Published in final edited form as:

Curr Urol Rep. ; 19(1): 13. doi:10.1007/s11934-018-0763-6.

The Role of the Genitourinary Microbiome in Pediatric Urology: a Review

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Abstract

Purpose of Review—In this review, we highlight the effects of the microbiome on urologic diseases that affect the pediatric patient.

Recent Findings—Perturbations in the urinary microbiome have been shown to be associated with a number of urologic diseases affecting children, namely urinary tract infection, overactive bladder/urge urinary incontinence, and urolithiasis.

Summary—Recently, improved cultivation and sequencing technologies have allowed for the discovery of a significant and diverse microbiome in the bladder, previously assumed to be sterile. Early studies aimed to identify the resident bacterial species and demonstrate the efficacy of sequencing and enhanced quantitative urine culture. More recently, research has sought to elucidate the association between the microbiome and urologic disease, as well as to demonstrate effects of manipulation of the microbiome on various urologic pathologies. With an improved appreciation for the impact of the urinary microbiome on urologic disease, researchers have begun to explore the impact of these resident bacteria in pediatric urology.

Keywords

Pediatric urology; Genitourinarymicrobiome; Urologic diseases; Urinary incontinence; Urolithiasis

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Compliance with Ethical Standards

Conflict of Interest

Daniel Gerber, Catherine Forster, and Michael Hsieh each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

From the 1950s, with the establishment of the standard urine culture, until approximately 10 years ago, the urinary tract was considered to be sterile under normal conditions. When the Human Microbiome Project began in 2008, with the goal to characterize the bacterial makeup of various body sites, the bladder was not included as a site of study. This exclusion was presumably due to the belief that the bladder was sterile, as well as the invasive nature of techniques necessary to avoid contamination with urethral organisms (e.g., suprapubic aspiration or bladder biopsy) [1–3]. While the bladder has fewer commensal bacteria than sites such as the skin and colon [4•], advances in bacterial assessment in the past decade, particularly 16S rRNA gene sequencing and expanded quantitative urine culture (EQUC) have shown that the bladder is far from sterile [4•, 5–7, 8•].

Sequencing is frequently used to study the microbiome in research settings. Briefly, sequencing involves amplification of the hypervariable region of the 16S rRNA bacterial DNA gene and comparison to a library of known 16S gene sequences in order to identify the bacterial species [2]. The limitation of sequencing is that it cannot differentiate DNA from living versus dead bacteria [9•], although any dead bacteria present were alive at some point and thus likely a component of the microbiome [1]. The improved precision of 16S rRNA sequencing has allowed for a new understanding of the role of specific microbes in the genitourinary tract. *Gardnerella* is an example of the evolving understanding of the origin of the genitourinary microbiome. *Gardnerella* was previously viewed as a pathogen associated with bacterial vaginosis, thought to only exist in urinary samples as a result of contamination during sample collection. However, studies have shown that *Gardnerella* can exist as a stable member of the bladder microbiome in asymptomatic women when samples are gathered by suprapubic aspiration or transurethral catheterization [6, 8•, 10]. Further, it has also been identified as a member of both the microbiome of the bladder as well as coronal sulcus in sexually inexperienced males, further supporting its role as a part of the urinary microbiome [11].

EQUC was developed in response to a growing need to culture species that were identified by sequencing, but not grown on standard urine cultures, using a technique that could be easily implemented and interpreted in a clinical setting [2, 8•]. EQUC involves plating urine on a variety of agars and incubating in various conditions, with a reporting threshold as low as 10 colony-forming units/mL [8•]. Hilt et al. have demonstrated good concordance between sequencing and EQUC, with over 80% of species identified by sequencing also found by expanded culture. Further, 92% of specimens that grew on EQUC were not identified using a standard urine culture protocol with a cutoff of $> 10^3$ colony forming units/mL [8•]. Multiple other studies have also demonstrated the superior sensitivity of EQUC compared to standard urine culture in cultivating a wide range of urinary bacteria [4•, 12].

Both 16S rRNA sequencing and EQUC have demonstrated that the urine is far from sterile and is indeed home to multiple microbial species. This paradigm shift has led to a new area of research into the role of the microbiome in commonly encountered urologic pathologies. However, it is first essential to understand the natural history of the urinary microbiome, and

the variations present with increasing age, and between genders. The acquisition of the microbiome in infancy is of particular relevance in pediatrics. Work done with infants suggests that the maternal microbiome strongly influences the neonatal microbiome and that the microbiome of the gut, oropharynx, and skin of the infant are affected not only by the maternal microbiome but also by the mode of delivery [13•, 14]. Moreover, a recent metatranscriptomic analysis of bacterial strains specific to mother-infant pairs suggests that gastrointestinal bacteria were not only transferred from the maternal gut to the infant gut environment, but that the bacteria adapted effectively to the infant gut [13•]. While no study to date has compared the urinary microbiome of parents and their children, Hickey et al. found that the vaginal microbiome of adolescent girls was not concordant with that of their mothers [15•]. Outside of the neonatal period, there continues to be limited data regarding age-related changes in the genitourinary microbiome. Lewis et al. examined the urinary microbiome in 16 healthy adults and found that while bacterial diversity did not correlate with age, there were some species present in the microbiome of all patients, while other species were found only in patients of particular age ranges [6]. Other work has examined the vaginal microbiome in relation to age, in particular around menarche. Most have found that *Lactobacillus* species are less common before menarche, but there is a rapid transition to the “adult-type” microbiome dominated by *Lactobacillus* [16••, 17, 18]. However, Hickey et al. recently found that the majority of vaginal microbiome samples showed a predominance of Lactobacilli in all ages, including premenarcheal girls, which is similar to previously reported microbiome makeup of both postmenarcheal girls and adult women. The transition to puberty, however, was associated with increased relative abundance of *Lactobacilli* and other lactic acid-producing bacteria, which results in acidification of the vaginal environment [15•]. Given the anatomic relationship between the vagina and the urinary tract, the association between puberty and maturation of the vaginal microbiome is certainly relevant to discussion of the urinary microbiome: A lower urinary pH has been shown to be associated with protection from urinary tract infection [19••, 20], suggesting that hormonal changes of puberty may also be associated with maturation of the microbiome of the urinary tract.

The differences in the urinary microbiome that are due to gender have not been as thoroughly studied as those due to age. While the genitourinary microbiome has been studied more extensively in females, comparative studies have found differences in the makeup of the microbiome between the sexes. Females tend to have a more heterogeneous population than males and typically have a microbiome dominated by *Lactobacillus* while the male microbiota tends to be dominated by *Corynebacterium* [6, 21].

Armed with a new appreciation of the genitourinary microbiome, researchers have begun to explore the role of these bacterial populations in urologic disease. Various studies have found a correlation with urologic cancers, sexually transmitted infections, interstitial cystitis, and chronic prostatitis [22–25]. This review, however, will focus on urologic pathologies that affect the pediatric patient, including urinary tract infection, urge urinary incontinence/overactive bladder, and urolithiasis.

Urinary Tract Infection

The introduction of technology that enabled exploration of the urinary microbiome has challenged the classical definition of a urinary tract infection (UTI). Since the 1950s, the definition of a UTI has been urine culture with growth of > 100,000 colony-forming units/mL, in addition to signs of inflammation in the urinary tract (e.g., pyuria) and the presence of typical symptoms [2]. The choice of > 100,000 cfu/mL was somewhat arbitrary, as there was little evidence to support this cutoff. Of note, in 2011, the American Academy of Pediatrics updated its guidelines for the diagnostic criteria of UTI in children 2–24 months old, lowering the bacterial burden to > 50,000 colony-forming units in the presence of signs of inflammation of the urinary tract [26]. With the discovery of the genitourinary microbiome, and the demonstration that most urinary bacteria identified by 16S sequencing can be cultured under expanded conditions, the previous concept of UTIs is almost certainly an oversimplification. As a symptomatic UTI is the result of alteration in the makeup of the urinary microbiome, researchers have suggested that the terms “urinary tract infection” and “asymptomatic bacteriuria” be replaced by the term “dysbiosis” [2].

A number of studies suggest that the microbial composition and diversity of the urinary microbiome is related to the development of a urinary tract infection, with an association between decreased microbial diversity and incidence of UTI [4•, 19••, 27]. There are a number of mechanisms that have been proposed by which native bacteria would prevent the overgrowth of uropathogens, including competition for nutrients and attachment sites, biofilm disruption, production of antimicrobial factors, immunomodulation, and regulation of gene expression [20, 28–34]. These mechanisms were tested by Chapman et al. who incubated *Escherichia coli* and *Enterococcus faecalis* with *Lactobacillus*, a well-represented member of the genitourinary microbiome, in the presence of human bladder epithelial cells. They found that uropathogen adherence was significantly reduced when incubated with *Lactobacillus* compared to when incubated alone, and that this reduction in adherence was associated with a lower pH. The authors postulate that these findings may be due to either decreased uropathogen adherence in a more acidic environment, or that the antimicrobial peptides (AMPs), products of the innate immune system promoted by *Lactobacillus*, are more active at an acidic pH [20]. Indeed, activation of AMPs is another role of the microbiome in UTIs. AMPs are products of the innate immune system that are produced by the urothelium either constitutively or in the presence of bacteria [19••, 35]. A number of AMPs have been identified in the urinary tract, and several mouse studies have demonstrated that their absence allows for increased representation of uropathogens including *Staphylococcus* and *E. coli* in the urine [35, 36]. AMPs are reliant on proteases produced by the epithelium for activation [19••, 37], and this process is highly dependent on pH [37]. Nienhouse et al. further examined urinary protease activity in relation to AMP function and found that protease activity was significantly higher in patients with bacteriuria. As proteases released from the urothelium are known to be pH dependent, and many of the components of the urinary microbiome decrease pH through production of lactic acid, it is possible that proteases from the host microbiome help activate AMPs to promote protection from uropathogens [19••].

Patients with neurogenic bladder are at high risk for recurrent UTI. Therefore, these patients are an important group to consider when assessing the impact of the microbiome on infection risk. Groah et al. found that the urinary microbiome in patients with neurogenic bladder consists of bacteria generally considered to be uropathogens, including *E. coli*, *E. faecalis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [38]. Bossa et al. recently demonstrated in a study of patients with neurogenic bladder and chronic catheterization that there is significant variation in the bacterial makeup of the microbiome of patients with neurogenic bladder. However, these communities are highly stable as demonstrated by reversion to baseline bacterial makeup after resolution of a UTI or discontinuation of probiotics [39]. Groah et al. suggest a number of possible causes for change in urinary microbiome with neurogenic bladder, including altered perineal bacterial makeup secondary to fecal incontinence and/or the use of bowel care regimens, or changes in gut microbiota that alter the ability of particular bacteria to colonize the urinary tract [38].

The relationship between dysbiosis and UTIs suggests that alteration of the urinary microbiome, through the use of probiotics, may have therapeutic effects. Probiotics could serve as either alternatives or adjuvant options to antibiotics, and would certainly be of interest in the era of antibiotic resistance. Researchers have explored the concept of bacterial interference using either *E. coli*, *Lactobacillus*, or *Bifidobacteria* species. This typically involves direct inoculation of the bladder with desired bacteria and results in a rate of successful colonization around 80% [27, 40]. The use of *E. coli* is of particular interest as researchers have employed a strain, HU2117, that has been genetically altered with deletion of the papG gene, which encodes for the P-fimbriae adhesion molecule. Trials of bacterial interference with this strain have shown protection from symptomatic UTI in neurogenic bladder [40–42]. However, benefits have not been seen in patients with chronic indwelling catheters despite a similar rate of colonization with probiotics [27, 39].

The relationship between the urinary microbiome and urinary tract infection is the most obvious and well-studied one. Given our new understanding of the lack of urinary sterility at baseline, and observed changes in both bacterial composition and antimicrobial peptides with UTI, it seems that we must shift our understanding of urinary infection from a binary perspective to that of a spectrum, as suggested by Brubaker et al [2]. The necessity of this shift in perspective is demonstrated by Bossa et al., who found that change in composition of the urinary microbiome was present before clinical diagnosis of UTI [39]. Future work will focus on the pathophysiological role of the microbiome, and whether it can be manipulated to either prevent or treat UTIs.

Urge Urinary Incontinence/Overactive Bladder

Overactive bladder (OAB) is a poorly understood disorder believed to originate from abnormal neuromuscular signaling that results in spasms of the detrusor muscle. This detrusor spasm can lead to episodes of urge urinary incontinence (UUI) during which the desire to urinate cannot be delayed, leading to leaking episodes [43•]. However, detrusor overactivity is reported in only 58% of patients with UUI [44].

Further, 40% of women treated with anticholinergic medications aimed at inhibiting detrusor irritability show minimal or no response [45]. Given the new appreciation for the impact of genitourinary microbiome on urologic health, researchers have begun to explore its role in OAB/UUI.

While there is wide variability in data reported in the literature, the presence of bacteria in urine, as well as of particular bacterial species, have shown to be associated with incidence, severity, and treatment response of UUI. The presence and amount of cultivatable bacteria are directly correlated with the presence and severity of UUI [7, 46]. Further, the presence of specific species in urine, by both sequencing and EQUC, have been shown to be predictive of UUI: *Gardnerella*, *Staphylococcus*, *Streptococcus*, *Actinomyces*, *Aerococcus*, *Corynebacterium*, *Oligella*, and others have all been reported more commonly in patients with UUI, while *Lactobacillus*, *Prevotella*, and others are reported more commonly in patients without UUI [4•, 10, 43•]. Karstens et al. note that of the nine species found to be overrepresented in urine of patients with UUI, five are known uropathogens that are not routinely detected by routine cultures [43•.]

Although *Lactobacillus* has been shown to be more common in patients without UUI, there are differences in *Lactobacillus* species between patients with UUI and healthy controls. *L. gasseri* is more common in UUI patients, while *L. crispatus* is more common in controls [4•]. As *L. crispatus* acidifies the vagina to a greater extent than another *Lactobacillus* species, including *L. gasseri* [47], it is possible that a more acidic bladder environment limits growth of pathogens that cause irritative symptoms [4]. This will be an important area of future research given the potential for therapeutic use of *Lactobacillus* probiotics in UUI.

The majority of studies to date report that greater genitourinary biodiversity is associated with both reduced incidence and severity of UUI, as well as improved treatment response [4•, 10, 43•, 48]. One notable study by Thomas-White et al. found that women with lower microbial diversity had less severe UUI symptoms and greater response to solifenacin treatment [7]. Although the reason for this finding is unclear, future work will focus on elucidating the relationship between UUI and the microbiome.

Urolithiasis

The role of the urinary microbiome is well established in the formation of struvite stones [49]. However, more recently, several studies have suggested that the microbiome may play a role in the formation of all stones. Up to 32% of calcium oxalate stones demonstrate bacterial growth when cultured [50, 51]. However, the cultured bacteria are unique to the stone: Calcium oxalate stones have been shown to harbor a microbiome that is independent of the urinary microbiome in children with urolithiasis. Recent work by Barr-Beare et al. using sequencing technologies demonstrated significant bacterial diversity in calcium oxalate stones from pediatric patients, including *Enterobacteriaceae*, *Gardnerella*, and *Lactobacillus*. However, in this series, sequencing of urine from the upper tract only found bacteria in one patient. While authors note that this discrepancy could be partially explained by the ability of the antibiotics to more effectively target bacteria in urine than those within the biofilm of the stones, authors postulate that calcium oxalate stones harbor a microbiome

that is independent from that of the surrounding urine. Further, they postulate that the bacteria within the stone's microbiome contribute to stone formation by altering urine supersaturation [16••]. Taken together, these data suggest that it may be possible that the stone microbiome could be predictive of stone recurrence; however, this possibility has yet to be studied.

In addition to the role of the genitourinary microbiome in pediatric urolithiasis, the microbiome of other body sites may also contribute to urologic disease. The gastrointestinal microbiome includes *Oxalobacter formigenes*, a Gramnegative obligate anaerobe that metabolizes oxalate, reducing plasma oxalate levels to impact formation of oxalate-containing stones [52, 53]. Colonization with *O. formigenes* has been shown to be associated with a 70% reduction in the risk of stone formation [53, 54] and a lower number of stone episodes in colonized patients [53]. Moreover, plasma and urinary oxalate have been shown to be significantly lower in colonized patients compared to those without *O. formigenes* colonization [53]. The theoretical basis for this is that the bacteria metabolize oxalate, thus lowering plasma and urine levels. However, work with rat models suggests that interaction between the bacteria and the colonic mucosa may also reduce urinary oxalate through induction of enteric secretion of oxalate [55].

Given the relationship between colonization with *O. formigenes* and stone formation, researchers have explored the utility of probiotics in reducing stone formation. There are conflicting data on the effect of *Oxalobacter* probiotics on urinary and plasma oxalate levels, as only one study demonstrates the benefit of probiotics on oxalate levels [56]. However, three subsequent studies, including a recently completed phase II/III study, failed to demonstrate benefit of oral *Oxalobacter* for urine oxalate, plasma oxalate, or incidence of stone episodes [57–59]. These studies, including Duncan et al., found that *Oxalobacter colonization* was not established, with a loss of benefit once oral probiotics were discontinued [56] or oxalate intake was reduced [59]. More recently, a study in rats found that fecal transplants containing the full microbiome including oxalate-degrading species rather than probiotics made up of subsets of oxalate-degrading bacteria had a pronounced effect on oxalate excretion. Further, the effect was preserved even after elimination of oxalate from the diet. Authors contend that establishment of a diverse community of bacteria containing various oxalate-metabolizers was the basis for the improved efficacy as it allowed for a cohesive balance of microbial interactions [60••].

Future directions in this area should address the impact of antibiotic exposure and low oxalate conditions on *Oxalobacter* colonization. Patients who receive frequent antibiotics have been shown to have a reduced rate of *O. formigenes* colonization [53, 61, 62]. This has particular relevance to pediatric urology given the frequency with which patients with anatomic abnormalities of the genitourinary system and recurrent infections are given prophylactic antibiotics. *Oxalobacter* has been shown to be resistant to amoxicillin, augmentin, ceftriaxone, and vancomycin [63], so it might be reasonable to consider using these antibiotics for prophylaxis where possible in order to avoid impacting *O. formigenes* colonization status, and ultimately, rates of stone formation. As *Oxalobacter* uses oxalate to meet both energy and carbon needs it has been suggested that low oxalate diets employed after a stone episode could impact colonization status [52]. Future research should address if

enteric oxalate secretion independent of diet is enough to sustain *O. formigenes* colonization, though the findings of Miller et al. are encouraging in this area [60••].

Future Directions

We are still in the infancy of our understanding of the role of microbiota in human health and disease. This is particularly true of the urinary environment, as it was less than 10 years ago that the Human Microbiome Project deemed the bladder not worth examining due to its presumed sterility. Now, in addition to the underlying bacterial milieu, we know that it is likely that other microorganisms are also present in the urinary tract. As Ackerman et al. point out in a recent review, our understanding of the urinary microbiome is not complete without consideration of resident fungal species. Although the urinary mycobiome has a relative minority of fungal species compared to bacteria in the bladder, the role of these fungi and their relationship to the other resident microbes will be an important area of future work. Continuing improvement in detection methods will allow us to better understand how the mycobiome interacts with host cells as well as the resident bacteria [64]. Further, the newly elucidated role of the urinary microbiome in various urologic pathologies suggests that both probiotics and dietary modification may have a therapeutic impact on recurrent UTI, urge incontinence, and urolithiasis. As we continue to expand our knowledge of the impact of the microbiome in these areas that affect the pediatric patient it will be important to increase the number of studies that examine pediatric patients to confirm applicability to this population. The urinary microbiome, mycobiome, and potentially virome have previously had an underappreciated role in human health. New technologies that enable the study of these communities will continue to advance our understanding of their impact on human health and disease, and open new therapeutic avenues for these previously refractory conditions.

Acknowledgments

The authors would like to thank Hans Pohl, M.D., for reviewing the manuscript.

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microbiome. Hickey et al. do note, however, that pubertal development is associated with more representation by lactic acid-producing bacteria in the vaginal microbiome, with a corresponding decrease in vaginal pH. This suggests that the acquisition of the female “adult-form” microbiome is more of a maturation of the microbiome, rather than a transition in the species representation as had been previously asserted.

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