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OVERACTIVE BLADDER (U LEE AND S ADELSTEIN, SECTION EDITORS)

The Bladder is Not Sterile: an Update on the Urinary Microbiome

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Abstract

Purpose of Review The article discusses (1) techniques used to study bacterial urinary microbiota; (2) existence of non-bacterial urinary microbiota; (3) associations between changes in urinary microbiota and various benign lower urinary tract disorders.

Recent Findings Urine harbors a diverse microbial community that resides within it. A multitude of studies have identified differences in these communities associated with urologic conditions, suggesting that microbial communities may maintain normal bladder homeostasis. Technological advances in analytic approaches have improved our understanding of the urinary microbiome. The choice of urine sampling method (voided, catheterized, or aspirated) will significantly influence microbiome findings. Sex and age highly influence urinary microbiota; in addition to rigorous inclusion criteria, microbial studies must be sufficiently powered to overcome the substantial interindividual variability of urinary microbiota. Regardless of these complicating factors, studies have identified microbial patterns correlating with both urologic diagnoses and treatment responses.

Summary Without a clear understanding of the variability of and exogenous influences on the urinary microbiota in the absence of disease, it has been challenging to reveal the microbial patterns responsible for disease pathophysiology. Host mechanisms in response to the urinary microbiome are also poorly understood. Additional research can address whether the manipulation of urinary microbiota will benefit lower urinary tract health.

Keywords Urinary microbiome · Benign lower urinary tract disorders

Introduction

The healthy bladder is not sterile. Urine harbors a complex microbial community even in healthy, asymptomatic individuals. This microbial community is thought to perform critical functions in bladder homeostasis, with potential roles in the maintenance of urothelial integrity, protection against infection, regulation of neurotransmission, and promotion of normal immune function [1]. Shifts in resident urinary microbiota towards different communities that do not perform these beneficial functions are termed dysbiosis, such imbalances have been implicated in dysfunction of nearly every organ system, from the central nervous system to the genitourinary tract (Fig. 1). Highly sensitive, culture-based, and state-of-the-art culture-independent techniques have opened a window into understanding these communities (reviewed in [2]). Detection of a microbial community in urine specimens, however, cannot presume an identical microbial community within or on tissue of either the lower (bladder, prostate, urethra) or upper (ureter, renal pelvis) urinary tract. In comparison with other body sites, there remains a substantial gap between curiosity about urinary microbes and our understanding of their role in urologic symptomatology. Of the literature identified preparing this review, approximately half of PubMed-indexed articles concerning the urinary microbiota were reviews lacking primary data. While a decade of study has revealed fundamental roles for the microbiota in health and disease in other systems, multiple technical and operational challenges specific to the urinary tract have challenged progress to understanding the urinary microbiome's specific functions.

This article is part of the Topical Collection on *Overactive Bladder*

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Technical Challenges

Deep sequencing of variable regions within the 16S rRNA locus (commonly referred to as next-generation sequencing

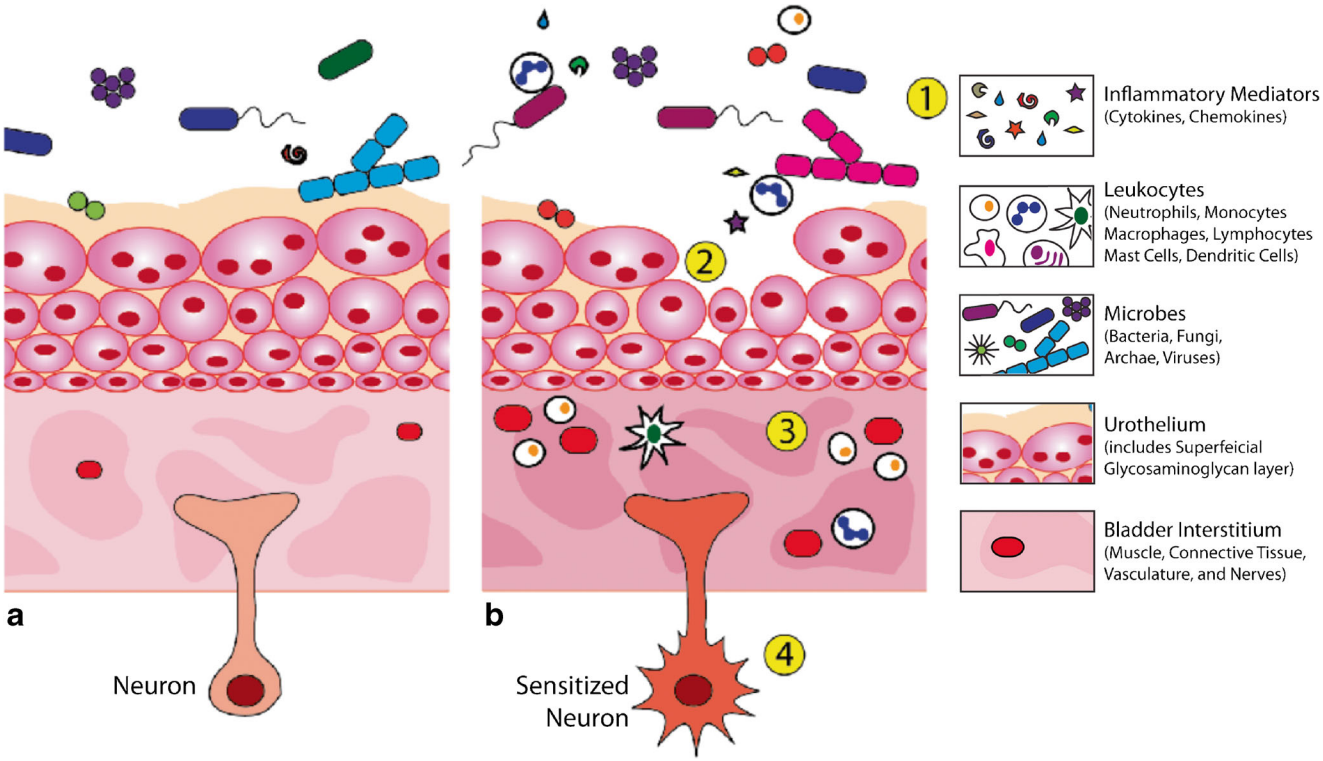

Fig. 1. The proposed role of urinary dysbiosis in the development of urinary pathology. (A) In a healthy bladder, a symbiotic microbiota maintains the urothelial barrier, prevents pathologic infection, and preserves normal neurotransmission. (B) In patients with bladder dysfunction, a dysbiotic urinary microflora alters bladder function and results in pain. (1) Pathobiont microbes, enriched in urinary dysbiosis, interact with a susceptible host immune system to induce inflammation. (2) Infiltration of immune cells and inflammatory mediator production (cytokines and chemokines) in concert with loss of the pro-homeostatic, microbially associated factors leads to a breakdown of the urothelial barrier and increased tissue permeability. (3) Host inflammation leads to increased vascularity, tissue edema, and leukocyte recruitment. (4) These fundamental changes in the bladder microenvironment lead to changes in both sensory and motor neurotransmission, leading to sequelae such as altered pain thresholds or detrusor muscle overactivity

(NGS)) remains the most common methodology to examine bacterial populations. Several important limitations to this technology are particularly relevant to the analysis of urinary populations, and urine presents unique technical challenges requiring special consideration before applying standard techniques and pipelines used for other biological samples. In contrast to stool, which contains ample bacteria, urine contains low numbers of microbes and significant host material in shed urothelial cells. To date, numerous studies have demonstrated a significant influence on the results of population analyses of collection technique, sample volume, DNA extraction methodologies, choice of consensus sequencing region and database for taxonomic assignment, and computational analysis for data interpretation [3••, 4•, 5, 6]. Even in higher biomass samples, such as stool, such choices influence results, producing disparate findings for similar studies with small variations in analytic approaches [7–10].

Standardized collection methods and analytic approaches may improve the relatability and scalability of independent studies, but as technology develops, continual refinement will be needed to address the limitations of culture-independent microbial profiling. Given the low biomass of urine samples, the intrinsic error rates of standard NGS can produce significant inaccuracy. In addition, contaminants, such as environmental, skin or vaginal microbes, and urothelial cells, can be amplified to levels similar to those of the community under analysis, creating considerable confounders [6]. The use of larger volume samples and more stringent lysis conditions improves microbial community representation [5, 11]. There have also been intriguing advances, such as PacBio circular consensus sequencing (CCS); sequencing and error-correcting of full-length bacterial 16S rRNA genes provides high-fidelity species-level microbiome data unobtainable with standard NGS [12]. Regardless of method, recognition of the biases in multiple decisions inherent for any individual study prompts caution in relying on any single study as conclusive in our exploration of the urinary microbiome and reinforces the need for confirmatory analyses using alternative approaches.

Defining Pathologic Changes in the Urinary Microbiota

To an extent, early studies of urinary microbes bypassed the iterative nature of scientific exploration, attempting to identify

pathognomonic bacteria for urologic diseases without first establishing the techniques needed for the study of urinary populations, reaching consensus on what defines normal or even that urinary microbiota represent the bladder environment.

Problematic is the proximity of the lower urinary tract (LUT) to higher biomass sites, such as the vagina, through which urine is typically sampled. Only one study looked at the microbiome of urinary samples collected by suprapubic aspirates [4•]. Large differences were found between urinary microbial communities collected by suprapubic aspiration from those seen in voided samples from matched patients, with voided samples exhibiting more similarity to matched vaginal samples. Catheterized samples were far more similar to aspirates, but these catheterizations were performed after sterile, surgical preparation of the urethra. Even under these conditions, catheterized samples contained more vaginal commensals (e.g., *Lactobacillus*, *Prevotella*, *Atopobium*) than aspirated samples. Operating room–based catheterization represents a different scenario than clinic-based catheterization, which will likely contain more genital flora. Suprapubic aspiration is impractical for routine studies, voided samples contain substantial contaminant microorganisms from neighboring urogenital sites, and catheterization reduces but does not eliminate that sampling contamination [13].

Also, there is no guarantee that the urinary microbiome, even when sampled by aspiration, reflects the bladder microbiome. In the gut, the microbial content of stool does not mirror that of the colonic mucosa [14, 15]. Microbes present within bladder tissue or at the bladder-urine interface are presumably selected for different environmental pressures than those in urine; the bladder-associated microbiome likely differs from urinary microbiota. This debate returns us to the question of why we study the urinary microbiome. Most studies seek biomarkers of disease in the hopes that recognizable alterations in urinary flora can provide clinically useful information about diagnosis, prognosis, or causative pathology. The appropriate urine specimen to study may therefore differ for the question being asked; for this reason, the study of voided urine, while generally less specific for the LUT, may serve a more valuable purpose if the goal is identifying diagnostic or prognostic, non-invasive biomarkers. Suprapubic aspirates, while likely to be most representative of the true LUT urinary microbiome, may still be insufficient to understand bladder pathophysiology if the disease is mediated at the microbe-urothelial interface.

A recent comparison of bacteria isolated from gastrointestinal, vaginal, and urinary samples revealed strong similarities between the vaginal and urinary microbiota, which were distinct from the gastrointestinal microbiota. Whole-genome phylogenetic analyses of bacterial strains isolated from the vagina and bladder within single subjects were similar and differed from isolates catalogued from stool. The authors suggested the possibility of interlinked urogenital microbiota, at least in women, which may reframe how we understand microbiota-related pathologies and potential interventions [16••].

Defining urotypes

In addition to technical challenges, difficulties defining experimental and control populations confound many current studies. Some of the best evidence linking the urinary microbiome exist for urgency incontinence, a condition that is more objectively defined than a subjectively defined symptom cluster lacking objective diagnostic criteria, such as interstitial cystitis/bladder pain syndrome (IC/BPS) or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Given substantial interindividual variability and intraindividual variation over time, large sample groups and longitudinal samples, particularly with monitoring of symptom fluctuations, may be necessary to overcome these limitations.

Instead of attempting to examine hundreds of taxa independently, several research groups have separated patients into low and high diversity groups, observing associations of these categories with urologic conditions from bladder cancer to IC/BPS [2, 17–19, 20•, 21, 22•, 23, 24]. Most studies, however, did not utilize an age-matched control cohort; the higher diversity group tended to reflect an older patient population for which age and menopausal status, not disease, may be the dominant influence. At least in women, age and menopausal status are associated with urinary microbial differences [17, 25–28], the magnitudes of which can exceed those associated with disease states. Rigorous matching is needed to avoid artifactual findings, particularly in age-associated conditions such as overactive bladder (OAB). Consensus on appropriate ways to structure both subject and control populations will allow faster progress in understanding types of dysbiosis.

The role of microbial diversity as a measure of disease must take these baseline differences into account. For younger women, in whom the microbiome is less diverse, increasing diversity may be the pathologic state; for older women, who are more diverse at baseline, decreased diversity may represent dysbiosis. Thus, while diversity measures can be helpful descriptors, their meaning is often confused or overstated. Many indices factor in both evenness and richness, which can lead to false equivalency between bacterial communities. The need for diversity measures to describe differences in urinary communities reflects a lack of simple associations of diseases with single organisms and emphasizes the complex interdependency between disease, host health and immune status, and the complex resident microbial community (including bacteria and the frequently neglected fungi, protozoa, viruses, and archaebacteria).

Non-bacterial organisms

While bacteria are the best-studied population within the urinary tract, we are beginning to see early explorations of other organism classes there, such as fungi and viruses.

Fungi Viable fungi in urine were identified as early as the 1850s, with alterations in fungal composition noted in diabetes and renal disease [29]. Yet 150 years later, there has been little improvement in our understanding of urinary fungi. Several reports examining urinary microbiota using expanded culture conditions identified viable Candida species [30, 31•, 32], reiterating the viability of fungi from urinary samples obtained by catheterization. Targeted analysis of fungi using multi-locus PCR coupled with electrospray ionization/mass spectrometry revealed increased fungal DNA during symptomatic flares in patients with IC/BPS [33, 34•]. Fungal community profiling by NGS using the ITS1 region of fungal rRNA, however, revealed that the taxa detected in previous studies are only a small component of the complement of genera present in urinary specimens [35, 36].

Multiple issues are responsible for the lack of progress in fungal characterization, including inadequate fungal reference databases, challenges in the isolation and processing of these more robust organisms, and an erroneous belief that these organisms are not critical in human disease. An increasing number of diseases are being linked to fungal dysbiosis, implicating a profound impact on health and disease [37]. In addition, microbiota are a collective community; the importance of synergistic and antagonistic associations with other microbial components has likely been underestimated.

Viruses Several studies have attempted to identify viral DNA from urine and link these to urinary symptomatology [38]. Viruses, however, lack conserved sequences to permit high-throughput amplification of conserved regions; it is necessary to perform either targeted detection of known viruses or mass DNA sequencing with subsequent viral identification after genomic reconstruction (metagenomic sequencing). Given the low urinary biomass and contaminating DNA, viral DNA may be a needle in the host DNA haystack. One study examining human papilloma viruses could not identify any associations with LUT symptomatology [39]. Another examined the correlation of JC polyoma viruses (JCPyV) with glomerulosclerosis, suggesting a protective effect for JCPyV [40]. Several recent studies were able to distinguish viral sequences from JCPyV and BK and Torque teno virus from urine after whole genome sequencing [41, 42]. As these reports examine urine, not tissue, such approaches may miss important viral reservoirs. Viral peptide mapping using mass spectroscopy–mediated proteomics avoids the pitfalls of genomic sequencing and has identified multiple novel viral proteins in urine, which occur at different frequencies in healthy subjects and patients with several renal diseases [43].

Bacteriophages In the urine, genetic sequences of numerous bacteriophages, viruses that kill bacteria, were found within the genetic sequences of urinary bacteria, suggesting their presence within the urinary microbiome. Bacteriophages play a fundamental role in modulating bacterial communities [44]. A few recent studies have examined this population more comprehensively; analysis of genomic sequences obtained from prior studies revealed a diverse abundance of phages, many of which had little homology to previously described phages [45, 46•]. In addition, abundance of bacteriophage gene sequences differed between asymptomatic subjects and individuals with urinary symptoms, implicating an impact of phages on bladder health [46]. This influence on bacterial microbiota suggests obvious therapeutic potential; bacteriophages have already been proposed as a treatment for complicated urinary tract infection (UTI) [47] as well as biofilm encrustation of indwelling urinary devices [48].

The Urinary Microbiome in Disease

The preponderance of evidence implicates urinary microbes as at least a bystander in or a modifier or promoter of LUT disease. As with all studies of non-malignant LUT conditions, a strict definition of the patient population under study with rigorous inclusion and exclusion criteria is essential for success. A substantial proportion of women will objectively have urinary symptoms, but not complain of these when asked [49, 50]. Given the high prevalence of LUT symptoms, contamination of control groups with symptomatic patients may result in inconclusive or false negative results. For urologic research in general, better and more careful specification of study populations and careful screening of asymptomatic patients using multiple modalities will improve future research.

Urinary Incontinence Most studies have focused on urgency urinary incontinence (UUI). Only one has primarily addressed stress urinary incontinence (SUI) and did not find any associations of microbiota with SUI symptoms, but did not compare these women to an age-matched control group [51].

Microbiota of UUI are by far the best studied, with multiple studies observing associations of the urinary microbes with symptoms [19, 20•, 24, 26, 30, 52, 53]. The specific differences, however, are not consistent between studies, with little overlap or conflicting results between different research groups. One interesting study examined baseline differences in urinary microbiota prior to treatment with solifenacin, an anticholinergic given for UUI, linking increased *Actinomyces*, *Corynebacterium*, and *Streptococcus* abundances with improved responses to medication [20]. These data provide an intriguing potential prognostic factor for individualized UUI treatment decisions and suggest medications, such as beta3-agonists, may be able to modify the urinary microbiome.

Studies from multiple groups implicate variations in *Lactobacillus* species identity and abundance in UUI. *Lactobacillus* predominance appears to associate with healthy controls, while Lactobacilli within a more diverse population are associated with disease, despite no significant differences in lactobacillus abundance across populations [26]. *Lactobacillus* species also segregate with UUI, particularly *Lactobacillus gasseri* with UUI and *Lactobacillus crispatus* with healthy controls [30]. Control and UUI populations differed significantly in age, exogenous sex hormone usage, and body mass index, however, which likely impact these associations. While further larger-scale studies will be necessary to clarify UUI-associated microbial changes, a role for lactobacilli in health and disease appears likely.

Genitourinary Pain (IC/BPS and CP/CPPS) For genitourinary pain syndromes, a single pathogen or microbial pattern is unlikely to cause all types of genitourinary pain. In a study by the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, bacterial composition of voided urine from men with CPPS differed significantly from healthy controls, with *Burkholderia cenocepacia* overrepresented in affected subjects [54]. A second study of voided samples reiterated differences between CPPS and controls, with overrepresentation of *Clostridia* and *Bacteroides* and underrepresentation of *Bacilli* in affected patients [21].

In women, a single, small study of catheterized urine samples revealed decreased bacterial diversity, with reduced *Lactobacillus* levels and increased proinflammatory cytokines in IC/BPS compared with controls [22•]. Three studies examining voided urine samples in women with IC/BPS were unable to identify differences in individual taxa between control subjects and IC/BPS participants [55–57], but did observe trends towards differential abundances of several species, such as increased *L. gasseri* and lower *Corynebacterium* prevalence in IC/BPS patients. Earlier studies using both voided and catheterized specimens are similar, visualizing overall changes in diversity without unique disease-associated species. In all these studies, quantity and species variations in the individual *Lactobacillus* spp. were common although typically not significant and frequently contradictory between studies. In studies of the vaginal microbiota, *Lactobacillus* species and strain variations can highly influence disease [58, 59], which may explain this ambiguity and confusion. Collectively, these studies implicate a critical role for *Lactobacilli* in IC/BPS, but the nature of this role remains undefined.

An additional MAPP study of women with IC/BPS revealed increased levels of detectable fungi, particularly *Candida* spp., without changes in bacterial community patterns [34]. A follow-up study examining fungal taxa in detail revealed greater fungal diversity and increased fungal burden in IC/BPS subjects with increased urinary symptom severity [33]. Unfortunately, neither study compared IC/BPS with controls. Altered levels of *Lactobacilli* may implicate a possible connection; selective *Lactobacillus* species will inhibit Candida growth and hyphae formation [60, 61]. Another potential confounder is that IC/BPS is often treated with antibiotics empirically, especially during painful flares; antibiotics can result in fungal overrepresentation within microbial communities.

These contradictory and inconclusive studies of genitourinary pain only promote confusion regarding any role for or correlation with urinary microbial communities. Direct comparisons are challenging due to a lack of consistency in study structure and subject differentiation, making strict, homogeneous definitions of study populations necessary to differentiate pathologic correlates. We cannot expect conclusive findings from microbiome or any field of study until we meet this standard.

Urinary Tract Infection (UTI) Our established diagnostic and management paradigm for UTI has come under fire with the revelation of a complex, symbiotic microbiome within the healthy genitourinary tract [62]. No consensus exists for a strict definition of UTI [63]; significant debate still surrounds the bacterial colony-forming unit-based thresholds that define infection by standard clinical urine culture. This uncertainty is compounded by the discovery that urinary bacteria can be detected in the urine of nearly all subjects, including those without urinary symptoms [64]. Thus, according to the strict definition, all individuals have "bacteriuria."

Asymptomatic bacteriuria has been implicated as protective against recurrent UTI [65]; better understanding of the urinary microbiome suggests a mechanism for this clinical observation. Multiple asymptomatic bacteriuria strains exhibit reduced virulence and effective inhibition of subsequent colonization with uropathogenic *Escherichia coli* strains [66, 67]. Interspecies bacterial antagonism is an emerging theme in UTI prevention, particularly with growing antibiotic resistance. Certain *Enterobacteriaceae*, including some asymptomatic bacteriuria *E. coli* strains, secrete a siderophore, escherichelin, that inhibits *Pseudomonas aeruginosa* from causing symptomatic infection [68]. In a comparative genomic study of *E. coli* isolates from women with recurrent UTI, the virulence potential of diverse bacterial strains significantly impacted UTI risk and outcome, stressing the importance of strain-level differences in bacterial uropathogenicity [69••]. Differences in virulence potential of *E. coli* strains similarly influence infection outcomes in prostatitis. Certain strains are associated with acute infection with subsequent clearance while others lead to persistence and pain [70]. Similar studies have noted significant strain effects for other species, which must reframe our concept of uropathogens and bacteriuria.

Novel diagnostic methods have capitalized on culture-independent DNA amplification–based molecular techniques for the improved detection of bacterial pathogens in urine from symptomatic patients. Such methods have the potential

to rapidly identify causative organisms. While there is evidence that these methods may be able to recognize antibiotic susceptibility [71], avoiding inappropriate or delayed treatment, it is unclear how more sensitive detection methods will impact the accuracy of UTI diagnosis. Current understanding stresses that urinary bacteria are present in the healthy state, and that certain bacteria, even some historically considered uropathogens, may be beneficial to bladder health. Thus, more sensitive bacterial detection in the absence of clinical context may be associated with diagnostic confusion and unnecessary overtreatment. As urinary frequency and urgency are associated with multiple urologic conditions, dysuria remains a more accurate diagnostic marker than any bacterial, threshold-based measure [72]. Context and judgement are important, especially in light of evidence refuting the concept that any strict detection threshold or particular species is pathognomonic for UTI.

Early data also suggest a role for the vaginal microbiota in recurrent UTI [73]. In an animal model of recurrent UTI in which animals are chronically colonized with uropathogenic *E. coli*, urethral inoculation with *Gardnerella* will prompt healthy mice to redevelop an *E. coli* UTI [74•]. These data support the concept that endogenous microbes affecting the local, visceral environment modulate disease independent of frank infection.

Effects of Antibiotics on the Urinary Microbiome No studies have directly examined the longitudinal effects of antibiotics on the healthy microbiome. While most studies of the urinary microbiome attempt to control for antibiotic effects by excluding patients with recent use, it is unclear what period free from treatment is needed to prevent confounding. The stable gut microbiome can be permanently perturbed by just two courses of ciprofloxacin, resulting in a stable alternative composition [75]. While causality is unclear, repeated antibiotic use has been linked to a variety of localized and systemic conditions such as depression, schizophrenia, and allergy/atopy [76–78].

With regard to the urinary microbiome, a few studies have implicated systemic antibiotic effects in this biological niche. In a small study of 27 subjects, prior antibiotic use was associated with decreased *Lactobacillus* and *Finegoldia* and increased *E. coli* and *Parabacteroides* [79•]. In women given oral metronidazole for bacterial vaginosis, the relative abundances of BV-associated pathogens such as *Gardnerella vaginalis*, *Atopobium vaginae*, and *Sneathia amnii* decreased, but did not disappear, and remained at levels not significantly different from those seen in asymptomatic controls [80]. After metronidazole treatment, increases in *Lactobacillus iners* were seen in vaginal and urinary specimens, and *Lactobacillus crispatus*, which commonly predominates in healthy, pre-menopausal women, became undetectable in urinary specimens after treatment despite persistence in vaginal samples.

In renal transplant recipients, urine samples taken 1 month after surgery demonstrate increased bacterial diversity as well as increased relative abundances of *Enterococcus*, *Escherichia coli*, *Gardnerella*, and *Prevotella* [18, 81]. As antibiotic suppression is standard for patients post-transplant, these changes were suspected to be due to daily antibiotic usage, but one cannot rule out effects of the metabolic and inflammatory dysregulation of chronic renal failure, transplantation surgery stress, and immunosuppression.

While it is unclear if such genitourinary dysbiosis is the result of antimicrobial drug use or a risk factor for urinary tract infections, data suggest that antimicrobials modify the urinary microbiome, possibly selecting for more pathogenic bacteria with increased antibiotic resistance. The long-term consequences of these shifts are unclear but urge caution when considering therapeutic antimicrobial interventions. Furthermore, antibiotics for non-urologic indications may have unintended alterations to urinary microbiota.

Genitourinary Malignancies Intravesical instillation of *Bacille Calmette-Guerin* (BCG), a live mycobacterium strain, has long been used for therapeutic manipulation of urinary microbiota as treatment of non-muscle invasive bladder urothelial carcinoma, demonstrating the potential effect of bacteria on cancer progression. Several studies identified pre-treatment differences in urinary communities between patients with bladder cancer and healthy controls [82, 83]. Increased levels of Fusobacterium, which is pro-tumorigenic in colonic malignancies [84, 85], were identified in patients with bladder cancer in comparison with controls [82]. While correlative at this point, the tumor promotional potential of the urinary microbiome promotes an interesting hypothesis for the gender differences observed in bladder cancer incidence [86]. Healthy women at baseline have a higher abundance of *Mycobacteria* and other *Actinomycetes* [87], which have been hypothesized to have an inhibitory effect on cancer promotion or progression. While immature, hints of evidence suggest certain urinary microbial profiles are associated with risk of cancer recurrence, progression, and treatment responses [83].

Urolithiasis Urinary stone disease is highly prevalent, affecting almost 10% of the US population, and linked to metabolic disorders, such as atherosclerosis, obesity, and diabetes mellitus [88]. The relationship between the gut microbiota, oxalate metabolism, and stone formation has been well described in animal models and human populations [89–92]. The role of urease-producing bacteria in the generation and promotion of struvite stones has long been accepted, but only in the past few years, however, have culture-independent techniques been applied to further our understanding of the role of urinary bacteria in urolithiasis.

Several studies have identified an enriched, altered group of bacteria in association with urinary stones, suggesting specific genera contribute to the urolithiasis pathophysiology [93]. Stone cultures yielded bacterial and even fungal growth, even

after surgical removal and perioperative antibiotics, indicating that microbes are viable within the stone fragments. While these bacteria are not urease-producing, many of the isolated genera promote crystal aggregation in vitro [94–96] which promotes stone formation [97]. Bacteria may also decrease citrate levels in urine via bacterial production of citrate lyase, which also promotes stone formation [98]. While urinary microbiota likely function in the promotion of urolithiasis, additional research is needed to understand its role in nidus formation and stone promotion as well as any possible protective role for alternative genera in the larger urinary milieu.

Conclusions and Future Directions

Advances in microbiome science promote a vision of the future in which tailored adjustments in an individual's resident communities can be exploited to improve health and combat disease. The substantial knowledge gap between this potential future and our present, however, requires a deeper dive into the basic host-microbe and microbe-microbe interactions within the LUT, including a characterization of microbial variability over time; the impact of environmental stressors, dietary intake, and medications on urinary microbial composition; and the consequences of microbial shifts on LUT function, all of which are unstudied. Also, increased granular understanding of host response mechanisms to the urinary microbiome, both in healthy and disease states, is lagging behind the burgeoning numbers of descriptive urinary microbiome studies. While cataloguing studies describe the constituents, from bacteria to bacteriophages, of the urinary microbiome in healthy and diseased hosts, it is "mission critical" to frame these taxonomical studies as to why and how the host responds (or does not respond) to these microbial communities.

Compliance with Ethical Standards

Conflict of Interest A. Lenore Ackerman has no conflict of interest. Toby C. Chai has no conflict of interest.

Human and Animal Rights and Informed Consent The authors did not perform any studies with human or animal subjects in this review article.

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