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Uropathogen and host responses in pyelonephritis

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Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections seen in clinical practice. The ascent of UTI-causing pathogens to the kidneys results in pyelonephritis, which can trigger kidney injury, scarring and ultimately impair kidney function. Despite sizable efforts to understand how infections develop or are cleared in the bladder, our appreciation of the mechanisms by which infections develop, progress or are eradicated in the kidney is limited. The identification of virulence factors that are produced by uropathogenic *Escherichia coli* to promote pyelonephritis have begun to fill this knowledge gap, as have insights into the mechanisms by which kidney tubular epithelial cells oppose uropathogenic *E. coli* infection to prevent or eradicate UTIs. Emerging data also illustrate how specific cellular immune responses eradicate infection whereas other immune cell populations promote kidney injury. Insights into the mechanisms by which uropathogenic *E. coli* circumvent host immune defences or antibiotic therapy to cause pyelonephritis is paramount to the development of new prevention and treatment strategies to mitigate pyelonephritis and its associated complications.

Introduction

Urinary tract infections (UTIs) are painful, costly and potentially harmful infections that are commonly caused by uropathogenic *Escherichia coli* (UPEC). Infections that are restricted to the bladder are termed cystitis and are associated with symptoms of dysuria, urinary frequency, haematuria or foul-smelling urine. By contrast, pyelonephritis is a severe and sometimes life-threatening infection that develops when bacteria ascend into the kidney from the bladder or invade the kidney from the bloodstream. Although pyelonephritis is commonly caused by UPEC, other Gram-negative and Gram-positive pathogens —

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Author contributions

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Competing interests

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including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus* spp. — can also cause pyelonephritis. Acute pyelonephritis can cause kidney injury, urosepsis or even death. Chronic or recurrent pyelonephritis can trigger kidney scarring and impair kidney function. A growing body of data suggests that the bladder and kidney engage adaptive or innate immune responses to prevent UTI and minimize tissue injury. However, despite considerable research into the mechanisms that underlie the pathogenesis of cystitis and the host immune responses through which the bladder protects itself against uropathogens[1–5], efforts to better define the pathogenesis of pyelonephritis and the corresponding antibacterial defences of the kidney are relatively new. Recent discoveries have uncovered intrinsic immune defence mechanisms of kidney epithelial cells, the protective and damaging effects of infiltrating immune cells, and provided insights into the way by which UTI defences are orchestrated in different regions of the kidney to eradicate invading pathogens.

In this Review, we discuss the clinical relevance of pyelonephritis and the mechanisms employed by uropathogenic bacteria to establish and promote kidney infection. We also highlight host–pathogen interactions and the innate immune responses that are activated in the kidney to eradicate infection. Greater appreciation of the antibacterial defences of the kidney and the mechanisms employed by UPEC to circumvent host defences may facilitate the development of new personalized treatments for pyelonephritis.

Risk, outcomes and treatment of pyelonephritis

Cystitis and pyelonephritis have shared and distinct causes. Risk factors for cystitis, such as sexual activity, personal history of UTI or family history of UTI also predispose individuals to pyelonephritis. In children, bowel and bladder dysfunction, vesicoureteral reflux (VUR), and congenital anomalies of the kidney and urinary tract (CAKUT) also increase the risk of pyelonephritis[6–8]. Diabetes, pregnancy, use of bladder catheterization, urolithiasis and immunocompromised states also increase pyelonephritis risk across the lifespan[7–13].

Unlike cystitis, pyelonephritis is generally associated with systemic signs of inflammation. Specifically, localized symptoms including dysuria, increased frequency of urination, foul-smelling urine and incontinence are associated with bladder involvement, whereas fever is a marker of renal parenchyma involvement. However, consensus on the diagnostic criteria for pyelonephritis is lacking. Hence, pyelonephritis is defined functionally, by the presence of bacteria in the kidney following their ascent from the bladder or spread from the bloodstream (referred to as haematogenous pyelonephritis). Unlike cystitis, in which uropathogens are restricted to the bladder, uropathogens in the context of pyelonephritis can enter the bloodstream from the kidney to cause bacteraemia and urosepsis. Among hospitalized adults, 42% of sepsis cases originate as UTIs and urosepsis remains a notable cause of mortality in the elderly[14–16].

Prompt antibiotic treatment is the cornerstone of pyelonephritis treatment. However, even with antibiotic use, kidney scarring is a potential sequela that can lead to hypertension and chronic kidney disease (CKD)[1]. However, in the last 30 years, Gram-negative bacteria, including *E. coli*, have developed mechanisms to evade the bactericidal effects

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of antibiotics — including β-lactams and carbapenems, fluoroquinolones, polymyxins and aminoglycosides — that are routinely prescribed to treat UTIs. *E. coli* infections now account for half of the estimated global burden of antibiotic resistance, and up to 90% of *E. coli* strains are now resistant to at least one antibiotic[2]. Antibiotic misuse, overuse and suboptimal infection prevention practices have accelerated the prevalence of antibiotic resistance among UPEC. The mechanisms developed by UPEC to resist antibiotics are complex and varied, and include modifications in outer membrane proteins that decrease antibiotic accessibility, an enhanced capacity to enzymatically degrade antibiotics (for example, through the expression of β-lactamases or carbapenemases), modifications of antibiotic target sites, or the ability to eliminate antibiotics[17,18] (Fig. 1). The prevalence of antibiotic-resistant *E. coli* highlights a need to interrogate the mechanisms by which UPEC establish pyelonephritis and the endogenous host responses that are activated to clear them from the kidney. Better understanding of these host–pathogen interactions may guide the development of new therapeutics that offer benefits over current antibiotics.

UPEC virulence factors

UPEC belong to a group of *E. coli* clones referred to as extraintestinal pathogenic *E. coli* (ExPEC) that have adapted to colonize and cause disease in extraintestinal host sites. Four UPEC phylogroups (A, B1, B2 and D) have been characterized based on their acquisition of pathogenicity-associated islands, which are large genetic elements (>10 kilobases) that encode virulence-associated genes that are absent in non-pathogenic *E. coli* species[19,20]. UPEC virulence factors can broadly be classified as cell surface structures or secreted toxins that promote colonization and disease progression in the kidney or bladder (Table 1). Cell surface virulence factors include adhesins, polysaccharides, flagella, and iron scavengers. Secreted toxins include α-haemolysin (hlyA), cytotoxic necrotizing factor 1 (cnf1), vacuolating autotransporter cytotoxin (vat), toll-interleukin-1 receptor domain-containing protein C (TcpC), and secreted autotransporter toxin (sat). The roles of these virulence factors in the pathogenesis of cystitis have been reviewed elsewhere[20–25].

Despite the dedication of considerable effort to understanding the virulence factors used by UPEC to establish cystitis, our understanding of the UPEC virulence factors needed to establish pyelonephritis is incomplete. Available data show that some virulence factors are expressed at comparable levels in UPEC strains that cause asymptomatic bacteriuria, cystitis or pyelonephritis. However, other virulence factors are more highly expressed by UPEC strains that are more frequently identified with pyelonephritis. For example, type 1 pili, which promote attachment to kidney or bladder uroepithelial cells, are similarly expressed across UPEC strains; however, the majority of pyelonephritis-causing UPEC strains produce P fimbriae, which facilitates attachment to kidney epithelial cells. To a lesser degree, α-haemolysin, F1C/S fimbriae and aerobactin are more commonly expressed in pyelonephritis-causing UPEC strains[20,23,25] (Table 1). As described below, the divergent expression patterns of these virulence factors provide insights into the mechanisms by which UPEC establish pyelonephritis.

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Targeting the collecting duct

To establish pyelonephritis, UPEC must ascend the ureters and invade the collecting tubules[26–28] — a component of the kidney tubules that are composed of principal cells (PCs) and intercalated cells (ICs). PCs are appreciated for their role in ion and water transport[29]. ICs regulate acid–base homeostasis and have historically been categorized into three subtypes – type-A (A-IC), type-B (B-IC), and non-A, non-B. A-ICs secrete hydrogen into the tubular lumen via an apical vacuolar H^+-ATPase (V-ATPase) and regenerate bicarbonate using a basal chloride–bicarbonate transporter. B-ICs secrete bicarbonate via an apical chloride–bicarbonate transporter, pendrin, and express a basolateral H^+-ATPase[29,30] (Fig. 2a). However, data from single-cell RNA-sequencing (sc-RNAseq) analyses suggest that additional IC subtypes may exist[31].

Data from preclinical murine models show that UPEC establish pyelonephritis by attaching to the luminal surface of A-ICs[32,33]. Binding to these kidney epithelia is dependent on the ability of UPEC to produce type 1 or P-pili. P-pili bind glycosphingolipids in the kidney whereas the type 1 pilus adhesin, FimH, binds desmoglein-2 — a mannosylated junctional protein that is expressed by collecting duct cells[34]. Sc-RNAseq data show that desmoglein-2 is abundantly expressed in murine and human ICs[35,36]. UPEC mutants that lack type 1 pili or the P-pilus PapG adhesin have an attenuated capacity to colonize murine or human kidney cells[37,38].

After binding to ICs, UPEC are internalized and invade the interstitium in a process that is facilitated by complement activation. Binding of type 1 fimbriae-expressing UPEC to kidney tubular cells engages CD46, a complement regulatory protein that promotes the production of the complement components C3 and C4 (ref. 39). C3 activation further enhances the internalization of UPEC. Mice that are deficient in C3 are resistant to pyelonephritis[39,40]. UPEC also translocate across tubular epithelial cells by two additional mechanisms. First, cytolytic UPEC strains express *hlyA* and *sat* (which encode the cytotoxins α-haemolysin and a secreted vacuolating cytotoxin, respectively) that activate caspase 3 and cause tubular cell apoptosis and kidney injury. Cellular injury compromises cellular junctions and facilitates paracellular bacterial translocation[41–43]. Second, non-cytolytic UPEC strains that lack *hlyA* and *sat* do not impair cellular junctions, but rather are internalized following remodelling of the actin cytoskeleton, after which they transcellularly translocate across kidney epithelial cells via lipid rafts and a clathrin-mediated process controlled by Toll-like receptor 4 (TLR4)[41,44] (Fig. 2b).

Following their migration into the nephron, UPEC form microcolonies within the lumens of proximal tubules. Here, UPEC can trigger components of the innate immune response, including monocyte chemoattractant protein 1 (MCP-1; also known as CCL2) and RANTES (also known as CCL5) through TLR signalling[45,46]. In addition, cytotoxic UPEC strains secrete sub-lytic levels of α-haemolysin, which induce low-frequency oscillations in intracellular calcium concentrations. These intracellular calcium fluctuations result in the release of cytokines such as IL-6 and IL-8, which contribute to the pro-inflammatory environment[46]. Sustained calcium entry can also activate caspase-mediated pathways that cause tubular injury, apoptosis and paracellular bacterial dissemination[47,48].

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Host responses to UPEC infection

Innate immune responses

In response to UPEC infection, kidney epithelia engage their pattern recognition receptors, including TLRs, nucleotide oligomerization domain (NOD)-like receptors (NLRs), and mannose binding lectins. These pattern recognition receptors elicit inflammatory chemokines and cytokines that communicate with neutrophils and monocytes or macrophages to orchestrate the recruitment of immune cells for the purpose of eliminating UPEC (Fig. 2c).

Type 1 pili, P-pili and LPS on the surface of UPEC activates TLR4-independent immune responses and TLR4-driven responses involving NF-κB and downstream extracellular signal-regulated kinase (ERK) 1/2, mitogen-associated protein kinase (MAPK) and c-jun N-terminal kinase (JNK) that converge on specific transcription factors[32,49,50]. In the kidney, these pro-inflammatory cascades result in the production of cytokines from kidney epithelial cells, such as type I interferons, IL-6 and TNF, and chemokines such as IL-8, CCL3 (also known as MIP-1α), RANTES and MCP-1, which activate and recruit inflammatory cells for the eradication of UPEC. The importance of TLR4 to the host immune response is demonstrated by the inability of *Tlr4*-knockout mice to elicit immune responses in response to UPEC infection. Consequently, bacteria proliferate inside the lumens of kidney tubules and potentiate renal abscess formation[51]. Similarly, chimeric mice that express an inactivating *Tlr4* mutation in their intrinsic kidney cells show impaired UPEC clearance and increased kidney abscess formation in response to UPEC infection. If UPEC are not contained within an abscess, mice succumb to disseminated infection[52].

TLR4 closely regulates immune defences and imbalances in these responses impact pyelonephritis outcomes. For example, when P-pili-expressing UPEC strains bind kidney epithelial cells, TLR4 engages type I interferons to facilitate pathogen clearance through activation of the transcription factors interferon regulatory factor (IRF)-3 and IRF-7. Mice that are deficient in *Irf3* hyperactivate *Irf7*-driven immune responses and develop pyelonephritis, renal abscesses and urosepsis following UPEC challenge[53,54]. By contrast, mice that are deficient in *Irf7* do not show alterations in UPEC susceptibility, whereas combined *Irf7* and *Irf3* deletion reverses the pyelonephritis susceptibility of *Irf3*-deficient mice. Moreover, polymorphisms in the promoter region of *IRF3* that confer reduced transcription factor activity have been identified in humans with pyelonephritis, whereas attenuating *IRF7* promoter polymorphisms correlate negatively with infection risk[53,54]. These studies suggest that a balance in TLR4 and type I interferon responses influence susceptibility to UPEC infection.

Available data suggest that pattern recognition receptors other than TLR4 may also contribute to the host immune response to pyelonephritis. TLR2 acts synergistically with TLR4 to activate NF-κB[55]. TLR5 is expressed in the medullary collecting duct and is activated by flagellin. *Tlr5* deletion promotes bacterial internalization, UPEC colonization, and suppresses the production of chemoattractant cytokines that recruit neutrophils to the site of infection[56,57]. A polymorphism in *TLR5*, which encodes a variant that negates flagellin-induced signalling, is associated with increased UTI risk[58]. Expression of TLR11

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is restricted to mice and *Tlr11* deficiency is associated with increased bacterial burden in the kidney following the induction of experimental UTI[59]. Although less frequently studied than TLRs, NLRs may also contribute to bacterial clearance in the context of pyelonephritis. *Nod1*-deficient mice are more susceptible to UPEC infection than wild type mice and demonstrate impaired neutrophil migration to the infected kidneys[60]. *Nod2*-deficient mice do not show impaired UPEC clearance but have a propensity to develop kidney abscesses following experimental UTI[61]. The involvement of different TLRs and NLRs in response to invading pathogens suggests that the interplay between pattern recognition receptors may be critical for the induction of protective immune responses following UPEC infection.

Antimicrobial peptides

In addition to activating innate immune signalling, host cells also eradicate invading pathogens by producing antimicrobial peptides and proteins. In the urinary tract, antimicrobial peptides are produced by the bladder urothelium, ureters, kidney epithelial cells and immune cells. In the kidney, ICs and PCs express antimicrobial peptides but ICs are viewed as the main source of production[1,62,63]. These antimicrobial peptides and proteins, including defensins, cathelicidin, metal binding proteins, ribonucleases and uromodulin, facilitate pathogen clearance by disrupting the integrity of the bacterial membrane, opsonizing bacteria, scavenging the nutrients needed for bacterial replication, preventing bacterial attachment, or modulating host responses. The utility of antimicrobial peptides in pyelonephritis antibacterial defences has been demonstrated in cultured human and mouse kidney cells, ex vivo in human and mouse urine samples, and in genetic mouse models[1,33,64–79] (Table 2). These functional and proof-of-concept studies are complemented by findings from clinical studies, which show that individuals who experience recurrent cystitis have suppressed concentrations of urinary antimicrobial peptides[76,80–82] (Table 2). In addition, genomic studies in children suggest that UTI susceptibility correlates with copy number variations or polymorphisms in antimicrobial peptide genes[75,83,84]. Thus, insufficient expression of antimicrobial peptides may augment UTI risk. Detailed prospective studies are needed to determine whether altered antimicrobial expression affects pyelonephritis severity or outcomes.

Intercalated cells

Transcriptome analyses of human collecting duct cells suggest that UPEC exposure induces a shift in ICs towards an A-IC-like subtype[31]. Murine ICs also show increased V-ATPase mRNA expression following UPEC exposure[31]. These findings suggest that ICs may differentiate during infection — potentially to enhance urinary acidification, which inhibits bacterial replication. In support of this concept, one published dataset showed that mice acidify their urine during UTI; however, a later study did not replicate this finding[33,85]. Moreover, other studies have shown that the introduction of LPS into the lumen of rodent or rabbit collecting ducts or thick ascending limbs impairs urinary acidification through TLR4-mediated inhibition of bicarbonate absorption and/or hydrogen secretion[86,87]. The discordant results reported by different studies may stem from differences in the infecting UPEC strain, bacterial inoculum, or variations in experimental technique. However, urinary acidification or pH may not contribute notably to UTI defence as two studies suggest that urine pH neutralization does not affect UPEC clearance[88,89].

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In contrast to extracellular acidification, available evidence suggests that intracellular acidification can facilitate bacterial clearance. Exposure of ICs to UPEC induces the expression of genes that are associated with phagosome maturation[31]. In addition, single tubule microperfusion experiments and intravital microscopy studies show that murine ICs can phagocytose UPEC. Once internalized, phagosome acidification creates a microenvironment that kills UPEC[31]. These findings indicate that ICs may have an important role in UTI prevention. They also implicate the need to identify new approaches that facilitate A-IC differentiation, IC-mediated phagocytosis and acidification to treat pyelonephritis.

However, and as noted earlier, UPEC internalization by ICs is required to establish pyelonephritis. Thus, further insights are also needed to determine whether the process of UPEC internalization actually confers an advantage to UPEC and whether this process may potentiate pyelonephritis or UPEC survival within kidney tubules. In line with this suggestion, it is important to note that the induction of systemic metabolic acidosis through ammonium chloride supplementation exacerbates pyelonephritis in rodents[89]. Although in vitro and in vivo studies suggest that such acidosis enhances hypoxia-inducible factor (HIF)-1-mediated immune responses, it also predisposes rodents with VUR to pyelonephritis[89–91]. Acidosis may augment UTI by disrupting uroepithelial barriers, modulating cytokine production and immune cell recruitment, thereby impairing neutrophil bactericidal activity or suppressing antimicrobial peptide functions[1,89].

The importance of ICs to the host response to UPEC is highlighted by the finding that reduced IC populations augment UPEC susceptibility. Mice that lack carbonic anhydrase 2 — an enzyme that is responsible for the generation of IC hydrogen gradients — have a 50% reduction in IC numbers and an impaired capacity to clear UPEC from the kidneys and bladder, independent of serum bicarbonate concentrations or urine pH[88]. Moreover, pyelonephritis risk increases when kidneys from carbonic anhydrase 2 knockout mice are transplanted into control syngeneic mice, suggesting that deficient kidney carbonic anhydrase 2 expression and reduced IC numbers are a risk factor for UTI[92]. In addition, ablation of IC populations by genetic deletion of the transcription factor *Tfcp2l1* in murine collecting ducts, limits bacterial clearance following experimental UTI[33]. These results demonstrate that ICs are critical for UTI defence (Fig. 2b).

Dendritic cells and macrophages

In addition to epithelial cells, immune cell populations also have important roles in UPEC clearance during pyelonephritis. An intricate network of resident dendritic cells and macrophages survey the kidney interstitium, by probing glomerular and tubular self-antigens, danger signals and pathogen-associated molecular patterns[5,93–96]. The sentinel role of these dendritic cells and macrophages enables them to orchestrate TLR4-mediated antibacterial defence programmes. For example, macrophages respond by activating NF-κB signalling and murine $CD11c^+$ dendritic cells respond by producing CXCL2 (MIP-2; the functional homologue of human IL-8) to recruit neutrophils to the site of infection. Ablation of $CD11c^+$ dendritic cells decreases neutrophil recruitment and augments pyelonephritis in mouse kidneys[52,97].

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Two populations of macrophages are engaged during pyelonephritis — resident kidney macrophages, which self-renew in situ and originate mostly from the yolk sac, and bone marrow-derived macrophages, which originate from circulating monocytes and are recruited to the renal interstitium upon injury or infection. As described above, kidney-resident $CX3CR1^{+}/F480^{+}$ macrophages act as sentries that alert other immune phagocytes, such neutrophils, to the bacterial insult[96,97]. A similar role has been attributed to resident bladder macrophages, which produce chemokines to attract neutrophils and monocyte-derived $Ly6C^{+}$ macrophages to the site of infection[98,99].

The recruitment of circulating, monocyte-derived $Ly6C^{+}$ macrophages to the kidney, particularly the renal medulla, is dependent on the C-C chemokine receptor type 2 (CCR2) and its chemokine C-C motif ligand 2 (CCL2). In mice with pyelonephritis, $Ly6C^{+}$ macrophages have a pro-inflammatory (M1) profile, characterized by the expression of inducible nitric oxide synthase (iNOS) and inflammatory cytokines such as TNF and IL-1β[100–102]. The depletion of blood monocytes in these mice prevents the recruitment of $Ly6C^{+}$ macrophages to the kidney and decreases inflammatory cytokine and chemokine expression. Notably, the bacterial burden in the kidney decreases after monocyte depletion, suggesting that monocyte-derived $Ly6C^{+}$ macrophages may have dual roles as drivers of inflammation and of bacterial dissemination during pyelonephritis[101]. Notably, neutrophil depletion also increases the number of M1 macrophages, enhances bacterial burden and promotes tubulointerstitial nephritis and renal scarring in UPEC-infected mice[101]. Depletion of recruited $Ly6C^{+}$ macrophages in neutropenic mice reduced these detrimental sequelae, indicating that dysregulated macrophage-dependent inflammation promotes kidney injury[101]. In support of this concept, monocyte-derived macrophages contribute to progressive kidney disease in the context of sterile inflammation[103,104]. The pro-inflammatory and profibrotic factors elicited by macrophages during pyelonephritis are unclear.

In human kidney tissues, two resident mononuclear phagocyte populations have been identified. $CD14^{+}$ cells are primarily located in the medulla where they might sense bacteria that ascend from the bladder into the kidney[100]. In support of this proposal, $CD14^{+}$ cells demonstrate greater phagocytic activity and produce higher levels of IL-8 and TNF than $CD14^{-}$ cells when challenged with UPEC[100]. The functional contribution of these cells to pyelonephritis resistance remains to be elucidated.

Neutrophils

Neutrophils promote bacterial killing by releasing reactive oxygen species (ROS) and antimicrobial peptides, phagocytosing UPEC, and producing neutrophil extracellular traps through (NETosis) to capture and eliminate UPEC (Fig. 2c). The recruitment of neutrophils to infected kidneys is initiated when UPEC stimulate tubular epithelial cells, resident dendritic cells or macrophages to secrete granulocyte-recruiting chemokines such as IL-8 (or CXCL2 in mouse), which act via the CXC chemokine receptors CXCR1 and CXCR2 (ref. 105). Similar to neutrophil-depleted mice[101,105], *Cxcr2* knockout mice develop pyelonephritis, renal abscesses and kidney scarring when challenged with UPEC, eventually succumbing to sepsis owing to delayed neutrophil recruitment and inefficient bacterial

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killing[106]. Clinical studies also support a protective role for IL-8 and its receptor. Children who are prone to pyelonephritis show lower CXCR1 expression in neutrophils than age-matched controls[107]. Moreover, polymorphisms in *IL8* and *CXCR1* are associated with an increased risk of pyelonephritis[108–110]. This body of evidence suggests that variations in the genes that drive neutrophil chemotaxis influence pyelonephritis susceptibility and severity.

Impairment of immune cell responses

In addition to possessing virulence factors that help to establish infection, UPEC genomes also encode virulence factors that interfere with neutrophil and mononuclear phagocyte function and may render the host susceptible to pyelonephritis (Table 1). For example, the secretion of Toll/interleukin-1 receptor (TIR) domain containing-protein C (TcpC) by UPEC subverts host defences by binding to the adaptor protein, myeloid differentiation factor 88 (MyD88), and blocking TLR signalling, thereby preventing NF-κB activation in macrophages[111]. Mutation of TcpC attenuates UPEC virulence and renal abscess formation[112,113]. In addition, TcpC also blocks activation of the NACHT leucin-rich repeat PYD protein 3 (NLRP3) inflammasome, which serves a role in pathogen recognition in the cytoplasm. Consequently, TcpC blunts IL-1β secretion by UPEC-infected macrophages[112].

Beyond its effects on macrophages, evidence supports a role for TcpC in suppressing the antimicrobial mechanisms of neutrophils. Specifically, TcpC inhibits NETosis by acting as an E3 ubiquitin ligase to enhance the ubiquitination and proteasomal degradation of the NETosis-related protein PAD4[114] (Fig. 2c). In addition, TcpC inhibits the LPS-elicited production of ROS and pro-inflammatory cytokines[114].

It has long been known that the secreted toxin, HlyA, is a potent leukocidal protein, as evidenced by the finding that low concentrations evoke membrane permeability defects in neutrophils, resulting in ATP release, granule exocytosis, loss of phagocytic killing and cell death[115]. Although these effects were initially presumed to result from its pore-forming ability, we now know that HlyA triggers the activation of serine proteases in macrophages, resulting in degradation of the cytoskeletal scaffolding protein, paxillin, along with components of the NF-κB signalling cascade[116]. In addition, HlyA triggers the NLRP3 inflammasome in a manner that is dependent on potassium efflux from the cell, resulting in increased IL-1β production, mitochondrial dysfunction and macrophage death through pyroptosis[117].

Finally, changes in UPEC morphology suppress host phagocyte activity. UPEC that express type 1 fimbriae interact with neutrophils in a mannose- and LPS-dependent manner, which induces neutrophil apoptosis. This apoptosis is abolished by blocking FimH-mediated attachment[118]. In addition, the PapG tip adhesin of P fimbriae protects UPEC from neutrophil killing through an unknown mechanism[119]. Although not defined in the kidney, bacterial filamentation limits UPEC phagocytosis in the bladder and in vitro, supporting the notion that alterations in morphology help UPEC to elude host immune responses[120,121].

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Regulation of immunity

The immune defences of the kidney are temporally and spatially regulated. Single-cell transcriptomic studies show that immune cell populations differ between fetal and adult human kidneys, and are asymmetrically distributed across the kidney. In adult kidneys, for example, T cells and B cells are enriched in the cortex, whereas neutrophils and mononuclear phagocytes are predominant in the medulla[100,122]. These data also show that the expression of immune genes is much higher in the adult kidney pelvic epithelium and medulla than in the fetal kidney epithelium. Immune genes that are enriched in the adult pelvic epithelium and medulla are involved in innate immune responses, and encode pattern recognition receptors, chemokines and antimicrobial peptides[122], suggesting that the defence capacity of the kidney is acquired in the postnatal period when the kidney is more likely to encounter pathogens. These findings also indicate that antibacterial defences in the kidney are strategically positioned to shield its most vulnerable regions from ascending pathogens[122]; however, and as described below, changes in the extracellular environment and hormone levels can affect kidney antibacterial defences.

The effect of sodium on kidney antibacterial defences

Available evidence suggests that the high interstitial sodium concentration in the medulla strengthens the antibacterial defences of the kidney. In the context of pyelonephritis, this hypersaline environment promotes nuclear factor of activated T cells 5 (NFAT5)-dependent cytokine production by kidney tubular cells, which stimulates the recruitment of neutrophils and $CD14^+$ mononuclear phagocytes that phagocytose UPEC (Fig. 2c). Disruption of the renal sodium gradient in mice or humans triggers aberrant cytokine production, reduces the recruitment of mononuclear phagocytes to the medulla, and increases pyelonephritis risk[100], suggesting that a critical relationship exists between the microenvironment within the medulla and UTI defences.

Despite this protective role, subsequent experiments have shown that a high salt intake increases pyelonephritis susceptibility via two mechanisms. First, the increased production of urea, which is needed to establish a medullary osmotic gradient in the context of increased sodium excretion, suppresses neutrophil antimicrobial functions. Second, a decrease in mineralocorticoid production, which is needed to enhance sodium excretion, promotes the accumulation of aldosterone precursors with glucocorticoid activity. The ensuing hyperglucocorticoidism impairs the ability of mouse neutrophils to kill UPEC. Concordantly, humans who consume a high salt diet develop suppressed plasma aldosterone, increased plasma corticosterone concentrations and decreased neutrophil antimicrobial capacity[123]. Together, these studies indicate that although the hypersaline environment of the medulla might prepare the kidney to sense and eliminate bacteria, high salt intake or hypernatraemia may in fact suppress UPEC defences and increase pyelonephritis risk.

Hormonal regulation of antibacterial defences

Hormones, including vasopressin, insulin and sex hormones, also affect the immune defences of the kidney.

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Vasopressin.—Arginine vasopressin (AVP), which is released from the pituitary in response to hypernatraemia or hypovolaemia, binds to the vasopressin V2 receptor (AVPR2) expressed on the basolateral surface of PCs to increase intracellular cAMP production and induce aquaporin 2-mediated water reabsorption. However, AVP also promotes sodium chloride reabsorption via the epithelial sodium channel (ENaC) and activates the cAMP-sensitive cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. Preclinical data indicate that pharmacological antagonism of AVPR2 stimulates the production of cytokines by kidney epithelial cells to facilitate bacterial clearance in UPEC-infected mice. (Fig. 2b). Diamino-8-D-arginine vasopressin (dDAVP), a AVPR2 agonist, suppresses TLR4-mediated NF-κB activity, chemokine secretion, neutrophil recruitment and augmented UPEC susceptibility through a mechanism that requires functional CFTR channels[124].

Insulin.—In the kidney, insulin regulates gluconeogenesis, blood flow, electrolyte and mineral balance, and vascular resistance[125]. Interestingly, the expression of some antimicrobial peptides may also be dependent on the bioavailability of insulin. For example, levels of *Bd1*, which encodes the antimicrobial peptide β-defensin 1, are lower in kidneys from rodents with type 1 and type 2 diabetes than in those from healthy control animals. Of note, kidney expression of *Defb11* and urinary β-defensin 1 levels were restored in diabetic models following insulin treatment[126,127]. Similarly, insulin induces expression of the iron scavenging protein lipocalin 2 and ribonucleases in kidney epithelial cells via PI3K–AKT signalling[81,85]. Last, cohort studies show that individuals with diabetes have lower serum and urinary concentrations of the antimicrobial peptides ribonuclease 7, lipocalin 2, cathelicidin and β-defensin 1 than healthy controls[81,85,128]. In a mouse model of localized insulin resistance, deletion of the insulin receptor in the collecting duct and ICs promoted localized cellular insulin resistance and increased cystitis susceptibility by suppressing antimicrobial peptide production, but did not cause hyperglycaemia or glucosuria[85]. These data suggest that insulin and its receptor regulate host responses and impact UTI risk. In addition, they suggest that hyperglycaemia and/or glucosuria are not the only factors that promote UTI in individuals with diabetes. Given the clinical impact of diabetes on UTI susceptibility, further investigation into the mechanisms by which insulin signalling regulates UTI responses is warranted. Although not comprehensively studied in the kidney, an impact of deregulated glucose control on cystitis antibacterial defences has been demonstrated[10,129].

Sex hormones.—Sex and sex hormones also shape immune responses[5,130–132]. Male mice develop pyelonephritis and form renal abscesses more frequently than female mice following a UTI[133], suggesting that androgens may potentiate UTI. In support of this concept, UTI severity increases in male and female mice treated with testosterone. Elevated testosterone levels prime the kidney for fibrosis by increasing local TGFβ production and promoting macrophage polarization towards a pro-fibrotic M2 phenotype[134]. By contrast, castration of male mice reduces UTI severity and pharmacologic or genetic inhibition of androgen receptor signalling reduces UTI risk[133–136]. These studies identify potential roles for mediators within the TGFβ superfamily in renal fibrosis, but further studies are

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warranted to identify specific ligands, receptors and cellular sources to substantiate these roles.

The influence of oestrogens on pyelonephritis is less frequently studied. Although ovariectomy in female mice does not make a sizable difference in pyelonephritis susceptibility, treatment of mice with an oestrogen receptor agonist facilitated UPEC clearance from the kidney[135,137]. By contrast, ovariectomized mice are predisposed to cystitis. Oestrogen therapy may improve UTI outcomes by boosting the expression of antimicrobial peptides, strengthening the urothelial barrier, changing the vaginal microbiome, and suppressing inflammation[130,138,139]. As more is learned about the effects of sex on UTI, new interventions may be applied to modulate hormonal signalling to reduce infection susceptibility or kidney scarring.

Therapeutic approaches

Targeting UPEC for pyelonephritis therapy—Given the rising prevalence of antibiotic-resistant uropathogens and the potentially devastating effect of pyelonephritis, sizable efforts are underway to develop new UTI therapies. Our newfound appreciation of the mechanisms used by UPEC to develop and propagate UTI has led to the development of vaccine-based approaches to boosting immune responses (Fig. 3a). Currently, four vaccines — Uro-vaxom, SolcoUrovac, Uromune, and ExPEC4V — have shown promise in randomized control trials[2,140–142]. The immunogens on which these vaccines are based include whole-cell heat-killed bacteria, bacterial extracts and antigenic bacterial cell wall components. In addition, bacterial iron acquisition proteins have also been assessed as vaccine antigens. Murine studies show that these prophylactics protect against cystitis and pyelonephritis; moreover, clinical isolates from patients with acute pyelonephritis show that iron acquisition antigens elicit immune responses and represent viable vaccine targets[143–146]. Finally, FimH is being investigated as a vaccine immunogen with promising results in cystitis and pyelonephritis prevention in preclinical models[147].

Beyond vaccines, approaches to minimizing UPEC attachment represents another promising strategy for preventing cystitis and pyelonephritis (Fig. 3a). Cell culture and murine studies have demonstrated the ability of D-mannose and globotriose to prevent binding of type 1 or P-pili to urothelial and kidney tubule cells, respectively[41,148]. Clinically, prophylactic administration of D-mannose reduces recurrent UTIs and prospective studies to test its benefits are ongoing[148–150]. This preliminary success with D-mannose has led to the development of synthetic mannosides — high-affinity receptor analogues for FimH on type I pili. In mice, mannosides prevent the binding of type 1 fimbriae to mannosylated desmoglein-2 on kidney epithelial cells[34]. Small molecules called pilicides represent another strategy for minimizing UPEC attachment by inhibiting the assembly of pili, which as noted above, have a role in the pathogenesis of cystitis and pyelonephritis[151,152]. Cranberry extracts have also been tested to prevent UPEC attachment in vitro and in vivo, but results from these experiments are inconclusive and their impact on improving pyelonephritis outcomes is unclear[153].

Preventing bacterial colonization and internalization are other approaches to limiting infection. Probiotics such as lactobacillus can limit the growth and survival of pathogenic

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and antibiotic-resistant bacteria by releasing lactic acid, hydrogen peroxide, and bacteriocin. The utility of probiotics as anti-UPEC therapies has been reviewed elsewhere but is understudied in pyelonephritis[153,154]. Cholesterol-lowering drugs such as statins prevent UPEC invasion by altering the composition of lipid rafts[41]. In addition to preventing UPEC internalization, statins can modulate inflammation, which affects UTI-associated morbidity[41,155–157].

New bactericidal approaches also show promise in UTI. Bacteriophages are highly effective at introducing their genetic material into host cells and can promote bacterial lysis. Prospective clinical studies have evaluated the use of bacteriophage instillation following kidney or prostate surgery to reduce UPEC titres. In addition to no adverse effects, these studies have reported a significant reduction in urinary UPEC concentrations[158,159]. Bacteriophages are also effective against biofilms and multidrug-resistant pathogens, and can restore antibiotic sensitivity[160–162]. Alternatively, antimicrobial peptides, which directly lyse UPEC and disrupt bacterial biofilms, show potential as UTI therapies. Further studies are needed to determine if synthetic antimicrobial peptides can be safely administered in a cost-effective manner to treat or prevent UTI[1]. Although in the early phases of development, bacteriophage therapies and antimicrobial peptides represent promising antibiotic alternatives or adjuvants for cystitis and pyelonephritis and warrant further exploration.

For the first time in almost two decades, a new antibiotic has been developed and shows promise for UTI treatment. Gepotidacin targets DNA gyrase and topoisomerase IV and has bactericidal activity against *E. coli*, including UPEC strains that are resistant to fluoroquinolones. Studies in rat models of pyelonephritis show that it is effective in reducing UPEC bladder and kidney burden[163]. Phase III clinical trials ended enrolment early owing to overwhelming evidence of efficacy in treating uncomplicated UTI[164,165].

Engaging kidney immune responses

Boosting kidney immune mechanisms to control infection may represent an alternative therapeutic approach to preventing or treating pyelonephritis (Fig. 3b). Given the importance of the collecting duct in shaping UTI responses, strategies that enhance bacterial phagocytosis or UPEC acidification, TLR4-mediated inflammatory defences, or antimicrobial peptide secretion may represent new therapeutic avenues. For example, targeting developmental programmes that regulate IC and PC fate may alter collecting duct phenotypes to enable UPEC clearance[166]. Alternatively, silencing of the transcription factor, IRF-7, represents an opportunity to transiently reduce toxic inflammation and minimize kidney injury[53]. Lessons learned from advances in therapeutic mRNA delivery contend that similar strategies could be utilized to deliver *IRF7* small interfering RNA or other target small interfering RNAs to dampen pro-inflammatory signalling following an infection. Moreover, our understanding of the response of the collecting duct to UPEC infection insinuates that medications such as the AVPR2 antagonist tolvaptan, insulin-sensitizing agents, or sex hormone therapy might lead to antibiotic-conserving therapies[81,124,130]. Last, repurposing clinically available drugs such as histone deacetylase inhibitors may reduce cystitis or pyelonephritis sequelae by augmenting antimicrobial peptide expression[167].

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Conclusions

The clinical ramifications of pyelonephritis are vast and represent a global challenge given the increasing prevalence of antibiotic-resistant UPEC and limited availability of approaches with which to treat or prevent kidney infections. This unmet need underscores a need for greater insights into mechanisms used by UPEC to establish pyelonephritis and the responses activated in the kidney to combat infection. Greater understanding of the mechanisms by which UPEC impacts the kidney and the epithelial or immune cells that impart immediate and sustained protection may provide a foundation on which to develop new immunotherapies for pyelonephritis.

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Key points

- Uropathogenic *Escherichia coli* (UPEC) is the most common bacterial cause of pyelonephritis; virulence factors expressed by UPEC promote survival in the kidney by expediating cellular invasion and neutralizing host defences.
- Within the collecting duct, intercalated cells are targeted by UPEC; intercalated cells respond by activating acid–base machinery, phagocytosing bacteria, producing cytokines and chemokines and releasing antimicrobial peptides.
- An intricate network of macrophages and dendritic cells, in close proximity to collecting tubules, survey the renal interstitium and respond to UPEC by producing neutrophil and monocyte chemoattractants to combat bacteria during pyelonephritis.
- Extracellular environmental factors and endogenous hormones influence or control important antibacterial defences in the kidney.
- The increasing prevalence of antibiotic-resistant uropathogens highlights an urgent need for new therapeutic approaches that conserve antibiotic use and mitigate the morbidity associated with pyelonephritis; better understanding of host–pathogen interactions in the kidney may aid these efforts.

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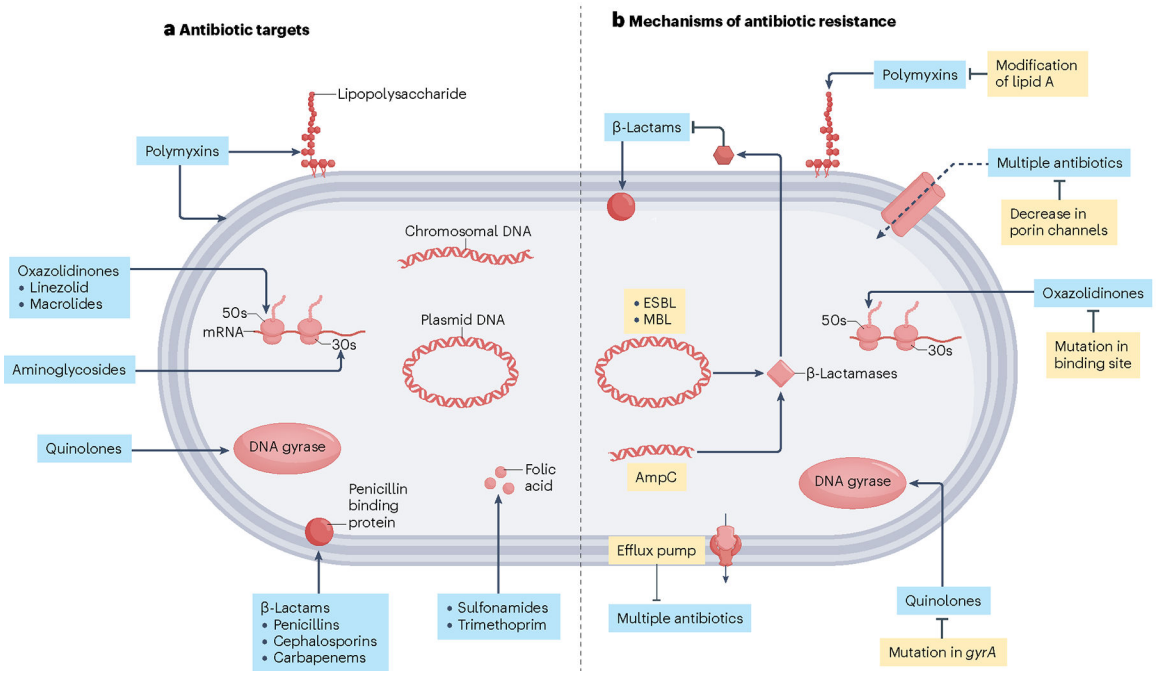

Fig. 1 |. Antibiotic targets and antibiotic resistance strategies deployed by uropathogenic *Escherichia coli*.

a, Targets of antibiotics used to treat uropathogenic *Escherichia coli* and Gram-negative uropathogens. Polymyxin antibiotics target outer membrane phospholipids and lipopolysaccharides of Gram-negative bacterial cell membranes. Oxazolidinones inhibit bacterial protein synthesis by blocking the large ribosomal subunit. Similarly, aminoglycosides inhibit bacterial protein synthesis by blocking the small ribosomal subunit. Quinolones target enzymes involved in bacterial DNA synthesis. β-lactam antibiotics prevent bacterial cell wall synthesis. Sulphonamides prevent folic acid synthesis in bacteria by targeting dihydropteroate synthase. **b**, Antibiotic resistance strategies adapted by uropathogenic *E. coli* and Gram-negative uropathogens. Bacteria can resist β-lactam antibiotics through the expression of β-lactamases, which disrupt the structure of the antibiotic and render it ineffective. They can also increase the expression of efflux pumps, which facilitate the removal of antibiotics from the bacterial cell interior. Conversely, bacteria can suppress expression of porin channels, reducing access to the bacterial cell interior to antibiotics. Bacteria can adapt to the selective pressure of antibiotics by modifying lipid A so that polymyxins no longer recognize their substrate. They can mutate their DNA or protein synthesis machinery so that oxazolidinones and quinolones can no longer bind their targets, respectively. ESBL, extended-spectrum β-lactamase; MBL, metallo-β-lactamase. Figure adapted from ref. 17, Springer Nature Ltd.

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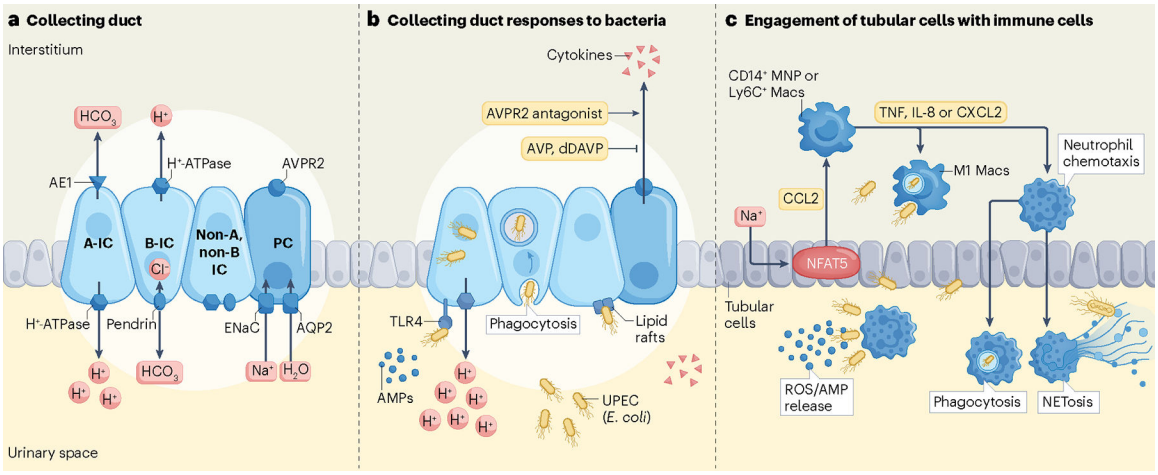

Fig. 2 |. Antibacterial responses of the kidney collecting duct to uropathogenic *Escherichia coli*.

a, Collecting ducts are composed of principal cells (PCs) and three types of intercalated cells (A-IC; B-IC; and non-A, non-B IC) that express cell-specific transport proteins. A-IC express an apical H^+-ATPase that interfaces with the urinary space and a Cl^-/HCO_3^- exchanger AE1 along their basolateral surface. B-IC express pendrin, a Cl^-/HCO_3^- exchanger, at their apical membrane and a basolateral H^+-ATPase. In the connecting segment, non-A, non-B ICs express both H^+-ATPase and pendrin at their apical surface. PCs express the epithelial sodium channel (ENaC), aquaporin 2 (AQP2) and an arginine vasopressin receptor (AVPR2). **b**, During pyelonephritis, uropathogenic *Escherichia coli* (UPEC) preferentially target the ICs in the collecting duct. UPEC are sensed by pattern recognition receptors, such as TLR4, on the surfaces of ICs. In addition, UPEC may access the interior of ICs via lipid rafts. ICs respond to uropathogens by releasing antimicrobial peptides (AMPs), cytokines and A-IC secrete protons into the urine. ICs also phagocytose UPEC. The role of PCs during pyelonephritis is less defined, but it is believed that activation of AVPR2 by vasopressin can induce secretion of cytokines by PCs and suppress endogenous dDAVP, ultimately reducing water reabsorption and increasing urine volume. **c**, (Top) In response to UPEC, the medullary sodium gradient orchestrates immune defences by stimulating NFAT5 in tubular epithelial cells to produce CCL2. CCL2 recruits human $CD14^+$ mononuclear phagocytes (MNP) or mouse $Ly6C^+$ macrophages (Macs) to the medulla where they differentiate into M1 macs and eliminate UPEC. Medullary epithelial cells and macs also secrete granulocyte-recruiting chemokines such as IL-8 (human), CXCL2 (mouse), or TNF to attract neutrophils to the source of infection. (Bottom) Neutrophil transepithelial migration into the urinary space combats UPEC by producing reactive oxygen species (ROS) and AMPs, phagocytosing UPEC and producing neutrophil extracellular traps (through NETosis).

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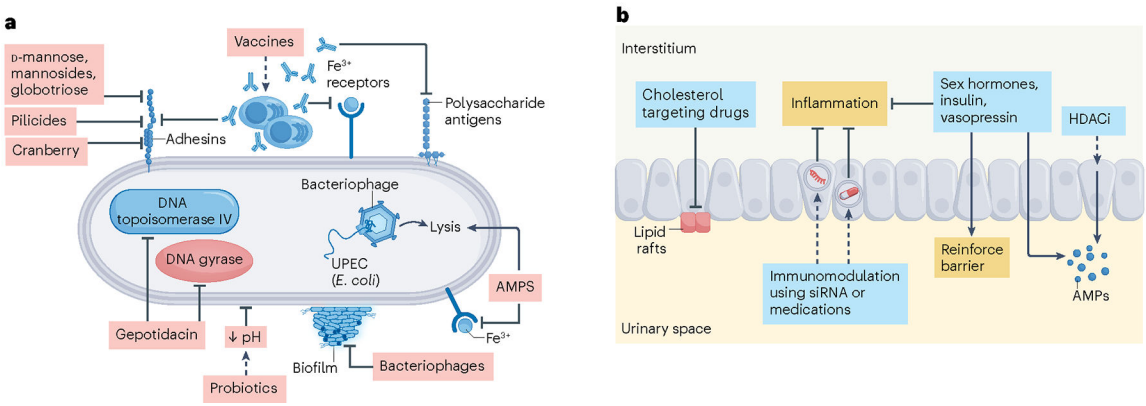

Fig. 3 |. Emerging and potential antibiotic-conserving therapeutics for pyelonephritis.

a, Vaccines induce immunity against uropathogenic *Escherichia coli* (UPEC) bacterial antigens including adhesins and polysaccharide antigens, such as FimH and lipopolysaccharide (LPS), respectively. Vaccines can also induce immunity against iron receptors. Exogenous mannose or its derivatives, pilicides and cranberry extracts can reduce UPEC binding to host cells. Gepotidacin is a novel antibiotic with a dual-targeting mechanism of action, disrupting both DNA gyrase and topoisomerase IV in bacteria. Probiotics can prevent UPEC attachment to host cells by altering the microenvironment, including pH. Bacteriophages can inhibit biofilm formation and induce lysis of cells that they infect. Antimicrobial peptides (AMPs) are small, cationic peptides that can directly kill bacteria by disrupting their cell membranes or by sequestering metal ions that are required for bacterial enzyme activity. **b**, Host defences against urinary tract infections (UTIs) can be boosted in a number of ways. Cholesterol targeting drugs can prevent UPEC from utilizing lipid rafts to gain entry into host cells. Immunomodulation of pro-inflammatory factors including interferon regulatory factor 7 (Irf7) has been shown to reduce pyelonephritis in rodent models (dashed line). Additionally, hormones including oestrogen, insulin and vasopressin suppress the inflammatory responses that contribute to pyelonephritis and strengthen the epithelial barrier. These hormones may also boost expression of AMPs. Finally, histone deacetylase inhibitors (HDACi) increase AMP production and reduce UTIs in rodent and cell-culture models. siRNA, small interfering RNA.

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Tables 1 |

UPEC virulence factors and their role in UTI pathogenesis

Virulence factor	Positive isolates from cystitis cases (%)[a]	Positive isolates from pyelonephritis cases (%)[a]	Positive commensal isolates (%)[a]	Role in cystitis	Role in pyelonephritis
Secreted toxins					
Alpha haemoLysin (HLyA)	39	54	17	Activates caspase 1/caspase 4 and the NLRP3 infLammasome[168] Promotes exfoliation of bladder urotheLiaL ceLLs[116]	Activates caspase 3 apoptosis and permits paraceLLuLar UPEC translocation[41–43] Induces haemolysis of tubular cells[169] Activates the NLRP3 infLammasome[117] Induces IL-6 and IL-8 production[46]
Cytotoxic necrotizing factor (Cnf1)	49	43	5.5	Rearranges the actin cytoskeLeton, which promotes bacterial internaLization[170] Induces apoptosis, which induces exfoLiation[171]	Unknown
Secreted autotransporter toxin (Sat)	Not reported	55	22	Induces vacuoLation[172]	Induces vacuoLation and ceLL detachment[172]
ToLL/interLeukin-1 receptor domain-containing protein C (TcpC)[111]	21	40	8	Unknown	Inhibits TLR4 by degrading MyD88 (ref. 113) Promotes inflammation by inducing MIP-2 in kidney ceLLs[173] Inhibits NETosis[114]
Adhesins					
Type I fimbriae	64	47	53	Bind uropLakins via FimH[174] Activate TLR4[175]	Binds desmogLein-2 via FimH[34] Activates TLR4 (ref. 32) Activates compLement via CD46 (refs. 39,40)
Type P fimbriae	31	81	13	Unknown	Binds gLycosphingoLipids via PapG[34]
Type S/F1C fimbriae	14	23	6.2	Unknown	Binds gLycosphingoLipids[176]
Afa/Dr adhesins	19	16	8.2	Unknown	Binds type IV coLLagen and decay-acceLerating factor (DAF)[177]
Iron acquisition					
Aerobactin	51	74	34	Sequesters iron (siderophore)[178]	
Immune evasion					
K1 capsule	Not reported	28	22	Involved in IBC formation[179]	Resists phagocytosis and compLement[180] Host immunomoduLation[181]

[a]Data compiled from multiple references[20–25,111]. IBC, intracellular bacterial communities; UPEC, uropathogenic *Escherichia coli;* UTI, urinary tract infection.

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Table 2 |

Antimicrobial peptides and proteins of relevance to UTIs

Name	Source of production	Antimicrobial activity	In vivo preclinical evidence of UTI relevance	Clinical evidence of UTI relevance
β-defensins 1–3 (HNP1–3)	Collecting duct Immune cells	Bactericidal	Humanized *DEFA1A3* mice are protected from UPEC[65]	*DEFA1A3* copy numbers correlate with UTI and pyelonephritis risk[83]
β-defensin 1	Nephron Collecting duct	Bactericidal	*Defb1* knockout mice develop *Staphylococcal* bacteriuria[182] *Defb1* knockout mice and controls have similar UTI risk[67]	Urine concentrations increase with pyelonephritis but not cystitis[183] Urine concentrations increase in pregnancy[183]
Cathelicidin (LL-37)	Nephron Collecting duct Immune cells	Bactericidal Chemoattractant Promotes NET formation	*Camp* knockout mice on 129/SvJ background show increased UPEC burden (strain CFT073)[184] *Camp* knockout mice on C57BI/6 background show decreased UPEC burden (strain UTI89)[66]	Urinary production increases with cystitis and pyelonephritis[66]
Hepcidin	Nephron Collecting duct	Bacteriostatic Ferroportin inhibitor (iron export)	*Hamp* knockout mice have increased UPEC burden[69,185]	Not tested
Lactoferrin	Collecting duct	Bacteriostatic Iron chelation	Oral lactoferrin treatment reduced UPEC bladder and kidney burden in mice[186]	Urinary production increases with UTI[187]
Pentraxin-related protein 3	Kidney epithelia Immune cells	Promotes opsonization and phagocytosis	*Ptx3* knockout mice have increased UPEC burden and renal abscess formation[75]	Urinary production increases with cystitis and pyelonephritis[75]
Lipocalin 2 (NGAL)	Nephron Intercalated cells Immune cells	Bacteriostatic Iron chelation	*Lcn2* knockout mice have increased UPEC burden[33,68]	Urinary production increases with UTI[33,68] Girls with recurrent UTI have suppressed urinary concentrations Women with recurrent cystitis have elevated urinary concentrations[68]
Ribonuclease 4	Collecting duct Immune cells	Bactericidal	Not tested	Girls with recurrent UTI have suppressed urinary concentrations[76]
Ribonuclease 7	Intercalated cells	Bactericidal	Humanized RNASE7 mice are protected from UPEC[73]	Girls with recurrent UTI have suppressed urinary concentrations[73] RNASE7 polymorphism correlates with UTI risk[84]
Uromodulin (Tamm-Horsfall protein)	Nephron	Prevents UPEC attachment	*Lcn2* knockout mice have increased UPEC burden[77]	UTI risk correlates with urine concentrations[82] UMOD variants correlate with UTI risk[188]

UPEC, uropathogenic *Escherichia coli;* UTI, urinary tract infection.