Clinical implications of the microbiome in urinary tract diseases

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Purpose of review
The purpose of this review is to outline and evaluate the most recent literature on the role of the microbiome in urinary tract diseases.

Recent findings
High throughput molecular DNA sequencing of bacterial 16S rRNA genes enabled the analysis of complex microbial communities inhabiting the human urinary tract. Several recent studies have identified bacterial taxa of the urinary microbiome to impact urinary tract diseases including interstitial cystitis, urgency urinary incontinence or calcium oxalate stone formation. Furthermore, treatment of urinary tract infections by antibiotics globally impacts community profiles of the intestinal microbiota and might indirectly influence human health. Alternative treatment options like application of probiotics for the treatment of urinary tract infections are currently under investigation.

Summary
The urinary microbiome and its relationship to urinary tract diseases is currently under comprehensive investigation. Further studies are needed to shed light on the role of commensal microbiota for urinary tract infections.

Keywords
antibiotics, probiotics, urgency urinary incontinence, urinary microbiome, urinary tract infections

INTRODUCTION

The field of human microbiome science is currently undergoing a period of explosive growth of interest. The understanding about the complex microbial communities colonizing our human body have been recognized as major factors for the development of various diseases and determinant for profound pathogen–host interactions [1,2]. Although densely populated microbial ecosystems of the human gut, skin, mouth or the vaginal microbiota have been studied on a large scale, microbial communities inhabiting the lower and upper urinary tract are less extensively studied today. In healthy individuals, urine was originally considered a sterile body fluid based on the lack of cultivable cells detectable by routine urine culture techniques. With the advent of modern molecular high throughput DNA sequencing techniques such as 16S ribosomal RNA (rRNA) gene or whole metagenome sequencing, slowly or fastidiously growing aerobic and anaerobic bacteria were detectable as part of a unique commensal flora colonizing the urinary tract which is most likely to have a profound effect on overall urologic health and urinary tract homeostasis [3,4]. Studies analyzing the urinary microbiome showed that a diverse bacterial community is inhabiting the urinary tract with the predominant genera being *Lactobacillus*, *Prevotella* and *Gardnerella* [5,4]. However, the role of these commensal bacteria and its importance in the emergence or prevention of urinary tract infections (UTIs) remains to be fully elucidated. In this review, we want to outline and evaluate recent studies on the role of the microbiome in urinary tract diseases.

METHODS AND LIMITATIONS OF ANALYZING THE URINARY TRACT MICROBIOME

Nowadays, ultra-deep DNA sequencing analysis of bacterial 16S rRNA genes is most commonly used for the determination of bacterial community profiles. The 16S rRNA is an essential part of the protein

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KEY POINTS

- Analysis of the bacterial 16S rRNA genes by high throughput sequencing enables the analysis of the human urinary microbiome, but some methodological limitations could narrow clinical interpretation.
- Significant differences of the urinary tract microbiome were observed between women with urgency urinary incontinence (UUI) and a control cohort. An increase in UUI symptom severity is associated with loss of microbial diversity.
- Gastrointestinal colonization with *Oxalobacter formigenes* decreases the risk of kidney stone recurrence. Application of *O. formigenes* to gnotobiotic mouse models showed oxalate degradation and its application as a probiotic is currently studied.
- Application of prophylactic antibiotics in uncomplicated urinary tract infections alter the human microbiota and may promote antibiotic resistance. Prevention of urogenital infections can be achieved by probiotics as an alternative to antibiotics or cotreatment.

biosynthesis machinery of bacterial cells and thus highly conserved among all bacterial species. Because of hypervariable regions in the DNA sequences of these genes, taxonomic classification can be achieved by comparison with publicly accessible 16S rRNA databases. Although the application of these molecular methods enabled the microbiome field to make a great leap forward, some methodological limitations should be highlighted which could potentially narrow clinical interpretation. Recent inter-laboratory studies have demonstrated that results obtained by different protocols and centres vary significantly and lacked reproducibility [6,7]. High throughput sequencing protocols may be biased regarding the implemented DNA extraction method [8], the used sequencing platform [9] 16S rDNA PCR primers [10] or the implemented bioinformatic approach downstream of DNA sequencing. Further standardization is urgently needed prior to the transition to clinical diagnostics [11].

Most of the studies investigating the urinary microbiome are based on the collection of midstream urine into a sterile container or through a transurethral catheter. Thus, contamination of the collected sample by bacteria inhabiting the lower genito-urinary tract such as the distal urethra might not be completely avoidable [3]. Still more, members of the urinary tract microbiome coincide with predominant taxa of the vaginal flora such as bacteria from the genus *Lactobacillus* [12]. Sterile alternatives like suprapubic bladder puncture (Fig. 1) might be more adequate but elaborate sampling techniques [13]. Additionally, laboratory reagents were found to be contaminated among others by the bacterial genera *Lactobacillus*, *Escherichia*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, which are also members of the urinary tract microbiota [14]. Sensitive detection methods such as ribosomal 16 rRNA gene analysis could be prone to result in false

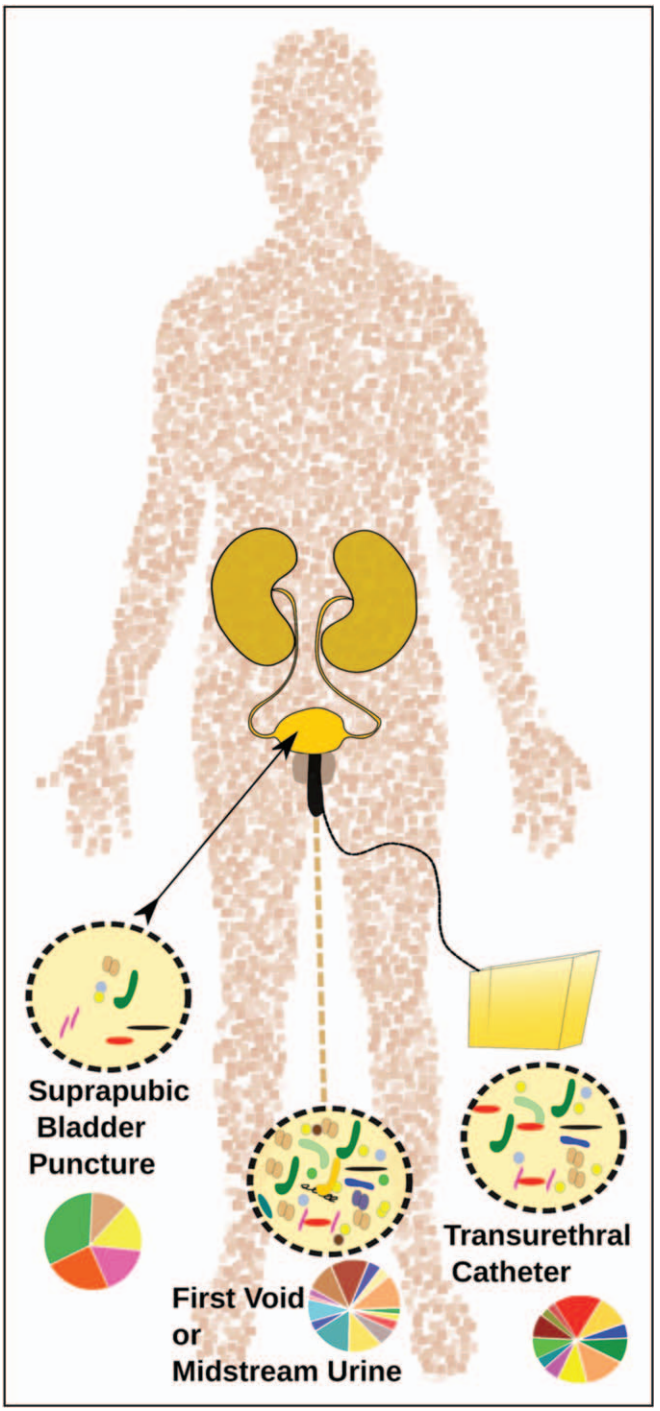

FIGURE 1. Importance of sampling techniques for studies about the microbiome of the urinary tract.

positive bacterial taxa for urinary colonization. Thus, especially when analyzing samples with low bacterial load like urine, obtained microbiota profiles should be interpreted carefully.

THE ROLE OF THE MICROBIOTA IN URINARY TRACT DISEASES

Despite possible limitations, several studies have been carried out in recent years to investigate the role of the microbiota inhabiting the human urinary tract. Along with uropathogens as causative agents of UTIs, additional complex host–microbe interactions might have to be considered in diagnosis and treatment of UTI in future, as pathogens such as uropathogenic *Escherichia coli* (UPEC) might propagate as part of a preceded urinary tract dysbiosis. Today, very little is known about the role of the urinary microbiota in the suceptibility to acute UTIs.

The role of the urinary microbiota in chronic urinary tract disorders, however, has been part of recent studies. For example, decreased diversity along with an increased abundance of the bacterial genus *Lactobacillus*, the urinary microbiome has been linked to interstitial cystitis [15]. Analyzing the gut microbiota in female interstitial cystitis patients and healthy controls by 16S rRNA gene sequencing and qPCR quantification, significantly reduced levels of *Eggerthella sinensis*, *Collinsella aerofaciens*, *Faecalibacterium prausnitzii*, *Odoribacter splanchnicus* and *Lactonifractor longoviformis* were identified in interstitial cystitis patients as potential biomarkers modulating chronic pelvic pain [16▪]. Additionally, significantly elevated glycaraldehyde levels were observed in interstitial cystitis patients.

The urinary microbiome and urgency urinary incontinence

Urgency urinary incontinence (UUI) of females is a poorly understood urinary condition characterized by symptoms that overlap urinary infection, including urinary urgency and increased frequency with urinary incontinence.

By analyzing urine collected by transurethral catheter from women with UUI and a control cohort, Pearce *et al.* [17] found significant differences of the urinary tract microbiome by both sequence and culture techniques comparing both groups. The UUI microbiome displayed increased *Gardnerella* and decreased *Lactobacillus* abundance by sequence analysis. Additionally, nine genera (*Actinobaculum*, *Actinomyces*, *Aerococcus*, *Arthrobacter*, *Corynebacterium*, *Gardnerella*, *Oligella*, *Staphylococcus* and *Streptococcus*) were more frequently cultured from the UUI cohort and there were significant quantitative differences of *Lactobacillus species*.

Urinary microbiome diversity has been associated with response to solifenacin – an orally administered anticholinergic medication used to treat UUI [18]. The authors reported that a lower diversity of cultivatable bacteria was associated with response to low dose of solifenacin and women requiring higher doses or nonresponders to have increased diversity of cultivable bacteria, though this association with the diversity of microbiota was not detected by sequence analysis of the microbiota for reasons not defined yet.

In a most recent similar study by Karstens *et al.* [19], the relative abundance of 14 bacteria significantly differed between control and UUI samples. Furthermore, the increase in UUI symptom severity was found to be associated with a decrease in diversity of the urinary microbiome in women with UUI.

Although the results of the three publications need to be validated in larger studies, they provide evidence that the diversity of the urinary microbiome may have clinical relevance. Although the microbiological findings of the studies are difficult to compare because of different cohorts, sampling and analysis details, the microbiome differences between healthy controls and UUI patients suggest a potential role for the urinary microbiome in female urinary health. Given the significant overlap of UUI symptoms with those of UTIs, it is important to further evaluate the functional role of microbial components in the pathophysiology of UUI.

Microbiome and calcium oxalate stone formation

A number of risk factors influence the development of calcium oxalate kidney stones, including urinary oxalate excretion. By analyzing large epidemiological cohorts it was found that small increases in oxalate excretion, even in ranges considered to be normal, significantly enhance the risk of kidney stone development. In addition, dietary oxalate consumption decreased calcium intake results in increased urinary oxalate excretion and stone risk. Thus, a low calcium, high oxalate diet may place an individual at risk for calcium oxalate kidney stones.

Oxalobacter formigenes (*O. formigenes*) is a gram-negative, anaerobic bacterium in the gut of humans and other mammalian species whose main carbon and energy sources are derived from oxalate metabolism. Enteric colonization with *O. formigenes* could thereby decrease intestinal oxalate and decrease

oxalate absorption and its urinary excretion, potentially decreasing the risk of calcium oxalate stone formation. In line with this concept, Kaufman *et al.* [20] observed that gastrointestinal colonization with *O. formigenes* was associated with a 70% decrease in the risk of kidney stone recurrence. A review of worldwide data indicated that 38–77% of a normal population and only 17% of stone formers are colonized with *O. formigenes* [20], further suggesting that colonization of calcium oxalate stone formers may be an efficacious method for limiting calcium oxalate stone risk. Jiang *et al.* [21] performed a study in which *O. formigenes* colonized and noncolonized patients were administered diets controlled in calcium and oxalate contents. Urinary calcium and oxalate excretion were significantly altered by the dietary changes in *O. formigenes* colonized and noncolonized individuals. However, on a low calcium (400 mg daily)/moderate oxalate (250 mg daily) diet *O. formigenes* colonization significantly decreased oxalate excretion.

On the basis of these initial findings *O. formigenes* colonization was thought to be an efficacious method for limiting calcium oxalate stone risk. However, challenges exist in the preparation of *O. formigenes* as a successful prophylactic probiotic due to it being an anaerobe with fastidious growth requirements.

Probiotic supplements that claim to contain *O. formigenes* can be ordered over the Internet from different vendors such as PRO Lab, Ltd and Sanzyme, Ltd. However, results from a recent analysis indicated that these supplements do not contain detectable living *O. formigenes*, pointing to difficulties of manufacturing and/or stabilizing *O. formigenes* for probiotic use [22]. A recent randomized, placebo-controlled, double-blind, multicentre study showed that ingestion of a lyophilized enteric coated capsulated preparation of *O. formigenes*, did not result in a significant reduction in urinary oxalate excretion [23] which could have been because of problems with bioavailability of the supplement or viability of *O. formigenes* in this formulation. Most recently, Li *et al.* [24▪] showed in gnotobiotic mouse models that *O. formigenes* as a probiotic has limited impact on the composition of the resident microbiota but providing efficient oxalate degrading function. To improve the clinical applicability of *O. formigenes* Ellis *et al.* [25] examined in-vitro properties expected of a successful probiotic strain. The data show that a special *O. formigenes* strain (OxCC13) is able to persist in the absence of oxalate, is aerotolerant, and survives for long periods when freeze-dried or mixed with yogurt. Clinical studies with this promising *O. formigenes* strain should now be performed to evaluate the efficacy of prophylaxis of kidney stones with a robust probiotic.

Role of antibiotics

As most of the antibiotics available on the market have a broad spectrum of action, they impact not only on pathogenic bacteria, but also on healthy microbiota. Beside this, additional direct harmful effect of antibiotics on, for example, epithelial cells and the selection and spread of antibiotic-resistant microorganisms have been demonstrated. Because antibiotic-mediated dysbiosis of gut and other microbiota may promote obesity, diabetes, inflammatory bowel disease, allergies and asthma in the long term, therapeutic choices should always consider collateral damage in addition to curative effectiveness.

Although recent treatment guidelines for uncomplicated UTIs discourage fluoroquinolone prescription because of collateral damage to commensal microbiota, these antibiotics are frequently prescribed. Stewardson *et al.* [26▪] analysed gut microbiota compositions at three time points from ambulatory patients with UTIs treated with ciprofloxacin or nitrofurantoin, patients not requiring antibiotics and household contacts of ciprofloxacin-treated patients. In contrast to nitrofurantoin, ciprofloxacin had a significant global impact on the gut microbiota. Ciprofloxacin treatment resulted in a reduced proportion of *Bifidobacterium* (Actinobacteria), *Alistipes* (Bacteroidetes) and four genera from the phylum Firmicutes and an increased relative abundance of Bacteroides and the genera *Blautia*, *Eubacterium* and *Roseburia*. Substantial recovery occurred 4 weeks after therapy cessation. In contrast, nitrofurantoin treatment correlated with minor changes such as a reduced relative proportion of the genus *Clostridium* and an increased proportion of the genus *Faecalibacterium*. Thus, the study supports use of nitrofurantoin over fluoroquinolones for treatment of uncomplicated UTIs to minimize microbiome shifts.

The influence of prophylactic antibiotics on the urinary tract microbiome of kidney transplant recipients has been analysed recently by Modena *et al.* [27]. Urine samples from patients after kidney transplantation with prophylactic trimethoprim-sulfamethoxazole treatment and healthy controls were collected and analysed by metagenomic sequencing. The urine microbiome of kidney transplants was markedly different, displaying decreased microbial diversity, and increased abundance of potentially pathogenic species compared with healthy controls. A significant decrease in Actinobacteria and an increase in Firmicutes, dominated

by *Enterococcus faecalis* accompanied by an increase in the Proteobacteria, mainly *Escherichia coli*, was observed. The increased abundance of genes for enzymes such as dihydrofolate synthase that are not inhibited by trimethoprim-sulfamethoxazole, are consistent with a selection of antibiotic-resistant bacteria in the urinary microbiota by the prophylactic regimen. On the basis of these findings, the evaluation and development of optimal prophylactic regimens that do not promote antibiotic resistance is clearly warranted.

Probiotics for treatment and prevention of urogenital infections in women

As comprehensively summarized in a recent review [28▪] several clinical studies focused on the potential benefit of probiotics for the therapy and prophylaxis of bacterial vaginosis, UTIs, vulvovaginal candidiasis and human papillomavirus (HPV) infections. The 20 studies analysed were heterogeneous in terms of design, intervention, outcomes and scientific quality.

Probiotic lactobacilli, particularly *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri*, as single strains or different combinations have been choices for treating and preventing urogynecologic infections. The probiotic interventions ranged from 5 days to 12 months, dosages ranged from 10^4 to 10^{10} CFUs and oral capsules as well as beverages or different forms of vaginal applications were tested.

Although clinical practice recommendations were limited by the strength of evidence, probiotic interventions appear to show some effectiveness in treatment and prevention of urogenital infections as an alternative or cotreatment. Of importance, none of the probiotic interventions were associated with serious adverse events. If used as cotreatments, other evidence suggests that antibiotic and probiotic interventions should be separated by at least 2–4 h to avoid the destruction of the live microorganisms in the gastrointestinal tract. Clearly, more well-designed clinical research studies with larger cohorts are needed on probiotics used to treat or prevent urogenital infections in women before reliable recommendations can be included in the clinical guidelines.

CONCLUSION

A large number of studies have been conducted in recent years to elucidate the role of the microbiome in urinary tract diseases. Further controlled cohort studies are needed to answers unsolved questions.

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Conflicts of interest

There are no conflicts of interest.

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- ▪▪ of outstanding interest

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