Review

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The Urinary Microbiome: A Pediatric Urological Perspective

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The human microbiome is currently being studied with increasing interest. The microbiome refers to the microorganisms living in the body and their genetic information. The human body is known to contain 1.3 to 10 times more microorganisms than human cells. The Human Microbiome Project was started in 2007 to characterize the human microbiome and analyze its role in human health and diseases. Based on the recent microbiome literature, alterations in the microbiome are associated with several non-urological diseases in pediatrics, such as infantile colic, necrotizing enterocolitis, asthma, atopy, obesity, type-1 diabetes, autism, atopic dermatitis, psoriasis, and bronchial asthma. While some urinary microbiome studies (including prostate cancer, bladder cancer, interstitial cystitis, urge urinary incontinence, overactive bladder, stone disease, and urinary tract infections) have been conducted in adults, there are very few pediatric urinary microbiome studies. This study reviews the role of the urinary microbiome in urinary tract diseases from a pediatric urological perspective.

Keywords: Microbiota; Urology; Urinary tract; Child

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INTRODUCTION

The human microbiome is currently being studied with increasing interest. Millions of microorganisms live in the human body and affect its homeostasis in several ways. The terms microbiome and microbiota are universally described to literally mean all living organisms in an area. The term 'microbiome', which was first used by Lederberg and McCray [1], is a combination of microbes living in the body and the biome (ecosystem). The microbiome, therefore, refers to the microorganisms living in the body and their genetic information. Conversely, microbiota refers to groups of microorganisms [2,3]. Understanding the complex microbial community that inhabits our bodies is recognized as a determinant of the pathogenesis of various diseases and profound pathogen-host interactions [4,5].

The Human Microbiome Project (HMP) was started in 2007 to characterize the human microbiome and analyze its role in human health and diseases. The project initiated large-scale studies on the microbial ecosystems of the human gut, skin, mouth, or vaginal microbiota [6-8]. Unfortunately, the urinary tract (UT) was not formerly explored in the HMP since it was considered unethical to obtain bladder biopsies or suprapubic aspirates from healthy individuals to characterize the bladder microbiome while avoiding sample contamination with microorganisms from the urethra [9,10]. Moreover, since the 1950s, with the establishment of the standard urine culture, until approximately 10 years ago, the UT had been considered sterile under normal conditions, and hence microbial communities inhabiting the UT have

been less extensively studied [4,7-11]. It is well known that the gut microbiome plays an important role in several body functions, including nutrient processing and assimilation, defense against pathogenic microbes, and even stimulation of angiogenesis [5]. The ecosystem changes are linked to the causes of several gut diseases in children. Based on the recent microbiome literature, alterations in the microbiome have been associated with the causation of several non-urological diseases in pediatrics, such as infantile colic, necrotizing enterocolitis, asthma, atopy, obesity, type-1 diabetes, autism, atopic dermatitis, psoriasis, bronchial asthma, caries, periodontitis, and chronic rhinosinusitis [5].

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 have been detected as part of a unique commensal flora colonizing the UT [4,12]. Recently, there has been a shift in the outdated concept of sterile urine in healthy individuals, and interest in the role of the urinary microbiome (UM) is growing. While some UM studies such as prostate cancer, bladder cancer, interstitial cystitis (IC), urinary urge incontinence (UUI), overactive bladder (OAB), urinary stone disease, and urinary tract infection (UTI) have been conducted in adults [6,9,11], there are still very few UM studies in children. Therefore, based on the recent literature on the UM in the UT, this review focuses on the role of the UM from a pediatric urological perspective.

MAIN BODY

1. Limitations of Sample Collection and Analysis

The urine collection methods employed in clinical practice are clean-catch midstream urine, first-void urine, suprapubic aspiration, or intermittent transurethral catheterization. The techniques used characterize the UM and determine the microbial diversity detected [9]. Urine sampling methods are frequently debated in any study related to UM. Most studies investigating the UM were based on collecting clean-catch midstream urine into a sterile container or through a transurethral catheter. These collection methods have potential urethral or perineal contamination since contamination by bacteria inhabiting the lower genitourinary tract, such as the distal urethra or perineum, might not be completely avoidable [4]. Wolfe et al. [13] compared different urine collection methods to discern bacteria present in the bladder. They concluded that due to minimal genitourinary contamination, the best methods are suprapubic aspiration and transurethral catheterization. However, although suprapubic aspiration is less vulnerable to genitourinary contamination, it is a more invasive procedure.

Additionally, consideration should be given to laboratory reagents contaminated by the bacterial genera *Lactobacillus*, *Escherichia*, *Bifidobacterium*, *Enterococcus*, and *Streptococcus*, which are members of the UT microbiota [4].

2. Limitation of Microorganism Detection Methods and Their Interpretations

Traditionally, the detection of microorganisms in the UT was based on standard urine cultures in clinical microbiology laboratories. These methods only allow the detection of aerobic and fast-growing bacteria such as *Escherichia coli*, and had significant limitations for the detection of anaerobic microorganisms characterized by slow growth or bacteria with complex nutrient needs [9]. Nowadays, ultra-deep DNA sequencing analysis of bacterial 16S rRNA genes is most commonly applied to determine the bacterial community profiles. Although these molecular methods have enabled the enhanced development of the microbiome field, some methodological limitations, which could potentially narrow clinical interpretation, need to be emphasized [4]. Sensitive detection methods such as bacterial 16S rRNA gene analysis are prone to produce false positive bacterial taxa for urinary colonization. Thus, the obtained microbiota profiles should be interpreted carefully, especially when analyzing samples with a low bacterial load like urine [4]. Besides, the study design and methodology currently used in different studies are quite heterogeneous, complicating the overall integration of the microbiome changes observed. Recent inter-laboratory studies have demonstrated that results obtained by different protocols and centers vary significantly and lack reproducibility [4]. High throughput sequencing protocols may be biased regarding the implemented DNA extraction method, the used sequencing platform, 16S rDNA PCR primers, or the bioinformatics approach downstream of DNA sequencing [4]. Therefore, taking into consideration all the above problems, further standardization of methods with larger sample sizes is necessary, prior to the transition to clinical diagnostics or therapy.

3. Urological Disease and UM

To date, the human microbiome in the urological field has been less extensively reported in adults as well as pediatrics. The microbiome studies recently reported for UT diseases in adults include chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), IC/bladder pain syndrome (IC/BPS), UUI/OAB, urinary stone disease, sexually transmitted infections (STI), UTI, prostate cancer, bladder cancer, and renal cancer [6]. However, unlike microbiome studies in gastrointestinal and non-gastrointestinal disorders as well as in UT disease in adults, few pediatric studies in the urological field have been conducted to assess the role of UM in health and disease. Therefore, this study examines and compares how and to what extent UM studies are different in children compared to adults.

1) Benign urological diseases and UM

Several studies in benign urological diseases have investigated the UM in relation to UTI, urinary stones, UUI, OAB, neurogenic bladder (NB), lower urinary tract symptoms, STI, IC/BPS, and CP/CPPS in adults. However, literature reviews have revealed that there are very few studies on UM in pediatric urological benign diseases.

(1) UTI: Since the establishment of the standard urine culture in the 1950s until approximately 10 years ago, the UT had been considered sterile under normal conditions [7-11]. This perspective is now altered with the discovery of the genitourinary microbiome and the demonstration that most urinary bacteria identified by 16S sequencing can be cultured under expanded conditions [10,12]. As symptomatic UTI is the outcome of an altered UM makeup, some researchers have suggested that the terms "UTI" and "asymptomatic bacteriuria" be replaced by the term "dysbiosis" [14].

The relationship between UM and UTI is obvious and relatively well-studied. Several studies suggest that the microbial composition and diversity of the UM are related to the development of a UTI, with an association between decreased microbial diversity and the incidence of UTI [10,15-17]. Studies analyzing the UM reported that the UT is inhabited by a diverse bacterial community, with the predominant genera being *Lactobacillus*, *Prevotella*, and *Gardnerella* [4]. However, the role of these commensal bacteria and their importance in the emergence or prevention of UTIs remains to be fully elucidated.

A study of patients with indwelling urinary catheters suggested that microbial diversity plays a protective role in the development of UTIs, which are caused by dysbiosis of the commensals [16]. However, the benefits of the HU2117 *E. coli* strain have not been seen in patients with chronic indwelling catheters despite a similar rate of colonization with probiotics [16,18].

Yoo et al. [19] conducted a human bladder microbiome study to investigate the effect of *Gardnerella* on recurrent UTI (rUTI). The *Gardnerella*-dominant or *Lactobacillus*-dominant groups expressed rUTI with symptoms in the presence of risk factors such as the degree of *Gardnerella* proliferation or causative agents of bacterial vaginosis. Depending on other risk factors, the presence of *Gardnerella* in the urine is considered to be related to rUTI.

The majority of UM studies have focused on adult subjects, and pediatric reports are scarce. Mapping the UM in the pediatric population has just begun [12]. Kinneman et al. [20] assessed the UM in 85 children younger than 48 months, with and without UTI. They demonstrated that UM was identified in every child, and altered microbiome diversity and composition were observed in subjects with a standard culture-positive UTI. They also observed that antibiotic administration was effective in the UM only for a short time. Kassiri et al. [21] examined the UM in 20 prepubertal boys (aged 3 months-8 years; median age 15 months) with and without prior antibiotic exposure. The majority of patients presented with *Staphylococcus* and *Varibaculum* species and, to a lesser extent, *Peptoniphilus* and *Actinobaculum*. Several of the detected genera have been previously identified in the urine of adult men. However, urinary microbial communities profiled in children differed from those described in adults. This study also presented that the composition of the UM in children may begin to develop early in life and evolve over time, achieving stability in adulthood.

(2) UUI and OAB: OAB is a poorly understood disorder, but is thought to be multifactorial and is believed to originate from abnormal neuromuscular signaling and functioning of the detrusor muscle [10,22]. In the pediatric population, OAB not only burdens the child's development but also negatively impacts the family [12]. OAB is a condition where frequent urination is required, which often leads to UUI. The mechanisms of OAB are not fully elucidated, but there are potential links to the involvement of the microbiome. Since

anticholinergics or beta-3 agonists that inhibit detrusor contraction and promote bladder relaxation are ineffective in many patients, this indicates other etiologies besides neuromuscular dysfunction, including UM [22,23].

Several studies of the UM in adults have been conducted in patients with UUI and OAB, and have reported significant differences in bacterial urine compositions between adult female UUI and healthy controls. These differences are reported to affect the symptom severity and treatment responses [15,23-25]. Compared to controls, patients with UUI exhibit higher *Gardnerella* and fewer *Lactobacillus* sequence profiles. Additionally, culture tests using expanded quantitative urine culture (EQUC) showed nine genera, including *Actinobaculum*, *Actinomyces*, *Aerococcus*, *Arthrobacter*, *Corynebacterium*, *Gardnerella*, *Oligella*, *Staphylococcus*, and *Streptococcus*, were commonly found in UUI patients [10,15]. The more frequently cultured organisms were *Lactobacillus gasseri* from UUI urine and *Lactobacillus crispatus* from control urine [10,15,23,24]. In another study, Pearce et al. [15] compared the baseline UM of women with and without UUI. They identified statistical associations between UUI and several bacterial species, including *Actinomyces neuii*, *Actinotignum schaalii*, *Aerococcus urinae*, *Corynebacteria coylae*, *Corynebacteria riegelii*, *Oligella urethralis*, and *Streptococcus anginosus*. Several of these species are considered uropathogens; however, most are not detected in standard urine cultures. This study also presented that while *L. crispatus* was associated with the non-symptomatic controls, *L. gasseri* was associated with UUI. Recently, Karstens et al. [23] found that the relative abundance of 14 bacteria significantly differed between healthy and UUI patients. They further suggested that persistent low-grade infections by bacteria that are not commonly detected by routine cultures could potentially be responsible for the irritative symptoms of UUI. This could justify the potential for the therapeutic use of *Lactobacillus* probiotics in UUI and OAB.

Other studies have investigated the clinical associations between UM and different UUI treatments. In 40% of UUI women treated with anticholinergics, there was minimal or no response [26]. The diversity of UM has been associated with the response to solifenacin for the treatment of UUI. The presence and number of cultivatable bacteria are directly correlated with the presence and severity of UUI [24,27]. Thomas-White et al. [24] found that a lower diversity of cultivatable bacteria is associated with a positive response to a low dose of solifenacin.

In summary, the studies published to date have demonstrated a clear role of the UM in adults with UUI/OAB, and in the response to UUI/OAB treatment. However, there is uncertainty about the possible associations between UM and OAB symptoms in the pediatric population due to a lack of reports concerning UM in pediatric UUI/OAB.

(3) Urinary stone: The role of the microbiome in urolithiasis is relatively well-established. Recently, numerous culture-independent UM studies have been published in an attempt to elucidate whether the UM contributes to urinary stone formation [28]. More recently, several studies have suggested that UM may play an important role in urinary stone formation [10]. Stern et al. [29] presented that compared to 6 controls, 23 patients with kidney stones had higher levels of *Bacteroides* and less *Prevotella*. Tang et al. [30] recently analyzed the characteristics of gut microbiomes in 13 patients with multiple kidney stones. They found an abundance of proinflammatory bacteria and fewer anti-inflammatory bacteria compared to 13 matched healthy controls.

Up to 32% of calcium oxalate stones demonstrate bacterial growth when cultured. However, the cultured bacteria may be unique to the stone. Calcium oxalate stones have been shown to harbor a microbiome that is independent of the UM in children with urolithiasis [10]. Barr-Beare et al. [31] demonstrated significant bacterial diversity in children with calcium oxalate stones, including *Enterobacteriaceae*, *Gardnerella*, and *Lactobacillus*. They postulated that calcium oxalate stones harbor a microbiome, and the bacteria within the stone's microbiome contribute to the stone formation by altering urine supersaturation.

Several studies revealed an inverse relationship between intestinal colonization with *Oxalobacter formigenes* and the development of calcium oxalate stones [32-34]. Kaufman et al. [34] reported that colonization with *O. formigenes* was associated with a 70% reduction in urolithiasis risk in adult patients with recurrent calcium oxalate stones. Barr-Beare et al. [31] also demonstrated that kidney stones are associated with the *Enterobacteriaceae* species, such as *E. coli*.

However, there are still uncertainties about differences obtained in the results between studies due to experimental factors (such as sample collection, storage, DNA extraction, sequencing, or data analysis) or biological factors (such as

geography, ethnicity, stone phenotype, or some other regional factors) [28].

Taken together, several studies proposed that bacteria present in calcium oxalate deposits contribute to calcium oxalate renal disease. Furthermore, the UM may play an important role in urinary stone formation in both children and adults.

(4) NB: It is well known that patients, especially children with NB, have a high risk of recurrent UTIs. UM variations were observed using 16S rRNA sequencing analysis for patients with NB and normal bladder function. Urine in the healthy control bladders was significantly predominant in *Lactobacillus* and *Corynebacterium* genera, whereas other bacterial genera, including *Klebsiella*, *Enterococcus*, and *Escherichia*, were more frequently found in the NB urine [35].

Groah et al. [36] demonstrated that the UM in patients with NB comprised uropathogenic bacteria, including *E. coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. They also suggested possible causes for changes in the NB UM, which showed an altered composition of perineal bacteria due to fecal incontinence and/or the use of bowel care regimens or changes in gut microbiota that alter the ability of certain bacteria to colonize the UT. Bossa et al. [18] recently demonstrated a significant variation in the bacterial composition of the microbiome in patients with NB. They further showed a return to baseline bacterial composition after the resolution of a UTI or discontinuation of probiotics. Forster et al. [37] presented that *Enterobacteriaceae* are the most predominant bacteria along with *Staphylococcus*, *Streptococcus*, and *Enterococcus* in the UM of 34 children with NB. They also observed that the route of catheterization might affect the composition of the UM. Children who catheterize their urethra have a higher proportion of *Staphylococcus*, while the UM of patients who catheterize through a Mitrofanoff was composed of *Enterobacteriaceae*.

2) Urological cancers and UM

Dysbiosis of the microbiome contributes to carcinogenesis through several mechanisms, including DNA damage, production of carcinogenic metabolites, and stimulation of inflammation [38]. Numerous studies have focused on the relationship between the host microbiome and cancer susceptibility in systems other than the UT, such as gastric, colorectal, or hepatobiliary cancer [9,39]. However, the role of UM in UT cancers has yet to be elucidated. According to the reports of a few studies on urological cancers in adults thus far, UM is mainly associated with prostate cancer, bladder cancer, and renal cancer.

(1) Prostate cancer: Many pathogenic microorganisms including endogenous *Enterobacteriaceae*, such as *E. coli* and *Pseudomonas* spp., are known to infect the prostate [6]. Inflammation in the prostate plays a vital role in the generation of prostate cancer, and cytokines such as interleukin (IL)-6 and IL-8 have been associated with the propagation of prostate cancer [40]. Compared to healthy controls, the level of *Bacteroides massiliensis* was elevated, whereas *Faecalibacterium prausnitzii* and *Eubacterium rectale* were reduced in the gut microbiota of adult patients with prostate cancer [41]. Cavarretta et al. [42] also reported that a high abundance of *Propionibacterium* spp., predominantly *Propionibacterium acnes*, is consistent with the pro-inflammatory role of *P. acnes*, thus supporting reports of its association with prostate cancer. According to the Feng et al. study [43], *Escherichia*, *Propionibacterium*, *Acinetobacter*, and *Pseudomonas* were the most abundant genera in radical prostatectomy tissue of adult patients with prostate cancer. Besides, Yu et al. [44] also reported that adults with prostate cancer had a lower number of *E. coli* in urine samples, and no significant differences were observed in *Enterococcus* numbers for urine and prostatic secretion samples.

However, unlike studies in adults, to date there are no reports on the relationship between UM and any prostate disease (including benign diseases and malignant tumors such as sarcoma) in children.

(2) Bladder cancer: Some studies have demonstrated that the UM is associated with bladder cancer transformation and metastatic progression. It has further been suggested that dysbiosis of the UM produces a chronically inflammatory urothelial microenvironment which leads to bladder cancer [45]. However, it remains unknown whether the UM affects the development or progression of bladder cancer, or whether bladder cancer is responsible for the alternation of the UM [6]. Some studies indicate that the bladder microbiome may change the extracellular matrix (ECM) to promote or inhibit urothelial carcinogenesis. The ECM regulates tissue homeostasis and maintains the onset and progression of cancer, including bladder cancer [6].

Conversely, Hayatsu and Hayatsu [46] reported that the *Lactobacillus casei* present in the UM prevents the production of carcinogens and mutagens by intestinal bacteria and the excretion of mutagens in urine. Some studies also demonstrated that *L. casei* reduces superficial bladder cancer recurrence in adults [47,48]. Wu et al. [25] analyzed midstream urine from 31 adult patients with bladder cancer and 18 healthy controls. They found an abundant presence of *Acinetobacter*, *Anaerococcus*, and *Sphingobacterium* species in bladder cancer. *Herbaspirillum*, *Porphyrobacter*, and *Bacteroides* species were detected in bladder cancers at a high risk of recurrence and progression. More recently, Ahn et al. [49] demonstrated that among the six genera of *Cutibacterium*, *Peptoniphilus*, *Sphingomonas*, *Staphylococcus*, *Micrococcus*, and *Moraxella*, there was a significant increase in *Micrococcus* sp. In bladder cancer compared to non-cancer.

There is a well-documented association between chronic schistosomiasis and bladder squamous cell carcinoma [6,50,51]. Adebayo et al. [50] demonstrated that certain UM, such as *Fusobacterium*, *Sphingobacterium*, and *Enterococcus* sp., distinguishes the patients with urogenital schistosomiasis from healthy individuals.

In terms of urothelial cell carcinoma (UCC), Xu et al. [52] studied the changes in the UM community of UCC patients in comparison to healthy individuals. They presented an enrichment of Streptococcus in urine in a preliminary study of the UM involving a small number of patients with UCC.

Unfortunately, unlike studies in adults, there has been no research in children to study the relationship between UM and bladder cancer or UCC.

(3) Renal cancer: Unlike UM studies in prostate cancer and bladder cancer in adults, very few studies have been conducted for renal cancer. In a recent study about the microbiome in 5 benign renal and malignant renal tissues in adults, Heidler et al. [53] are the first to demonstrate a plethora of microorganisms with significant differences between the two groups. They isolated 3 domains, 15 phyla, 16 classes, 19 orders, 27 families, 28 genera, and 30 species of microorganisms. *Trachelomonas volvocinopsis*, *Pseudoalteromonas haloplanktis*, *Halomicrobium mukohataei*, *Aeromonas salmonicida*, and *Mycoplasma mycoides* were abundantly found in renal cell carcinoma tissue. Microorganisms that appeared solely in renal cancer tissue were *Cyanophora paradoxa*, *Spirosoma navajo*, *Phaeocystis antarctica*, *Euglena mutabilis*, and *Mycoplasma vulturii*. More recently, Ahn et al. [49] performed a metagenomic analysis of urinary DNA in 12 patients with renal cancer. They demonstrated that among the six genera of *Cutibacterium*, *Peptoniphilus*, *Sphingomonas*, *Staphylococcus*, *Micrococcus*, and *Moraxella*, the *Micrococcus* sp., *Cutibacterium acnes*, *Cutibacterium granulosum*, *Peptoniphilus lacydonensis*, and *Staphylococcus epidermidis* were significantly increased in renal cancer compared to non-cancer.

Unfortunately, there are no reports on the association between UM and renal cancer, especially Wilms' tumor, in children.

(4) Other genitourinary cancers: There have been no studies associating UM and other genitourinary cancers, such as testis cancer, penile cancer, urethral cancer, etc., in both adults and children. Therefore, there needs to be an escalated interest in the UM study of these genitourinary cancers.

3) Role of probiotics and diet in pediatric urological diseases

There have been recent attempts for the therapeutic application of probiotic microorganisms as a treatment of disease in clinical practice. Probiotics have also been applied to modify the intestinal microbiome. Various clinical trials, including fecal microbiota transplantation, have been performed to study the role of certain beneficial strains in UTI, bladder cancer, and urinary stone formation [9].

To date, antibacterial therapy is the primary basic management for UTIs in children. However, it is inevitable that the UM undergoes alterations during UTI and antibiotic therapy. Long-term use of broad-spectrum antibiotics negatively affects the beneficial bacterial flora in the host, with the consequential selective overgrowth of pathogenic bacteria, eventually resulting in bacterial resistance [54]. Therefore, probiotics such as *Lactobacillus*, cranberries, and D-Mannos have been used as an alternative or adjuvant therapy for the prevention and treatment of UTIs in both children and adults [9].

Recently, the relationship between dysbiosis and UTI suggests that altering the UM through the use of probiotics may impart therapeutic effects. Probiotics using commensal bacteria could serve as either alternatives or adjuvant options to antibiotics for preventing antibiotic resistance [10]. Trials of bacterial interference with probiotics of the HU2117 *E. coli* strain have shown protection from symptomatic UTI

in NB [10].

According to recent cancer studies in adults, probiotics such as the oral *L. casei* strain *Shirota* could be effective for the prevention and treatment of non-invasive bladder tumors [47,55]. Ohashi et al. [56] reported that habitual intake of lactic acid bacteria reduces the risk of bladder cancer in adult patients. However, to date, there have been no reports that probiotics are efficacious for the preventing and treating pediatric malignant tumors.

Siener et al. [32] demonstrated that *O. formigenes* reduces urinary oxalate by decreasing intestinal absorption. Given the relationship between colonization with *O. formigenes* and stone formation, researchers have explored the efficacy of probiotics in reducing stone formation [10]. Some studies described *O. formigenes* as a probiotic with the potential to treat hyperoxaluria. However, there is conflicting data on the effect of *Oxalobacter* probiotics on the prevention of urinary oxalate stones [10].

Dietary factors such as cranberry, D-Mannos, and fermented milk products are also known to reduce the risk or incidence of recurrent UTIs by altering the properties of the genitourinary bacterial flora [57-60]. Therefore, dietary habits that may change the UM may be important factors associated with urological disorders, especially UTIs in children.

FUTURE PERSPECTIVE

We are still in the early stages of understanding the role of UM in human health and genitourinary diseases, especially in pediatric patients. It is important for us to expand our knowledge of the impact of UM in urological areas, especially in children. New diagnostic technologies and therapeutic avenues on the UM in pediatric urological diseases will continue to advance our understanding of the impact of UM on pediatric health and disease. The newly elucidated role of UM in the pediatric urological area suggests that both probiotics and dietary modification probably exert a therapeutic influence on pediatric urological diseases, including recurrent UTI, UUI/OAB, NB, and urolithiasis.

Previous microbiome studies in the gut and non-urological, non-gut diseases in pediatrics (such as infantile colic, necrotizing enterocolitis, asthma, atopy, obesity, type-1 diabetes, autism, atopic dermatitis, psoriasis, bronchial asthma, caries, periodontitis, and chronic rhinosinusitis) can expose new strategies for the prevention and management of these diseases by several applications such as immunomodulatory, anti-inflammatory, or growth-promoting action and antibacterial substances production [5]. More prospective research unearthing the characterization of the human microbiome in the pediatric urological area might help to develop new microbiome-based biomarkers to provide information about the diagnosis, disease severity, or treatment response, and also utilize these bacterial derivatives as therapeutic or preventive agents to control various pediatric urological diseases [9].

CONCLUSIONS

The UM and its relationship to UT diseases are currently under comprehensive investigation. Recently, improved techniques such as EQUC and high throughput molecular DNA sequencing of bacterial 16S rRNA genes have resulted in the discovery of a significant and diverse microbiome. However, there are insufficient studies evaluating the role of UM in the pediatric urological area. It is also important to consider the variations observed in the bacterial, fungal, and viral genera described for the UM in different studies; this could be attributed to differences in the gender, sample size, and urine collection methods and techniques used to study the UM [9].

Since initiation of the HMP, it is now known that urine is not sterile. The differences in the UM between healthy individuals and urological patients suggest that urinary dysbiosis may constitute an etiological factor in several urological disorders in both children and adults, with potential diagnostic and therapeutic implications in the near future.

Further larger scale, well-designed, prospective investigations to characterize the pediatric urological UM are required. This will enable the development of new microbiome-based biomarkers for the diagnosis and treatment, and also new microbiome-based-bacterial derivatives as therapeutic or preventive agents to control various pediatric urological diseases.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article

was reported.

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AUTHOR CONTRIBUTIONS

S.D.L. participated in data collection and analysis and wrote the manuscript. J.M.C. participated in the study design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Lederberg J, McCray AT. 'Ome sweet 'omics -- a genealogical treasury of words. The Scientist 2001;15:8.
2. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev 2012;70(Suppl 1):S38-44.
3. Berg G, Rybakova D, Fischer D, Cernava T, Vergès MC, Charles T, et al. Microbiome definition re-visited: old concepts and new challenges. Microbiome 2020;8:103. Erratum in: Microbiome 2020;8:119.
4. Hiergeist A, Gessner A. Clinical implications of the microbiome in urinary tract diseases. Curr Opin Urol 2017;27:93-8.
5. Arora SK, Dewan P, Gupta P. Microbiome: paediatricians' perspective. Indian J Med Res 2015;142:515-24.
6. Lee KW, Song HY, Kim YH. The microbiome in urological diseases. Investig Clin Urol 2020;61:338-48.
7. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature 2012;486:207-14.
8. Human Microbiome Project Consortium. A framework for human microbiome research. Nature 2012;486:215-21.
9. Aragón IM, Herrera-Imbroda B, Queipo-Ortuño MI, Castillo E, Del Moral JS, Gómez-Millán J, et al. The urinary tract microbiome in health and disease. Eur Urol Focus 2018;4:128-38.
10. Gerber D, Forster CS, Hsieh M. The role of the genitourinary microbiome in pediatric urology: a review. Curr Urol Rep 2018;19:13.
11. Li JKM, Chiu PKF, Ng CF. The impact of microbiome in urological diseases: a systematic review. Int Urol Nephrol 2019;51:1677-97.
12. Kilis-Pstrusinska K, Rogowski A, Bienkowski P. Bacterial colonization as a possible source of overactive bladder symptoms in pediatric patients: a literature review. J Clin Med 2021;10:1645.
13. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol 2012;50:1376-83.
14. Brubaker L, Wolfe AJ. The new world of the urinary microbiota in women. Am J Obstet Gynecol 2015;213:644-9.
15. Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. mBio 2014;5:e01283-14.
16. Horwitz D, McCue T, Mapes AC, Ajami NJ, Petrosino JF, Ramig RF, et al. Decreased microbiota diversity associated with urinary tract infection in a trial of bacterial interference. J Infect 2015;71:358-67.
17. Nienhouse V, Gao X, Dong Q, Nelson DE, Toh E, McKinley K, et al. Interplay between bladder microbiota and urinary antimicrobial peptides: mechanisms for human urinary tract infection risk and symptom severity. PLoS One 2014;9:e114185.
18. Bossa L, Kline K, McDougald D, Lee BB, Rice SA. Urinary catheter-associated microbiota change in accordance with treatment and infection status. PLoS One 2017;12:e0177633.
19. Yoo JJ, Song JS, Kim WB, Yun J, Shin HB, Jang MA, et al. *Gardnerella vaginalis* in recurrent urinary tract infection is associated with dysbiosis of the bladder microbiome. J Clin Med 2022;11:2295.
20. Kinneman L, Zhu W, Wong WSW, Clemency N, Provenzano M, Vilboux T, et al. Assessment of the urinary microbiome in children younger than 48 months. Pediatr Infect Dis J 2020;39:565-70.
21. Kassiri B, Shrestha E, Kasprenski M, Antonescu C, Florea LD, Sfanos KS, et al. A prospective study of the urinary and gastrointestinal microbiome in prepubertal males. Urology 2019;131:204-10.
22. Antunes-Lopes T, Vale L, Coelho AM, Silva C, Rieken M, Geavlete B, et al.; EAU Young Academic Urologists (YAU) Functional Urology Working Group. The role of urinary microbiota in lower urinary tract dysfunction: a systematic review. Eur Urol Focus 2020;6:361-9.
23. Karstens L, Asquith M, Davin S, Stauffer P, Fair D, Gregory WT, et al. Does the urinary microbiome play a role in urgency urinary incontinence and its severity? Front Cell Infect Microbiol 2016;6:78.
24. Thomas-White KJ, Hilt EE, Fok C, Pearce MM, Mueller ER, Kliethermes S, et al. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J 2016;27:723-33.
25. Wu P, Zhang G, Zhao J, Chen J, Chen Y, Huang W, et al. Profiling the urinary microbiota in male patients with bladder cancer in

China. Front Cell Infect Microbiol 2018;8:167. Erratum in: Front Cell Infect Microbiol 2018;8:429.
26. Santos JC, Telo ER. Solifenacin: scientific evidence in the treatment of overactive bladder. Arch Esp Urol 2010;63: 197-213.
27. Sorrentino F, Cartwright R, Digesu GA, Tolton L, Franklin L, Singh A, et al. Associations between individual lower urinary tract symptoms and bacteriuria in random urine samples in women. Neurourol Urodyn 2015;34:429-33.
28. Kachroo N, Lange D, Penniston KL, Stern J, Tasian G, Bajic P, et al. Standardization of microbiome studies for urolithiasis: an international consensus agreement. Nat Rev Urol 2021;18: 303-11.
29. Stern JM, Moazami S, Qiu Y, Kurland I, Chen Z, Agalliu I, et al. Evidence for a distinct gut microbiome in kidney stone formers compared to non-stone formers. Urolithiasis 2016;44:399-407.
30. Tang R, Jiang Y, Tan A, Ye J, Xian X, Xie Y, et al. 16S rRNA gene sequencing reveals altered composition of gut microbiota in individuals with kidney stones. Urolithiasis 2018;46:503-14.
31. Barr-Beare E, Saxena V, Hilt EE, Thomas-White K, Schober M, Li B, et al. The interaction between enterobacteriaceae and calcium oxalate deposits. PLoS One 2015;10:e0139575.
32. Siener R, Bangen U, Sidhu H, Hönow R, von Unruh G, Hesse A. The role of Oxalobacter formigenes colonization in calcium oxalate stone disease. Kidney Int 2013;83:1144-9.
33. Lange D. Dietary habits may influence oxalate degradation by intestinal bacteria commentary on: the role of Oxalobacter formigenes colonization in calcium oxalate stone disease. Urology 2014;84:1263-4.
34. Kaufman DW, Kelly JP, Curhan GC, Anderson TE, Dretler SP, Preminger GM, et al. Oxalobacter formigenes may reduce the risk of calcium oxalate kidney stones. J Am Soc Nephrol 2008;19:1197-203.
35. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med 2012;10:174.
36. Groah SL, Pérez-Losada M, Caldovic L, Ljungberg IH, Sprague BM, Castro-Nallar E, et al. Redefining healthy urine: a cross-sectional exploratory metagenomic study of people with and without bladder dysfunction. J Urol 2016;196:579-87.
37. Forster CS, Panchapakesan K, Stroud C, Banerjee P, Gordish-Dressman H, Hsieh MH. A cross-sectional analysis of the urine microbiome of children with neuropathic bladders. J Pediatr Urol 2020;16:593.e1-8.
38. Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. CA Cancer J Clin 2017;67:326-44.
39. Neto AG, Bradshaw AD, Pei Z. Microbiome, a new dimension in cancer research. Ann Transl Med 2015;3:229.
40. Puhr M, De Marzo A, Isaacs W, Lucia MS, Sfanos K, Yegnasubramanian S, et al. Inflammation, microbiota, and prostate cancer. Eur Urol Focus 2016;2:374-82.
41. Golombos DM, Ayangbesan A, O'Malley P, Lewicki P, Barlow L, Barbieri CE, et al. The role of gut microbiome in the pathogenesis of prostate cancer: a prospective, pilot study. Urology 2018;111:122-8.
42. Cavarretta I, Ferrarese R, Cazzaniga W, Saita D, Lucianò R, Ceresola ER, et al. The microbiome of the prostate tumor microenvironment. Eur Urol 2017;72:625-31.
43. Feng Y, Ramnarine VR, Bell R, Volik S, Davicioni E, Hayes VM, et al. Metagenomic and metatranscriptomic analysis of human prostate microbiota from patients with prostate cancer. BMC Genomics 2019;20:146.
44. Yu H, Meng H, Zhou F, Ni X, Shen S, Das UN. Urinary microbiota in patients with prostate cancer and benign prostatic hyperplasia. Arch Med Sci 2015;11:385-94.
45. Friedrich V, Choi HW. The urinary microbiome: role in bladder cancer and treatment. Diagnostics (Basel) 2022;12:2068.
46. Hayatsu H, Hayatsu T. Suppressing effect of Lactobacillus casei administration on the urinary mutagenicity arising from ingestion of fried ground beef in the human. Cancer Lett 1993;73:173-9.
47. Aso Y, Akazan H. Prophylactic effect of a Lactobacillus casei preparation on the recurrence of superficial bladder cancer. BLP Study Group. Urol Int 1992;49:125-9.
48. Naito S, Koga H, Yamaguchi A, Fujimoto N, Hasui Y, Kuramoto H, et al.; Kyushu University Urological Oncology Group. Prevention of recurrence with epirubicin and lactobacillus casei after transurethral resection of bladder cancer. J Urol 2008;179:485-90.
49. Ahn HK, Kim K, Park J, Kim KH. Urinary microbiome profile in men with genitourinary malignancies. Investig Clin Urol 2022;63:569-76.
50. Adebayo AS, Suryavanshi MV, Bhute S, Agunloye AM, Isokpehi RD, Anumudu CI, et al. The microbiome in urogenital schistosomiasis and induced bladder pathologies. PLoS Negl Trop Dis 2017;11:e0005826. Erratum in: PLoS Negl Trop Dis 2017;11:e0006067.
51. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013;63:234-41.
52. Xu W, Yang L, Lee P, Huang WC, Nossa C, Ma Y, et al. Mini-review: perspective of the microbiome in the pathogenesis of urothelial carcinoma. Am J Clin Exp Urol 2014;2:57-61.
53. Heidler S, Lusuardi L, Madersbacher S, Freibauer C. The microbiome in benign renal tissue and in renal cell carcinoma. Urol Int 2020;104:247-52
54. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. J Infect Dis 2001;183 Suppl 1:S1-4.
55. Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, Naito S.

Preventive effect of a Lactobacillus casei preparation on the recurrence of superficial bladder cancer in a double-blind trial. The BLP Study Group. Eur Urol 1995;27:104-9.
56. Ohashi Y, Nakai S, Tsukamoto T, Masumori N, Akaza H, Miyanaga N, et al. Habitual intake of lactic acid bacteria and risk reduction of bladder cancer. Urol Int 2002;68:273-80.
57. Kontiokari T, Sundqvist K, Nuutinen M, Pokka T, Koskela M, Uhari M. Randomised trial of cranberry-lingonberry juice and Lactobacillus GG drink for the prevention of urinary tract infections in women. BMJ 2001;322:1571.
58. Kontiokari T, Laitinen J, Järvi L, Pokka T, Sundqvist K, Uhari M. Dietary factors protecting women from urinary tract infection. Am J Clin Nutr 2003;77:600-4.
59. Raz R, Chazan B, Dan M. Cranberry juice and urinary tract infection. Clin Infect Dis 2004;38:1413-9.
60. Foxman B, Cronenwett AE, Spino C, Berger MB, Morgan DM. Cranberry juice capsules and urinary tract infection after surgery: results of a randomized trial. Am J Obstet Gynecol 2015;213:194.e1-8.