Review Article

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Urobiome: An outlook on the metagenome of urological diseases

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The urinary tract likely plays a role in the development of various urinary diseases due to the recently recognized notion that urine is not sterile. In this mini review, we summarize the current literature regarding the urinary microbiome and mycobiome and its relationship to various urinary diseases. It has been recently discovered that the healthy urinary tract contains a host of microorganisms, creating a urinary microbiome. The relative abundance and type of bacteria varies, but generally, deviations in the standard microbiome are observed in individuals with urologic diseases, such as bladder cancer, benign prostatic hyperplasia, urgency urinary incontinence, overactive bladder syndrome, interstitial cystitis, bladder pain syndrome, and urinary tract infections. However, whether this change is causative, or correlative has yet to be determined. In summary, the urinary tract hosts a complex microbiome. Changes in this microbiome may be indicative of urologic diseases and can be tracked to predict, prevent, and treat them in individuals. However, current analytical and sampling collection methods may present limitations to the development in the understanding of the urinary microbiome and its relationship with various urinary diseases. Further research on the differences between healthy and diseased microbiomes, the long-term effects of antibiotic treatments on the urobiome, and the effect of the urinary mycobiome on general health will be important in developing a comprehensive understanding of the urinary microbiome and its relationship to the human body.

Keywords: Microbiome; Mycobiome; Urology

INTRODUCTION

Why urobiome?

Although it was previously believed that urine was a sterile substance, new research indicates that it contains a host of microorganisms. This has left the urinary microbiome relatively unstudied, as it was not a part of the Human Microbiome Project which aimed to identify and categorize the microbiomes of the human body in healthy individuals [1]. However, research suggests that the urinary microbiome is extremely diverse and may play a role in a host of urinary diseases [2-5]. While research remains relatively inconclusive, studies have indicated an association between certain bacterial and fungal species and various urinary diseases using new technologies like next-generation sequencing (NGS) and expanded quantitative urine culture (EQUC) that help identify a majority of the bacteria found in urinary microbiomes [6]. This review aims to provide a comprehensive un-

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derstanding of select bladder diseases and their respectively identified bacterial signatures using NGS- and EQUC-based analysis from data compiled through previous studies and reviews surrounding the subject.

HUMAN GENOME, MICROBIOME, AND MYCOBIOME

The human genome is complex and, although efforts have been made to fully sequence it, remains relatively unexplained regarding its mechanistic function. After the Human Genome Project, it was discovered that there was much left to understand regarding the human body, the relationship between DNA and protein function, and the interaction between these elements and the various microbiomes in the human body [7]. For urinary diseases specifically, the lack of research on the urinary microbiome has left much to be understood about its relationship with the human body. The microbiome, consisting of the microorganisms and their respective genomes that exist within a region of a host body, as well as their individual activity and formed micro-ecosystems, have been indicated to significantly affect the health of the host as changes occur due to situational and environmental factors [8]. Another factor to consider is the region's mycobiome, which is the fungal microbiota within an area. This also can significantly impact host health, as well as the microbiome of the region, making it important to investigate in combination with the bacterial microbiome [9].

Although the urinary microbiome and mycobiome remain relatively unstudied, there is significant evidence indicating that the microbiome and mycobiome of other regions, like the lungs and gut, heavily affect the overall health of the human body [10]. Evidence has linked lung and gut microbiome and mycobiome health to a host of issues, including asthma, colorectal cancer, alcoholic liver disease, cystic fibrosis, and hypoglycemia [10-13]. This type of linkage between microbial health and host health indicates that the urinary microbiome and mycobiome play a similarly important role in the overall health of the human body.

THE GASTROINTESTINAL MICROBIOME AND INDICATION OF UROBIOME SIGNIFICANCE

Traditionally, research has focused on the gut microbiota and its relationship to various disease. With its expansive surface area and constant processing of food, symbiosis of the gut microbiota have long been recognized as an important step in disease prevention [14]. Important for diseases related to diet and obesity, as well as atherosclerosis, Crohn's disease, ulcerative colitis, and autism, research has indicated that the balance of the gut microbiota is extremely significant to host health [14,15]. Studies have begun to indicate a similar importance for the health of an individual's urinary microbiota. With connections to prostate, gut, and renal health, dysbiosis of the urinary microbiota has indicated increased risk of various lower urinary tract diseases, prostate cancer, kidney disease, and increased risk of gastrointestinal dysbiosis as well [16-20]. Research of the urinary microbiome is thus extremely important in understanding these inter-system relationships and their effect on overall host health.

UROBIOME, MICROBIOME AND MYCOBIOME IN URINE

With the discovery of bacteria in urine, research into its relation to urological diseases began. Consequently, the urinary microbiome has become increasingly important, although it has been shown to vary significantly between individuals. This environment, consisting of all the bacterial microorganisms contained within the bladder, as well as the proteins and metabolites they produce, their genetic material, and the host proteins and metabolites within the region, has been shown to be increasingly more complex than previously believed [21,22]. Together, with the urinary mycobiome, which is all the fungal microbiota and its subsequent genetic material, proteins, and metabolites within the bladder, evidence suggests that the balance of a healthy individual's urinary biome is important to prevent and protect against many urinary diseases [23].

NEXT-GENERATION SEQUENCING METHOD AND CULTURE-BASED VALIDATION OF UROBIOME

The urinary microbiome is most effectively determined using a combination of NGS and EQUC. Because whole genome sequencing can be performed as a form of NGS, DNA NGS is generally performed using polymerase chain reaction amplification and 16S rRNA gene high-throughput sequencing, which allows the entire genome to be sequenced. Although this process is much better than standard diagnostic methods of urine analysis, there are still several limitations [6]. This includes an inability to distinguish closely related bacterial taxa, confirm bacterial viability, and link the genotypic resistance to a specific organism [6]. In addition, bacterial abundance can be determined by 16S rRNA sequencing, but not precisely [6].

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EQUC is also important because it can detect bacterial growth as low as 10 CFU/mL by plating a urine sample on various media at different temperatures and under various atmospheric conditions for a longer period, resulting in detection of up to 92% of bacteria species not otherwise detected on a standard urine culture. This contrasts with the standard urine culture, which was designed to grow specific *Escherichia coli* pathogens and can only detect about 33% of bacterial growth [6,24].

Both EQUC and NGS are important analysis techniques because they each provide data that the other one may not [2,25]. Although sequencing allows for the bacterial populations to be studied, more specific technology must be used to determine the functional ability of these microbes, indicating that the specific metabolites, and not the species of microbe, are what will drive future research and therapies [17].

MICROBIAL DIVERSITY IN HUMAN URINE

There is substantial variation in an individual's microbial diversity, and the way subjects are grouped in studies may greatly affect the analysis of the results. For some populations, an increase in microbial diversity may be beneficial, and for others it may be harmful, which is why factors such as age and gender must be accounted for when organizing studies [26-29]. For example, studies have indicated that menopause causes a significant alteration in the female urinary microbiome. Although the *Lactobacillus* species is the most prevalent bacteria in pre-menopausal women, post-menopausal women have more significant levels of *Mobiluncus* and a general decrease in overall microbial diversity [1,30]. This change in the microbiome of a healthy female can greatly affect studies when age is not accounted for. Similarly, the female microbiome is very different than that of a male, which has a high amount of the species *Corynebacterium* in most control groups [2,25,31]. Overall, the most common bacterial species found in sampled urine include *Lactobacillus* and *Streptococcus*, with *Gardnerella*, *Staphylococcus*, and *Corynebacterium* following closely and *Alloscardovia*, *Burkholderia*, *Jonquetella*, *Klebsiella*, *Saccharofermentans*, *Rhodanobacter*, *Prevotella*, and *Veillonella* also noted as prevalent.

In patients over seventy years old, one study indicated that there was again a change in the microbiome, detecting *Proteiniphilum*, *Saccharofermentans*, and *Parvimonas* in the microbiome, which are species not commonly found in samples from younger individuals [2,6,25,31-34]. Table 1 shows a compiled list of these bacterial species commonly found in the healthy human urinary microbiome. Similarly, Table 2 is a list of the bacterial (and certain fungal) species commonly found in the urinary microbiome of individuals with the urinary diseases discussed in this article and is organized accordingly.

The sampling method also tends to affect the microbial diversity observed in urinary samples. Since there is not yet a standard method of collection and analysis for urine samples, it is often difficult to compare studies [6,35]. Urine has a very low concentration of microbes within each sample, resulting in a high potential for contaminant amplification that leads to significant error rates and confounders [35]. This can be combated by larger volume samples, stricter lysis conditions, and new sequencing techniques with higher fidelity. For women it is difficult to collect urine samples without vaginal contamination. Several studies aimed at determining the optimal sampling method have been performed. Results indicated that collecting female urine via a transurethral catheter most closely resembled samples obtained via suprapubic aspiration, suggesting that this may be a better collection method than midstream voided urine [35-37]. For men, a subsequent study indicated that the male bladder, like the female bladder, is a low biomass environment, making catheterization a preferred urine sample

Table 1. Bacterial prevalence in the urinary microbiome of a healthy individual

Genus[a]	Primary cohort[b]	Prevalence[c]	Reference
Alloscardovia		Rare	[2]
Burkholderia		Rare	[2]
Corynebacterium	Males	Common	[2,14,21]
Gardnerella		Frequent	[3]
Jonquetella		Rare	[2]
Klebsiella		Rare	[2]
Lactobacillus	Pre-menopausal females	Common	[1,2,20]
Mobiluncus	Post-menopausal females		[1,20]
Parvimonas	Individuals over 70		[2]
Prevotella		Rare	[2]
Proteiniphilum	Individuals over 70		[2]
Rhodanobacter		Rare	[2]
Saccharofermentans	Individuals over 70		[2]
Staphylococcus		Frequent	[3]
Streptococcus		Common	[2]
Veillonella		Rare	[2]

[a]:Bacterial species are listed alphabetically.
[b]:A primary cohort is only specified if there was a group of significance indicated in the reference article(s).
[c]:Prevalence is ranked from common to frequent to rare and is only noted if again specified within the references.

Table 2. Bacterial prevalence in the urinary microbiome for various urinary diseases

Urinary disease	Genus[a]	Primary cohort[b]	Prevalence[c]	Reference
Bladder cancer	*Acinetobacter*		Increased	[5,30]
	Burkholderia		Increased	[30,31]
	Corynebacterium	Males	Decreased	[5,30]
	Fusobacterium		Increased	[15]
	Klebsiella	Females	Increased	[30,31]
	Lactobacillus	Females		[30]
	Streptococcus		Increased	[2]
Benign prostatic hyperplasia	*Enterococcus*	Males		[32]
	Escherichia	Males	Decreased	[32]
	Micrococcus	Males		[32]
	Pantoea	Males		[32]
	Pseudomonas	Males		[32]
	Serriatia	Males		[32]
	Staphylococcus		Increased	[32,35]
Urgency urinary incontinence	*Actinobaculum*	Females	Increased	[3,5]
	Actinomyces	Females	Increased	[3,5]
	Areococcus	Females	Increased	[3,5]
	Arthrobacter	Females	Increased	[3,5]
	Corynebacterium	Females	Increased	[3,5]
	Gardnerella		Increased	[2]
	Lactobacillus		Decreased	[2,3,16]
	Oligella		Increased	[3]
	Staphylococcus		Increased	[3]
	Streptococcus	Females	Increased	[3,5]
Overactive bladder syndrome	*Corynebacterium*			[20]
	Lactobacillus		Decreased	[2,20]
	Proteus	Females	Increased	[5,20]
	Staphylococcus	Females	Increased	[5,20]
	Streptococcus			[20]
Interstitial cystitis/bladder pain syndrome	*Candida*[d]		Increased	[2,23]
	Lactobacillus	Females	Increased	[2,3,5,23]
	Saccharomyces[d]		Increased	[2,23]
Urinary tract infection	*Atopobium*	Females	Decreased	[16]
	Finegoldia	Females	Decreased	[16]
	Gardnerella	Females	Decreased	[16]
	Lactobacillus	Females	Decreased	[1,16]
	Sneathia	Females	Decreased	[16]

[a]:Bacterial species are listed alphabetically.
[b]:A primary cohort is only specified if there was a primary group in which this species was found indicated in the reference article(s).
[c]:Prevalence is considered increased or decreased in comparison to the controls from that same study and is left blank if not specified by the reference, or if no significant difference was observed.
[d]:Indicative of two fungal species which were discussed in the literature that deviate from the bacterial species that make up most of the table.

collection method [35]. Another study also concluded that suprapubic aspiration and transurethral catheterization are the two best forms of sample collection because they avoid contamination from the genitals [2]. Trials have also indicated that, in males specifically, there is a difference in the beta microbial diversity when comparing voided and catheterized samples. It was hypothesized that this was likely due to the difference in urethra length between males and females, which likely allows for a greater difference between the bladder and the urethra microbiome in males than in females. This difference between collection methods in males raises the question of which would act as a stronger diagnostic method for diseases like bladder cancer because, while one may better represent the urinary microbiome, this may

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not be the best functional representation of urological microbes for therapeutic purposes. And voided urine has been served for initial identification of diagnostic, prognostic, and non-invasive biomarkers for diseases primarily at the microbe-urothelial interface [26,38,39].

MICROBIAL DIVERSITY IN BLADDER CANCER

The taxa *Fusobacterium*, *Sphingobacterium*, and *Enterococcus* are present in schistosomiasis-induced bladder cancer patients [17,40]. This type of bladder cancer is also more prevalent in individuals with strains of bacteria that can mediate the formation of N-nitrosamines. Chronic urinary tract infections (UTIs) are hypothesized to leave an individual predisposed to developing bladder cancer, but there is conflicting epidemiological evidence surrounding this. It has not been determined whether the presence of these microbes is a result of or a cause for bladder cancer. One hypothesis is that the extracellular matrix is influenced by the urinary microbiome, which may either help prevent or induce cancer depending on the microbes present. This would be similar to the influence of microbiomes in intestinal cancer. However, studies have conflicting support for this hypothesis. Biofilms may be a cause for chronic inflammation in the genitourinary system, among other places, which has been indicated to correlate with a higher risk of developing cancer due to their interactions with epithelial cells. There is also evidence to suggest that the urinary tract's microbiome hosts commensal microorganisms, and the interaction between these microbes and bladder cancer cells may affect tumorigenesis [41,42]. The presence of some species, like *Lactobacillus*, have been indicated to help aid in the prevention of disease in some women, dissuading from the growth of other, more commonly harmful species. However, the growth of too much of a commensal organism, like *Lactobacillus*, can become harmful to the surrounding environment by decreasing the overall microbial diversity, which has been indicated to promote tumorigenesis [2].

Although bladder cancer is much more common in men, it is much more deadly in women [41,43]. While this is likely affected by factors related to social inequality, it may also be due to the microbial differences between male and female urinary tracts. For bladder cancer, the genetic difference between male and female patients remains unknown. One specific example is the activity of glutathione-S-transferase M1, which affects the metabolizing of carcinogens. Studies also indicated that increased age, parity, premenopausal status, and use of estrogen and progestin are all associated with a lower risk of developing bladder cancer. In females, the *Lactobacillus* species is extremely common in the urinary microbiome, while in males *Corynebacterium* is most prevalent. Additionally, one study indicated that females with bladder cancer had higher levels of *Klebsiella* in urine samples than healthy women, and an increase in *Burkholderia* for bladder cancer patients was observed regardless of gender [41,44].

It has been suggested that 20% to 30% of cancers, like gastric cancer, liver cancer, urinary bladder cancer, cholangiocellular neoplasia, and cervical cancer are related to recurring microbial infections [41,45]. Evidence has also indicated that abnormal microbiomes have been correlated with a higher risk of cancer, but it is unclear what the "normal" microbiome of the urinary tract is specifically. Various bacteria have been indicated to play a role in the relationship between bladder cancer and the urinary microbiome, but studies vary in the specific species associated. In one, it was an increase in *Streptococcus* in cancerous patients. In another, it was *Fusobacterium nucleatum*, which has known associations with carcinogenesis. [41,42] This bacterium is gram-negative and anaerobic and is known to induce a chronic inflammatory response by promoting the beta-catenin pathway. There are several geneses also associated with bladder cancer, with one of significance being *Acinetobacter*, which consists of several gram-negative, anaerobic species that are indicated to impair immune response to bovine papillomavirus type 2 and thus increase susceptibility to carcinogenesis.

The microbiome has a promising predictive ability for urinary cancer, with dysbiosis showing evidence of a relationship to anticancer therapy and a potential to predict Bacillus Calmette–Guerin (BCG) therapy response. *Lactobacillus iners*, which is more prevalent in females, may also play a role in BCG efficacy due to the competition between them for fibronectin binding [41,46]. One notable difference in the urinary microbiome of individuals with urothelial cell carcinoma was an increase in *Streptococcus*. Associations between bladder cancer and *Mycobacterium tuberculosis* from the BCG vaccine have also been made, but the mechanistic reason for its success in bladder cancer inhibition remains unsure [2,47,48].

BCG is used for bladder cancer treatment via direct insertion, but the induced immune response may be due to the interaction of BCG with urinary bacteria, and BCG may be competing with other bacteria, like *L. iners*, for fibronectin-binding positions, potentially reducing its treatment efficacy (Fig. 1) [17,46,49]. BCG has been regularly used to deter cancer progression, and studies before treatment indicate that patients with bladder cancer were more likely to have increased levels of *Fusobacterium* [26,42]. Healthy

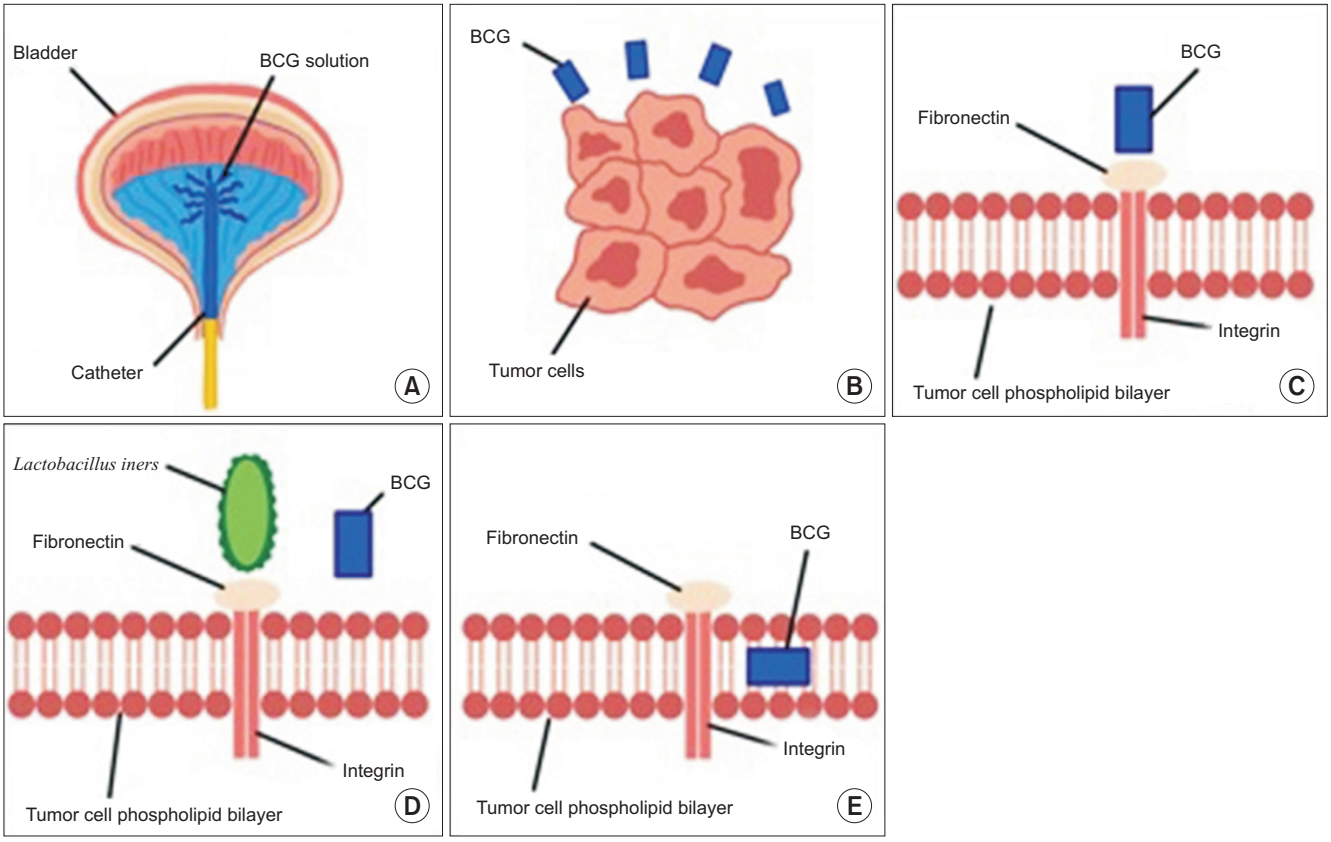

Fig. 1. The hypothetical mechanistic pathway of BCG in the bladder. (A) BCG is injected into the bladder via a catheter. (B) BCG identifies and attatches to tumor cells which activates a variety of pathways, including the binding of fibronectin. (C) BCG binds to the fibronectin of a tumor cell and will subsequently be absorbed into the bilipid layer. (D) BCG binding may sometimes be blocked by *Lactobacillus iners*, reducing the drug's efficacy. (E) If the BCG effectively blinds to the fibronectin, it is absorbed into the bilayer and promotes an immune response to destroy the tumor cell. BCG, Bacillus Calmette–Guerin.

women generally have higher levels of *Mycobacteria* and other *Actinomycetes*, which are suspected to help impede cancer progression, and some studies suggest that certain urinary microbial profiles may leave an individual predisposed to malignancies and affect treatment response [26,50]. Additionally, *Lactobacillus casei* was previously believed to reduce the recurrence of bladder cancer, but human studies were stalled due to complications [17,51,52]. However, with new technology in the microbiome field, these studies should be reinvestigated because of their promising potential, and the *L. casei* strain Shirota may be a viable for non-muscle-invasive bladder tumors [17,51,52].

Antibiotic treatments of patients with bladder cancer reduced the progression-free and overall survival of immunotherapy-treated patients, indicating that an alteration of the patient's microbiome may lead to a better therapeutic result [17]. The presence of certain bacteria (species of *Mycoplasma* and *Proteobacteria*) can metabolize the chemotherapy drug gemcitabine, rendering it ineffective. Other bacteria can reactivate irinotecan, causing drug toxicity. There is also evidence that certain bacteria can affect the efficacy of immunotherapy [17,53].

MICROBIAL DIVERSITY IN BENIGN PROSTATIC HYPERPLASIA (BPH)

BPH may be correlated to an increase in *E. coli* in prostatic secretion, a decrease in *E. coli* in urine, and an increase in *Enterococcus* in the seminal fluids, but it is unknown whether these changes in the microbiome are the cause for BPH or are a result of prostate cancer treatment [54,55]. Several studies have indicated a correlation between chronic prostate inflammation and BPH, implicating that the urinary microbiota may play a role in its development due to the increase in proinflammatory cytokines observed in the urinary microbiome of individuals diagnosed with BPH [56]. Additionally, this study suggested that inflammasomes may have a role in BPH development due to their involvement with activation of the immune system's inflammatory response [56,57]. Factors such as oxidative stress, DNA dam-

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age, and signaling involving nuclear factor-κB (NF-κB) and cyclooxygenase-2 (COX2) have also been indicated to play a role in BPH onset and development [54,58-60]. The species *Staphylococcus*, *E. coli*, *Micrococcus*, *Enterococcus*, *Serratia* spp., *Pseudomonas aureginosa*, and *Pantoea* spp. were all identified in 22% to 28% of BPH samples from a study of 36 individuals, with the relatively high rate of 11.1% for *E. coli* matching the findings of previous studies, making this the most common bacteria associated with BPH [54,58].

Although more individuals are being diagnosed with BPH, its overall severity has decreased with the usage of oral medication, leading to a reduction of surgical cases [54]. This combination therapy using an alpha-blocker and a 5-alpha-reductase inhibitor help reduce inflammation of the prostate to relax the organ and minimize BPH symptoms [61,62]. This management of the chronic inflammation associated with BPH further indicates its importance in the disease pathology, suggesting that inflammation is not only a correlated factor, but possibly a causative factor as well [61,63].

MICROBIAL DIVERSITY IN URGENCY URINARY INCONTINENCE (UUI)

Although UUI is the most frequently studied bladder disease, there is little consistency or overlap between results. One study suggested that increased prevalence of *Actinomyces*, *Corynebacterium*, and *Streptococcus* correlated with better responses to medication. Another study suggested that the *Lactobacillus* species dominates the urinary microbiome in healthy controls, while diseased groups are more likely to have Lactobacilli within a diverse microbiome [6,64]. This is especially interesting because *Lactobacillus* species are more common in the female urinary system, and young women tend to have less diverse urinary microbes, while older women tend to have more diverse ones [26]. Another study showed evidence that patients with evidence of bacterial DNA in their urine had fewer episodes of UUI on a daily basis than those with no reported urinary bacterial DNA. In this study, *Actinobaculum*, *Actinomyces*, *Areococcus*, *Arthrobacter*, *Corynebacterium*, *Gardnerella*, *Oligella*, *Staphylococcus*, and *Streptococcus* were more prevalent in those experiencing UUI, and *Lactobacillus* was once again present in decreased amounts. However, the use of either NGS or EQUC altered whether there was a significant overall difference in microbial diversity of the urinary system for those experiencing UUI and healthy individuals, although evidence suggests that there is some type of microbial component to UUI [6,26,31,64].

In the studies regarding UUI, women generally tended to have lower *Lactobacillus* and higher *Gardnerella* counts when experiencing this disease. Research concluded that there was a correlation between UUI symptom severity and decreased urinary microbial diversity, and one study additionally suggested that the use of solifenacin to treat UUIs was more effective when women had a lower microbial diversity in the urinary system [2,25].

MICROBIAL DIVERSITY IN OVERACTIVE BLADDER SYNDROME (OAB)

OAB, characterized by frequent urination, urinary urgency, and difficulty controlling bladder contractions, is a syndrome with a multitude of possible pathologies [65]. Sampling has indicated that in at least some cases, the urinary microbiome may play a role in OAB. In one study analyzing the urinary microbiome in females, the most prevalent bacteria found in both healthy and OAB urinary microbiomes were *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Lactobacillus*. There was also a statistically significant difference in the prevalence of *Lactobacillus* and *Proteus* between the control and the OAB samples, with *Lactobacillus* being much more prevalent in healthy individuals and *Proteus* in OAB samples [65].

It is hypothesized that the presence of *Lactobacillus* bacteria in the urinary tract, especially in women, may help prevent OAB because it promotes a more acidic environment that prevents more virulent bacteria from growing there [2]. Although the specific role of the microbiome is not yet known in relation to OAB, preliminary trials for several antimuscarinics and intradetrusor botulinum toxin injections have indicated that patients who respond to these treatments usually have a reduced microbial diversity in their urinary tract [66]. Furthermore, there is a possibility that the urinary microbiome is related to brain function, similar to the gut microbiome, which may affect neurotransmitter release and immune system stimulation to affect an individual's risk of experiencing OAB [66].

MICROBIAL DIVERSITY IN INTERSTITIAL CYSTITIS AND BLADDER PAIN SYNDROME (IC/BPS)

Not much is fully understood about the role microbes play in IC/BPS. Studies suggest that there is a decrease in diversity for the urinary microbiome in individuals suffering from IC/BPS, but an increase in levels of the *Lactobacillus* species [2]. One study also suggests that the level of

inflammatory cytokines is increased in those affected with IC/BPS. However, there is not enough conclusive evidence to show that bacteria play a role in IC/BPS development, and some studies have even concluded that no significant role for the urinary microbiome can be determined for IC/BPS susceptibility [1,6]. On the other hand, there may be an overall decrease in the urinary microbiome diversity for individuals suffering from IC/BPS, but an increase in levels of the *Lactobacillus* species, as well as the level of inflammatory cytokines in those affected with IC/BPS, with one study additionally concluding that an increase in *Lactobacillus* levels was associated with an increase in IC/BPS severity [6,33].

Some studies have also indicated that increased amounts of fungi in the bladder may influence IC/BPS [2,33]. Although there was no significant difference in bacterial species composition when comparing patients with IC/BPS to healthy individuals, symptom flares indicated increased levels of the fungal species *Candida* and *Saccharomyces*, but subsequent studies did not observe a similar conclusion. Testing for IC/BPS is unfortunately extremely limited because 16S NGS is unable to detect eukaryotic microbes, and EQUC cannot identify several types of fungi, resulting in many negative tests using the current diagnostic standards due to culture testing inconsistency [6,33].

MICROBIAL DIVERSITY IN CHRONIC URINARY TRACT INFECTIONS

Although acute UTIs are primarily caused by *E. coli*, when UTI is chronic and persistent, it is likely caused by a different microbe, which is why standard urine culture often misses this as a diagnosis [6,26,67]. Chronic lower urinary tract symptoms are likely caused by the formation of biofilms, which protect harmful bacteria from helpful immune mechanisms while simultaneously promoting mutations. It has been indicated that chronic UTIs can be perpetuated by treatment through antibiotics because the formation of biofilms can aid in increased resistance as well [6,36,68]. It was previously believed that bacteriuria caused UTIs, but evidence suggests that asymptomatic bacteriuria may help prevent chronic UTIs by inhibiting the growth of certain *E. coli*, especially those which are shown to be antibiotic resistant. Current diagnostic methods for UTIs are effective, and further specification for diagnosis is unnecessary and would likely result in overtreatment [26].

No longitudinal studies regarding the urinary microbiome and antibiotics have been performed. However, a general decrease in *Lactobacillus*, *Finegoldia*, *Gardnerella*, *Atopobium*, and *Sneathia* species were observed from various studies [26,69]. One study in particular saw that after treatment from metronidazole, *Lactobacillus crispatus* was completely undetectable in urinary samples, despite being one of the most prevalent bacteria in the urinary samples of healthy young females [1]. The lack of *Lactobacilli* likely increased post-menopausal susceptibility to recurrent UTIs [1,70].

Although antibiotic treatment is a popular method to combat UTIs, it has been associated with long-term problems by promoting antibiotic resistance. Probiotics, prebiotics, and diet alterations have been proposed as alternative preventative and general treatment methods to avoid this problem. This includes administration of the *Lacticaseibacillus rhamnosus* GR-1, *Limosilactobacillus fermentum* RC-12, and *Limosilactobacillus reuteri* B-54 for UTIs [2,17]. The risk of recurrent UTIs can be reduced using estrogen replacement treatment, which increases the *Lactobacillus* population in the vagina and likely the urinary tract as well [1,71,72]. Although certain *Lactobacillus* species may aid in UUI treatment, the presence of the specific *Lactobacillus delbrueckii* and *Lactobacillus gasseri* are indicated to be associated with increased UTI and UUI severity [2,36]. Another treatment method that has been investigated to replace antibiotic treatment is the consumption of cranberry juice supplements, although studies indicated that supplement use showed no significant decrease in UTI risk. However, intake of higher doses of D-mannose, which is found in cranberries, may be effective in UTI risk reduction [2,25].

MICROBIOME CAN BE ALTERED BY PROCEDURES AND MEDICATIONS

There are several current potential procedures aimed at altering the urinary microbiome of individuals with urinary disease. For bladder cancer, *M. tuberculosis* from the BCG vaccine has shown success in inhibiting the spread of bladder cancer despite the mechanistic understanding of this process remaining unknown [2,47,48]. Additionally, *L. casei*, specifically the Shirota strain, has had promising results in preliminary testing regarding its ability to reduce the recurrence of non-muscular invasive bladder tumors [17,51,52]. In BPH, relative success has come from a combination therapy treatment using alpha-blockers and 5-alpha-reductase inhibitors, but 12.6% of patients that receive this therapy still observe clinical progression and 5% still require surgery [61,62]. UUI has most often been treated using solifenacin, a bladder relaxant, but its success has been indicated to be tied with the patient's relative urinary microbial diversity [2,25,73]. Similarly, treatment for OAB has been indicated to

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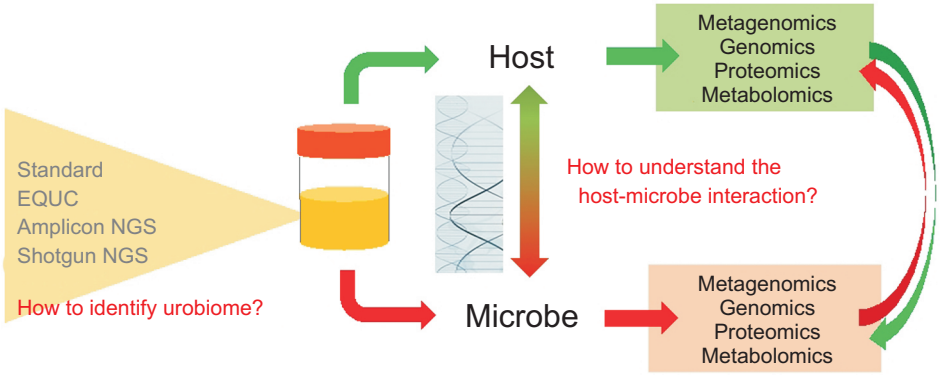

Fig. 2. New challenges in the urobiome. EQUC, expanded quantitative urine culture; NGS, next-generation sequencing.

depend on the patient's urobiome diversity, with preliminary trials using antimuscarinics and intradetrusor botulinum toxin injections showing potential primarily in individuals with reduced diversity [66]. Widespread clinical procedures and drug treatments for those with IC/BPS have been difficult to identify. A distinct pattern connecting the urinary microbiome to these diseases remains unknown, making the development of an effective treatment difficult as well [1]. For UTIs, a common treatment method involves the administration of antibiotics, but studies indicate that treatment using the *L. rhamnosus* GR-1, *L. fermentum* RC-12, and *L. reuteri* B-54 may be better options [2,17]. Estrogen replacement therapies and D-mannose supplements have also shown potential in reducing the risk of recurrent UTIs [1,71,72].

CONCLUSIONS

As research about the urinary microbiome and mycobiome continue, evidence regarding its relationship to urinary disease will expand and improve. Methods like NGS and EQUC remain relatively limiting in their ability to analyze microorganisms present within the bladder microbiome, but they are still much improved from previous techniques. The use of antibacterial treatments for various bladder diseases and their effects on the balance of bacteria in a healthy bladder must be researched further to help elucidate whether changes in the urinary microbiome are primarily causative or correlative with bladder disease.

Current limitations and future plans

The potential benefits of understanding the urinary microbiome are numerous. Despite the current limitations due to lack of previous research, difficulties in standardizing sampling techniques and analysis methods, and problems with defining the scope of the urinary microbiome, much progress has already begun in the field. New challenges in this field include to develop the better identification methodologies of microbiome and to understand the pathological function of micro- and mycobiome include multi-omics-based and host-microbe interaction (Fig. 2) [8]. With further research and technological development, the relationship between the urinary microbiome and mycobiome health and the health of the human body will be understood, allowing for more specific clinical treatment of a variety of urinary diseases and a potential connection to diseases not directly associated with the urinary environment. It can also provide a stronger understanding regarding the use of antibiotics and their effects on the microbiomes of the body, as well as the potential efficacy of other treatments, including the use of probiotics and dietary supplements, in regard to various urinary diseases.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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