). Because biopsy is not typically performed in patients with UTI, urine cytology and metabolic analysis are often necessary to correlate findings in mouse models to human disease. This has recently

been accomplished by metatranscriptomic analyses of UPEC isolated directly from the urine of patients with UTIs [24]. Knowledge gained from numerous mouse models of UTI pathogenesis have proven to be translatable to humans. After reaching the mouse bladder lumen, UPEC adhere to the surface of superficial umbrella cells using thin adhesive filaments called type 1 pili (Figure 1). Following attachment, bacteria are internalized into epithelial cells, where they multiply within this protected intracellular niche to form large biofilm-like intracellular bacterial communities (IBCs). Here, UPEC can evade Toll-Like receptor 4 (TLR-4)-mediated expulsion and replicate in the urothelial cell cytoplasm before fluxing out back into the bladder lumen and adhering to nearby cells [25,26]. Formation of IBCs is the result of clonal expansion of a single bacterium, enabling the infection to sidestep efficient host clearance of extracellular bacteria while decreasing of the overall genetic diversity of the population [27]. Although the kinetics of attachment, internalization, and IBC formation in humans may differ from their mouse counterparts, the presence of IBCs has been verified in several human adult and pediatric UTI studies [28–31].

In response to infection of naïve C3H/HeN mice with human UTI isolates, host secretion of interleukin-6 (IL6), IL-17, tumour necrosis factor (TNF), C-X-C motif chemokine 1 (CXCL1), CXCL2 and CXCL5 results in extensive neutrophil recruitment to the urothelium [32–35]. This response is followed by the recruitment of monocytes and the induction of programmed cell death and exfoliation, which results in an initial decrease in bacterial burden in the bladder but also exposes the underlying transitional epithelium [36,37]. Histologically, these processes are accompanied by lymphonodular hyperplasia and loss of terminal markers of differentiation on mouse superficial facet cells, both of which have also been observed in humans with rUTI [38]. In mouse models, this immune-mediated exfoliation can lead to bacterial attachment and invasion of the transitional epithelium, which results in the formation of small quiescent intracellular reservoirs (QIRs) that persist even after resolution of bacteriuria that are capable of seeding rUTI [39]. Although the presence of QIRs has yet to be observed in humans, these models suggest that exfoliation can be a double-edged sword wherein the initial bacterial UPEC burden is reduced at the expense of long-term persistence and recurrence.

Upregulation of cyclooxygenase 2 (COX2) expression in urothelial cells and neutrophils in response to UTI is associated with neutrophil transmigration across the bladder epithelium, and is crucial for the associated mucosal damage that ensues [40]. Following this transmigration in mouse models of infection, persistent high-titer bacteriuria accompanied by severe immunopathology and ablation of the terminally differentiated superficial umbrella cells can develop[39,41]. Chronic cystitis is a prolonged infection observed in mice that is characterized by persistent high-titer bacteriuria with high bladder bacterial burdens. Histologically, this is manifested as chronic inflammation with lymphonodular hyperplasia of the submucosa and urothelial hyperplasia with loss of terminal differentiation [41]. Placebo studies have shown that 50% of infections in humans do not resolve in the absence of effective antibiotic treatment, and histological features of chronic cystitis observed in mice have also been observed in humans [38,42]. Studies of serum biomarkers in premenopausal women with acute cystitis caused by UPEC reveal that chronic and recurrent cystitis can be predicted 24 hours post-infection by increased levels of the serum biomarkers IL-5, IL-6, CXCL1 and granulocyte colony-stimulating factor (G-CSF) [40,41]. Similarly, in the sera of

young women with acute cystitis, increased levels of soluble biomarkers involved in myeloid cell development and chemotaxis were predictive of future UTI recurrence [41].

An episode of acute cystitis can result in either a protective response or render the host more susceptible to recurrences. In a mouse model of rUTI, the dynamics of TNF signalling activation differed when the initial infection was chronic (sensitized) compared with self-limiting (resolved). Inoculation of mice who had experienced a prior bladder infection with UPEC resulted in an earlier onset of TNF-mediated inflammation compared with age-matched naïve mice. This early-phase TNF signaling was shown to decrease colonization by facilitating rapid recruitment of neutrophils and exfoliation of infected bladder cells. By contrast, mice who suffered from chronic UTI following initial infection experienced a prolonged TNF signaling period, which exacerbated inflammation and worsened infection. Thus, the regulation of TNF dynamics and COX-2 expression (Box 1) could explain how a prior infection can modulate susceptibility to future infections [43].

Chaperone-usher pathway pili.

Chaperone-usher pathway (CUP) pili are proteinaceous extracellular appendages found on the outer membrane of Gram-negative bacteria. The *E. coli* pangenome encodes 38 distinct CUP pili, and individual *E. coli* genomes encode an average of 12 CUP operons, each of which likely mediates binding to a specific molecular receptor on biotic or abiotic surfaces [44]. The binding specificity of each pilus type is typically conferred by a two-domain adhesin located at the distal tip of a fiber comprising hundreds of major structural subunits (Fig 2a). P pili and type 1 pili are two CUP pili that have well-established roles in UTI pathogenesis. The PapG adhesin at the tip of P pili binds to a Galα1-4Gal core on globoside glycolipids in the kidney and contributes to pyelonephritis, whereas the FimH adhesin at the tip of the type 1 pilus binds mannosylated glycoproteins on superficial epithelial cells with stereochemical specificity (Fig 2a) [36,45–47]. This type-1-pilus-mediated adhesion mediates colonization and invasion of the bladder epithelium and facilitates the formation of the biofilm-like IBCs (discussed above).

In the past few years, a wealth of new information has emerged about the role of other CUP pilus types in UTI pathogenesis [48,49]. For example, expression of the Fim-like (Fml) pilus, which is enriched in the genomes of clinical UPEC isolates, was shown to occur in the urine of women with UTI, and in mice, the presence of the Fml pilus confers a fitness advantage to UPEC in the inflamed bladder [50–52]. The Fml pilus adhesin FmlH recognizes GalNAc-containing receptors expressed on inflamed bladder tissue, Gal(β1-3)GalNAc in the core 1 Thomsen-Friedenreich antigen, and core 2 *O*-glycans found on glycoproteins excreted by the kidney epithelium [52].

CUP pili also facilitate the colonization of numerous other habitats, such as the gastrointestinal tract. The observation that UPEC strains isolated from the urine of women with UTI often concurrently dominate the rectal florae of those patients suggests that CUP adhesins with tropism for the gastrointestinal tract may influence the urovirulence of a strain [18]. Genomic analyses of clinical UTI isolates causing recurrence revealed a strong enrichment for the carriage of the F17-like pilus, also known as the Ucl pilus, relative to the general *E. coli* pangenome [53]. In a mouse model of gastrointestinal colonization, deletion of

the *ucl* operon was associated with a stark competitive defect, validating its role in colonization of this habitat [53]. The F17-like adhesin, UclD, uses a canonical β-sandwich topology to bind *O*-glycans on epithelial cells in the lower crypts of the colon [53]. Phylogenetic analyses indicate that UPEC acquired the *ucl* operon from *Proteus mirabilis*, and that strains encoding this operon were subsequently better adapted for gastrointestinal persistence and seeding of the urinary tract [53]. Additionally, FimH also has a role in gastrointestinal colonization by binding the upper crypts of the intestine in a mannose-dependent manner [53].

Structural and kinetic analyses revealed that UPEC can engage in a low affinity FimH-mediated multivalent bonding with mannosylated *N*-glycans on host cells within the bladder under static conditions, whereas the shear forces induced by rapid urine flow induce a high affinity catch-bond conformation that prevents bacterial expulsion in the urine stream [54]. Recent biophysical analysis has also revealed that soluble FimH adhesin exists in two distinct conformational states that engage with mannose via unique binding modes with markedly different affinities [55]. In addition to the catch-bond mechanism, positively-selected residues identified by prior genetic studies were also found to modulate the allosteric interconversion between these low- and high-affinity states [55–57]. In the high-affinity state, the FimH adhesin is able to transition between a wide range of conformational states, enabling the tethering of bacteria to the luminal surface of the bladder. Allelic variants favoring the high-affinity conformation bind bladder cells with high affinity and invade transitional epithelial cells *in vitro*, but are attenuated in *in vivo* mouse models of cystitis [55]. Thus, it seems that evolutionary selection has fine-tuned conformational states of the FimH adhesin in solution to modulate molecular tethering to receptors that are crucial for virulence in UTIs.

The role of type 1 rod dynamics has also been the subject of recent investigation. The major subunits of many CUP pili are assembled into a helical rod that is capable of unwinding into a linear fiber upon the application of external force to dampen the force at the adhesin-receptor interface, providing a ‘shock absorber’ effect which prevents bacterial dissociation from host tissues [58,59] (Fig 2a). Because interactions formed between neighboring pilin subunits within the helical rod dictates the strength of this unwinding force, the biomechanical properties of individual pili seem to be evolutionarily selected to optimize adhesion within different habitats [60]. For example, P pili have adapted to the constant urine flow in the kidney by requiring a greater force to unwind, whereas the CS20 fimbriae that mediate intestinal attachment in enterotoxigenic *E. coli* (ETEC) strains are more easily unwound to accommodate the short, intense forces bacteria experience in the intestine [60]. Structural and biophysical studies of the type 1 pilus rod revealed that residues responsible for the interaction between adjacent pilus subunits involved in winding and unwinding are subject to purifying selection. Mutation of these residues leads to substantial attenuation during *in vivo* infection in a mouse model [61,62]. Thus, the unwinding dynamics of the pilus rod has a crucial function in host-pathogen interactions. Conversely, the high rate of polymorphism among outward-facing residues of the rod reflects high rates of positive, diversifying selection, which are likely to aid in immune evasion [61].

Finally, the role of the outer-membrane usher has also been subject to extensive biochemical and structural investigation. Recent high-resolution structures of the P pilus and type 1 pilus ushers in complex with their respective chaperone-adhesin complexes have revealed a putative mechanism by which the chaperone-adhesin complex is transferred from the amino-terminal to the carboxy-terminal periplasmic domain of the usher where the donor strand exchange reaction is subsequently catalyzed [63,64]. For P pili, transfer requires formation of a tripartite interface between the incoming PapD chaperone and the N-terminal and C-terminal domains of PapC usher. The PapC-PapD-PapG structure revealed a potential mechanism for how bicyclic 2-pyridone compounds are able to inhibit pilus biogenesis by blocking interactions necessary for the formation of the tripartite complex [65]. Such compounds hold promise in the treatment of infectious diseases.

Metal sequestration.

Sequestration of metals and other trace minerals by the host is perhaps the best-studied example of nutritional immunity [66,67]. For example, the host response to infection includes increased expression of several iron-binding proteins, including lactoferrin and lipocalins, to sequester iron away from invading pathogens [68–70]. To combat this, bacterial pathogens have evolved various genes that encode siderophores **[G]** [71](Fig 2b). Metabolomic characterization of *E. coli* during colonization has elucidated the interspecies diversity in metal acquisition systems between *E. coli* isolated from various habitats [72]. Clinical UPEC isolates preferentially express two siderophores, yersiniabactin and salmochelin, at higher levels than gastrointestinal *E. coli* isolates, which suggests that optimization of iron uptake in the bladder environment is important for UPEC pathogenesis [73]. Yersiniabactin binds extracellular iron and copper ions and delivers them to the FyuA outer membrane receptor (Fig 2b), which is associated with UPEC virulence in the bladder and is required for the establishment of septicemia [71,74–76]. UPEC strains lacking the *fyuA* gene experience a bladder and kidney colonization defect relative to a wild-type control [77]. Expression of an essential peptide for yersiniabactin synthesis , high-molecular weight protein 2 (HMWP2) encoded by the *irp2* gene, is regulated by environmental iron levels and is associated with increased UPEC virulence and antibiotic resistance[74,78]. HMWP2 associates with HMWP1 to catalyze the formation of yersiniabactin via a series of biosynthetic precursors in the presence of low iron levels. Further metabolomic and biochemical characterization of the siderophore biosynthetic pathways has revealed metabolic precursors that may have secondary functions, such as microbial antagonism [79]. For example, the production of escherichelin, a product of the yersiniabactin biosynthetic pathway, interferes with pyochelin iron uptake in *Pseudomonas* aeruginosa[79]

Two-component systems.

α-hemolysin (HlyA) is a proteinaceous pore-forming toxin that assembles into a water-filled channel that perforates the outer membrane of host cells, leading to lysis and cell death that increases nutrient availability to pathogens [80]. HlyA is found in approximately 50% of recurrent UTI clinical isolates from women aged 18-39 versus 37% and 31% of isolates from first and second UTIs, respectively. This represents an enrichment over isolates found in the peri-urethral and fecal environments from women in those same age groups, where prevalence is 26% and 15%, respectively [81]. HlyA is known to cause damage to urothelial

and renal tubular cells, promoting exfoliation and inflammatory changes [82–84]. Expression of *hlyA* in *E. coli* is regulated, in part, by the CpxR-CpxA two-component signal transduction system [84] (Fig 2c). Dysregulation of the CpxR-CpxA system alters the expression profiles of several virulence factors, including *hlyA*, as deletion of the response regulator CpxR in UPEC results in overexpression of hemolysin and loss of bacterial fitness in mouse models of acute and chronic cystitis [84]. Mechanistic studies have revealed that HlyA contributes to activation of the host inflammasome by activating the caspase-1 and caspase-4. The CpxR-CpxA system itself is regulated by the interaction of CpxP with unfolded pilus subunits, further highlighting the complex interplay between regulators of bacterial virulence [85,86]. Additional two-component systems known to affect UPEC virulence include the YdpA-YdpB and BtsS-BtsR systems, which together upregulate transporter genes in acute and chronic mouse models of UTI and in response to the presence of environmental biomolecules such as pyruvate [87,88]. The QseB-QseC system, which is composed of the QseB response regulator and QseC kinase, is known to regulate UPEC virulence factors in response to high levels ferric iron [89–91]. Finally, recent work has shown that crosstalk between the QseB-QseC and the PmrA-PmrB two-component systems has a role in antimicrobial resistance [92,93].

Biofilms.

Formation of biofilms by UPEC isolates is associated with increased bladder fitness and antimicrobial resistance. In a prospective study of 105 patients with *E. coli* UTIs, biofilm production was noted in 62% of UPEC isolates versus only 1% of control isolates, and was associated with higher rates of antimicrobial resistance [94]. Recent studies have revealed that the reduced oxygen tension found in the bladder, coupled with the presence of terminal electron receptors within urine, facilitates the preferential expression of *E. coli* biofilms [95]. The expression of other factors, such as the quinol oxidase cytochrome *bd*, facilitates biofilm complexity and resistance to extracellular stressors by altering the abundance of extracellular matrix components [96].

Curli fibers are a functional amyloid that forms the major proteinaceous component of many Gram-negative bacterial biofilms (Fig 2d) [97,98,99,100]. The presence of curli fibers within these biofilms provide a competitive advantage in mouse models of UTI by promoting adhesion to bladder epithelial cells [101,102]. This adhesion is further augmented by the presence of phosphoethanolamine cellulose concurrently produced by UPEC [102]. Structural, biochemical and biophysical analyses have elucidated the molecular mechanisms of curli biogenesis by *E. coli* and other pathogens. Curli comprise repeating amyloidogenic CsgA subunits that are assembled into sodium dodecyl sulphate-resistant and protease-resistant extracellular fibers by several periplasmic and extracellular assembly factors. Specifically, CsgE is a periplasmic chaperone that interacts with unfolded CsgA via a series of charge-charge interactions to facilitate the transport of CsgA to and through the nonameric CsgG outer membrane pore [103](Fig 2d). The concerted efforts of CsgE and the chaperone-like protein CsgC prevent premature CsgA polymerization in the periplasm [104] (Fig 2d). Following transport to the CsgG pore, CsgA is secreted across the outer membrane where curli fiber formation is facilitated by CsgB, which is held to the outer membrane by CsgF. CsgB nucleates the folding of CsgA into its fibrillar β-solenoid conformation, resulting in a

nucleation-elongation reaction that promotes fiber polymerization [105]. Following a one-step nucleation process, elongation of individual curli fibers proceeds through stop-and-go dynamics and can be inhibited by CsgC [106]. Further, forward-genetic screening examining curli production has revealed a number of genes essential for curli biosynthesis in a human pyelonephritis isolate that had not been previously characterized [107]. Four genes with newly confirmed roles in curli biosynthesis (*purF*, *purD*, *purM* and *purK*) belong to the purine biosynthesis operon, and one gene (*yrfF*) controls the Rcs phosphorelay system important for colonic acid biosynthesis [108,109].

Catheter-associated UTI

Although UPEC are the most common causative agents in both community-acquired and CAUTI, the proportion of infections attributed to Gram-positive bacteria and fungi are enriched in CAUTI [14,110]. Although reported prevalence rates vary depending on geographical location, sampling technique and diagnosis cutoffs, a recent multistate study in the United States revealed that *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (ESKAPE) are the most common causative healthcare-associated UTI pathogens after *E. coli* [110], and many of these species are commonly associated with nosocomial infections and antibiotic resistance [111,112]. As with UPEC, the proliferation of antibiotic-resistant bacterial strains in the healthcare environment has increased the cost and complexity of treating these infections [113]. Efforts to reduce the incidence of CAUTI by limiting the frequency and duration of catheter use have been successful to some extent[114,115]. However, strict adherence to clinical guidelines to limit the screening and treatment of asymptomatic bacteriuria in the absence of symptomatic indications during catheterization, along with new treatment approaches, are needed to limit the total disease burden and proliferation of antimicrobial resistance among CAUTI strains [116]. Recent studies examining the interplay of host response to indwelling urinary catheters and bacterial virulence factors have revealed hitherto unappreciated mechanisms of catheter colonization as described below.

Catheterization alters bladder ecology.

Implantation of a urinary catheter in the bladder is accompanied by various immunological and histological changes, including edema, epithelial exfoliation, metaplasia and inflammation [117,118]. in a mouse model of urinary catheterization, edema occurs within three hours of bladder implantation, and continues for up to 24 hours post-implantation (as indicated by the thicker bladder outline (Fig 3))[119]. An inflammatory response mediated by interleukins IL-1β, IL-6, IL12 p40 and IL-17 is also observed and can be partially inhibited by treatment with glucocorticoids (Fig 3). The physiologic response to catheterization also includes the release of fibrinogen into the bladder lumen, which coats the catheter [120]. A study conducted in a swine model of sterile catheterization revealed a Toll-like receptor 9 (TLR9)-nuclear factor-κB (NF-κB)-mediated increase in neutrophil migration into bladder tissues driven by increased levels of circulating mitochondrial DNA (which is associated with tissue damage and organ dysfunction)[121]. This results in bladder congestion, edema and hemorrhage that can be partially mitigated by coating catheters with chloroquine and *N*-acetylcysteine to decrease the levels of circulating cytokines[121]. In sum, catheterization

alters the bladder ecology and creates an environment that is more amenable to colonization by common CAUTI pathogens, including *Enterococcus* and *Staphylococcus* species.

Enterococcus.

Enteroccocus faecalis and *E. faecium* are Gram-positive bacteria responsible for up to 17% of nosocomial CAUTIs [110,113]. Over 85% of *E. faecium* and 7% of *E. faecalis* clinical CAUTI isolates tested were found to be resistant to vancomycin [113]. Using a mouse foreign-body implantation model of UTI (Supplementary figure S1), it was found that *Enterococci* utilize endocarditis- and biofilm-associated pili (Ebp) to form biofilms on catheters and overcome the neutrophil-mediated immune response [119] (Fig 3). Ebp pili are tipped with a fibrinogen-binding adhesin known as EbpA, which binds to the fibrinogen coating the catheter. Mutation of the N-terminal metal-ion-dependent fibrinogen-binding domain of the EbpA adhesin, or blockade with antibodies raised against the N-terminal domain of EbpA, prevents bacterial adhesion to fibrinogen-coated catheters *in vitro* and in mouse models of infection [120]. Enterococci also use fibrinogen as a nutrient source, enabling their growth in urine [120]. Enterococcal virulence is dependent on two secreted proteases, GelE and SprE, which are upregulated during infection to cleave the α-, β- and γ-chains of fibrinogen to potentiate biofilm formation (Fig 3) [122]. This provides further evidence that *Enterococcus* species utilize fibrinogen as both a binding platform and food source during biofilm formation and growth. Activation of SprE by host proteases was further shown to promote bacterial dissemination in the kidneys, whereas inhibition of either host or bacterial proteases was able to curb bacterial dissemination [120]. Ebp pili are also important for cell-cell aggregation and interspecies gene transfer, facilitating the spread of genetic elements that encode antibiotic resistance and other virulence factors [123].

Staphylococcus aureus.

Although *S. aureus* accounts for fewer than 3% of all positive urine cultures, *S. aureus* bacteriuria is more likely to progress to bacteremia than bacteriuria caused by other pathogens [14,113,124,125] Additionally, *S. aureus* is a major cause of asymptomatic bacteriuria and UTI during pregnancy in many developing countries [126]. The frequency of complications, combined with methicillin-resistance rates of up to 86%, has prompted researchers to investigate the pathogenesis of *S. aureus* UTIs [113,125]. Urinary catheterization is the most important risk factor for complicated methicillin-resistant *S. aureus* (MRSA) UTI [125,127]. As with *Enteroccocus* CAUTI, interaction with fibrinogen is key for MRSA pathogenesis in various habitats [128]. However, MRSA strains do not encode pili, but instead use microbial surface components recognizing adhesive matrix molecules (MSCRAMMS) to mediate binding to fibrinogen-coated catheters [129] (Fig 3). Specifically, clumping factor B (ClfB) has been shown to have an active role in MRSA CAUTI pathogenesis, while ClfA is important for systemic infection [130,131]. Further, MRSA potentiates catheter-mediated inflammation by up-regulating the expression of IL-1α, IL-1β, IL-6, and TNF to promote fibrinogen release and bacterial persistence in the bladder in a mouse model of CAUTI [130](Fig 3).

Proteus mirabilis.

Although short-term community- and catheter-associated UTIs are mostly caused by monomicrobial infections, most CAUTIs in patients catheterized for long periods (>30 days) are polymicrobial in nature [132,133]. *p. mirabilis,* a Gram-negative bacterium that is nearly ubiquitous in soil and water, is a common constituent of CAUTI polymicrobial communities in chronically catheterized patients [133,134]. *p. mirabilis* uses various canonical virulence factors to establish infection in the bladder, including flagella and adhesive mannose-resistant *Proteus-like* MR/P fimbriae [135] (Fig 3). In addition, *P. mirabilis* expresses high levels of urease, an enzyme that can hydrolyze urea to ammonia and raise the pH of urine to facilitate the formation of kidney stones [135]. Although carriage of this enzyme is not uncommon in uropathogens, *P. mirabilis* urease activity generates a substantially higher urinary pH than any of its uropathogenic counterparts, enabling the formation of crystalline biofilms that provide a protective niche during antibiotic treatment, promoting recurrence [136]. Recent work has revealed that, like UPEC, *P. mirabilis* invades the superficial umbrella cells of the bladder and forms IBCs in the urothelium [137].

However, these IBCs are smaller and more scarce than their UPEC counterparts. Instead, most *P. mirabilis* bacteria form large extracellular clusters in the lumen of the bladder that function as the nidus for the formation of bladder stones [137]. These clusters are dependent on both MR/P fimbriae and urease (Fig 3). Additionally, coinfection with *Escherichia, Enterococcus, Klebsiella* or *Pseudomonas* species in a mouse model of CAUTI enhances the urease activity of *P mirabilis,* resulting in increased disease severity and dissemination to other organ systems [138].

Emerging therapeutics

The mechanistic insights provided from the interdisciplinary studies outlined above have informed the development of novel antibiotic-sparing therapeutic approaches for the treatment and prevention of UTIs. These approaches, which target both bacterial and host processes, are outlined briefly below and summarized in Table 2. Given that 9% of all antibiotic prescriptions in the ambulatory setting in the United States are written for the treatment of UTI, UTIs are at the forefront of the antibiotic resistance problem [139]. Thus, there is an urgent need to develop antibiotic-sparing therapeutics for the treatment of UTI.

Glycomimetic anti-adhesives.

Many common uropathogens have developed various strategies to adhere to host tissues to colonize specific habitats and cause infection. Pilus-mediating binding of UPEC and other Gram-negative bacteria to sugar moieties on glycoproteins can be inhibited by presence of glycomimetic small molecules that competitively outcompete interactions with the receptor by occupying the binding pocket of the fimbrial adhesin. Mannosides are high-affinity mannose analogues that bind the FimH adhesin at the tip of type 1 pili, preventing adhesion to mannosylated glycoproteins in the bladder and colon [53,140–142]. Elucidation of the structure-activity relationship has enabled the development of mannosides that inhibit FimH function with a 10^6-fold higher potency than α-D-mannose and have considerably improved the bioavailability and half-life of these compounds [143,144]. Studies examining the role of conformational dynamics in FimH-glycan interactions have revealed new classes of

mannose analogues, the flexibility of which exploits known conformational changes within binding loops of the adhesive domain to further increase affinity [145]. Mannosides are orally bioavailable and treatment reduces bladder bacterial burdens by up to 4-logs in mouse models of UTI after just 6 hours post infection [144].

The gastrointestinal tract is the ultimate reservoir for UPEC, and FimH and has been shown to have a critical role in maintaining colonization of this habitat in mice. Depletion of this reservoir within a given host would be expected to reduce the rate of recurrent UTIs. In mice, oral administration of mannosides selectively extirpates UPEC from the urinary and gastrointestinal habitats simultaneously. Unlike antibiotics that reduce the complexity of the gut microbiota, mannosides do not alter the structure of the native microbiota in mouse models of acute cystitis and gastrointestinal colonization[53]. Interestingly, those same experiments revealed that, while UPEC was selectively depleted, the abundance of intestinal Enterobacteriaceae in the healthy microbiota was not affected by mannoside treatment, which led the authors to speculate that not all *E. coli* strains express type 1 pili in the gastrointestinal environment[53].

Recent studies have also focused on the use of small-molecule receptor analogues for the inhibition of Fml pilus-mediated adhesion to GalNAc-containing receptors expressed in the epithelia of kidneys and chronically infected bladders[52]. These high-affinity ‘galactosides’ have a 2000-fold higher affinity for FmlH than GalNAc and can reduce bacterial bladder and kidney in infected mice by approximately 1.5-logs [146]. When given to mice in combination with mannosides, galactosides can reduce kidney burdens below the limit of detection and reduce bladder burdens to a greater degree than monotherapy with galactosides or mannosides[146]. These data suggest that glycomimetic compounds hold promise as antibiotic-sparing therapeutics for the prevention and treatment of acute, chronic and recurrent UPEC UTI. However, the efficacy of small molecular inhibitors of adhesion need to be fully assessed in humans; a candidate mannoside has been selected for clinical development in humans [147].

Immunization.

Active vaccination has proven highly efficacious in reducing the incidence and severity of UTI in several animal models. Vaccination with the type 1 pilus adhesin, FimH, has been shown to greatly decrease the incidence of UTI in mouse models of infection and in nonhuman primates [148]. Vaccination with the N-terminal adhesive domain of FmlH decreases the bacterial burden 2-3 days post-infection in a mouse model of chronic cystitis [52]. Boosting the humoral immune response using the truncated flagellin (FliC) protein from enteroaggregative *E. coli* (EAEC) as an adjuvant has also shown promise in a mouse model of immunization [149].

A phase 1a/1b clinical trial was recently completed to evaluate the safety and IgG response to immunization with the FimCH chaperone-adhesin complex in both healthy women and women experiencing at least five UTIs in the 24 months prior to enrollment [150]. Co-administration with a synthetic adjuvant, phosphorylated hexaacyl disaccharide (PHAD), led to a 152 to 330-fold increase in antibody titers at 7 months and a 50-fold sustained increase in antibody titers at month 12 with no severe systemic reactions. In the 8 months following

FimCH immunization, women in the rUTI cohorts experienced a 72% decrease in total UTI recurrence relative to the 8 months prior to the immunization. Based on these results, and the lack of serious complications arising from vaccination, the administration of the FimCH vaccine has been allowed by the FDA for compassionate use for individuals suffering from rUTI who no longer respond to standard of care, including last-line carbapenem agents [150].

Outer-membrane iron-acquisition proteins, including siderophores and heme receptors, also represent promising vaccine candidates against *E. coli* UTIs [151]. These proteinaceous outer-membrane targets are required for urinary tract colonization by UPEC, expressed during infection and immunogenic during experimental infection [51,152]. A recent study focusing on four antigens, including the yersiniabactin receptor FyuA, the heme receptor Hma, a catecholate receptor IreA and aerobactin receptor IutA, revealed the presence IgG titers against these antigens in females with a history of UTI and in males with no history of UTI [151]. These data suggest that exposure to these antigens at other body sites create subprotective baseline immunologic responses that could be augmented by the administration of multivalent vaccines.

Vaccination also holds promise against Gram-positive bacterial pathogens during CAUTI. Immunization of mice with the EbpA N-terminal domain ($EbpA^{NTD}$) can reduce bladder and catheter bacterial burdens and protect against CAUTI caused by diverse enterococcal strains, including vancomycin-resistance isolates [120,153]. Antibodies generated by $EbpA^{NTD}$-vaccinated mice can prevent bacterial adhesion to fibrinogen-coated catheters *in vitro*, and passive antibody transfer can reduce bladder and catheter bacterial burdens in mice with preexisting infections and naïve mice following subsequent bacterial challenge [153]. It was also found that catheter-induced inflammation of the bladder increases the levels of serum antibody present in the bladder lumen, even in the absence of infection. Exposure of mice to *E. faecalis* results in an antibody response ~1000-fold lower than $EbpA^{NTD}$ vaccination and is not protective against subsequent challenge infection [153]. These results suggest active and passive immunity may hold promise as both a therapeutic and prophylactic option for the treatment of CAUTI. Vaccines against MRSA that have been promising in mouse models have not proven efficacious in clinical trials, although a newer four-component vaccine has successfully completed phase I clinical trials with no safety concerns [154]. Thus, immunization against proteinaceous mediators of bacterial adhesion is a promising approach against various Gram-positive and Gram-negative uropathogens.

Immunomodulatory therapies.

Mouse studies have implicated inflammation-induced remodeling of the bladder epithelium as a mediator of increased UTI susceptibility (Box 1). In humans with persistent bacteriuria and rUTI, biopsies revealed B-cell and plasma cell migration with development of lymphoid nodules in the submucosa [38]. Cyclooxygenase-1 (COX-1) and COX-2 are responsible for the synthesis of proinflammatory prostaglandins, which are the target of the nonsteroidal anti-inflammatory (NSAID) drug class, including naproxen, ibuprofen, aspirin and indomethacin [155]. COX-2 activity predominates at sites of inflammation, is increased in neutrophils and urothelial cells in the human and mouse bladders during acute cystitis, and is strongly predictive of progression to chronic cystitis [40] (Box 1). COX-2 can be selectively inhibited

using celecoxib to avoid gastrointestinal ulceration associated with nonspecific inhibition of COX-1. Inhibition of COX-2, but not COX-1, reduces the severity of acute cystitis and decreases the incidence of chronic infection in mouse models of cystitis by preventing mucosal damage and enabling the regeneration of the bladder epithelium [40]. These data are supported by human trials showing some efficacy of oral NSAID use for symptomatic resolution of UTIs in human patients[156]. Although women treated with NSAIDs alone generally report a longer duration of symptoms and some require subsequent antibiotic use, NSAIDs seem to be a possible treatment option for symptomatic treatment in women who wish to avoid antibiotic use [157,158]. Unfortunately, a side effect profile that includes chronic interstitial nephritis and gastrointestinal ulceration may limit the use of nonselective NSAID therapy in women suffering from chronic rUTIs.

Host factors that facilitate the inflammatory response of the bladder to UPEC colonization have also been examined as putative therapeutic targets. One such target, hypoxia-inducible factor 1α (HIF-1α, is a master transcriptional activator that has been shown to have a major immunomodulatory role under hypoxic conditions in mouse models of bacterial infection [159]. Stabilization of HIF-1α by the novel drug candidate AKB-4924 (initially developed for inflammatory bowel disease) in *in vivo* mouse models of infection decreases the host inflammatory response to infection by reducing the levels of IL-1 β, IL-6, IL-8 (CXCL8) and CXCL1 [160]. This, in turn, reduced the level of epithelial hyperplasia in the bladder and the subsequent bacterial burden. If these findings are translatable to humans it would provide insight into the possibility of repurposing AKB-4924 for the treatment of UTI.

Probiotics.

Although probiotic use is most commonly associated with the gastrointestinal tract, the clinical use of antibacterial competition and interference has also been implicated in the urinary tract. The efficacy of asymptomatic urinary tract colonization with *E. coli* isolate 83972 as an antibiotic-sparing alternative for the prevention of rUTI has been shown in various patient populations and was endorsed by the European Urology Guidelines [https://uroweb.org/wp-content/uploads/EAU-Extended-Guidelines-2015-Edn..pdf] in 2015 [161–163]. The ability of E83972 to maintain stable colonization of the urinary tract for prolonged periods without the development of symptomatic UTI has been attributed to robust biofilm formation and rapid growth in urine, poor expression of flagella and lack of functional P pili or type 1 pili. Three alternative asymptomatic bacteriuria isolates that can outcompete E83972 *in vivo* in the absence of urovirulence have recently been identified based on these criteria [164]. These strains belong to different phylogroups and display different sets of urovirulence factors, and their increased fitness in urine is thought to be due primarily to the expression of nutrient uptake and metabolite synthesis pathways [165,166]. Although the competitiveness of these strains against various uropathogenic bacterial isolates remains under study, the use of asymptomatic colonization of the urinary tract with avirulent strains to exploit bacterial interference holds promise in the prevention of UTI. Additional studies aimed at engineering *E. coli* strains to contain CsgA fusion proteins have shown that fusion of bioactive molecules to the CsgA curli subunit can enhance bacterial interactions with intestinal mucins and promote persistence [167]. This work suggests that directed bioengineering, together with microbiological characterization of existing members of the

beneficial host microbiota, may lead to the development of therapeutic strains with enhanced probiotic activity for the prevention of UTI. Additional potential for probiotic use extends into the vaginal microbiome. A recent literature review suggests that probiotics containing *Lactobacillus* species, in conjunction with vaginal estrogen creams, may have clinical use in the prevention of UTI [168].

Other approaches.

The targeting of other virulence factors known to participate in the host-pathogen interaction of uropathogens has also been proposed. Because CpxA and CpxR are highly conserved among many Gram-negative bacterial species and are known to regulate various virulence factors, CpxA inhibitors have potential use in the treatment of UTIs [169]. The differential expression of siderophores, including yersiniabactin and salmochelin, in UPEC isolates suggests that targeting metal sequestration systems may also be effective in selectively attenuating virulence in the bladder, especially given the high degree of cross-talk between siderophore systems [72]. Additionally, treatment of human bladder epithelial cells with exogenous human lactoferrin reduces UPEC adherence and increases neutrophil-mediated bacterial killing [70]. The therapeutic promise of this approach is further supported by murine studies showing increased clearance of UPEC *in vivo* with a single extravesicular treatment of human lactoferrin. However, the precise mechanism by which these effects occur requires further study.

The unique pathogenesis of CAUTI also presents several enticing antivirulence targets. The role of both MR/P fimbriae and urease in mono- and polymicrobial CAUTI suggests that targeting these central virulence factors may represent a viable antibiotic-sparing therapeutic option. Furthermore, our increasing understanding of the inflammatory changes that follow catheterization and subsequent bladder infection have suggested that an immunomodulatory approach may be efficacious for the treatment of some CAUTIs to decrease the morbidity and mortality associated with MRSA bacteriuria.

Outlook

Community- and healthcare associated UTIs affect millions of women worldwide every year. In the United States, hospitalizations for a primary diagnosis of UTI result in a total cost of $2.8 billion USD annually, in addition to 1 million visits to the emergency department and 7 million office visits [170]. Development of UTI in hospitalized patients, especially those with an indwelling urinary catheter, extends the duration of patient stay and increases the complexity of patient treatment. Additionally, urosepsis comprises 25% of all adult sepsis cases, and is associated with an overall mortality rate of 20-40% [171]. Patients suffering from a symptomatic UTI are commonly treated with broad-spectrum antibiotics; however the increase in antibiotic resistance and the impact of antibiotics on the gut microbiota raises the need for alternative antibiotic-sparing therapeutics that can selectively treat UTIs. A detailed mechanistic understanding of UTI pathogenesis is crucial for our ability to develop novel antimicrobial agents to effectively treat UTIs.

Although UTIs are caused by diverse pathogens harboring various virulence factors, we are gaining a greater appreciation for the complexity of the host-pathogen interface during

colonization and infection. Through metabolomic and gene expression studies, we are beginning to understand the dynamic regulation of bacterial virulence throughout the infectious cycle, which enables us to pinpoint determinants of bacterial virulence and target them appropriately. Such treatments include small-molecular inhibitors of adhesion, inhibitors of nutrient update and immunization against bacterial antigens. Similarly, studies of animal models of infection and human immunology have identified components of the human immune response that influence infection outcome, which suggests a possible role for immunomodulatory therapy in the treatment of UTIs.

Although there is still much work that needs to be done to translate our structural and functional understanding of urovirulence factors to effective antibiotic-sparing therapeutics for humans, new breakthroughs hold promise to improve the lives and the health of millions of women suffering from this disease. Additionally, the strategies that have been developed to translate an understanding of UTI pathogenesis into therapeutic leads may be applicable to the treatment of other infections, enabling us to expand our therapeutic arsenal by targeting bacteria in the precise habitat in which they cause infection or reside asymptomatically as a reservoir.

Display Items

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Urinary meatus
The opening of the urethra through which urine exits in males and females, sometimes referred to as the external urethral orifice.

Uncomplicated cystitis
an isolated infection of the bladder and/or lower urinary tract without signs or symptoms of upper tract or systemic infection in a patient without significant comorbid conditions, such as pregnancy or structural urinary tract abnormalities.

Complicated cystitis
An infection of the upper urinary tract leading to upper tract signs or systemic symptoms, or any urinary tract infection in pregnant women, immunocompromised patients, or patients with functional urinary tract abnormalities.

Pyelonephritis
An infection of the renal pelvic, calices and/or cortex.

catheter-associated UTIs (CAUTIs)
Catheter-associated urinary tract infection.

Fibrinogen
A glycoprotein released into the bladder lumen in response to inflammation and infection, and can coat urinary catheters and serve as a nidus for bacterial binding.

Umbrella cells
Also known as facet cells, umbrella cells are large, polarized superficial cells that line bladder lumen.

C3H/HeN mice
An inbred mouse strain commonly used for the study of a variety of disease processes, including UTI.

Lymphonodular hyperplasia
Enlargement of mucosal lymphoid nodules seen via histology.

Bacteriuria
The presence of bacterial in a urine system not attributable to contamination. Can be symptomatic or asymptomatic.

Nutritional Immunity
Sequestration of nutrients by a host organism to prevent colonization and proliferation by pathogens.

Siderophores
Low-molecular-weight compounds secreted by the host systems to bind metal ions and transport them across cellular membranes.

Biofilms
Large collections of microbial organisms embedded within a complex extracellular matrix comprising polysaccharides, proteinaceous fibers, and extracellular DNA

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Box 1: Understanding host susceptibility

Many established risk factors, such as female sex and sexual activity, are consistent with the hypothesis that mechanical transfer of resident pathogens from the perianal and perineal areas to urinary meatus leads to inoculation of the urinary tract [3,18]. However, the molecular basis for other reported associations has only recently begun to be appreciated. Although it has long been known that a history of urinary tract infections (UTIs) is the greatest independent risk factor for the development of a UTI, the recent establishment of a mouse model of recurrent cystitis has provided fresh insight into the molecular mechanism for susceptibility (Supplementary figure S1). Transurethral infection of C3H/HeN mice with uropathogenic *Escherichia coli* (UPEC) isolate UTI89 resulted in two distinct outcomes; development of persistent, chronic cystitis or spontaneous resolution of infection within 14 days [173]. These findings are consistent with the range of infection outcomes observed in untreated human UTIs [15]. Outcome of the initial infection was strongly correlated with the response of the mouse to a second bacterial challenge following antibiotic treatment and a period of convalescence. Mice that spontaneously resolved the first infection were resistant to subsequent UTI, whereas mice that had previously progressed to chronic cystitis were more susceptible to severe recurrent UTI, even if the challenge strain differed from the initial sensitizing strain [173]. Examination of the bladder epithelium revealed that the superficial umbrella cells of sensitized mice were smaller than those from resolved mice or age-matched adult naive controls and lacked markers of terminal differentiation [173]. Furthermore, proteomic and transcriptomic analyses revealed that infection can leave a molecular imprint on the bladder, even after clearance of the infection with antibiotic therapy, which can predispose to recurrent UTI (rUTI). Susceptibility to recurrent UTI was strongly correlated with the expression of cyclooxygenase-2 (COX-2) following challenge infection and could be reduced by treatment with a selective COX-2 inhibitor. This is consistent with previous observations showing that activation of a host-pathogen checkpoint influences the outcome of infection [40,174].

Recent studies have also revealed a molecular basis for the association between vaginal dysbiosis and increased UTI risk [175]. Transient colonization with *Gardnerella vaginalis*, an organism that is overgrown in patients with bacterial vaginosis, triggered activation of latent UPEC reservoirs in a mouse model of infection [176]. *G. vaginalis* colonization leads to the exfoliation of superficial umbrella cells by inducing apoptosis in the bladder epithelium. These effects were not seen with transient colonization of *Lactobacillus crispatus*, a normal component of a healthy vaginal microbiota with proven efficacy in reducing the risk of rUTI [177]. Additionally, *G. vaginalis* causes kidney inflammation in an interleukin-1α (IL-1α)-dependent manner, predisposing colonized animals to UPEC-dependent pyelonephritis [176]. Together, these findings provide mechanistic support for previous observations noting the overlap between bacterial vaginosis and pyelonephritis risk factors [178,179].

Box 2: Understanding uropathogenic *Escherichia coli* urovirulence based on host and pathogen factors

Despite our growing appreciation for the function of different virulence factors during uropathogenic *Escherichia coli* (UPEC) urinary tract infection (UTI) pathogenesis, the identification of genomic signatures that can reliably predict urovirulence has proven challenging. Analysis of 43 *E. coli* strains and collected from women suffering from recurrent UTIs and 46 reference strains spanning multiple clades revealed a set of 'core' genes that comprise 60%-75% of the total genome, whereas the remaining 25%-40% varied between strains [23]. Pathogenic *E. coli* strains are traditionally categorized into one of several pathotypes, each of which contains a combination of virulence factors that facilitates a specific pathogenic cascade within a defined host habitat [180]. Although *E. coli* strains within a given pathotype are often clustered into the same phylogenetic groups based on population genetic studies, this is not the case for UPEC [181]. For example, over 50% of *E. coli* strains isolated from patients with UTIs in the United States and Europe belong to clade B2, whereas 25%-50% belong to clades A, B1 and D [182,183]. Additionally, this proportion is subject to a high degree of geographic variation; 42% of urinary isolates from East Asia belong to group D, whereas fewer than 30% belong to B2 [184]. Although studies have shown that B2 strains typically encode a greater number of putative urovirulence factors than strains from other groups, analysis of virulence factor carriage alone does not elucidate a clear genomic signature of urovirulence [23,166,182,185]. However, analysis of *E. coli* metabolic activity during colonization, coupled with the expression pattern of certain genes participating in core bacterial functions during growth in liquid culture *in vitro*, may effectively predict uropathogenic potential [23,186]. A systematic, interdisciplinary study of diverse clinical urine-associated *E. coli* isolates revealed a new conceptual model of UTI in which UTI risk and outcome are determined by a combination of dynamic host susceptibility determinants and diverse bacterial urovirulence phenotypes that are driven not only by variations in gene content but by differential expression of conserved functions [23].

Putative virulence factor carriage and expression also correlate with antibiotic resistance [75,78]. A study examining the relationship between virulence factor carriage and resistance to fluoroquinolones in UPEC revealed that resistance is negatively associated with the carriage of specific virulence factors, including P pili and α-hemolysin [182]. Specific associations, such as that between ciprofloxacin susceptibility and the carriage of *papG-II*, *hylA* and *kpsMTK1* (a capsule gene), have also been described [187]. Based on these data, it seems that the ability of individual *E. coli* strains to colonize the urinary tract is dependent on the interaction between bacterial determinants of virulence and individual host susceptibility factors. Further studies suggest that recurrent UTI and persistent bladder colonization by diverse pathogens may also be associated with poor treatment response in patients with overactive bladder [188]. These studies illustrate the layers of complexity that underlie urovirulence, and underscore the need for continued interrogation of the *E. coli* genome and transcriptome in UTI isolates.

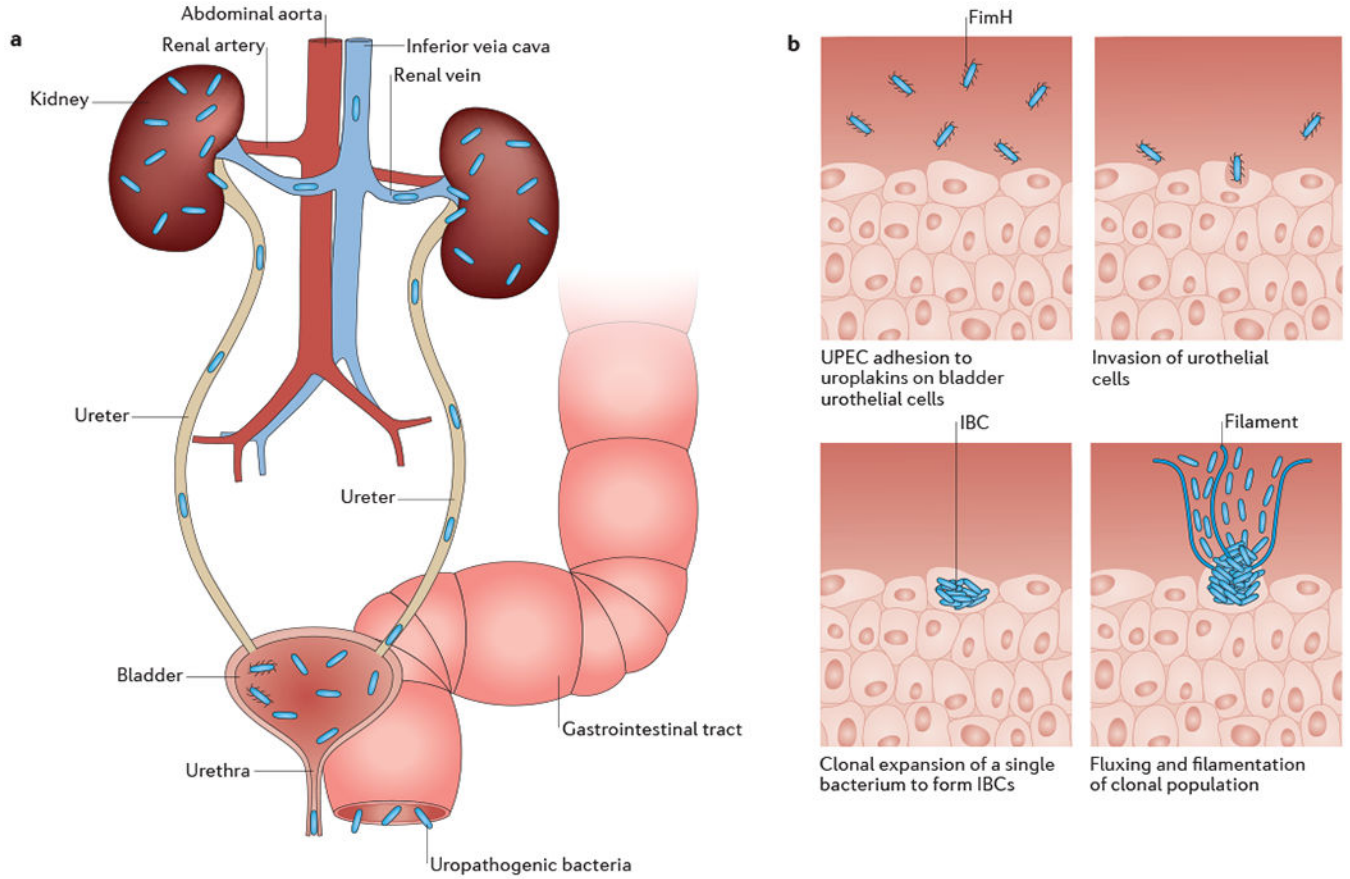

Figure 1: Overview of urinary tract infection pathogenesis by UPEC.

A) Uropathogenic *Escherichia coli* (UPEC) colonizing the gastrointestinal tract, perineum or vagina inoculate the urethra and ascend into the bladder. Isolated infections of the bladder are termed cystitis, and result in the classic urinary tract infection (UTI) symptoms, such as urinary frequency, urinary urgency, dysuria, and suprapubic tenderness. Bacteria can also ascend the ureters to the kidneys, where they cause a kidney infection termed pyelonephritis that can result in fever, chills and flank pain. Finally, bacteria can invade the bloodstream, causing bacteremia that can eventually lead to septic shock. **B)** In the bladder, individual bacteria adhere to and invade superficial urothelial umbrella cells in a FimH-dependent manner. There, they undergo clonal expansion to form biofilm-like intracellular bacterial communities (IBCs), where they can evade mechanical and immunological clearance mechanisms by the host. Bacteria then form filaments that flux out of the umbrella cells into the bladder lumen, where they can subsequently bind to neighboring cells and begin a new infection cycle.

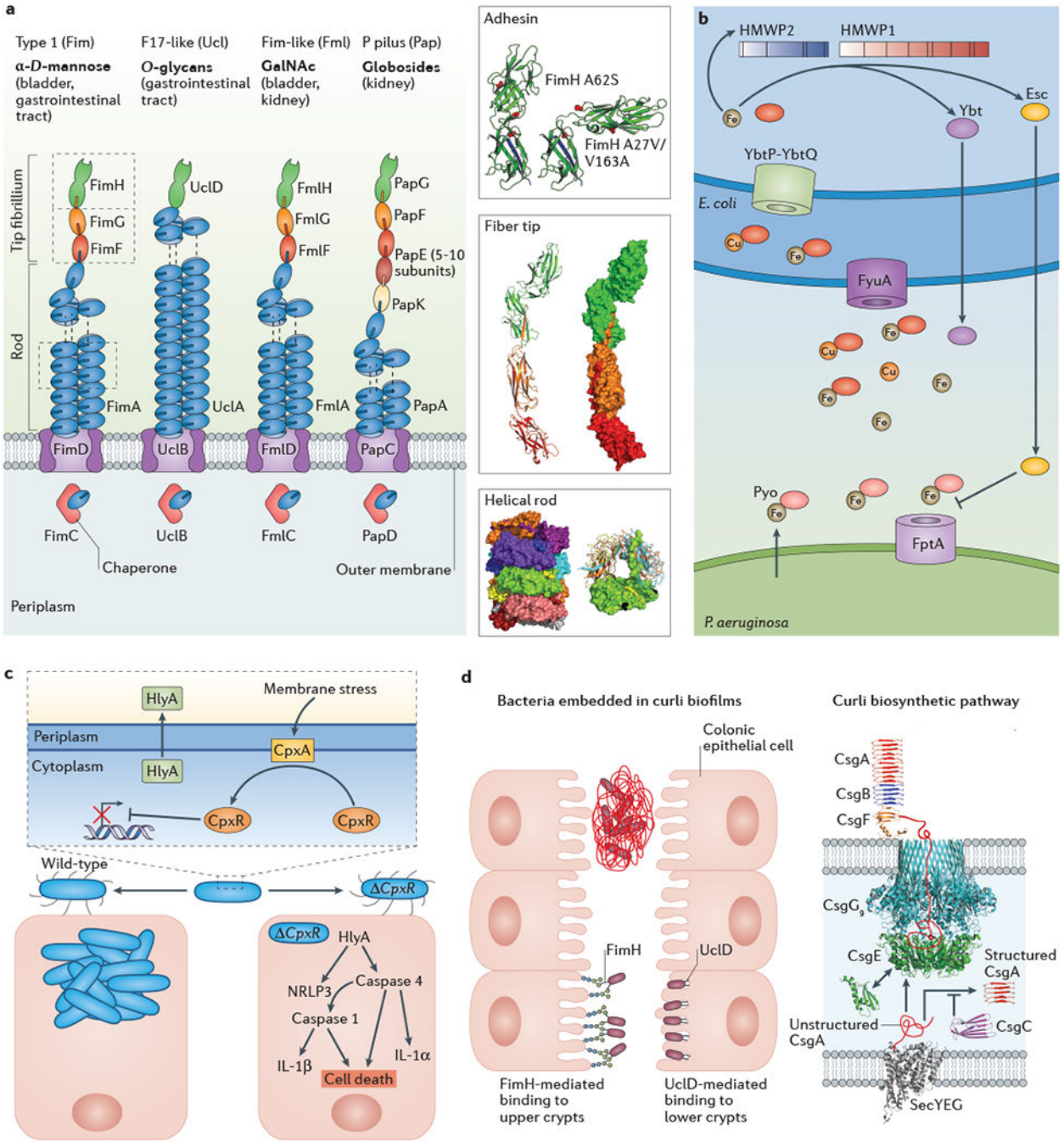

Figure 2: Bacterial urovirulence factors.

A) Four chaperone-usher pathway pili, including type 1 pili (also known as Fim pili), F17-like pili (also known as Ucl pili), Fim-like pili (also known as Fml pili) and P pili (also known as Pap pili), are involved in urinary tract infections (UTIs) through various mechanisms (left panel). Type 1 pili bind mannosylated uroplakins in the bladder and mannosylated mucous components in the gastrointestinal tract using the two-domain FimH adhesin. F17-like pili bind *O*-glycans in the gastrointestinal tract, helping to establish a reservoir for recurrent UTIs. Fim-like pili bind GalNAc moieties found in the inflamed

bladder and kidneys, which promotes chronic UTIs. P pili bind globosides in the kidney and are essential for progression to pyelonephritis. Biophysical studies performed on type 1 pili revealed the importance of conformational dynamics in the adhesin and pilus rod during infection (right panel). More specifically, positive selection at positions 27, 62, and 163 influencesconformational changes in the FimH adhesion that modulate the affinity of the binding pocket for mannose residues on the bladder epithelium. The FimHA62S variant preferentially adopts a 'tense' conformation with a low affinity for mannose, whereas the FimHA27V/V163A variant preferentially adopts a 'relaxed' conformation with high affinity for mannose. In the wild type protein, it is thought that an equilibrium between these species is necessary for maximum virulence. These adhesin subunits are noncovalently linked to the pilus tip and rod via a process known as donor strand exchange, wherein a donor strand from the preceding pilus completes the beta strand of the immunoglobulin-like fold. Furthermore, interactions between neighboring subunits within the Fim pilus rod when it adopts its native helical conformation modulate the force-length characteristics of the type 1 pilus in response to external force. Alterations to these interactions lead to an attenuation of virulence in mouse models of cystitis.

B) Uropathogenic *Escherichia coli* (UPEC) preferentially express a subset of siderophores, including yersiniabactin (Ybt), during infection of the bladder. Ybt binds extracellular iron and copper ions and delivers them to the FyuA outer membrane receptor for internalization. The YbtP-YbtQ complex then transports the copper-Ybt complexes to the cytoplasm. The presence of depressed cytoplasmic metal levels upregulates transcription of the HWMP1 and HWMP2, which subsequently synthesize Ybt. A metabolic byproduct of the HMWP1/2-mediated Ybt synthesis pathway, escherichelin (Esc), engages in interbacterial competition by disrupting prochelin (Pyo)-mediated iron uptake through the FptA outer membrane transporter in *Pseudomonas aeruginosa*. **C)** Periplasmic CpxA (the kinase of the CpxA-CpxR two component system) can sense protein misfolding (periplasmic stress) in *E. coli during UTI*, leading to the induction of genes responsible for the production of the pore-forming toxin α-hemolysin (HlyA) by the CpxR response regulator. Approximately 40-50% of UPEC strains encode HlyA, which contributes to activation of the NRLP3 inflammasome, caspase-1 and caspase-4, interleukin-1β (IL-1β) and IL-1α, leading to urothelial cell death in the host. Deletion of the CpxR in UPEC results in overexpression of HlyA and loss of bacterial fitness in mouse models of acute and chronic cystitis by triggering the caspase-mediated inflammatory cell death pathway and early vigorous urothelial exfoliation. Such vigorous exfoliation overcomes the ability of wild type UPEC to subvert the exfoliation response and invade underlying epithelium. **D)** Mechanisms of gastrointestinal colonization and curli biofilm formation. In the gastrointestinal environment, *E. coli* can embed themselves in a complex extracellular matrix containing curli fibers, cellulose and DNA fragments to promote persistence and prevent clearance. Curli comprise repeating amyloidogenic CsgA subunits that are assembled into extracellular fibers. The periplasmic chaperone CsgE interacts with unfolded CsgA to facilitate the transport of CsgA to and through the nonameric CsgG outer membrane pore. CsgE and the chaperone-like protein CsgC prevent premature CsgA polymerization in the periplasm. Following transport to the CsgG pore, CsgA is secreted across the outer membrane where curli fiber formation is facilitated by CsgB, which is held to the outer membrane by CsgF. CsgB nucleates the folding of CsgA resulting fiber polymerization. The FimH adhesin at the tip of type 1 pili

and UclD adhesin at the tip of F17-like pili also mediate binding to the intestinal crypts, helping to establish a stable gastrointestinal reservoir.

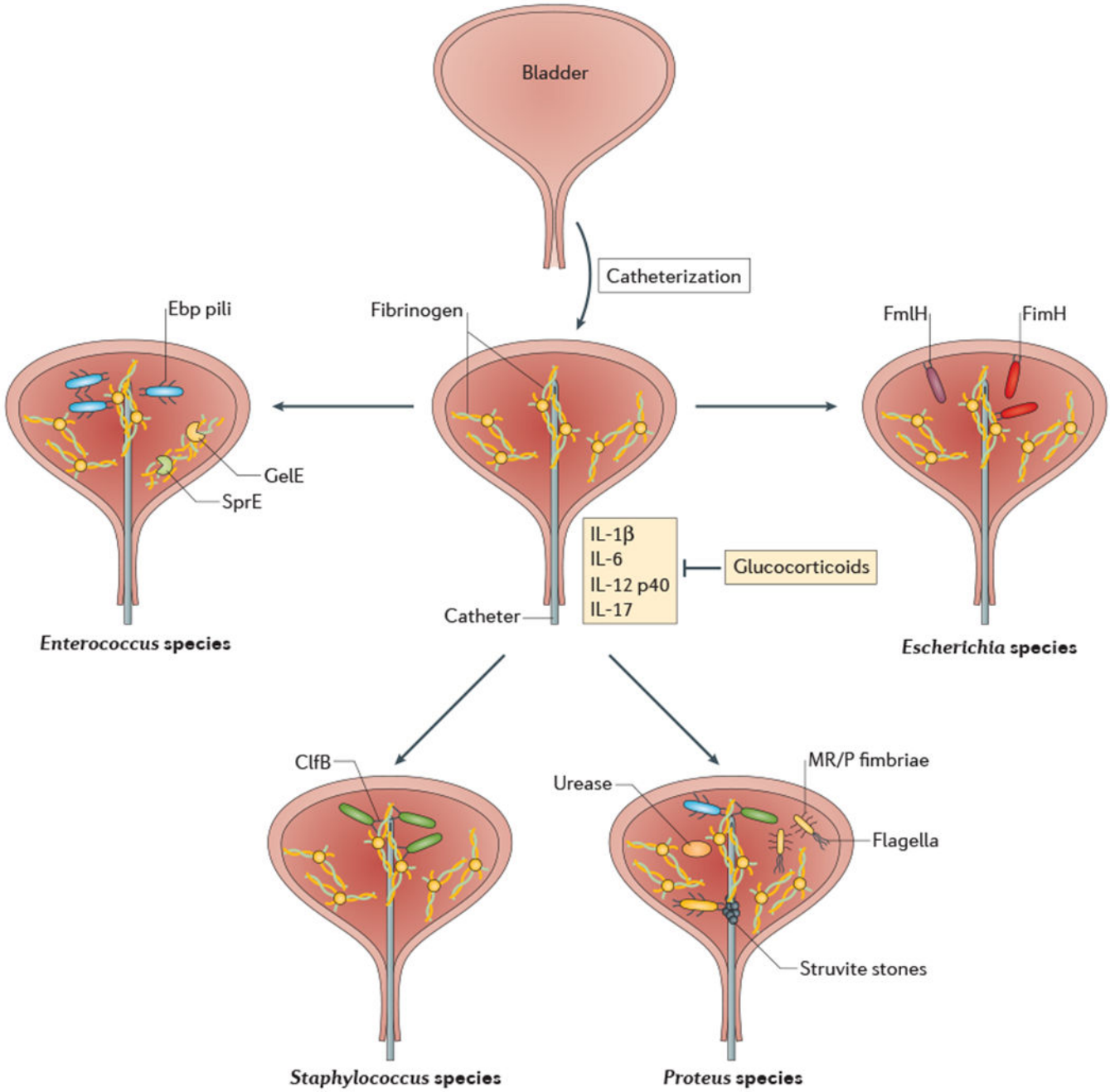

Figure 3:

Pathogenesis of catheter-associated urinary tract infections**.** Introduction of a urinary catheter into the bladder environment results in inflammation and the generation of fibrinogen, which is deposited on the urinary catheter. Urinary pathogens exploit this fibrinogen deposition in various ways. *Enterococci* utilize Ebp pili to bind directly to the fibrinogen, and the GelE and SprE proteases to promote fibrinogen cleavage and urinary growth using fibrinogen as a food source. *Staphylococci* use clumping factor B (ClfB) to adhere to the fibrinogen-coated catheter and potentiates bladder inflammation to promote persistence. *Proteus* species use various virulence factors, including mannose-resistant

Proteus-like (MR/P) fimbriae, flagella and urease to promote the formation of bladder stones and crystalline biofilms that permit bacterial persistence in the catheterized bladder. Finally, *Escherichia coli* use the FimH adhesin to bind fibrinogen coating the catheters.

Table 1:

Classification of urinary tract infections, symptoms, host risk factors and treatment. [Au: would it work to set the table up as indicated?]

Infection type	Cystitis (bladder infection)	Pyelonephritis (kidney infection)
Classification	Uncomplicated UTI Complicated UTI	Complicated UTI
Symptoms	- Painful urination (dysuria) - Urinary frequency - Urinary urgency - Suprapubic pain - Bloody urine (hematuria)	- Fever - Flank pain - Costovertebral angle tenderness
Host risk factors	- Positive individual UTI history - Positive family history - Sexual activity - New sexual partner - Postmenopausal age - Vaginal dysbiosis - Recent antibiotic use	- Positive individual UTI history - Positive family history - Sexual activity - New sexual partner - Postmenopausal age - Vaginal dysbiosis - Recent antibiotic use - Anatomic urogenital abnormalities - Vesicoureteral reflux - Diabetes mellitus - Pregnancy - Catheterization - Urolithiasis - Immunosuppression - History of pyelonephritis
Diagnosis	- Positive urine culture - Pyuria - Hematuria *	- Positive urine culture - Pyuria - Hematuria *
Treatment (low MDR risk **)	Empiric treatment with nitrofurantoin or fosfomycin	Treatment with ciprofloxacin or levofloxacin
Treatment (high MDR risk **)	Treatment with oral β-lactams and fluoroquinolones until culture sensitivity results are acquired	Treatment with ceftriaxone or ertapenem and trimethoprim-sulfamethoxazole, Augmentin or a 3rd-generation cephalosporin

MDR, multi-drug resistant; UTI, urinary tract infection.

* Diagnosis and treatment of acute simple cystitis is often alone, although symptoms are not considered diagnostic.

** MDR risk factors include an extended stay at a hospital facility, recent travel to India, Israel, Spain and Mexico, and/or recent antibiotic use.

Table 2:

Emerging therapeutics for the treatment of urinary tract infections.

Therapeutic	Bacterial target	Disease target	Evidence
Anti-adhesives			
Mannosides	UPEC; type 1 pili (FimH)	Acute cystitis	Reduced bacterial burdens in a mouse bladder following treatment and as a prophylactic agent in mouse models[53,143,144]
Galactosides	UPEC; Fim-like (Fml) pili	Chronic cystitis and/or pyelonephritis	Reduced bacterial burden in the bladder and kidney in mouse models of chronic UTIs[146,172]
Vaccine			
FimCH	UPEC expressing type 1 pili	Acute and chronic cystitis	Phase 1 clinical trial showing no safety concerns and a reduction in total UTI recurrence in treatment cohort; approved for compassionate use as an investigational intervention[150]
$EbpA^{NTD}$	Enterococcus species	CAUTI	Reduces bacterial titers in the bladder and catheter adhesion in *in vitro* mouse models[153]
Four-component (Two capsular polysaccharides, mutated α-toxin, clumping factor A)	*Staphylococcus* species	Staphylococcal UTI	Shown efficacy in mouse models, has completed phase I clinical trials with no safety concerns[154]
Other			
NSAIDs	UPEC	Chronic and recurrent cystitis	*In vivo* mouse models demonstrate reduced bladder remodeling, and human clinical studies demonstrate effective resolution of symptoms with a reduction in overall antibiotic used when used in place of antibiotics[40,156–158]
HIF-1α inhibition (AKB-4924)	UPEC		Decreased UPEC adherence and invasion of cultured human uroepithelial cells, decreased inflammation and bacterial load in mouse models of infection[160]
Probiotics	UPEC	rUTI	Reduced frequency of infection in a variety of patient populations with rUTI[161–163]
Lactoferrin	UPEC	Cystitis	Reduction in UPEC adherence to human bladder epithelial cell lines and reduced mouse bladder bacterial burdens following treatment with exogenous lactoferrin[70]

CAUTI, catheter-associated urinary tract infection; MRSA, methicillin-resistant *Staphylococcus aureus*; NSAIDs, nonsteroidal anti-inflammatories; NTD, amino-terminal domain; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.