REVIEW ARTICLE

The microbiome and host mucosal interactions in urinary tract diseases

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The urinary tract consists of the bladder, ureters, and kidneys, and is an essential organ system for filtration and excretion of waste products and maintaining systemic homeostasis. In this capacity, the urinary tract is impacted by its interactions with other mucosal sites, including the genitourinary and gastrointestinal systems. Each of these sites harbors diverse ecosystems of microbes termed the microbiota, that regulates complex interactions with the local and systemic immune system. It remains unclear whether changes in the microbiota and associated metabolites may be a consequence or a driver of urinary tract diseases. Here, we review the current literature, investigating the impact of the microbiota on the urinary tract in homeostasis and disease including urinary stones, acute kidney injury, chronic kidney disease, and urinary tract infection. We propose new avenues for exploration of the urinary microbiome using emerging technology and discuss the potential of microbiome-based medicine for urinary tract conditions.

Mucosal Immunology (2021) 14:779–792; <https://doi.org/10.1038/s41385-020-00372-5>

INTRODUCTION

It has become apparent that the microbiome in humans is involved in both the maintenance of health and susceptibility to disease at mucosal sites.¹ While the substantial focus has been placed on understanding the microbial communities that inhabit the gastrointestinal tract,^{2–4} increasing evidence supports the existence of a genitourinary microbiome.⁵ Findings obtained from urine microbial culturing,^{6,7} bacterial genomic sequencing,^{8–10} and metagenomics^{11,12} have highlighted differences in the urinary microbiome in those individuals with urological conditions compared to healthy individuals. This overturns the long-standing belief that the bladder is a sterile environment.^{7,8,13} The increasing availability of 16S ribosomal RNA (rRNA) amplicon sequencing and improved efficiency of shotgun metagenomic and whole-genome sequencing has facilitated a more in-depth understanding of the microbiome. It has now been shown that the urine in the bladder contains a variety of Gram-positive organisms such as *Lactobacilli*, *Streptococci*, and *Staphylococci*.¹⁴ Application of these emerging technologies may shed light on host–microbe interactions within the urinary tract during health and disease.

Despite the significant health and financial burden of recurrent urinary tract infection (rUTI), antibiotic therapy remains the mainstay treatment, contributing to the rise of multidrug-resistant uropathogens.^{15–17} Therefore, there is a clear unmet need to develop novel diagnostic approaches and therapeutic interventions to complement existing antimicrobial therapies. This review aims to summarize the current insights into the

gastrointestinal, vaginal and urinary, microbiomes, and how they may influence the pathogenesis of urinary tract diseases. We also discuss emerging tools and technologies for the urinary microbiome field and how these methods could be integrated to enable the development of novel therapeutic interventions and obtain a better understanding of host–microbe responses in maintaining urinary tract health.

INTERCONNECTION BETWEEN THE GASTROINTESTINAL AND URINARY MICROBIOTA

The gastrointestinal tract is commonly considered to be the origin of most bacterial infections in the urinary tract. *Escherichia coli* is the dominant uropathogen and establishes reservoirs inside the bladder lining. Nielsen et al. investigated reservoirs of *E. coli* from UTI patients compared to healthy individuals with no history of UTI and found that mutations in the bacterial adhesion molecule FimH, were significantly associated with UTI.¹⁸ The fecal flora is also a common reservoir of uropathogenic *E. coli* (UPEC). Magruder et al. assessed the link between the gastrointestinal microbiome and the likelihood of contracting a UTI. They report that the abundance of uropathogens in the gastrointestinal tract is associated with an increased risk of developing a UTI.¹⁹ Analysis of fecal specimens and urine supernatants via metagenomic sequencing identified a 1% relative abundance of *E. coli* in the gastrointestinal tract as an independent risk factor for UTI.¹⁹ To assess the dynamics of pathogen persistence in the urinary tract,

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Received: 30 June 2020 Accepted: 3 December 2020

Published online: 4 February 2021

Thänert et al. assessed uropathogen carriage in both the urinary and gastrointestinal tracts of patients with rUTI.²⁰ Clonal tracking demonstrated that transmission of uropathogens between gastrointestinal reservoirs and the urinary tract were common and that UTI was commonly preceded by an intestinal bloom of uropathogens in the same individual.²⁰ These relationships are not limited to pathogenic bacteria. Combined culturing and high-resolution genomic analysis identified similarities between the vaginal and the urinary microbial strains (*Lactobacillus* spp., *E. coli*, and *Streptococcus anginosus*), further supporting the existence of an interlinked urogenital microbiome in women.⁵

INTERCONNECTION BETWEEN THE VAGINAL AND URINARY MICROBIOTA

Vaginal and urinary microbial communities and the relationship between these mucosal sites are becoming increasingly well-characterized and have been recently reviewed.²¹ Both culturing assays and metagenomic analyses have revealed the existence of an interconnected urogenital microbiota.^{5,22} This relationship is further supported by studies from Komesu et al. who demonstrate associations between the vaginal and urinary microbiota, with *Lactobacillus* commonly found at both sites.²³ Transurethral inoculation of the common vaginal microbe *Gardnerella vaginalis*, to the bladder of mice that have recovered from a UPEC infection, demonstrated that *G. vaginalis* can cause urothelial exfoliation, which in turn leads to the exposure of latent *E. coli* reservoirs.²⁴ Mice that went on to experience *Gardnerella*-induced rUTI had more severe primary infection with *E. coli* compared to animals that did not experience rUTI with *G. vaginalis*, suggesting that the severity of primary *E. coli* UTI may be a key determinant of rUTI risk upon secondary exposure.²⁴

Analysis of the urinary microbiota of healthy individuals compared to women with bacterial vaginosis, demonstrated that oral antibiotic treatment significantly reduced the relative abundance of some bacterial species including *G. vaginalis*, *Atopobium vaginae*, and *Sneathia amnii*, while increasing others such as *Lactobacillus iners*.²⁵ Antimicrobial therapy was also associated with differences in the beta-diversity, but not alpha-diversity of the genitourinary microbiota.²⁶ These data suggest that intervention with antimicrobial drugs alters the composition of the genitourinary microbiota²⁶ and highlights the need to study how the manipulation of the urogenital microbiome may impact on treatment strategies for urinary tract diseases.

INTERACTION BETWEEN THE GASTROINTESTINAL MICROBIOTA AND THE URINARY TRACT IN HOMEOSTASIS

Fundamental studies in *Drosophila* have revealed that renal filtration of bacterial peptidoglycan, derived from the microbiota, promotes homeostasis and prevents deleterious host immune-driven responses.²⁷ *Drosophila* deficient in the transcription factor Kruppel Like Factor 15 (*Klf15*) lacked nephrocytes and displayed increased resistance to infection, compared to their wild-type counterparts, but *Klf15*-deficient flies had a shorter lifespan.²⁷ In wild-type flies, functional nephrocytes were able to effectively remove peptidoglycan from the circulation.²⁷ These fundamental studies in *Drosophila* have since been advanced by experimental rodent models and clinical human studies, showing that the kidneys may sense changes in the levels of metabolites derived from the gastrointestinal microbiome.²⁸ However, the role of this gastrointestinal–kidney axis in the pathogenesis of urinary tract disease remains to be elucidated.

The gastrointestinal microbiota also impacts urinary bladder physiology under homeostatic conditions.²⁹ Mice harboring minimal bacteria, as a result of treatment with broad-spectrum antibiotics and mice completely lacking a microbiota through rederivation in germ-free facilities, exhibit reduced urinary bladder

size and weight that was not associated with any obvious histological changes to the bladder.²⁹ Transcriptomic analysis of the mouse bladder identified 97 genes that were differentially expressed in germ-free mice compared to specific pathogen-free mice.²⁹ These included genes regulating circadian rhythm, e.g., downregulation of aryl hydrocarbon receptor nuclear translocator like (*Arntl*); extracellular matrix proteins, e.g., upregulation of matrix metalloproteinase 12 (*Mmp12*); and neuromuscular synaptic transmission, e.g., upregulation of Tyrosine hydroxylase.²⁹ Microbiota-deficient mice displayed no change in the expression of genes in the bladder that are known to be responsible for recognizing microbes or their products.²⁹ These data indicate that the gastrointestinal microbiota has an impact in multiple physiological processes in the urinary tract under homeostatic conditions.

ALTERATIONS IN THE GASTROINTESTINAL AND VAGINAL MICROBIOME ARE ASSOCIATED WITH DISEASES OF THE URINARY TRACT

In addition to contributing to urinary tract homeostasis, substantial emerging evidence demonstrates a strong association between the urinary, gastrointestinal and vaginal microbiomes, and various urinary tract diseases (Table 1).

Kidney stone disease

In the last 50 years, the incidence of kidney stone diseases has increased by more than fourfold.³⁰ In the urinary tract, oxalate can combine with calcium to form calcium oxalate stones, which make up the vast majority of kidney stone episodes in humans.³¹ The enzymes that are required to metabolize oxalate are absent in mammals, who instead rely on their gastrointestinal microbiota for oxalate metabolism.³²

Mouse models of fecal transplant suggest that the gastrointestinal microbiome may modulate the risk of developing urinary stones.³³ Fecal transplantation from Zucker lean rats into germ-free mice by oral gavage, reduced both oxalate and calcium levels in the urine, which were negatively correlated with the abundance of the *Clostridiaceae* family.³³ Furthermore, fecal transplant increased the expression of the oxalate transporter gene, Solute Carrier Family 26 Member 6.³³ Miller et al. assessed oxalate excretion in rats following the oral transfer of fecal-derived microbes from a donor animal that was adapted to a high oxalate diet.³⁴ Transplantation of feces containing oxalate-degrading bacteria to rats was found to increase oxalate metabolism,³⁴ whereas rats that received a fecal transplant had a more diverse bacterial network, consisting of more oxalate-degrading bacteria, than controls.³⁴ Further studies demonstrated that antibiotic exposure in mice caused a disruption in the gastrointestinal microbiota and oxalate metabolism following fecal transplantation, however, the microbial population was able to recover with time.³⁵ In contrast, exposure to a high fat and high sugar diet resulted in a loss of the transplanted bacteria population and decreased oxalate metabolism.³⁵

In humans, the fecal microbiota composition of patients that experience kidney stones also showed reduced bacterial diversity compared to healthy controls.³⁶ In these patients a reduction in *Oxalobacter formigenes*, a known oxalate-metabolizing bacterial species, was observed compared to healthy controls.³⁷ In similar clinical studies, *Gardnerella* dominated in healthy controls, whereas *Staphylococcus* was dominant in non-hypertensive kidney stone patients. There was an overrepresentation of *Sphingomonas* in hypertensive kidney stone patients.³⁸ Increased use of antimicrobials in urinary stone disease patients has also been associated with changes in both the gastrointestinal microbiome and metabolome, further implicating the gastrointestinal microbiome in kidney stone disease.³⁹ These studies show that the gastrointestinal microbiota can be disrupted by both diet and

Table 1. The microbiome in urinary tract diseases.

Disease	Experimental approach	Key results	Outcomes and significance	Reference
Kidney stone disease Urinary stone disease—mouse model of fecal transplantation	Investigation of the gastrointestinal microbial community and the effect of fecal transplantation on urinary stone risk parameters in a murine model. Microbiome analysis by 16S rRNA gene amplification and sequencing.	Post-fecal transplantation (4 weeks): ↓ Urinary calcium (>50%) ↓ Urinary oxalate levels ↓ <i>Clostridiaceae</i> family ↑ Urinary pH ↑ Cecum Solute Carrier Family 26 Member 6 (<i>Slc26a6</i>) expression	Fecal transfer from donor to recipient mice can impact urinary parameters associated with stone formation.	³³
Kidney stone disease and hypertension—a clinical study	Investigation of the relationship between the urinary microbiome and blood pressure in patients with kidney stone disease and hypertension by 16S rRNA sequencing and PCR.	Patients with kidney stone disease had an altered urinary microbiome compared to healthy controls. Predominant species identified: Healthy controls: <i>Gardnerella</i> Non-hypertensive kidney stone disease: <i>Staphylococcus</i> Hypertensive kidney stone disease: <i>Sphingomonas</i>	Patients with kidney stone disease and hypertension have a unique urinary microbiome. Monitoring alterations in the microbiome may be advantageous in determining disease progression and/or severity.	¹⁴⁷
Urinary stone disease—an animal model	Fecal transplant <i>via</i> oral gavage of microbes isolated from a high oxalate diet-adapted mammalian herbivore.	Fecal transplant animals had enhanced oxalate metabolism, which increased bacterial diversity.	Targeted bacteriotherapy may be used to reduce urinary oxalate excretion in patients at high risk of recurrent calcium oxalate stones.	³⁷
Acute kidney injury (AKI) AKI—mouse model	Broad-spectrum antibiotic-induced depletion of microbiota and induction of AKI <i>via</i> renal ischemia-reperfusion injury in mice.	Depletion of the gastrointestinal microbiota attenuated renal damage and dysfunction after renal ischemia-reperfusion injury. Microbiota depletion reduced the number of kidney resident F4/80 ⁺ macrophages compared to controls.	Antibiotic-induced depletion of the gastrointestinal microbiota protects against renal ischemia-reperfusion injury. Reducing the macrophage response by targeting microbiota-derived mediators may be a promising therapeutic avenue.	⁴¹
AKI—clinical and mouse model	Murine model of ischemia-reperfusion injury, bacterial analysis by 16S rRNA sequencing, and analysis of chiral amino acid by 2D high-performance liquid chromatography (HPLC).	Gastrointestinal microbiota protected against renal tubular injury. AKI-induced gastrointestinal dysbiosis and altered metabolism of D-amino acids. Serum D-serine levels were significantly correlated with decreased kidney function in human AKI patients.	Gastrointestinal-derived D-serine has a protective effect in AKI and can be used as a serum biomarker for disease progression.	⁴²
Chronic kidney disease (CKD) CKD—clinical study (adult cohort)	Urine and plasma biochemical analysis and PCR.	Increased mRNA expression of decapentaplegic homolog 3 (SMAD3) and NLR Family Pyrin Domain Containing 3 (NLRP3) and increased serum transforming growth factor-beta (TGF-β)1 and interleukin-1β protein levels, were associated with increased microbiota-dependent metabolite trimethylamine N-oxide (TMAO) levels.	Serum and/or urine TMAO along with key immune factors may be used as biomarkers to evaluate CKD progression.	⁵⁵
CKD—clinical study (children and adolescent cohort)	Gastrointestinal microbial composition and TMAO metabolic pathway were assessed by mass spectrometry and PCR. Correlation with blood-pressure and vascular abnormalities in children with early-stage CKD was evaluated.	Children with later stage CKD had a lower urinary level of TMAO. Urinary TMAO levels were correlated with an abundance of the genera <i>Bifidobacterium</i> and <i>Lactobacillus</i> . CKD in children with abnormal ambulatory blood pressure had a lower abundance of <i>Prevotella</i> .	Early detection and stratification by microbial signatures may assist in preventive care to improve cardiovascular outcomes in children with CKD.	⁵⁷
CKD—clinical study (adult cohort)	Short Chain Fatty Acids (SCFAs) were measured in plasma using targeted liquid chromatography-mass spectrometry.	Increased plasma levels of valerate, an SCFA generated by the gastrointestinal microbiota, was found in patients with CKD; this increased level of	Improving stratification of cardiovascular risk in patients with CKD based on plasma levels of SCFAs	¹⁹⁰

Table 1. continued				
Disease	Experimental approach	Key results	Outcomes and significance	Reference
CKD—clinical study (adult cohort)	Pilot study characterizing urinary microbiome in patients with non-dialysis-dependent CKD.	plasma valerate positively correlated with cardiovascular disease. Midstream urine microbiomes are diverse in adults with CKD; however, diversity was lower with reduced glomerular filtration rate, and more advanced CKD.	may help identify patients with the subclinical disease to facilitate early intervention. Association between microbiome diversity and kidney function. Further studies are required to determine whether kidney disease influences the microbiome or vice versa.	¹⁹¹
CKD—rat model	Adenine diet-induced CKD in rats followed by a lactulose-containing diet. Renal function, uremic toxins, and gastrointestinal microbiota were assessed.	Lactulose containing diet improved levels of serum blood urea nitrogen and creatinine, whilst tubulointerstitial fibrosis and microbiota-derived serum indoxyl sulfate levels were suppressed compared to the control diet group.	Lactulose by diet modifies gastrointestinal microbiota and improves CKD disease progression by suppressing uremic toxin production.	¹⁹²
Urinary tract infection (UTI) Recurrent (rUTI)—mouse model	Mouse model of cystitis with two different clinical uropathogenic (UPEC) isolates, UTI89, a cystitis isolate, or CFT073, pyelonephritis isolates.	Mice infected with CFT073 and treated with antibiotics were protected against reinfection with the same isolate but not secondary infection with UTI89. Mice infected with UTI89 were highly susceptible to a secondary infection with either strain. Depletion of T cell subsets impaired bacteria clearance and increased susceptibility to rUTI.	UPEC has to strain-specific implications to the host and a more advanced understanding of host–pathogen interactions is required for improved effective drug development.	¹⁹³
Neurogenic bladder Neurogenic bladder—a clinical study	Urinary microbiome analyzed by 16S rRNA pyrosequencing, from asymptomatic patients with and without a neurogenic bladder.	Neurogenic bladder patients are more likely to have bacteriuria than patients without a neurogenic bladder. Female patients with neurogenic bladder had a greater abundance of <i>Lactobacillus crispatus</i> in their urine while male patients had <i>Staphylococcus haemolyticus</i> . In both sexes, patients with neurogenic bladder had greater proportions of <i>Enterococcus faecalis</i> , <i>Proteus mirabilis</i> , and <i>Klebsiella pneumoniae</i> .	16S pyrosequencing was able to identify unique, phenotype dependent urinary microbiome populations, even in asymptomatic cohorts. The <i>Lactobacillus</i> community in urine from females with neurogenic bladder differed depending on bladder function.	⁷⁵
Neurogenic bladder—a clinical study	Urine was collected from both healthy controls and healthy subjects with neurogenic bladder. Urinalysis, urine culture, and NextGen16S rDNA sequencing were used with further metaproteomic analysis.	The bacterial taxa showing the highest relative abundance and change, include <i>Lactobacillales</i> , <i>Enterobacteriales</i> , <i>Actinomycetales</i> , <i>Bacillales</i> , <i>Clostridiales</i> , <i>Bacteroidales</i> , <i>Burkholderiales</i> , <i>Pseudomonadales</i> , <i>Bifidobacteriales</i> , and <i>Coriobacteriales</i> .	The healthy urine microbiome is characterized by an abundance of <i>Lactobacillales</i> in women and <i>Corynebacterium</i> in men. Integrating 16S rDNA sequencing with metaproteomics can improve diagnostics and more targeted use of therapeutics in patients with neurogenic bladder.	⁷²
Neurogenic bladder—clinical study (children cohort)	Urinary microbiome analysis by 16SrRNA sequencing, from children with neurogenic bladder (both with UTI, and asymptomatic bacteriuria)	Children with neurogenic bladder had an increased abundance of members of the <i>Enterobacteriaceae</i> family. However, there was no difference between those with UTI and those with asymptomatic bacteriuria.	In children with neurogenic bladder, it is difficult to distinguish between UTI and asymptomatic bacteriuria. Analysis of the urinary microbiome may provide further differentiation to aid incorrect diagnosis. Although with only a small cohort further work is needed to determine if the urinary microbiome varies in larger cohorts.	⁷⁴

exposure to antimicrobials, both of which may increase susceptibility to kidney stone disease.

Acute kidney injury

Acute kidney injury (AKI) is a potentially fatal, global challenge in critical care medicine. It is also a known risk factor for the development of chronic kidney disease (CKD).⁴⁰ The loss of the gastrointestinal microbiota by oral antibiotics has been shown to be protective against experimental AKI in mice.⁴¹ This is thought to be regulated through renal resident macrophages and microbiota-derived metabolites^{41,42} as antibiotic-mediated depletion of the gastrointestinal microbiota decreased renal resident macrophages, reduced AKI-induced renal damage, and maintained the integrity of the renal tubules.⁴¹ Mice that received a transplant of fecal material from antibiotic-treated mice, were no longer protected against AKI-induced renal damage⁴¹ suggesting specific antibiotic-mediated bacterial composition may be driving the detrimental response. Therefore, direct microbiome targeting or amelioration of the microbiota-induced macrophage-driven inflammatory response may be a promising therapeutic strategy in AKI.

The impact of the gastrointestinal microbiota on AKI may be mediated, at least in part, by specific microbial metabolites such as D-amino acids that can be altered in response to dysbiosis.⁴² Detectable levels of D-serine were found in the mouse kidney and the activity of D-amino acid oxidase was reduced following AKI in mice.⁴² In human AKI patients, there was a correlation between circulating D-serine levels and decreased kidney function.⁴² These data shed new light on the interactions between the kidney and gastrointestinal microbiota-derived metabolites in AKI. Further investigation into the role of the gastrointestinal microbiome in AKI, may enable the development of microbiome-derived preventative or therapeutic interventions for AKI.

Chronic kidney disease

Patients with CKD may lose up to 90% of their kidney function before presenting with symptoms. It is a life-threatening condition with dialysis and transplantation the mainstay treatment options for end-stage renal disease. There are a number of studies that demonstrate alterations in the gastrointestinal microbiota of human CKD patients are associated with disease progression. Alterations to the gastrointestinal microbiota are also strongly associated with chronic inflammation, which is a major contributor to the progression of CKD severity.^{43–46} The impact of the gastrointestinal microbiome in kidney transplantation, is also established but outside the scope of this review and has been recently reviewed.⁴⁷

In one CKD study, 190 microbial operational taxonomic units from the gastrointestinal microbiome, exhibited significant differences in relative abundance in late-stage CKD patients, compared with healthy controls.⁴⁸ The majority of these operational taxonomic units (159) belonged to the *Pseudomonadaceae* family. CKD patients had reduced *Lactobacillaceae* and *Prevotellaceae*, but 100-fold increased *Enterobacteria* and *Enterococci* species.⁴⁹ These same species are also increased in patients with late-stage CKD.⁴⁸ Moreover, during the later stages of CKD, the abundance of *Roseburia*, *Faecalibacterium*, *Clostridium*, *Coprococcus*, and *Prevotella* were decreased.⁵⁰ These bacteria are known to increase the production of the short-chain fatty acid butyrate, which has multiple known functions in maintaining gastrointestinal homeostasis, including, promoting regulatory T-cell differentiation,^{51,52} suppressing inflammation by inhibiting nuclear-factor-kappa B activity,⁵³ and enhancing epithelial tight-junction assembly.⁵⁴

In CKD patients, impaired kidney function and alteration in the gastrointestinal microbiota contributes to increased levels of the microbiota-dependent metabolite trimethylamine N-oxide (TMAO).^{44,55} Circulating TMAO is also a marker of renal

damage.⁵⁶ Indeed, this has been demonstrated by urine TMAO levels in a pediatric cohort of CKD patients, which was also correlated with a decreased abundance of *Lactobacillus* and *Bifidobacterium* in the gastrointestinal tract. Furthermore, children in this cohort with abnormal blood pressure had a lower abundance of *Prevotella*.⁵⁷

Progression of CKD also has deleterious effects on the gastrointestinal tract.^{58–60} CKD increases urease and uricase-producing bacteria, which in turn, raises the levels of the toxins urea and uric acid.⁶¹ Increased uremic toxin concentrations have been shown to drive dysbiosis of the gastrointestinal microbiota and contribute to CKD progression.^{62,63} Furthermore, ammonia, metabolized from urea by microbial urease, can damage the epithelial barrier in the gastrointestinal tract, enabling the release of gastrointestinal-derived uremic toxins.^{64,65}

Although the underlying mechanism of gastrointestinal dysbiosis in CKD remains to be fully elucidated, there is emerging evidence for a link between the gastrointestinal microbiota and microbial metabolites, specifically associated with the degree and progression of the disease.

Neurogenic bladder

Neurogenic bladder is a bladder dysfunction disorder caused by damage to the nervous system. Damage to the nerve cells in neurogenic bladder disorder may be a risk factor for bacteriuria.^{66–70} The role of the microbiome in the neurogenic bladder has been reviewed.⁷¹ Unlike healthy individuals,⁷² increased microbial diversity precedes the onset of UTI in neuropathic bladder patients.⁷³ A pilot study by Forster et al. found that children with neuropathic bladder disorder have urinary microbiomes that are predominantly composed of the *Enterobacteriaceae* family.⁷⁴ Other studies have also identified that the urinary microbiome of patients with neuropathic bladder consists of uropathogens, such as *Enterococcus*, *Klebsiella*, and *Pseudomonas* compared to non-uropathogenic bacteria identified in the urinary microbiome of people without a neurogenic bladder.^{71,72,75} Further studies investigating the impact of specific site microbiota on the severity of neurogenic bladder is warranted.

Recurrent urinary tract infection

The role of the healthy urinary microbiome in maintaining bladder homeostasis and preventing rUTI is an emerging field.^{76,77} It is known that the bladder of healthy individuals harbors non-uropathogenic bacteria, such as *Lactobacillus*, which are thought to be protective against rUTI.^{17,25,78–80}

A proportion of rUTI are thought to result from intracellular colonization by UPEC.⁸¹ Intracellular UPEC colonies are found within the bladder wall of mice and humans.^{82–84} In mice these intracellular colonies are dependent on both mouse strain and the strain of UPEC. Transient exposure of the bladder to vaginal *G. vaginalis* was associated with the release of uropathogenic bacteria from intracellular colonies in the bladder epithelium through exfoliation of the bladder mucosa resulting in reactivation and rUTI.²⁴ *G. vaginalis* was also associated more commonly with women with overactive bladder syndrome, than healthy controls.¹³ In animal models, the link between a fecal load of UPEC and rUTI is relatively well established.^{85–87} However, in humans, UPEC appears to be present in abundant quantities in the feces but, its direct causal relationship to rUTI in women is far from established.⁸⁸

Catheter-associated UTI (CAUTI) are a growing problem in patients of all ages, particularly in patients with underlying urological diseases. The involvement of the microbiome in CAUTI has been recently reviewed.⁸⁹ A more detailed investigation of catheter-induced changes to the urinary microbiome is warranted.

Although the relationship between the gastrointestinal microbiome and UTI in adults continues to be studied⁹⁰ equivalent data on the impact of the genitourinary microbiome in the early years

of life and changes through puberty are scant.⁹¹ rUTI is a common issue in girls with bladder bowel dysfunction and/or vesicoureteral reflux.^{92,93} The presence of UPEC in feces, as well as the formation of intracellular colonies in the bladder epithelium, have been reported to contribute to rUTI in children similar to adult women.⁹² Interestingly, aggressive treatment of constipation has a proven beneficial effect, although the mechanisms underpinning this remain uncertain.⁹¹ Microbiome-based studies focusing on better diagnosis and treatment need to be pursued in children to reduce morbidity due to overzealous antibiotic use and emerging antimicrobial resistance.

In line with a more advanced understanding of disease manifestation and improved treatment options, the ability to accurately detect infections is essential. Mouraviev and associates applied a next-generation DNA sequencing-based diagnostic algorithm (DecodEX) for detection and targeted antimicrobial treatment in males with neurogenic bladder or chronic prostatitis.⁹⁴ In phase II clinical study on men with chronic prostatitis, acute UTI, or chronic UTI, the diagnostic yield with DecodEX was 100% (44/44) while it was 29.5% (13/44) with the traditional urine culture test. In summary, an improved understanding of the genitourinary microbiota has provided us with a new lens to look through to address the important issue of rUTI in women, men, and children. Combining emerging microbiome technologies and bacteriotherapy with existing diagnostics and treatment strategies may offer new strategies for the management of rUTI.

TOOLS FOR IMPROVED ASSESSMENT OF THE URINARY MICROBIOME

Urine microbial culturing

Prior to the recent understanding of the urinary microbiome, the gold-standard urine culture test developed in the 1950s was the common procedure performed to determine the presence of a UTI. Uropathogens such as UPEC, *Enterococcus*, and *Staphylococcus* spp. in urine samples were enumerated on agar plates, and colony counts of $>10^4$ colony forming units (CFU)/mL was considered a positive test.⁷ Despite the complexity of the urinary microbiota being relatively well understood it remains unclear whether standard microbial culturing methods are adequate to capture species diversity or even sufficient to detect the presence of all UTIs.^{7,22} These findings highlight several challenges when exploring the urinary microbiome in health and disease states. Furthermore, the low-microbial biomass of the urinary microbiota ($<10^5$ CFU/mL) combined with the difficulty in isolating native health commensals and uropathogens that may require specific culture conditions emphasizes the need for better tools to gain advancement in the field.^{7,22}

The expanded quantitative urine culture (EQUC) was developed to enable the detection of previously unculturable bacteria or uropathogens that exist in low abundance.^{6,7} EQUC confirmed that urine contains viable bacteria that comprise the female urinary microbiome,⁷ and was able to detect 84% of all uropathogens in symptomatic patients, whilst conventional culturing was limited to 33% detection.⁶ This method has been adapted for streamlined use in clinical laboratories.⁶ Furthermore, multiple sequencing tools have been performed to further characterize the urinary microbiome to achieve in-depth sample and phylogenetic resolution (Fig. 1).

Whole-genome sequencing

Detailed measurements of individual bacterial isolates can be achieved by combining culturing with whole-genome sequencing (Fig. 1). The complete genome sequence can be used to detect gene contents, genetic mutations, and genomic rearrangements. Assembling sequence reads into complete genome sequences can be challenged by the presence of repetitive DNA regions, a

Fig. 1 Improved characterization of the urinary microbiome using culturing and sequencing tools.

In-depth functional and genomic characterization of the urinary microbiome can be achieved with tools and technologies that help improve sample resolution and phylogenetic resolution. Traditional microbial culturing by quantifying bacterial numbers on agar plates is now considered an inadequate approach to detect the presence of UTI due to the limited sample resolution that traditional microbial culturing can achieve. The expanded quantitative urine culture (EQUC) method is an improved microbial culturing technique that enables the detection of low abundance uropathogens and is the preferred method for routine UTI testing in clinical laboratories. EQUC can be combined with sequencing tools to further characterize the microbiota and enable an expanded reference genome database. Amplicon sequencing of 16S ribosomal RNA (rRNA) gene regions allows the taxonomic identification of microbial communities without the need for microbial culturing. Clonal typing of bacterial species within a sample can be achieved with targeted amplicon sequencing. Sequences from the entire genome can be characterized by metagenomic sequencing, which enables the characterization of the microbiome at a genomic and functional level. Long-read sequencing can improve whole-genome sequencing methods when assembling sequence reads. Using integrated multi-omics approaches can enhance the understanding of host-microbe interactions and functions. These culturing and sequencing methods could be integrated to improve the reference database and enable isolate banking from samples.

problem that can be overcome by using long-read sequencing platforms.^{95–97} As long-read sequencing technologies still suffer from low sequencing yields and relatively high sequencing error rates, they are often combined with short sequence reads in hybrid de novo assemblies to produce high-quality reference genomes.⁹⁸

Thomas-White et al. combined EQUC methods with whole-genome sequencing to characterize the phylogenetic diversity of bacteria from perimenopausal women with and without UTI.⁵ The authors were able to culture two-thirds of the genera detected by metagenomics, however, the anaerobes *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* were not able to be isolated with the EQUC method alone.⁵ This highlights the synergistic relationship between the vaginal and urinary microbiome and the need to harness genome sequencing technologies to achieve the isolate-level resolution needed for further investigations into the host-microbial signature within these sites.

Amplicon sequencing

High-throughput sequencing of genetic markers amplified via polymerase chain reaction (known as amplicon sequencing or metabarcoding) is a sensitive and cost-effective method to achieve low-resolution measurement of the microbial composition of a sample without the need to culture bacterial isolates. As the urinary microbiome research field emerged, multiple studies utilized 16S rRNA amplicon sequencing for insight into the taxonomic composition of bacterial communities that were associated with urological disorders.^{8,11,13} Urgency urinary incontinence (UUI) has been associated with differences in urinary microbial community diversity and composition within the female urinary tract when compared with healthy controls.^{8,11,13} Treatment success rates against UUI in females with the anticholinergic drug solifenacin,¹¹ and risk of post-operative infections⁹⁹ have also been linked to the urinary microbiome, warranting further studies in this field.

However, the low microbial biomass in urine samples poses an additional challenge to adequately define the microbiome composition. To avoid introducing bias, various sample collection methods have been proposed (voided urine, transurethral catheter, and suprapubic aspiration).¹⁰⁰ Sample collection can also be improved by the addition of a preservative (AssayAssure®) to conserve the microbial composition in single-voided urine samples from women.¹⁰¹ Importantly, collection and preservation methods for all specimens should be kept consistent across studies within the field to avoid confounders.¹⁰²

Where particular species of interest are known, targeted amplicon sequencing can be applied. Shevchenko et al. used a novel high-throughput method for clonal typing of multidrug-resistant *E. coli* in fecal and urinary samples from women and developed an algorithm called population level-allele profiler, which enables the simultaneous identification of alleles and their prevalence in both fecal and urine samples.¹⁰³ This method could potentially be adapted to clonal-type other bacterial species for future studies of population and transmission dynamics of the urinary microbiome; however, it is fundamentally dependent on pre-existing knowledge of the isolates of interest.

Despite the advances made in urinary microbiome research, 16S rRNA amplicon sequencing is often limited to family and genus level resolution (Fig. 1), presenting challenges to species-level identification, particularly in diverse polymicrobial samples, and offers no capacity to measure functional genes within the microbial community.^{104,105} Amplicon sequencing of the 16S rRNA can be complemented with EQUIC to assess the microbial composition of culture-negative urine samples from healthy individuals.^{9,10} These studies confirmed the presence of a microbial community in the healthy urinary tract, including an array of microorganisms that would normally remain undetected by conventional methods such as *Aerococcus uringe*, *Ureaplasma* spp, and the genera *Soehngenia*.^{9,10}

Metagenomic sequencing

Metagenomics is another culture-independent high-throughput sequencing method used to measure genetic material from microbes within a sample. Sequences from entire genomes within the genetic pool can be characterized at a higher taxonomic resolution and functional level (Fig. 1). This approach allows an in-depth analysis of the microbiome without inherent bias associated with specific genetic markers.

Community profiling using metagenomic sequencing in clinical samples could enhance the understanding of antibiotic resistance or virulence genes associated with uropathogens.^{26,106,107} In addition to higher resolution characterization of community composition. Moustafa et al. used metagenomic sequencing to detect the novel uropathogens *Ureaplasma*, *Alloscardovia*, and *Actinotignum*, which normally exist in low abundance in the urinary tract.¹²

In addition, the study identified eukaryotic fungal species (*Candida* spp) and the protozoan, *Trichomonas vaginalis* along with and viral organisms (e.g., Herpesvirus 6, human papillomavirus, polyomavirus), which would not be detected via 16S rRNA amplicon sequencing.¹² A similar approach, known as metatranscriptomics, allows characterizing of the actively transcribed genes of the microbial population, which can help to understand the gene expression of potential pathogens. For example, metatranscriptomes of the vaginal microbiota have revealed that the pathogen responsible for bacterial vaginosis, *G. vaginalis*, can subvert the effects of antibiotic therapy by expressing DNA-repair genes.¹⁰⁸ RNA-sequencing has been used to understand the expression of virulence genes in UPEC isolated from women with UTIs,¹⁰⁹ but to our knowledge, this technique is yet to be applied to understand community-wide patterns in the urinary microbiome. Although sequencing the entire DNA content has advantages, high sequencing depth is typically required to enable de novo assembly, especially in complex microbial communities. In urine samples, which contain low microbial biomass, contaminating human DNA can also constitute a substantial proportion of the sequence data regularly rendering this approach cost-prohibitive. This limitation may be overcome by enriching bacterial DNA in a sample while depleting human DNA.^{110,111} Another strategy is to target cell-free DNA (cfDNA), which are single-stranded DNA fragments from dead cells, many of which are host-cell or microbial-derived. Metagenomic analysis of cfDNA is an emerging technology that could improve the precision of diagnostic screening for UTI and provide insight into urinary microbiome dynamics. Urinary cfDNA metagenomics has recently been utilized to detect and monitor UTI in kidney transplant recipients.^{112,113} However, whether cfDNA sequencing is an efficient tool to assess the urinary microbiome remains to be further investigated. The low-biomass of microbial-cfDNA may lead to a higher risk of DNA contamination from external sources, resulting in false-positive rates during analysis.¹¹⁴ Thus, these limitations should be considered when applying cfDNA technology for the assessment of the urinary microbiome.

POTENTIAL FUTURE TREATMENT STRATEGIES TARGETING THE URINARY MICROBIOTA

The use of broad-spectrum antibiotics to treat urological disorders has led to the emergence of multi-antibiotic resistant uropathogens.¹⁵ Despite limited knowledge of the various factors that can modulate the urinary microbiota, multiple therapeutic approaches to promote microbiota restoration are now also being explored (Fig. 2).^{115,116} Switching from an eradication-of-infection approach to microbiota modulation or restoration may pave the way for more effective management strategies for urological diseases.

Biotherapeutics

Commercial probiotics are easily accessible and have become widely popular due to the benefits of promoting gastrointestinal microbiome health. Prophylactic probiotics have also been used to prevent gastrointestinal disorders such as antibiotic-associated diarrhea, colitis, and inflammatory bowel disease.¹¹⁷ Several benefits of probiotics include suppressing pathogen colonization,¹¹⁸ modulating host immunity,¹¹⁹ promoting cell proliferation,¹²⁰ and maintaining epithelial barrier integrity.^{121–124} Bacterial strains have been evaluated for their probiotic properties to treat rUTI, bladder cancer, and kidney disease. In a randomized clinical phase II trial, the *Lactobacillus crispatus* probiotic (Lactin-V) administered via intravaginal suppository led to a significant reduction in rUTI.¹¹⁵ The protective mechanism of *L. crispatus* is mediated by the secretion of lactic acid, which has broader implications in the genital tract against *Chlamydia trachomatis* and *Candida albicans* infection.^{125–127} Further screening of *Lactobacilli* isolates from the vagina of healthy women identified potential

Fig. 2 Modulating the microbiota as a future intervention strategy for urological diseases. Administration of broad-spectrum antibiotics is the mainstay treatment for recurrent UTI (rUTI). Prolonged exposure to antibiotics to eradicate infection has resulted in the development of multi-resistant uropathogens, which can disrupt the microbiome (dysbiosis), leading to an imbalance of native health commensals and uropathogens. Insight into the genitourinary and gastrointestinal microbiome has paved the way for potential therapeutics that target host-microbiome interactions in order to restore homeostasis by increasing commensals and reducing the uropathogenic population. Probiotics consisting of live *Lactobacillus* spp. can be orally administered to restore balance to the host microbiome following antibiotic exposure and has been widely implicated in gastrointestinal disorders. However, further validation of *Lactobacillus* probiotics in the context of urological disorders is essential. Multiple animal studies and patient trials have investigated establishing asymptomatic colonization of attenuated *Escherichia coli* to outcompete colonization of uropathogens. The same principle can be applied for catheterized patients, whereby catheters can be coated with attenuated *E. coli* isolates. Specific uropathogens such as uropathogenic *E. coli* (UPEC) can be genetically modified by targeting virulence and fitness genes that are essential for UPEC pathogenesis (e.g. fimbriae adhesins, pilus, biofilm formation machinery). These virulence factors can be directly inhibited by small synthetic molecular compounds such as mannosides, which functions as a fimbriae antagonist. The synergistic relationship between the female reproductive tract, urinary, and gastrointestinal tract can be harnessed to develop adequate microbiota transplantation (fecal microbiota transplant; FMT and vaginal microbiota transplant; VMT) and restoration. Modulating the host immune response by targeting host factors such as the cytokine interleukin-22 (IL-22), which has been shown to regulate commensal bacteria in the gastrointestinal and maintaining epithelial barrier integrity may also be an emerging form of therapy. However, the role of IL-22 in urological diseases should be fully characterized in future studies.

candidates for probiotics, including *L. rhamnosus*, *Lactobacillus helveticus*, and *Lactobacillus salivarius*.¹²⁸ However, a Cochrane review of nine studies with children and adult participants found no significant differences between placebo and probiotic groups in managing rUTI, although the variation of methods in these studies could be a confounding factor.¹²⁹ Hence, there is a need to further assess the efficacy of *Lactobacillus* spp. as probiotics in bladder health, especially considering that the vaginal and urinary microbiome are interconnected.⁵ Whether one or multiple strains of commensal bacteria are required to confer protection and restore the urinary flora remains to be explored.

Treatment of rUTI with asymptomatic *E. coli* has been investigated in murine models and trialed with patients.^{130–133} Attenuated *E. coli* isolates with a mutation in the fimbrial adhesins, are able to rapidly establish asymptomatic long-term colonization in the urinary tract and outcompete colonization of uropathogens without activating host immune responses.^{131,132,134} Further

studies with a larger cohort of patients and testing in immunocompromised individuals are required to further develop this form of therapy. Catheterized patients may benefit from *E. coli* HU2117 coated catheters, which was reported to lower incidence rates of UTI among subjects, although *E. coli* colonization in the urinary tract was not investigated in the study.¹³⁵ *E. coli* could also be bioengineered to modulate bacterial-host interactions such as enhancing biofilm formation and mucin production on epithelial surfaces by targeting bacterial fimbriae.¹³⁶

Microbiota transplantation and microbiome restoration

Fecal microbiota transplantation (FMT) has been used as an alternative approach for gastrointestinal microbiome restoration and increasing microbial diversity, particularly in patients with prolonged exposure to antibiotics.¹³⁷ FMT in patients with UTI and recurrent *Clostridium difficile* infection was associated with a decrease in the colonization of antibiotic-resistant pathogens (e.g.

vancomycin-resistant *Enterococci*)¹³⁸ and an increase in antibiotic susceptibility of the uropathogens, *E. coli*, and *Klebsiella*.¹³⁹ In another study, 16S rRNA profiling, and metagenomics were performed to assess the microbiome pre- and post-FMT in a patient with irritable bowel syndrome and rUTI.¹⁴⁰ In the post-FMT stool sample, there was a reduction in pathogenic strains and 12 new strains that were not detected in the pre-FMT sample were identified.¹⁴⁰ UTI symptoms in the treated group also did not reoccur 8-months following treatment.¹⁴⁰ However, FMT has not been successful in eradicating all pathogens, and a randomized control trial on FMT post-antibiotic usage observed no significant differences in the decolonization of multi-drug resistant *Enterobacteriaceae*.¹⁴¹

The microbiome restoration therapy RBX2660 formulated for the prevention of recurrent *C. difficile* infection is a standardized broad-spectrum microbiota suspension manufactured from healthy stool donors which can be delivered directly to the intestinal tract and is undergoing Phase III clinical trials.^{142–144} To date, one case-study trialing RBX2660 to treat rUTI has been reported, however, the treatment failed to abrogate uropathogens and the patient continued experiencing rUTI following exposure to broad-spectrum antibiotics.¹⁴⁵ It would be insightful to investigate the effectiveness of FMT and microbiome restoration therapies in managing diseases solely associated with the lower urinary tract because existing gastrointestinal disorders may have influenced the treatment success rate in this cohort.

The shared vaginal-urinary-microbial-axis could be a useful management strategy for UTI.⁵ Vaginal microbiota transplantation (VMT) has been trialed for recurrent bacterial vaginosis,¹⁴⁶ however, the potential relevance of this treatment to UTI has not been explored. Indeed, alterations of the female urinary microbiome can be influenced by multiple factors such as age, hormonal status, and mode-of-delivery^{80,147} which are important considerations when implicating VMT for UTI. Perhaps microbiota transfer and microbiome restoration therapies could be optimized to contain synthetic communities of health-associated *Lactobacilli* that are known to mediate host-microbe interactions and maintain mucosal immune homeostasis in the genitourinary tract.

Bacterial inhibitors

One of the critical factors of UPEC pathogenesis is its ability to adhere to the urothelium, which is mediated by the binding interaction between the bacterial type I pilus FimH adhesin to mannosylated glycoproteins expressed on host epithelial cells in the bladder.¹⁴⁸ Mannosides are a class of small molecular compounds that function as receptor analogs and bind to bacterial fimbriae to prevent UPEC-host cell interactions. The efficacy of mannosides in reducing bacterial burden in the kidney and bladder has been shown in murine infection models and ex vivo human kidney cells.^{149–151} In murine-models, mannosides have also been an effective preventative treatment measure for UPEC-associated CAUTI, including CAUTI caused by multi-drug resistant UPEC.^{151–153} Targeting FimH with mannosides can selectively deplete UPEC isolates that are genetically diverse in the gastrointestinal tract, and potentially reduce UTI incidence without disrupting the microbiota.⁸⁷ The inhibitory function was observed across multiple genetic backgrounds in mice and was tested against different UPEC isolates.⁸⁷

Emerging immunomodulatory therapies and microbiome mimetics

Although the distinct roles of the urinary microbiome have yet to be comprehensively defined, it is thought that the microbiota within this site is critical for homeostasis of the urinary tract, much like other mucosal regions such as the gastrointestinal and female reproductive tract.¹⁵⁴ These protective functions likely include maintaining the epithelial barrier, outcompeting pathogens for metabolites, and modulating the host immune responses.¹⁵⁵ The

high presence of specific resident immune cell populations such as tissue-resident macrophages¹⁵⁶ in the urinary tract may suggest the importance of host cell-microbial crosstalk in priming the immune response against infection.¹⁵⁷ However, further exploration of microbial-mediated mechanisms of homeostasis in the urinary tract is warranted.

Antimicrobial peptides are amongst the first line of defense against uropathogens that invade the urinary tract following the breakdown of the mucus and epithelial barriers.¹⁵⁸ Human bladder epithelial cells produce lactoferrins, which are iron-binding glycoproteins that drive neutrophil infiltration, resulting in lower bladder bacterial burden in a murine infection model.¹⁵⁹ The cathelicidin family of antimicrobial peptides, LL-37, mediates T lymphocyte, macrophage, and neutrophil responses during UTI.¹⁶⁰ Endogenous LL-37 activity in the urinary tract could be enhanced with the treatment of the synthetic antimicrobial, ceragenins, which could be an effective form of managing multi-drug resistant UPEC infection.^{161,162} A prolyl-hydroxylase inhibitor (GB-004, formerly known as AKB-4924), was proposed to regulate the transcriptional factor, hypoxia-inducible factor-1 α (HIF-1 α), thereby resulting in decreased bacterial burden coupled with a reduction in macrophage and neutrophil-mediated inflammation in a mouse model of UTI.¹⁶³ The authors propose that GB-004 could be compatible with other therapies for rUTI, however, this will need to be evaluated further.¹⁶³

During infection, activation of pattern-recognition receptors expressed on urothelial cells such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) bind to cognate microbial antigens that drive downstream signal transduction in cells, leading to secretion of pro-inflammatory cytokines and chemokines.¹⁶⁴ Murine UPEC infection models have identified the innate immune sensors, TLR4,^{165,166} TLR5,¹⁶⁷ and TLR11,¹⁶⁸ and the pro-inflammatory chemokine CXCL12, as important in bacterial clearance and infiltration of natural-killer and T lymphocytes in the bladder,¹⁶⁹ respectively. However, whether these findings can be implicated in human UTI, and their potential as diagnostic biomarkers or therapeutic targets remain to be investigated.

Screening of urine and plasma samples from UTI patients revealed an upregulation of tumor necrosis factor, interleukin (IL)-6, IL-8, IL-10, IL-17 at both the transcript and protein levels.¹⁷⁰ In addition, NLRP3, IL-1 β , and tumor growth factor- β (TGF- β) mRNA levels have been associated with the microbial nutrient metabolite TMAO in the urine and plasma of CKD patients.⁵⁵ The increased mRNA levels of the inflammasome components, NLRP3 and NLR4, have previously been detected in UTI patients.¹⁷¹ These findings suggest the involvement of the microbiota in regulating adaptive cellular immune responses and inflammation during urological disorders.

IL-22 is a member of the IL-10 cytokine superfamily with multifaceted functions in the gastrointestinal tract.^{172,173} The role of IL-22 in regulating commensal bacteria and modulating the expression of antimicrobial peptides in human urothelial cells^{174,175} may offer insight into the protective and immunoregulatory role of the interleukin during infection in the urinary tract. To date, clinical trial drugs targeting IL-22 have been developed to treat inflammatory diseases such as psoriasis and rheumatoid arthritis.^{176,177} With further studies, immunomodulatory therapies that inhibit or enhance IL-22 or other host immune receptors or cytokines/chemokines could potentially be applied to manage inflammation and/or control bacterial colonization during urological disorders.¹⁷⁸

Despite the activation of innate and adaptive immune responses during infection, rUTI remains a significant burden in human health. It is unclear how the adaptive and humoral immune response plays a role during repeated infection and whether the mechanisms of immune memory breakdown, resulting in recurrent infections. Furthering our understanding of immune memory after UTI could advance the development of

effective vaccines to target the humoral and adaptive immune response. Multi-subunit vaccines composed of UPEC antigens (e.g., fimbriae, hemolysin, and siderophore receptor) have been proposed as effective candidates in raising protective antibody titer levels in mouse models, however, the authors were unable to speculate whether this approach would be protective in patients.¹⁷⁹ A tetravalent bioconjugate vaccine, ExPEC4V, that targets the O-antigen of extraintestinal pathogenic *E. coli* lipopolysaccharide, showed promising results in a clinical phase 1b trial, and effectiveness was shown for rUTI in women.¹⁸⁰ Multi-strain vaginal mucosal vaccines (Urovac, Urostim, and Urvakol) have also been developed, but validation in human trials will need to be performed. The sublingual spray vaccine, Uromune, which consists of heat-inactivated whole bacterial lysates composing of uropathogens such as *E. coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Proteus vulgaris*¹⁸¹ have also been trialed in women with UTI. Compared to women that were treated with antibiotics, less than 10% of the immunized group developed UTI within 12 months post-vaccination. However, further studies are needed to evaluate these results.¹⁸²

CONCLUSIONS AND PERSPECTIVES

Our expanding knowledge of the urinary, gastrointestinal, and vaginal microbiomes and their roles in both homeostatic and disease conditions in the urinary tract provides a significant opportunity for microbiome-derived therapeutic interventions in conditions ranging from AKI and CKD to rUTI. However, the development and application of these therapeutic interventions are dependent on a rationalized application of emerging technologies and improved understanding of these systems at both the local and systemic level.

Emerging -omics technologies and microbial culturing have complemented one another in advancing our understanding of the urinary microbiome.⁵ Metabolomics, metaproteomics, and metatranscriptomics could be integrated in the workflow for specific assessment of patient cohorts and may enhance current diagnostic approaches. The application of genome-guided metagenomics, used commonly in the study of the gastrointestinal tract, integrates these approaches to enable causative validations of health and disease-associated genes within bacterial genomes¹⁸³ or between mucosal sites.⁵

While metagenomic analysis allows prediction of functional capacity, combination with transcriptomics to determine the response, and metabolomics to determine function is required. Genome-wide immune associations of the bladder transcriptome could be further investigated to identify host and bacterial markers to control infection. At the functional level, the investigation of polymicrobial communities can identify synergistic relationships during health and disease. For example, iron-uptake of UPEC is induced by a metabolic product from *E. faecalis*,¹⁸⁴ and urinary tract commensals such as the genera *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* are known to metabolize the extracellular glycosaminoglycan layer which coats the host urothelium.¹⁸⁵ Identifying the metabolic activities or microbiome-products could provide further insight into potential targets for treatment. Indeed, these -omics approaches offer distinctly invaluable information, combining these tools in future analyses could shed light on the human host and urinary microbiome.

To develop targeted therapies, it is essential to integrate the knowledge of microbiome genomics, transcriptomics, and functional capacity with host state and response; however, the host immune responses to the urinary microbiome has not been well defined. Application of genetically-engineered murine models, 3D organoid culture,¹⁸⁶ or microfluidic organ-on-a-chip models¹⁸⁷ in combination with sequencing tools such as single-cell sequencing,^{188,189} metagenomics and -omics approaches will prove essential in the advancement of this field. A focus on the

application of emerging microbiome technologies to the urinary microbiome has the potential to revolutionize the treatment of UTI or related complications of the lower urinary tract and could offer insight into the development of novel therapeutics and intervention strategies.

ACKNOWLEDGEMENTS

S.C.F. (1156333, 1181105, 1186371, and 1159239) and M.R.S. (1156898) are supported by the National Health and Medical Research Council Australia. S.C.F. (DP190101504) and M.R.S. (DE170100226) are supported by the Australian Research Council. S.C.F. is supported by the Victorian Government's Operational Infrastructure Support Program.

AUTHOR CONTRIBUTIONS

B.J.F., M.C., V.R.M., A.V.D., S.C.F., and M.R.S. wrote and edited the paper. M.R.S. conceptualized the review.

ADDITIONAL INFORMATION

Competing interests: S.C.F. is a scientific advisor to Microbiotica and Biomebank Australia. B.J.F., M.C., A.V.D., V.R.M., and M.R.S. declare no competing interests.

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