REVIEW

The clinical implications of bacterial pathogenesis and mucosal immunity in chronic urinary tract infection

Catherine C.Y. Chieng [1], Qingyang Kong [2], Natasha S.Y. Liou [1,3], Rajvinder Khasriya [2] and Harry Horsley [1,✉]

Urinary tract infections (UTIs) exert a significant health and economic cost globally. Approximately one in four people with a previous history of UTI continue to develop recurrent or chronic infections. Research on UTI has primarily concentrated on pathogen behavior, with the focus gradually shifting to encompass the host immune response. However, these are centered on mouse models of *Escherichia coli* infection, which may not fully recapitulate the infective etiology and immune responses seen in humans. The emerging field of the urobiome also inadvertently confounds the discrimination of true UTI-causing pathogens from commensals. This review aims to present a novel perspective on chronic UTI by linking microbiology with immunology, which is commonly divergent in this field of research. It also describes the challenges in understanding chronic UTI pathogenesis and the human bladder immune response, largely conjectured from murine studies. Lastly, it outlines the shortcomings of current diagnostic methods in identifying individuals with chronic UTI and consequently treating them, potentially aggravating their disease due to mismanagement of prior episodes. This discourse highlights the need to consider these knowledge gaps and encourages more relevant studies of UTIs in humans.

Mucosal Immunology (2023) 16:61–71; https://doi.org/10.1016/j.mucimm.2022.12.003

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections worldwide, with an upward trend of disease burden. Globally, more than 404 million individuals suffered from UTIs in 2019 (60% increase from 1990), with approximately 237,000 related deaths and 5.2 million disability-adjusted life-years. The highest number of reported cases was found in the age group between 30 to 34 years[1]. Nonetheless, UTI is far from uncommon in the pediatric population, with an estimated prevalence of 7.8% in children aged under 19 years[2]. UTIs affect mostly females in all age groups, with an estimated one-third getting at least one UTI by the age of 24 and a lifetime UTI risk of 60%[3]. The disease is complex and has heterogenous presentations, ranging from asymptomatic sub-clinical infection to life-threatening urosepsis. Acute UTI or cystitis, its commonest form, is typified by the onset of lower urinary tract symptoms (LUTS), namely urinary frequency, urgency, and pain[4]. For those with a history of UTI, the risk of recurrence is around 26% within 6 months in adults[5] and 17.3% in children aged between 2 to 6 years within 2 years[6]. Indeed, the propensity for UTI to recur is a particularly concerning manifestation of this disease[5,7–9]. A study utilizing pharmacy records for antibiotic dispensing for UTI similarly found that 20% of the patients had recurring prescriptions every year, with more than 50% having at least one repeat prescription in the 5-year study period[10]. These recurrent or chronic UTIs have a significant negative impact on quality of life and are especially difficult to treat[11–13].

Chronic UTIs are microbiologically and immunologically intricate. Little is known regarding the etiopathogenesis or the role of the host immune system in the natural history, progression, and resolution of this disease in humans. The pathogenesis of infection is muddied by the multiplicity of pathogenic and commensal organisms found in the urinary tract and their virulence mechanisms capable of circumventing immune detection[14]. The urinary bladder also appears to exhibit an atypical adaptive immune response to infection[15,16], leading to incomplete resolution of UTIs. The study of chronic UTI is further complicated by significant discrepancies between infection models with key distinctions within mouse strains[17] and between mice and humans[18,19]. These deficiencies in our knowledge of the infective etiology and host immune responses have hampered the current development of an effective vaccine that would have otherwise greatly reduced the health and economic costs of UTI[3].

Insufficient treatment of index or prior UTIs may also plausibly exacerbate chronic infection. The prevailing advice for index acute cystitis is that UTI is self-limiting, and therefore, only short courses of antibiotic treatment are necessary[20]. The ramifications of such guidelines could arguably lead to a heightened inflammatory response and microbial persistence. Even if treated with antibiotics, symptomatic and microbiological failure is remarkably high, with a significant chance of relapse[21,22]. Routine diagnostic tests such as the dipstick assay and standard urine culture

[1]Department of Renal Medicine, University College London, London, United Kingdom. [2]Department of Microbial Diseases, Eastman Dental Institute, University College London, London, United Kingdom. [3]EGA Institute for Women's Health, University College London, London, United Kingdom. ✉email: h.horsley@ucl.ac.uk

Received: 18 November 2022 Accepted 21 December 2022
Published online: 13 January 2023

commonly fail to detect persistent infection[23–25]. These tests, therefore, are arguably unfit for purpose in the context of chronic infection. Worryingly, if these 'gold standard' tests produce false negative results, this could lead to erroneous diagnoses of overactive bladder and interstitial cystitis or bladder pain syndrome[12], which by definition present with similar LUTS in the absence of an infection[26,27]. Because of this, patients with chronic UTI often describe a delay, sometimes up to 12 years[12,28], before receiving the right diagnosis and much-needed treatment. More diligent work is necessary to improve the management and care available for this patient cohort.

The term 'recurrent UTI' is used throughout this review when citing works that recruit study participants based on their history of UTI as stipulated in the European Association of Urology (EAU) guideline. This refers to three UTI episodes per year or two UTIs in the last 6 months[4]. However, in the author's specialist center for chronic UTI, we have observed that the patients are not entirely symptom-free in between flares of UTI events until they fully recover at the end of multiple consultations (more details in Section 'Is chronic urinary tract infection caused by shortfalls in the management of index or prior urinary tract infections?'), thus are regarded as having a 'chronic UTI.' This review discusses the challenges of understanding, diagnosing, as well as treating recurrent and chronic UTI and attempts to combine the often-disparate fields of microbiology and immunology. It will explore what is known about the pathogenesis, the bladder immune response, and clinical management of chronic UTI. With this discourse, we aim to highlight gaps in our current understanding and engage the wider research community in formulating novel research questions and study approaches in the field.

WHO ARE THE TRUE CULPRITS OF CHRONIC URINARY TRACT INFECTION?

Much information on the virulence mechanisms that lead to UTI has been learned from experimental infection models of *Escherichia coli* in mice, with little being studied in humans. Presently, classical uropathogens such as *E. coli*, *Enterococcus faecalis*, *Proteus mirabilis,* and *Klebsiella pneumoniae* have been recognized as such based on standard midstream urine culture and abundance. This, unfortunately, also forms the basis of guidelines for the diagnosis and treatment of recurrent UTI[4,29]. We are now aware that urine culture is flawed as urine is not sterile due to the presence of bladder resident microbiota[30,31], and more than half of the samples will produce absent or mixed growths[24,25]. The co-existence of pathogenic bacteria and commensals in the bladder necessitates a re-evaluation of previous research in the microbiology of UTI and hence, more rigorous research approaches in this area.

In a well-described murine model of acute UTI, uropathogenic *E. coli* (UPEC) is shown to invade the bladder urothelium and form biofilm-like intracellular bacterial communities (IBCs). These bacteria then flux out of the cells, bind to adjacent tissue, and repeat the invasion process. During egression, UPEC cells elongate, and this "filamentous" morphology has been shown to protect against neutrophil phagocytosis and detachment from the bladder wall during micturition. A small proportion of these bacteria can develop into quiescent intracellular reservoirs (QIRs) that reside deep in the murine mucosa. In mice, at least, these reservoirs are thought to divide rapidly once reactivated, signaling a subsequent round of symptomatic infection[32–35]. It is of note that IBC formation in Toll-like receptor 4-deficient C3H/HeJ mice is 10 times that found in their immunocompetent C3H/HeN counterparts[34]. Formation of IBCs and QIRs have been suggested as the mechanistic basis of chronic UTI; however, C3H/HeN mice harbor very few or no IBCs at subsequent infections in chronic infection studies. This could possibly be due to morphological changes in the urothelium, with thickening of the basal and intermediate layers. The luminal epithelium also lacks terminal differentiation markers such as cytokeratin 20 and uroplakin IIIa in the smaller and rounder superficial cells[17,36], potentially diminishing a urothelial-specific transmembrane protein necessary for UPEC tropism in the bladder[32].

In humans, only one study has detected similar bacteria-filled pods and filamentous bacteria in the urine of women with acute cystitis[37] described earlier. Other species isolated from urinary cells of patients with chronic UTI that showed cellular invasion capability *in vitro* are *E. faecalis*, *P. mirabilis,* and *Streptococcus anginosus*[38–40]. This intracellular behavior, coupled with the possible presence of QIRs in the bladder wall, may contribute to the high treatment failure and tenacity of infection seen in these patients. Antibiotics are unable to penetrate the highly specialized urothelial cell in sufficient doses to be therapeutic and, furthermore, are unable to eradicate QIRs that are not metabolically active or dividing[35,41]. These limitations have prompted the development of alternative drug carrier systems using ultrasound-activated microbubbles[42] or polymeric particles[43] for better intracellular delivery. However, if pathogens other than *E. coli* can be isolated in 50% of chronic UTI cases[44], further research is warranted to uncover contemporaneous pathogens which may express different virulence factors and exhibit novel invasive mechanisms.

In addition to our lack of understanding surrounding both causation and the mechanisms of infection, research in chronic UTI has, in recent years, been confounded by the discovery of the urinary microbiome[30]. A diverse ecology of mutually overlapping bacterial species is present in the urine of healthy individuals and patients with urinary symptoms, including bacteria that are conventionally regarded as uropathogens[25,31,39,45–48]. These data raise the question of whether the preponderance of *E. coli* isolated from earlier studies truly represents the majority of UTI cases as reported. A recent longitudinal study found that *E. coli* species abundance in the urine was similar between healthy controls and recurrent UTI patients, and in-depth phylogenetic analysis into the species did not show any difference in strains. A temporal spike in the number of *E. coli* in the gut also did not predict UTI in the patients[49]. This suggests that other bacterial species, or combinations of bacteria, may be causing disease in this patient cohort.

There is a dearth of information pertaining to the tissue localization of urinary microbiota. Cellular invasion may be a transient phenomenon found in health as well as disease. Therefore, it is possible that chronic UTI is a polymicrobial infection stemming from internal dysbiosis of bladder microbiota and/or infiltration from external environment. As with chronic otitis media, it is possible that commensals enhance the survival of pathogens in bladder biofilms[50]. Biofilm formation may be distinctly pathological but may also be a physiological phenomenon that is manifest in the normal healthy bladder. To accurately determine causative pathogens in chronic UTI, longitudinal analysis in both patients and healthy volunteers will need to be performed, aimed at characterizing the bladder microbiota in health and parsing the species that are pathogenic.

WHAT IS THE BLADDER MUCOSAL IMMUNE RESPONSE IN HEALTH AND URINARY TRACT INFECTION?

In contrast to the study of bacteria in the pathogenesis of UTI, there is a paucity of research into host immune factors, particularly the adaptive immune responses elicited in chronic UTI. The bladder sloughs off its epithelial layers upon infection as a bacterial clearance mechanism, and the cell regeneration that follows seems to limit inflammatory processes required for complete killing of pathogens. In mice, a prolonged infection has been attributed to this skewing of the type 1 and type 2 T helper cells (Th1/Th2) immune response. In addition to the T cell-mediated responses, humoral responses through production of antimicrobial factors and antibodies have also been described, albeit predominantly in mice. Translating research from animal models to humans is challenging, especially due to differences in the physiology and immune composition between the two species (Fig. 1). Vaccines and immunostimulants have been developed in an effort to harness a targeted immune response, but none has proven to be fit for widespread use thus far. As discussed in Section 'Who are the true culprits of chronic urinary tract infection?', causation has yet to be fully described or proven in this disease, potentially restricting the design of clinically efficacious prophylactic measures.

The kidneys, upstream of the bladder in the urinary system, have a robust dendritic cell–mediated immune response predominated by neutrophils and macrophages[51] with interferon-γ[52,53] as the key inflammatory cytokine during infection. A bladder infection also induces similar neutrophil-driven responses as a result of interleukin (IL)-8 produced by uroepithelial cells[54–57]. The role of broad-spectrum antimicrobial peptides that are either constitutively expressed or upregulated during UTI has also been described and comprehensively reviewed elsewhere[58]. Upon re-infection with the same bacteria, the kidneys are able to produce bacterial species-specific antibodies, but this secondary memory response is not observed where the prior infection is restricted to the bladder alone[15]. However, in another study, C57BL/6 mice were infected with UPEC, and the bacterial burden remained low post subsequent rounds of bacterial challenge. There were increased aggregates of CD4+ and CD8+ lymphoid infiltration in the bladder, with antigen-specific urinary immunoglobulin (Ig)G along with serum IgG and IgM detectable after 2 weeks. Adoptive transfer of serum and splenic T cells from pre-immune mice to naïve mice also conferred partial protection from infection, showing presence of both humoral and cellular adaptive immune responses[59].

It is also part of the immune response to shed epithelial cells to reduce bacterial burden and prevent further colonization in both mice and humans[32,38,60]. In adult mice, the urothelium turnover rate is slow. An umbrella cell lifespan is around 40 weeks[61], but within 6 hours of an acute bladder infection, the cells rapidly exfoliate and show early signs of regeneration[32]. In *E. coli*-infected mice, urothelial exfoliation is initiated by bacterial attachment to uroplakin Ia on the epithelial cells through type 1 pilus FimH adhesin[32,62]. However, further immune activation that stimulates epithelial cells to produce IL-6 for neutrophil recruitment is dependent on the presence of lipopolysaccharide[63], showing that the bladder is capable of recognizing a common antigen present in Gram-negative bacteria and mounting an immune response. In humans, epithelial shedding is evidenced by an increase in pro-apoptotic factors and a decrease in proliferation and cytoskeletal proteins[64].

The bladder, however, appears to exhibit a low propensity for inflammation during infection. In mice, the Th2 response has been found to predominate in the bladder in an effort to prioritize epithelial repair[16]. This Th2 response leads to IL-10 production by mast cells[15], which dampens the inflammatory response to infection so as to aid epithelial regeneration. When the immune response in the bladder is skewed from Th2 to Th1, bacteria are seemingly cleared without affecting the Th2 response[65]. It would appear plausible, therefore, that these phasic Th1/Th2-driven states of inflammation and tissue regeneration might play a significant role in the chronicity of infection. However, while Th1/Th2 play antagonistic roles that can be distinctly defined in mice, their effects are less clear-cut in humans, and various immune and non-immune cells have been found to produce IL-10[19]. In humans, both Th1 and Th2 cells produce IL-10, exerting autocrine effects, which suppress further antigen-stimulated cytokine production by inhibiting Th1 and Th2 cell proliferation[66]. In another study in mice with severe combined immunodeficiency, experimental infection with UPEC did not result in chronic UTI. Lacking mature lymphocytes, these mice also showed an impaired neutrophil response and absence of inflammation upon infection, indicating perhaps an unfavorable role of lymphocytes in the development of chronic UTI[17]. However, in patients with recurrent UTI, the level of growth factors for the myeloid lineage instead was elevated compared to UTI patients that did not experience recurrence[67]. It may be the case that in the human bladder, there exists a non-canonical Th2-biased or dysregulated inflammatory responses as observed in mice, but it remains to be investigated whether these are naturally occurring or only present in a subpopulation predisposed to developing this disease.

Research into immune responses to infection in the bladder has mostly been performed in mouse models inoculated transurethrally with large boluses of UPEC [approximately 10^8 colony-forming units (CFU)][16,36,65,68]. Although these studies have been useful in providing insights into the different facets of immune response in UTI, this initial bacterial load is unlikely to accurately reflect the natural history of infection in humans. Moreover, murine immunology is inherently different from that of humans[18,69], and therefore, the extent to which these findings can be translated clinically should be approached with these caveats in mind. Schistosomiasis, for example, which is a chronic parasitic infection with urinary or hepatointestinal manifestations, shows distinct immunological responses in mice and humans. Th1 response with an elevated interferon-γ level is elicited in mice during the acute phase, followed by a Th2 response with IL-10 production, resulting in tissue damage and decreased resistance to re-infection. However, it is lethal in mice in the absence of a Th2 response, resulting in an unchecked polarization towards Th1. In contrast, in humans, the presence of parasite-specific IgE and IL-4, indicating a Th2 response, confers protection against re-infection[70]. Further distinctions between these species can be seen when scrutinizing the gross circulatory white blood cell populations. The leukocytes in mice are lymphocyte-rich (75% to 90% lymphocytes, 10% to 25% neutrophils)[71], while in humans, they are neutrophil-rich (50% to 70% neutrophils, 30% to 50% lymphocytes). The shorter lifespan of the rodents also provides a hindrance to studying a chronic infection in humans, which could span an average of more than 5 years[12,25,28] for chronic UTI. Different mouse strains show divergent responses toward experimental infection with UPEC[17], with all C57BL/6 showing

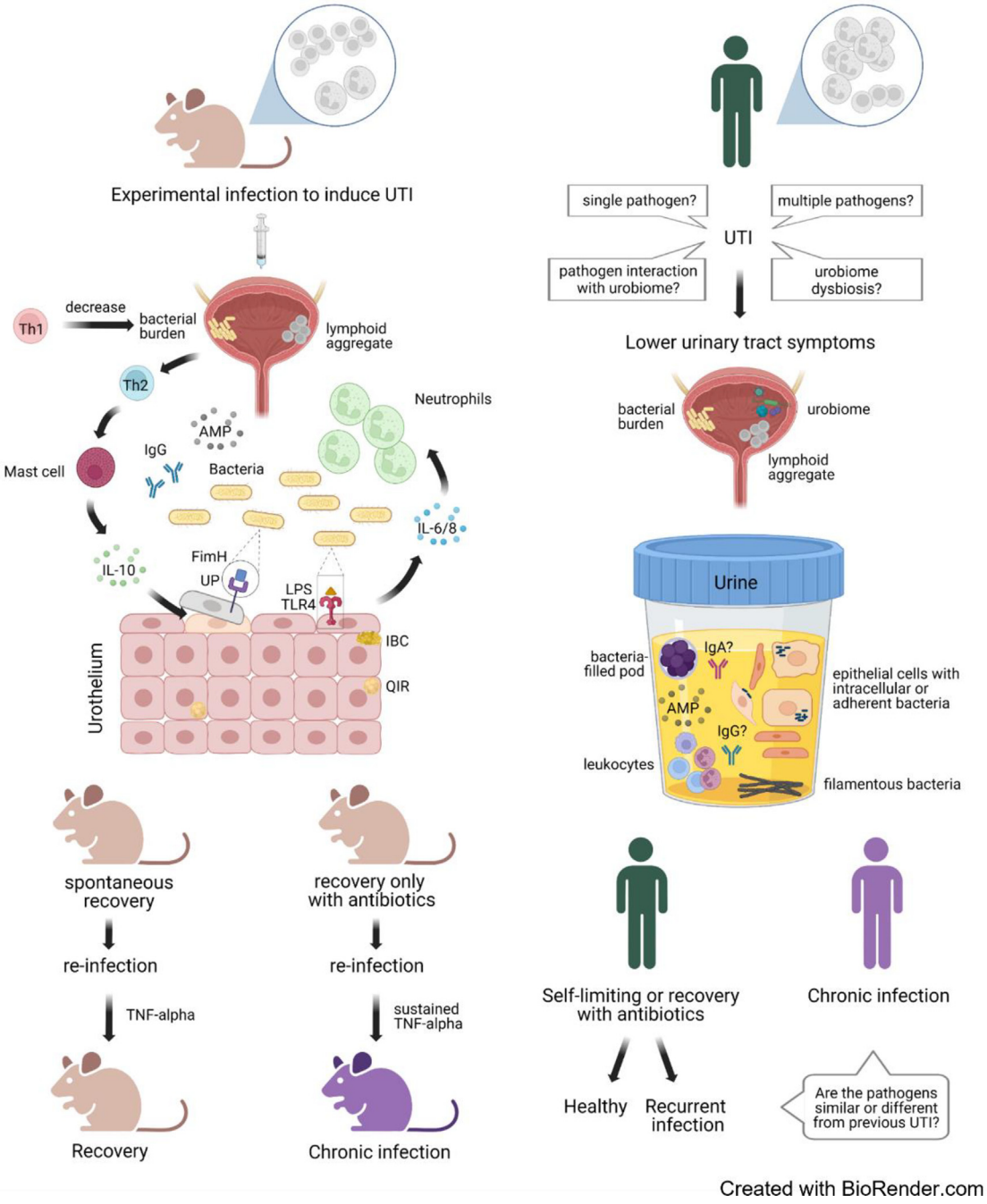

Fig. 1 The immunology and microbiology of UTI are intrinsically linked. The leukocyte population in mice is lymphocyte-rich. A large number of studies have been performed using murine models of UTI by direct bacterial inoculation into the urethra. Bacterial attachment to urothelial cells via FimH to uroplakin induces epithelial shedding but also paves the way for cellular invasion, forming IBC and QIR, which may seed subsequent infections. Immune activation following Toll-like receptor 4 binding with LPS produces chemokines (IL-6 or IL-8) that recruit neutrophils. Another innate immune response involves AMP secretion. Adaptive immune responses from antigen-specific IgG have also been described, while Th1/Th2 signaling drives bacterial clearance or epithelial regeneration. Upon re-infection, the mice either spontaneously recover or develop a chronic infection due to sustained TNF-α imprinted from prior infection. Conversely, in humans, the leukocyte population is neutrophil-rich. The discovery of the urobiome (which has not been described in mice) has complicated identification of causative pathogens in UTI, where infections are postulated to be a result of urobiome dysbiosis or pathogen interaction with resident bladder microbiota. It is also not known whether the disease is caused by single or multiple pathogens. Apart from lower urinary tract symptoms, signs of the disease are inferred from leukocyte counts and infected epithelial cells found in the urine. Other possible markers of disease are presence of bacteria-filled pods and filamentous bacteria. Production of antigen-specific antibodies has not been ascertained. Some individuals completely recover from UTI, but a subset of the population goes on to develop chronic or recurrent infections. AMP = antimicrobial peptides; IBC = intracellular bacterial communities; IL = interleukin; Ig = immunoglobulin; LPS = lipopolysaccharide; QIR = quiescent intracellular reservoirs; Th = T helper; TNF-α = tumor necrosis factor-alpha; UP = uroplakin; UTI = urinary tract infection.

Table 1. List of candidate vaccines or immunostimulants for use in recurrent or chronic urinary tract infection.

Name	Other names	Active ingredients	Administration route	Recommended dosage	Stage in clinical trial (identifier*)	References
Uro-Vaxom®	OM-89	Lyophilized lysate from 18 *Escherichia coli* strains	Oral	6 mg daily for 3 months, stop for 3 months, 6 mg daily for the first 10 days of each month for 3 months as booster	Phase 4 (NCT04049994)	77–79,113–115
Uromune®	MV140	*E. coli, Klebsiella pneumoniae, Proteus vulgaris* and *Enterococcus faecalis* in equal amounts of 10^9 inactivated whole bacteria/ml	Sublingual	2 puffs of 100 µl each (10^8 bacteria/puff), maintained under the tongue for 1–2 minutes and then swallowed, once daily for 3 months	Phase 2 (NCT04096820)	116–118
ExPEC4V	JNJ-63871860	Polysaccharide antigen from extraintestinal *E. coli* ExPEC4V serotypes O1A, O2, O6A and O25B	Intramuscular	Single dose	Phase 2 (NCT02546960)	119
Urovac®		Heat-killed bacteria consisting of 6 *E. coli* strains, *P. mirabilis, K. pneumoniae, Morganella morganii* and *E. faecalis*	Intravaginal	Three suppositories at weekly intervals, three suppositories at monthly intervals as booster	Phase II (NCT00261248)	120–122
StroVac	SolcoUrovac	Heat-killed bacteria consisting of 6 *E. coli* strains, *P. mirabilis, K. pneumoniae, M. morganii* and *E. faecalis*	Intramuscular	Three doses at weekly intervals, one dose 12 months later as booster	Information not available	123

* Identifier can be used to search for the clinical trial study on *clinicaltrials.gov*.

spontaneous recovery without apparent IBC formation and epithelial layer damage[32,33,59], as opposed to C3H/HeN which has a subgroup that does not recover without antibiotics[17,36,68]. Therefore, to truly understand mucosal immunity in the bladder, urinary and tissue-resident leukocyte populations along with their associated activating/inhibiting/effector cytokines will need to be measured and characterized in humans. Additionally, these factors could be clearly defined in the context of health (steady state) and disease (UTI) to elucidate immune homeostasis in the bladder and changes that occur during infection.

A vaccine for UTI is not yet available, but there have been multiple candidates for immunostimulants made of killed or inactivated bacteria over the years (Table 1). Among these, the most widely studied is oral Uro-Vaxom® (OM Pharma, Switzerland), made of lyophilized membrane proteins from 18 UPEC strains and marketed for recurrent UTI treatment and prevention. It was first described 40 years ago for treating rheumatoid arthritis[72], then used at a lower dose for treating recurrent UTIs[73]. It is required to be taken as a daily 6 mg oral capsule for 90 days, followed by three 10-day boosters per month after 3 months of rest. In Balb/c mice, oral gavage of Uro-Vaxom® in 1 mg/ml showed an increase in bacteria-specific serum IgG and IgA, but the same was observed in non-immunized mice, albeit at lower levels[74]. It is found to boost the immune response by stimulating B lymphocyte proliferation and macrophage activation in murine *in vitro* studies[75] and triggering the maturation of monocyte-derived dendritic cells in another human *in vitro* study[76]. In trials involving humans, it aided in symptom alleviation during recurrences[28,77]. However, in terms of its effect on preventing recurrences, it showed 17% to 35% reduction in the group that was administered Uro-Vaxom® compared to placebo 3 months after intake[77,78], reducing to 15% at 12 months[79]. A more recent study showed 50% decrease (from 3.1 to 1.5 UTI episodes per year) in 46 out of 79 recurrent UTI patients, but this was compared within group without a placebo control[80]. Even though the formulation was prepared from *E. coli*, it was only effective in 69% of the 38 patients that were culture-positive for *E. coli*. It was also effective in 23% of patients that showed growth of *Klebsiella*, *Proteus,* or others but was not effective in all 18 patients that showed mixed growth[80]. In these trials, it is still difficult to ascertain whether the primary outcome measures of reduction in symptoms and UTI episodes are of clinical significance. A longer follow-up period may be necessary to establish whether Uro-Vaxom® is as effective as antibiotics in preventing and treating chronic UTI.

Use of vaccines and immunostimulants are attractive non-antibiotic alternatives in the clinical management of UTI. However, the design of an efficacious formulation with the specific goal of preventing or treating a target health condition is technically difficult. There are multiple factors to be considered, including route of administration, dosage, duration, timing, type of adjuvant, and the identification of biomarkers that signal successful immunity. In the context of chronic UTI, the low efficacy of the candidates studied so far could be attributed to the more challenging aspect of selecting the correct immunizing antigen. Until now, causative bacteria and pathogen diversity in chronic UTI are still indeterminate, and the immune response that is present in the bladder is not fully characterized. Arguably, therefore, it would be difficult to rationally design a solution to target the elements of the immune system that need enhancing. Additionally, predictors of recurrence[17,67] can be validated to potentially identify individuals that would benefit the most from these

prophylactic measures. We must also be mindful that the bacterial formulations do not eventually result in immune imprinting, where the opposite undesirable effect of declining immune response towards a related emerging pathogen occurs, rendering them ineffective against the disease they are supposed to confer protection to[81,82]. It is imperative to have a better understanding of the immune responses to UTI in humans as they are closely intertwined with disease resolution (or persistence in the case of chronic infection) and may prove valuable therapeutic targets.

IS CHRONIC URINARY TRACT INFECTION CAUSED BY SHORTFALLS IN THE MANAGEMENT OF INDEX OR PRIOR URINARY TRACT INFECTIONS?

Although UTI frequently occurs in adult pre- and postmenopausal women, it is also a common pediatric infection with the incidence rate eight times higher in girls than in boys[83]. Symptoms are less specific, and urine collection is difficult in infants and children who are not yet toilet-trained[84], thus posing a challenge in confirming a UTI diagnosis. Different imaging modalities may be appropriate in newborns suspected of having a febrile UTI to exclude anatomical and functional anomalies[85], but are not recommended for children and adults with uncomplicated UTIs[4,85,86]. In more mature children and beyond, diagnosis of UTI is reliant on LUTS and urinalysis, which include urine culture and biochemical dipsticks, but these tests have been shown to be unreliable in recent years. Inadequate clinical management, in terms of a failure to arrive at an accurate UTI diagnosis and subsequent misuse of antibiotics, could possibly result in long-term ramifications affecting future susceptibility to UTI.

Based on the 2022 Guidelines on Urological Infections published by EAU[4], uncomplicated cystitis is diagnosed by a history of LUTS, including dysuria, frequency, and urgency without vaginal discharge. Urine culture is only recommended for individuals whose symptoms do not resolve or recur within 4 weeks after initial treatment. It is recommended as a diagnostic aid, with the result reported as positive only when more than 10^5 CFU/ml of a single known uropathogen is detected. The sample is dismissed as contaminated when there is mixed growth[29,87]. This microbiological threshold was first described in 1956 when 95% of 74 patients with pyelonephritis (kidney infection) showed urine culture bacterial counts of more than 10^5 CFU/ml while 90% of 595 asymptomatic controls (including diabetic, pregnant, and individuals with cystocele) had bacterial counts lower than 10^5 CFU/ml[88]. This value was later reduced to 10^2 CFU/ml in symptomatic individuals, although 50% of cultures that gave counts at or more than this new limit contained mixed growth[89], which would then make it difficult to meet the criteria of pure growth of a single known uropathogen. Despite the small sample size of diseased cases and limitations in these studies, these cut-off values, and the ranges in between, are still widely used for all infections of the urinary tract and in all patient groups regardless of gender, age, and co-morbidities. Moreover, urine culture is usually performed over 24 to 48 hours on chromogenic agar under aerobic conditions, which selects for *Enterobacteriaceae* species[29,90]. This is likely to miss bacteria requiring longer incubation times, higher concentrations of CO_2 for optimal growth and pathogenic anaerobes[46,91]. These inadequacies show standard urine culture to be an inaccurate diagnostic tool and that the presence (or absence) of growth does not directly indicate whether there is an infection or inform subsequent decisions to treat.

Even though the urinary biochemical dipstick assay has been discouraged in the diagnosis of UTI in adults[4], it is still recommended as a diagnostic aid in pediatric UTI[92,93]. Positive leukocyte esterase and nitrite are the two most-used markers of infection. An esterase-positive result suggests the presence of granulocytes, or pyuria (defined as leukocyte counts more than 10^5/ml), while Gram-negative enteric microbes, if present in significant numbers, convert nitrate to nitrite. Gram-positive uropathogens such as *Staphylococcus saprophyticus* and *Enterococcus* spp. do not produce nitrate reductase and would, therefore, not produce a nitrite-positive result. The sensitivity and specificity of the urine dipstick increase only when interpreted in combination with other positive measures, including leukocyte esterase, nitrite, blood, protein, a positive culture defined as more than 10^5 CFU/ml, and symptoms of dysuria, urgency, and frequency[23,94–96]. However, infections are unlikely to present with all these signs simultaneously[23,24]; therefore, the value of using dipsticks in the diagnosis of UTI has arguably become antiquated.

One of the treatment recommendations for pediatric and adult recurrent UTI involves continuous low-dose antimicrobial prophylaxis[4,92]. However, in the current global call for antimicrobial stewardship and efforts to impede further detrimental effects of antimicrobial resistance, this strategy may prove counterproductive. This was demonstrated in a randomized, open-label trial where participants receiving prophylaxis showed an increase in the number of antibiotic-resistant urinary bacterial isolates when compared to the control group, which was not on prophylaxis[97]. A recent *in vitro* study[98] modeling mucosal infection of the airway in cystic fibrosis found that isolates of *Pseudomonas aeruginosa* recovered from a challenge with a dose of twice the minimum inhibitory concentration (2X MIC) have a higher prevalence of resistance than those previously challenged with 5X MIC. The increased resistance was proposed to be mediated through natural selection, as elevated mutation rates were not observed in these species[99]. In the same study, interspecies interactions that occur in a polymicrobial environment also attenuated the efficacy of species-specific antimicrobials in *P. aeruginosa* and *Candida albicans*. Conversely, *Staphylococcus aureus* grown in biofilm with *C. albicans* has been found to show tolerance to multiple antibiotics tested[100]. When UTI persists, urine culture and antimicrobial susceptibility testing are recommended by EAU guidelines, assuming that the infecting organism is not susceptible to the initial antibiotic[4]. As recurrent UTI could plausibly be caused by more than one pathogen or a different pathogen each time, such as that seen in recurrent acute otitis media[101], more studies are necessary to establish the culprits responsible for chronicity of the infection.

The questionable accuracy of diagnostic tests and inappropriate use of antibiotics in managing prior UTIs, possibly tracing back to childhood years, may shape the response to subsequent UTIs. This phenomenon has been shown in a C3H/HeN mouse model of UTI, where two spontaneous outcomes from infection with UPEC were observed—a self-limiting/resolved group that recovered from the infection and a chronic/sensitized group that carried a sustained high bacterial load for more than 2 weeks; the latter group requiring antibiotics to recover. When these mice were subjected to a second infection, both groups showed rapid and heightened inflammatory response mediated by tumor necrosis factor-alpha (TNF-α), with the former group able to recover rapidly after 24 hours. However, TNF-α expression in

the latter group was maintained, and this resulted in prolonged inflammation and severe tissue damage[17,36,68].

It is plausible that similar immune mechanisms may contribute to chronic UTI in humans. Individuals with LUTS, suggestive of UTI, may be hesitant in seeking treatment[102], with up to 37% willing to delay commencing antibiotics upon visiting primary care[103]. For those that start antibiotic therapy, the National Institute for Health and Care Excellence (NICE) official guidelines recommend a short course of antimicrobials for acute cystitis[20], which may not be adequate to fully eradicate the infection-causing pathogens in some individuals. Indeed, if intracellular infection is responsible for chronic or recurrent UTIs, then short-course therapy may be unable to penetrate the bacterial reservoir. Therefore, the ability to identify patients that will go on to develop a chronic or recurrent form of the disease could signal the clinician to switch to a more prolonged antibiotic regimen. Common antibiotics prescribed for acute uncomplicated cystitis are nitrofurantoin, fosfomycin trometamol, pivmecillinam, and trimethoprim[4,104]. Unfortunately, to exacerbate matters, incidences of incorrect antibiotic prescription that deviate from national guidelines (error in indicating the type, dosage, or duration) can range from 34% to 90% regardless of medical proficiencies[83,105,106], while unnecessary use of broad-spectrum antibiotics for UTI in children reaches 32%[107]. These observations suggest that our current impression of UTI as a self-limiting infection may need a shift in paradigm. There have been suggestions that differences in gut microbiota[49] or disease mechanisms shown by different bacterial species or strain[108] may explain why some recover from UTI while others do not. Until the reasons for such disparity in host response or susceptibility are known, it remains possible that future or prolonged episode(s) of UTI in some people could be prevented by timely and potentially extended treatment of the index infection.

HOW CAN WE IMPROVE THE CLINICAL MANAGEMENT OF CHRONIC URINARY TRACT INFECTION WITH WHAT WE KNOW NOW?

We are now cognizant of the failure of standard urine culture and dipstick tests in recognizing UTI in the presence of LUTS. Pyuria is another aspect of urinalysis, often performed by microscopy of neat or spun urine. Leukocyte counts of more than 10^5/ml are suggestive of infection, but samples containing squamous epithelial cells are often disregarded as contamination from the perineal region[29], though this claim could not be substantiated. In one study[24], if diagnosis were made based on standard threshold values for culture and microscopic pyuria, only 30% out of 4375 urine samples showed fresh leukocyte microscopy count of more than 10 cells/μl or positive culture of a single known uropathogen at more than 10^5 CFU/ml. Those that matched both criteria were just 12%, signifying at least 70% of missed diagnosis depending on which criterion was adopted, even though the samples were all from symptomatic patients. Nonetheless, LUTS and pyuria (without any arbitrary threshold) in combination with first-generation antibiotics (cefalexin, trimethoprim, and nitrofurantoin) have been found to be valuable in the management of chronic UTI based on the clinical experience in the author's specialist center for chronic UTI[12,13].

As discussed in previous sections, certain bacterial species have been found to invade bladder epithelial cells[37,38], which are then shed as part of the immune response[60] in an effort to reduce the bacterial burden in the tissues. Studies have found that 75% to 91% of epithelial cells from urine samples stained positive with uroplakin III[38,109], which is a marker for urothelium extending from the renal pelvis to the urethra[110]. One of the authors has also previously observed the presence of uroplakin IIIa-expressing squamous cells in patients with chronic UTI (unpublished data, HH) (Fig. 2), along with extracellular bacteria coated with the same protein, suggesting that these squamous epithelial cells may not be contamination as previously thought, but rather a potential marker of disease state and progression. It has been shown that the number of leukocytes in the urine is directly proportional to the number of shed infected epithelial cells in a cohort of patients presenting with chronic UTI, which indirectly informs the severity of infection[38]. Leukocyte count is also the more sensitive surrogate marker for UTI compared to urine culture, correlating with voiding symptoms and pain[111]. Characterizing currently undescribed populations of white blood cells present in the urine of these patients, thus gaining insight into the immune responses in this chronic disease, is of paramount importance to this field of research and an important future goal.

Presently in the author's specialist center for chronic UTI, diagnosis of UTI is based on a 39-question LUTS inventory[111]

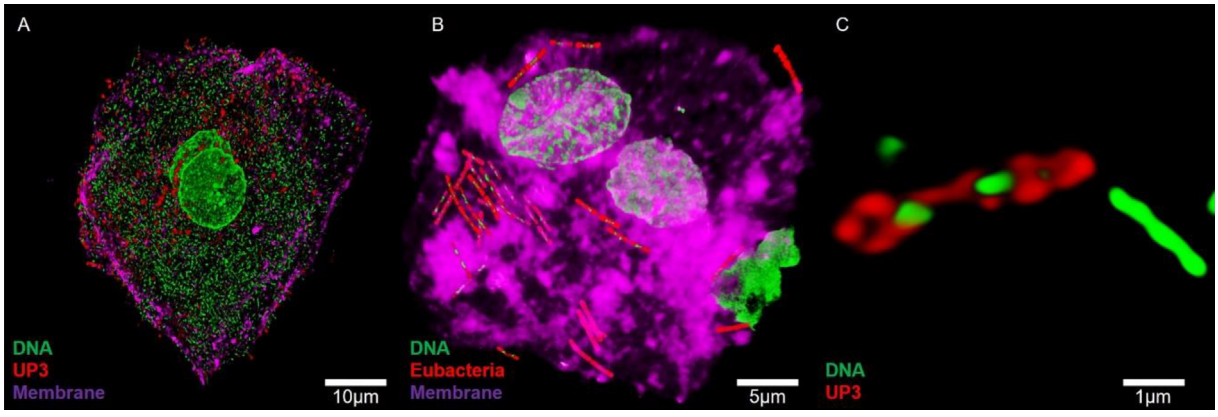

Fig. 2 Representative 3D rendered deconvolution laser-scanning confocal micrographs of epithelial cells, white blood cells, and bacteria found in the urine of patients with chronic urinary tract infection. (A) Squamous epithelial cell positive for UP3 (red) with significant number of adherent bacteria (DNA, green); (B) White blood cells with adherent or phagocytosed bacteria, suggesting activation of the immune response. Bacteria were labeled with eubacterial peptide nucleic acid-fluorescence *in situ* hybridization probe (PNA-FISH) (eubacteria, red); (C) Bacteria (DNA, green) enveloped in UP3 (red), signaling association with cells of the urinary tract. UP3 = Uroplakin III.

with urinary leukocyte and epithelial cell counts from freshly collected urine specimens. These measures are subsequently collected at each clinic visit, allowing the practitioner to monitor treatment success or failure. The therapeutic agents utilized are treatment dose, first-generation, narrow-spectrum antibiotics (cefalexin, trimethoprim, and nitrofurantoin), and a urinary antiseptic, methenamine hippurate. Treatment ceases when symptoms have reduced with zero pyuria, upon which drug withdrawal is trialed. Treatment is reinstated if relapse occurs, with most of the patients requiring multiple rounds to attain long-lasting symptom resolution. This published treatment protocol[12,13] was described in a study carried out over a ten-year period in 624 women diagnosed with chronic UTI. Across 273,762 treatment days, 83% showed a clinically significant reduction in symptoms and absence of pyuria at the end of the treatment. Importantly, prolonged courses of treatment dose antibiotics did not generate antimicrobial resistance. A randomized placebo-controlled trial for this novel regimen for the treatment of chronic UTI is planned for the near future. Thus far, however, this protocol appears to be clinically effective. The majority of this patient cohort (73%) shows signs of a chronic disorder, deduced from the persistent symptoms reported between episodic flares of UTI, and that only gradually abate on the road to recovery following treatment with antibiotics[12] (Fig. 3). This contrasts with the definition of recurrent UTI, described as two or more UTIs in 6 months or three or more UTIs in the past 1 year[4], suggesting a convalescent symptom-free period in between episodes. A prospective longitudinal study that samples LUTS scores and urinary cell counts from individuals with UTI may be able to elucidate if these two presentations are different from each other. It is unclear whether a rigid definition based on number of UTIs within a specified duration is meaningful in the clinical management of individuals who experience debilitating effects of repeated UTIs.

CONCLUDING REMARKS

Chronic and recurrent UTIs are responsible for significant suffering in those affected and account for substantial costs to healthcare systems globally. Non-antibiotic alternatives are continually being designed[112] for the treatment of UTI, but their development is in its infancy. The success of these therapies could be accelerated with detailed mechanistic knowledge of UTI pathogenesis and host immune responses. Studies in humans and/or based on human samples are crucial to our understanding of the clinical presentations of chronic UTI. This will aid in strategizing a more targeted approach in selecting antimicrobial agents or designing immunomodulatory therapies to treat these patients. To avoid antimicrobial treatment in the absence of infection or *vice versa*, a more accurate and faster diagnostic protocol is immensely important to identify individuals with infectious diseases. It is imperative that healthcare guidelines go hand in hand with scientific progress and advocate for more per-

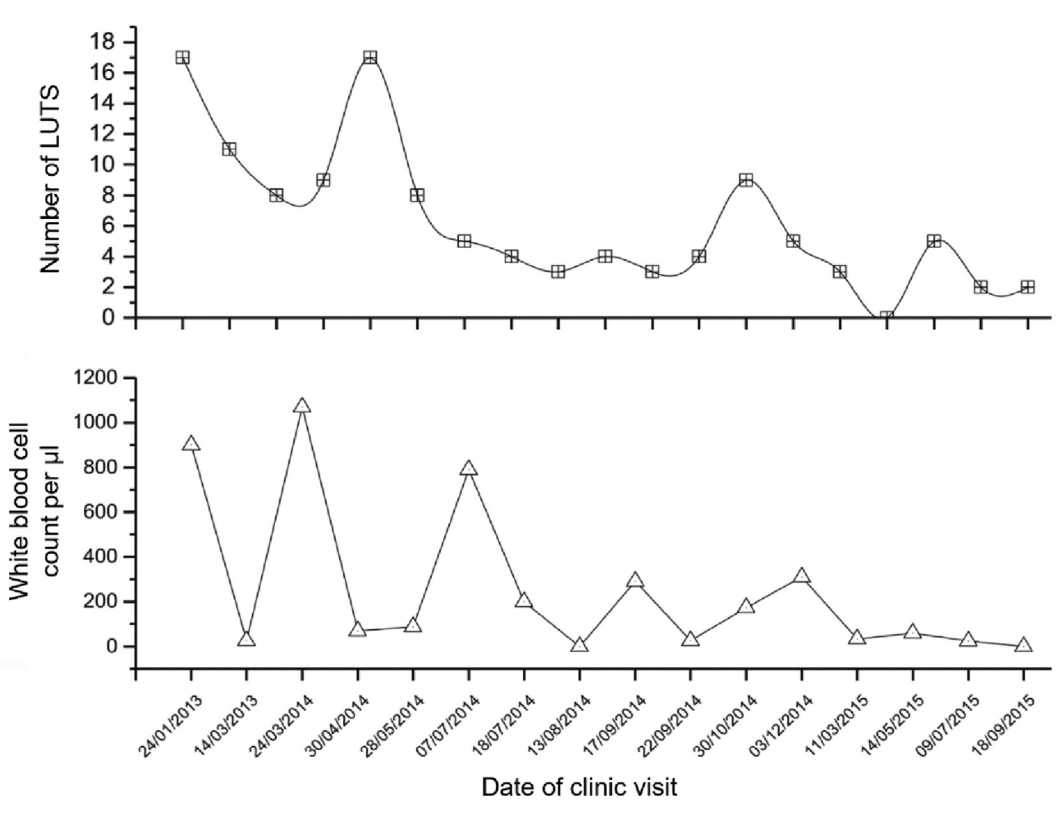

Fig. 3 The number of LUTS (upper graph) and urinary white blood cell count (lower graph) across an extended period of narrow-spectrum antibiotic treatment in a representative chronic UTI patient. Both graphs showed damped oscillation patterns with gradually diminishing magnitude of symptoms and pyuria. Note that symptoms were still reported between UTI episodes with accompanying pyuria; both parameters approached zero only towards the end of the treatment, indicating recovery. Figure adapted from Swamy, Barcella[12]. LUTS = lower urinary tract symptoms; UTI = urinary tract infection.

ceptive clinical management in adults and children. In the context of UTI, treatment with appropriate antibiotics at treatment doses and at the right time not only improves prognosis for the patients but also promotes antibiotic stewardship. With such reformative goals in mind, it is hoped that the incidence of chronic UTI can be reduced and, as a global research community, we can significantly improve the lives of those afflicted with this disease.

AUTHOR CONTRIBUTIONS

CC and HH conceptualized the manuscript. All authors contributed to the writing, review, editing, and final approval of the manuscript.

FUNDING

This work is funded by charitable donations to HH and RK.

COMPETING INTEREST

The authors declare no competing interests.

ACKNOWLEDGMENTS

The authors dedicate this manuscript to the memory of Professor James Malone-Lee. Prof's unwavering commitment to educating clinicians and scientists on applying science-backed approaches in the diagnosis and treatment of chronic urinary tract infection helped many suffering from this disease. We honor him as a mentor, leader, and friend.

REFERENCES

1. Zeng, Z., Zhan, J., Zhang, K., Chen, H. & Cheng, S. Global, regional, and national burden of urinary tract infections from 1990 to 2019: an analysis of the global burden of disease study 2019. *World J. Urol.* **40**, 755–763 (2022).
2. Shaikh, N., Morone, N. E., Bost, J. E. & Farrell, M. H. Prevalence of urinary tract infection in childhood: a meta-analysis. *Pediatr. Infect. Dis. J.* **27**, 302–308 (2008).
3. Foxman, B., Barlow, R., D'Arcy, H., Gillespie, B. & Sobel, J. D. Urinary tract infection: self-reported incidence and associated costs. *Ann. Epidemiol.* **10**, 509–515 (2000).
4. Bonkat, G. et al. EAU guidelines on urological infections. Edn. presented at the EAU Annual Congress Amsterdam, the Netherlands 2022. (EAU Guidelines Office, Arnhem, 2022).
5. Foxman, B. Recurring urinary tract infection: incidence and risk factors. *Am. J. Public Health* **80**, 331–333 (1990).
6. Keren, R. et al. Risk factors for recurrent urinary tract infection and renal scarring. *Pediatrics* **136**, e13–e21 (2015).
7. Hooton, T. M. Recurrent urinary tract infection in women. *Int. J. Antimicrob. Agents* **17**, 259–268 (2001).
8. Hunstad, D. A. & Justice, S. S. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. *Annu. Rev. Microbiol.* **64**, 203–221 (2010).
9. Pouwels, K. B., Visser, S. T. & Hak, E. Effect of pravastatin and fosinopril on recurrent urinary tract infections. *J. Antimicrob. Chemother.* **68**, 708–714 (2013).
10. Lelie-van der Zande, R. C., Bouvy, M. L. & Teichert, A. M. Patterns of recurring dispensing of guideline antibiotics for uncomplicated urinary tract infection in women over a 5-year period: longitudinal patterns of recurring dispensings of Dutch guideline UTI antibiotics. *Fam. Pract.* **38**, 286–291 (2021).
11. Renard, J. et al. Recurrent lower urinary tract infections have a detrimental effect on patient quality of life: a prospective, observational study. *Infect. Dis. Ther.* **4**, 125–135 (2014).
12. Swamy, S. et al. Recalcitrant chronic bladder pain and recurrent cystitis but negative urinalysis: what should we do? *Int. Urogynecol. J.* **29**, 1035–1043 (2018).
13. Swamy, S. et al. Cross-over data supporting long-term antibiotic treatment in patients with painful lower urinary tract symptoms, pyuria and negative urinalysis. *Int. Urogynecol. J.* **30**, 409–414 (2019).
14. Anderson, G. G., Martin, S. M. & Hultgren, S. J. Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes Infect.* **6**, 1094–1101 (2004).
15. Chan, C. Y., St John, A. L. & Abraham, S. N. Mast cell interleukin-10 drives localized tolerance in chronic bladder infection. *Immunity* **38**, 349–359 (2013).
16. Wu, J. et al. A highly polarized Th2 bladder response to infection promotes epithelial repair at the expense of preventing new infections. *Nat. Immunol.* **21**, 671–683 (2020).
17. Hannan, T. J., Mysorekar, I. U., Hung, C. S., Isaacson-Schmid, M. L. & Hultgren, S. J. Early severe inflammatory responses to uropathogenic *E. coli* predispose to chronic and recurrent urinary tract infection. *PLoS Pathog.* **6**, e1001042 (2010).
18. Mestas, J. & Hughes, C. C. W. Of mice and not men: differences between mouse and human immunology. *J. Immunol.* **172**, 2731–2738 (2004).
19. Rasquinha, M. T., Sur, M., Lasrado, N. & Reddy, J. IL-10 as a Th2 cytokine: differences between mice and humans. *J. Immunol.* **207**, 2205–2215 (2021).
20. National Institute for Health and Care Excellence Urinary tract infection (lower): antimicrobial prescribing. (National Institute for Health and Care Excellence, London, 2018).
21. Ikäheimo, R. et al. Recurrence of urinary tract infection in a primary care setting: analysis of a 1-year follow-up of 179 women. *Clin. Infect. Dis.* **22**, 91–99 (1996).
22. Foxman, B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am. J. Med.* **113**, 5S–13S (2002).
23. Khasriya, R. et al. The inadequacy of urinary dipstick and microscopy as surrogate markers of urinary tract infection in urological outpatients with lower urinary tract symptoms without acute frequency and dysuria. *J. Urol.* **183**, 1843–1847 (2010).
24. Kupelian, A. S. et al. Discrediting microscopic pyuria and leucocyte esterase as diagnostic surrogates for infection in patients with lower urinary tract symptoms: results from a clinical and laboratory evaluation. *BJU Int.* **112**, 231–238 (2013).
25. Sathiananthamoorthy, S. et al. Reassessment of routine midstream culture in diagnosis of urinary tract infection. *J. Clin. Microbiol.* **57**, e01452–e10518 (2019).
26. Engeler, D. et al. EAU guidelines on chronic pelvic pain. Edn. presented at the EAU Annual Congress Amsterdam 2022. (EAU Guidelines Office, Arnhem, 2022).
27. Harding, C. K. et al. EAU guidelines on management of non-neurogenic female lower urinary tract symptoms. Edn. presented at the EAU Annual Congress Amsterdam March 2022. ((EAU Guidelines OFfice, Arnhem, 2022).
28. Kim, K. S. et al. A prospective multi-center trial of *Escherichia coli* extract for the prophylactic treatment of patients with chronically recurrent cystitis. *J. Korean Med. Sci.* **25**, 435–439 (2010).
29. UK Standards of Microbiology Investigations. Investigation of urine. (Public Health, England, 2019). **B41**, 1–51.
30. Fouts, D. E. et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J. Transl. Med.* **10**, 174 (2012).
31. Hilt, E. E. et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J. Clin. Microbiol.* **52**, 871–876 (2014).
32. Mulvey, M. A. et al. Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* **282**, 1494–1497 (1998).
33. Mulvey, M. A., Schilling, J. D. & Hultgren, S. J. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect. Immun.* **69**, 4572–4579 (2001).
34. Anderson, G. G. et al. Intracellular bacterial biofilm-like pods in urinary tract infections. *Science* **301**, 105–107 (2003).
35. Justice, S. S. et al. Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Proc. Natl Acad. Sci. U. S. A.* **101**, 1333–1338 (2004).
36. O'Brien, V. P. et al. A mucosal imprint left by prior *Escherichia coli* bladder infection sensitizes to recurrent disease. *Nat. Microbiol.* **2**, 16196 (2016).
37. Rosen, D. A., Hooton, T. M., Stamm, W. E., Humphrey, P. A. & Hultgren, S. J. Detection of intracellular bacterial communities in human urinary tract infection. *PLoS Med.* **4**, e329 (2007).
38. Horsley, H. et al. *Enterococcus faecalis* subverts and invades the host urothelium in patients with chronic urinary tract infection. *PLoS One* **8**, e83637 (2013).
39. Khasriya, R. et al. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. *J. Clin. Microbiol.* **51**, 2054–2062 (2013).
40. Horsley, H., Dharmasena, D., Malone-Lee, J. & Rohn, J. L. A urine-dependent human urothelial organoid offers a potential alternative to rodent models of infection. *Sci. Rep.* **8**, 1238 (2018).
41. Schilling, J. D., Lorenz, R. G. & Hultgren, S. J. Effect of trimethoprim-sulfamethoxazole on recurrent bacteriuria and bacterial persistence in mice

infected with uropathogenic *Escherichia coli*. *Infect. Immun.* **70**, 7042–7049 (2002).
42. Horsley, H. et al. Ultrasound-activated microbubbles as a novel intracellular drug delivery system for urinary tract infection. *J. Control. Release* **301**, 166–175 (2019).
43. Lau, W. K. et al. Novel antibiotic-loaded particles conferring eradication of deep tissue bacterial reservoirs for the treatment of chronic urinary tract infection. *J. Control. Release* **328**, 490–502 (2020).
44. Amna, M. A., Chazan, B., Raz, R., Edelstein, H. & Colodner, R. Risk factors for non-*Escherichia coli* community-acquired bacteriuria. *Infection* **41**, 473–477 (2013).
45. Wolfe, A. J. et al. Evidence of uncultivated bacteria in the adult female bladder. *J. Clin. Microbiol.* **50**, 1376–1383 (2012).
46. Pearce, M. M. et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *MBio* **5**, e01283–e11214 (2014).
47. Moustafa, A. et al. Microbial metagenome of urinary tract infection. *Sci. Rep.* **8**, 4333 (2018).
48. Price, T. K. et al. The urobiome of continent adult women: a cross-sectional study. *BJOG* **127**, 193–201 (2020).
49. Worby, C. J. et al. Longitudinal multi-omics analyses link gut microbiome dysbiosis with recurrent urinary tract infections in women. *Nat. Microbiol.* **7**, 630–639 (2022).
50. Bair, K. L. & Campagnari, A. A. *Moraxella catarrhalis* promotes stable polymicrobial biofilms with the major otopathogens. *Front. Microbiol.* **10**, 3006 (2019).
51. Tittel, A. P. et al. Kidney dendritic cells induce innate immunity against bacterial pyelonephritis. *J. Am. Soc. Nephrol.* **22**, 1435–1441 (2011).
52. Shaikh, N. et al. Host and Bacterial Markers that Differ in Children with Cystitis and Pyelonephritis. *J. Pediatr.* **209**, 146–153.e1 (2019).
53. Steiner, S. E. et al. UPEC kidney infection triggers neuro-immune communication leading to modulation of local renal inflammation by splenic IFNγ. *PLoS Pathog.* **17**, e1009553 (2021).
54. Agace, W. W., Hedges, S. R., Ceska, M. & Svanborg, C. Interleukin-8 and the neutrophil response to mucosal gram-negative infection. *J. Clin. Invest.* **92**, 780–785 (1993).
55. Agace, W. W. The role of the epithelial cell in *Escherichia coli* induced neutrophil migration into the urinary tract. *Eur. Respir. J.* **9**, 1713–1728 (1996).
56. Hang, L. et al. Macrophage inflammatory protein-2 is required for neutrophil passage across the epithelial barrier of the infected urinary tract. *J. Immunol.* **162**, 3037–3044 (1999).
57. Haraoka, M. et al. Neutrophil recruitment and resistance to urinary tract infection. *J. Infect. Dis.* **180**, 1220–1229 (1999).
58. Spencer, J. D., Schwaderer, A. L., Becknell, B., Watson, J. & Hains, D. S. The innate immune response during urinary tract infection and pyelonephritis. *Pediatr. Nephrol.* **29**, 1139–1149 (2014).
59. Thumbikat, P., Waltenbaugh, C., Schaeffer, A. J. & Klumpp, D. J. Antigen-specific responses accelerate bacterial clearance in the bladder. *J. Immunol.* **176**, 3080–3086 (2006).
60. Choi, H. W. et al. Loss of bladder epithelium induced by cytolytic mast cell granules. *Immunity* **45**, 1258–1269 (2016).
61. Jost, S. P. Cell cycle of normal bladder urothelium in developing and adult mice. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **57**, 27–36 (1989).
62. Mysorekar, I. U., Mulvey, M. A., Hultgren, S. J. & Gordon, J. I. Molecular regulation of urothelial renewal and host defenses during infection with uropathogenic *Escherichia coli*. *J. Biol. Chem.* **277**, 7412–7419 (2002).
63. Schilling, J. D., Mulvey, M. A., Vincent, C. D., Lorenz, R. G. & Hultgren, S. J. Bacterial invasion augments epithelial cytokine responses to *Escherichia coli* through a lipopolysaccharide-dependent mechanism. *J. Immunol.* **166**, 1148–1155 (2001).
64. Jhang, J. F. et al. Deficits of urothelial cell proliferation, cytoskeleton, and barrier function protein expressions in patients with recurrent and persistent urinary tract infections. *Low. Urin. Tract Symptoms* **13**, 203–209 (2021).
65. Wu, J., Bao, C., Reinhardt, R. L. & Abraham, S. N. Local induction of bladder Th1 responses to combat urinary tract infections. *Proc. Natl Acad. Sci. U. S. A.* **118**, e2026461118 (2021).
66. Del Prete, G. et al. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J. Immunol.* **150**, 353–360 (1993).
67. Hannan, T. J. et al. Inhibition of cyclooxygenase-2 prevents chronic and recurrent cystitis. *EBioMedicine* **1**, 46–57 (2014).
68. Yu, L. et al. Mucosal infection rewires TNF signaling dynamics to skew susceptibility to recurrence. *Elife* **8**, e46677 (2019).
69. Rydell-Törmänen, K. & Johnson, J. R. The applicability of mouse models to the study of human disease. In *Mouse cell culture: methods and protocols* (Bertoncello, I., ed 3–22 (Springer, New York, 2019).
70. Molehin, A. J. Current understanding of immunity against schistosomiasis: impact on vaccine and drug development. *Res. Rep. Trop. Med.* **11**, 119–128 (2020).
71. Doeing, D. C., Borowicz, J. L. & Crockett, E. T. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clin. Pathol.* **3**, 3 (2003).
72. Rosenthal, M. & Plattner, S. The treatment of rheumatoid arthritis with OM-8930, a bacterial immunostimulating agent. *Z. Rheumatol.* **40**, 228–231 (1981).
73. Frey, C., Obolensky, W. & Wyss, H. Treatment of recurrent urinary tract infections: efficacy of an orally administered biological response modifier. *Urol. Int.* **41**, 444–446 (1986).
74. Huber, M. et al. Immunostimulation by bacterial components: II. Efficacy studies and meta-analysis of the bacterial extract OM-89. *Int. J. Immunopharmacol* **22**, 1103–1111 (2000).
75. Van Pham, T., Kreis, B., Corradin-Betz, S., Bauer, J. & Mauël, J. Metabolic and functional stimulation of lymphocytes and macrophages by an *Escherichia coli* extract (OM-89): *in vitro* studies. *J. Biol. Response Mod.* **9**, 231–240 (1990).
76. Schmidhammer, S. et al. An *Escherichia coli*-based oral vaccine against urinary tract infections potently activates human dendritic cells. *Urology* **60**, 521–526 (2002).
77. Tammen, H. Immunobiotherapy with Uro-Vaxom in recurrent urinary tract infection. The German Urinary Tract Infection Study Group. *Br. J. Urol.* **65**, 6–9 (1990).
78. Magasi, P., Pánovics, J., Illés, A. & Nagy, M. Uro-Vaxom and the management of recurrent urinary tract infection in adults: a randomized multicenter double-blind trial. *Eur. Urol.* **26**, 137–140 (1994).
79. Bauer, H.W. et al. A long-term, multicenter, double-blind study of an Escherichia coli extract (OM-89) in female patients with recurrent urinary tract infections. *Eur. Urol.* **47**, 542–548; discussion 548 (2005).
80. Brodie, A., El-Taji, O., Jour, I., Foley, C. & Hanbury, D. A retrospective study of immunotherapy treatment with Uro-Vaxom (OM-89®) for prophylaxis of recurrent urinary tract infections. *Curr Urol* **14**, 130–134 (2020).
81. Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. *Nat. Rev. Immunol.* **21**, 83–100 (2021).
82. Reynolds, C. J. et al. Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants. *Science* **375**, 183–192 (2022).
83. Kwok, W. Y. et al. Incidence rates and management of urinary tract infections among children in Dutch general practice: results from a nation-wide registration study. *BMC Pediatr.* **6**, 10 (2006).
84. Korbel, L., Howell, M. & Spencer, J. D. The clinical diagnosis and management of urinary tract infections in children and adolescents. *Paediatr Int Child Health* **37**, 273–279 (2017).
85. Expert Panel on Pediatric Imaging et al. ACR Appropriateness Criteria® urinary tract infection-child. *J. Am. Coll. Radiol.* **14**, S362–S371 (2017).
86. Expert Panel on Urological Imaging et al. ACR Appropriateness Criteria® recurrent lower urinary tract infections in females. *J. Am. Coll. Radiol.* **17**, S487–S496 (2020).
87. Centers for Disease Control and Prevention. Chapter 7: Urinary Tract Infection (UTI) Event. (National Healthcare Safety Network, 2022).
88. Kass, E. H. Asymptomatic infections of the urinary tract. *Trans Assoc Am Physians* **69**, 56–64 (1956).
89. Stamm, W. E. et al. Diagnosis of coliform infection in acutely dysuric women. *N. Engl. J. Med.* **307**, 463–468 (1982).
90. Miller, J. M. et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clin. Infect. Dis.* **67**, e1–e94 (2018).
91. Hochstedler, B. R. et al. Urinary microbiota of women with recurrent urinary tract infection: collection and culture methods. *Int. Urogynecol. J.* **33**, 563–570 (2022).
92. Radmayr, C. et al. EAU guidelines on paediatric urology. Edn. presented at the EAU Annual Congress Amsterdam, the Netherlands 2022. (EAU Guidelines Office, Arnhem, 2022).
93. National Institute for Health and Care Excellence Urinary tract infection in under 16s: diagnosis and management. *EDN*. (National Institute for Health and Care Excellence, London, 2022).
94. Patel, H. D., Livsey, S. A., Swann, R. A. & Bukhari, S. S. Can urine dipstick testing for urinary tract infection at point of care reduce laboratory workload? *J. Clin. Pathol.* **58**, 951–954 (2005).
95. Bellazreg, F. et al. Diagnostic value of dipstick test in adult symptomatic urinary tract infections: results of a cross-sectional Tunisian study. *Pan Afr. Med. J.* **33**, 131 (2019).
96. Mambatta, A. K. et al. Reliability of dipstick assay in predicting urinary tract infection. *J. Family Med. Prim. Care* **4**, 265–268 (2015).

97. Fisher, H. et al. Continuous low-dose antibiotic prophylaxis for adults with repeated urinary tract infections (AnTIC): a randomised, open-label trial. *Lancet Infect. Dis.* **18**, 957–968 (2018).
98. O'Brien, T. J., Figueroa, W. & Welch, M. Decreased efficacy of antimicrobial agents in a polymicrobial environment. *ISME J.* **16**, 1694–1704 (2022).
99. O'Brien, T. J. & Welch, M. A continuous-flow model for *in vitro* cultivation of mixed microbial populations associated with cystic fibrosis airway infections. *Front. Microbiol.* **10**, 2713 (2019).
100. Nabb, D. L. et al. Polymicrobial interactions induce multidrug tolerance in *Staphylococcus aureus* through energy depletion. *Front. Microbiol.* **10**, 2803 (2019).
101. Leibovitz, E. et al. Recurrent acute otitis media occurring within one month from completion of antibiotic therapy: relationship to the original pathogen. *Pediatr. Infect. Dis. J.* **22**, 209–216 (2003).
102. Leydon, G. M., Turner, S., Smith, H., Little, P. & UTIS team Women's views about management and cause of urinary tract infection: qualitative interview study. *BMJ* **340**, c279 (2010).
103. Knottnerus, B. J., Geerlings, S. E., Moll van Charante, E. P. & ter Riet, G. Women with symptoms of uncomplicated urinary tract infection are often willing to delay antibiotic treatment: a prospective cohort study. *BMC Fam. Pract.* **14**, 71 (2013).
104. Gupta, K. et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin. Infect. Dis.* **52**, e103–e120 (2011).
105. McEwen, L. N., Farjo, R. & Foxman, B. Antibiotic prescribing for cystitis: how well does it match published guidelines? *Ann. Epidemiol.* **13**, 479–483 (2003).
106. Chardavoyne, P. C. & Kasmire, K. E. Appropriateness of antibiotic prescriptions for urinary tract infections. *West. J. Emerg. Med.* **21**, 633–639 (2020).
107. Copp, H. L., Shapiro, D. J. & Hersh, A. L. National ambulatory antibiotic prescribing patterns for pediatric urinary tract infection, 1998–2007. *Pediatrics* **127**, 1027–1033 (2011).
108. Hopkins, W. J., Uehling, D. T. & Balish, E. Local and systemic antibody responses accompany spontaneous resolution of experimental cystitis in cynomolgus monkeys. *Infect. Immun.* **55**, 1951–1956 (1987).
109. Collins, L., Sathiananthamoorthy, S., Rohn, J. & Malone-Lee, J. A revalidation and critique of assumptions about urinary sample collection methods, specimen quality and contamination. *Int. Urogynecol. J.* **31**, 1255–1262 (2020).
110. Wu, X. R., Kong, X. P., Pellicer, A., Kreibich, G. & Sun, T. T. Uroplakins in urothelial biology, function, and disease. *Kidney Int.* **75**, 1153–1165 (2009).
111. Khasriya, R. et al. Lower urinary tract symptoms that predict microscopic pyuria. *Int. Urogynecol. J.* **29**, 1019–1028 (2018).
112. Kenneally, C., Murphy, C. P., Sleator, R. D. & Culligan, E. P. The urinary microbiome and biological therapeutics: novel therapies for urinary tract infections. *Microbiol. Res.* **259**, 127010 (2022).
113. Krebs, J., Stoyanov, J., Wöllner, J., Valido, E. & Pannek, J. Immunomodulation for primary prevention of urinary tract infections in patients with spinal cord injury during primary rehabilitation: protocol for a randomized placebo-controlled pilot trial (UROVAXOM-pilot). *Trials* **22**, 677 (2021).
114. Schulman, C. C., Corbusier, A., Michiels, H. & Taenzer, H. J. Oral immunotherapy of recurrent urinary tract infections: a double-blind placebo-controlled multicenter study. *J. Urol.* **150**, 917–921 (1993).
115. Wagenlehner, F. M. et al. A randomized, double-blind, parallel-group, multicenter clinical study of *Escherichia coli*-lyophilized lysate for the prophylaxis of recurrent uncomplicated urinary tract infections. *Urol. Int.* **95**, 167–176 (2015).
116. Lorenzo-Gómez, M. F. et al. Comparison of sublingual therapeutic vaccine with antibiotics for the prophylaxis of recurrent urinary tract infections. *Front. Cell. Infect. Microbiol.* **5**, 50 (2015).
117. Lorenzo-Gómez, M. F. et al. Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus prophylactic treatment with antibiotics. *Int. Urogynecol. J.* **24**, 127–134 (2013).
118. Yang, B. & Foley, S. First experience in the UK of treating women with recurrent urinary tract infections with the bacterial vaccine Uromune®. *BJU Int.* **121**, 289–292 (2018).
119. Frenck Jr, R. W. et al. Safety and immunogenicity of a vaccine for extra-intestinal pathogenic *Escherichia coli* (ESTELLA): a phase 2 randomised controlled trial. *Lancet Infect. Dis.* **19**, 631–640 (2019).
120. Hopkins, W. J., Elkahwaji, J., Beierle, L. M., Leverson, G. E. & Uehling, D. T. Vaginal mucosal vaccine for recurrent urinary tract infections in women: results of a phase 2 clinical trial. *J. Urol.* **177**, 1349–53; quiz 1591. (2007).
121. Uehling, D. T., Hopkins, W. J., Balish, E., Xing, Y. & Heisey, D. M. Vaginal mucosal immunization for recurrent urinary tract infection: phase II clinical trial. *J. Urol.* **157**, 2049–2052 (1997).
122. Uehling, D. T., Hopkins, W. J., Elkahwaji, J. E., Schmidt, D. M. & Leverson, G. E. Phase 2 clinical trial of a vaginal mucosal vaccine for urinary tract infections. *J. Urol.* **170**, 867–869 (2003).
123. Nestler, S. et al. Efficacy of vaccination with StroVac for recurrent urinary tract infections in women: a comparative single-centre study. *Int. Urol. Nephrol.* **53**, 2267–2272 (2021).