International Urology and Nephrology (2019) 51:1677–1697
https://doi.org/10.1007/s11255-019-02225-y

UROLOGY - REVIEW

The impact of microbiome in urological diseases: a systematic review

Joseph K. M. Li[1] · Peter K. F. Chiu[1] · Chi-Fai Ng[1]

Received: 1 June 2019 / Accepted: 4 July 2019 / Published online: 12 July 2019
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Abstract

Objective The term microbiome is used to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that share our body space, in which there were increasing evidences to suggest that they might have potential roles in various medical conditions. While the study of microbiome in the urinary system is not as robust as the systems included in the Human Microbiome Project, there are still evidences in the literature showing that microbiome may have a role in urological diseases. Therefore, we would like to perform a systematic review on the topic and summarize the available evidence on the impact of microbiome on urological diseases.

Methodology This review was performed according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement. After screening 589 abstracts and including additional studies (such as references from review papers), 76 studies were included for review and discussion.

Results Studies had suggested that there were correlations of microbiome of different body cavities (e.g., fecal, urinary and seminal fluid) with urological diseases. Also, different diseases would have different microbiome profile in different body cavities. Unfortunately, the studies on the association of microbiome and urological diseases were still either weak or inconsistent.

Conclusion Studies suggested that there might be some relationship between microbiome and various urological diseases. However, further large-scale studies with control of confounding factors should be performed under a standardized methodology in order to have better understanding of the relationship. Also, more standardized reporting protocol for microbiome studies should be considered for better communications in future studies.

Keywords Microbiota · Urology · Prostate cancer · Urolithiasis · Lower urinary tract symptom

Introduction

Our human body is inhabited by at least ten times more bacteria than the number of human cells in the body [1], and the term "microbiome" was initially used to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space [2]. Currently, the term microbiome has various definitions, with some groups limiting its use to the genes of bacteria, and other groups refer this to the metagenome, i.e., all genetic materials of a population including plasmids [3]. In turn, microbiota was defined as the microorganisms in a particular environment [4, 5].

The Human Microbiome Project (HMP) was started since 2007 and one of the aims of HMP was to characterize the human microbiome and its influence on disease [6]. Five body sites (the gastrointestinal tract, the mouth, the vagina, the skin and the nasal cavity) were chosen for kickoff of the HMP, yet the urinary tract was not chosen because urine was considered sterile. The study of microbiome in the urinary tract started in male urethral microbiome in patients with and without sexually transmitted infection [7]. Later, the study of microbiota in urine by culture-independent [8, 9] and culture-dependent [10, 11] methods was started and disproved the dogma of urine being sterile.

It was noted that the microbiota plays an important role in disease development and progression in different parts of the body. For example, inflammatory bowel disease, *Clostridium difficile*-associated colitis and colorectal cancer are associated with gut dysbiosis. However, the association of dysbiosis and urological diseases is not well studied in the

✉ Chi-Fai Ng
ngcf@surgery.cuhk.edu.hk

[1] SH Ho Urology Centre, Department of Surgery, The Chinese University of Hong Kong, Shatin, Hong Kong

literature, with mainly observational studies or cohort studies with small number of subjects involved. Therefore, the aim of this review is to give urologists an overview of how microbiome is associated with urologic diseases by summarizing the available evidence of the role of microbiota in urologic disease and outlining the future prospects for microbiome studies for urologic disease.

Methodology

A systematic literature search was performed using PubMed and Medline databases from inception until April 2019. Papers written in English were selected following the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement. A flowchart of the Systematic search process is shown in Fig. 1. The following keywords were included in this systematic review: "microbiome" or "microbiota" in combination with "prostate cancer, bladder cancer, kidney cancer, urolithiasis, urinary tract stone, Oxalobacter formigenes, urinary tract infection, bladder pain syndrome, painful bladder syndrome, interstitial cystitis, chronic pelvic pain syndrome, lower urinary tract syndrome, overactive bladder syndrome, urinary incontinence, infertility". The initial search identified 589 studies. Additional manuscripts, such as those referenced by reviews, were also reviewed (Fig. 1).

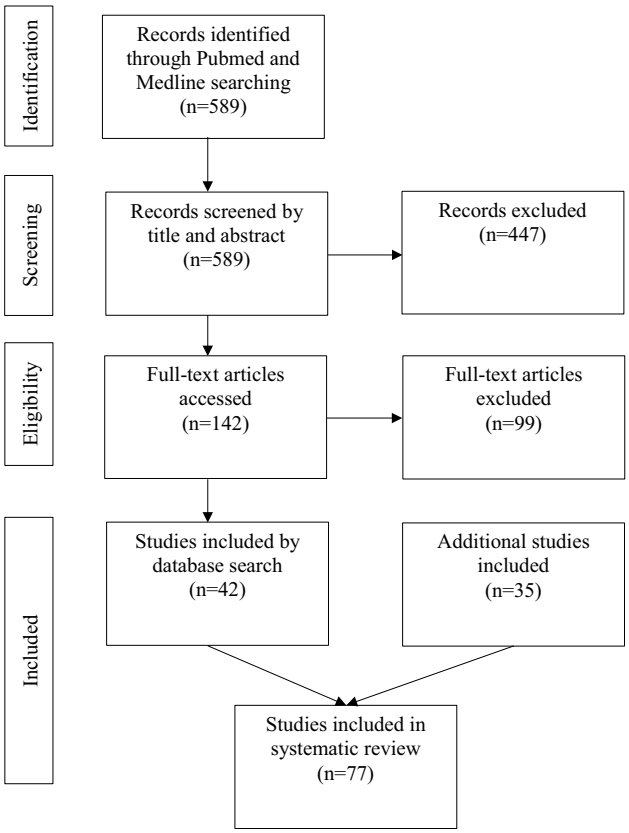

Fig. 1 Flow diagram of evidence acquisition in a systematic review on role of microbiome in urological diseases

Sample collection, microorganism detection and data analysis

It is well known that different collection methods will reveal different microbiota even within the same environment. Rectal swab will be different from the stool sampling, which will also be different from the mucosal biopsy. Therefore, it is very important to identify the methods for sample collection before we use the results from the studies.

Urine samples

For urine samples, the gold standard is suprapubic aspiration (SPA) of urine because it will avoid the contamination from the urethra and external genitalia. There are studies showing that catheterized urine has similar microbiota with urine sampling from SPA, and mid-stream clean catch urine (MSU) has microbiota similar to that of the external genitalia [8]. Although there is a higher chance of contamination by external genitalia, MSU has the advantage of being convenient and less invasive, when compared to SPA and catheterized urine.

Microorganism detection

Microorganisms can be detected by culture-dependent methods and culture-independent methods. Culture-dependent methods include those of standard culture, which are used to identify common uropathogens, and expanded quantitative urine culture (EQUC), which aims to detect organisms that are difficult to be cultured in standard culture using a greater volume of urine, incubation in varied conditions, and the use of extended incubation times [11]. Culture-independent methods include the sequencing of the 16S ribosomal RNA (rRNA), which has highly conserved regions with 9 different variable regions (V1–V9). By detecting the difference in the variable regions, different genus of the bacteria can be identified. The disadvantage of culture-independent methods is that they can only tell the bacteria has been present, but cannot tell whether the bacteria are viable. Also, the use of different variable regions cannot be compared directly; therefore, if two similar studies are using different variable regions in identifying bacteria, the results can be different.

Results from 16S rRNA sequencing and EQUC can be complementary for the identification of bacteria in urine, instead of being mutually exclusive [12].

Analysis of microbiome data

Diversity of the microbiome is used to standardize the result and to measure the differences between two groups. Alpha- and beta-diversities are two commonly used parameters. Alpha-diversity measures the species richness within a fine and homogeneous extent, whereas beta-diversity measures the extent of species composition among different communities [13].

Malignant urological conditions

The rising interests in relating microbiome with different aspects of cancer management started since the popularization of the next generation sequencing (NGS). Focuses of the field are how the microbiota contribute to the cancer development and progression, and also how it affects the treatment of cancer [14]. In this section, we will also summarize the evidence available in the literature on how microbiota affects the pathogenesis and treatment of common urological cancer.

Prostate cancer

The risk of prostate cancer is shown to be associated with obesity and inflammation [15]. As well, certain foods and nutrients may be associated with increased risk of prostate cancer [16]. The above risk factors are also associated with the gut microbiome [17] and, therefore, a hypothesis of the difference of gastrointestinal microbiome between healthy men, those with latent prostate cancer, and those with invasive prostate cancer was made [16]. Recently, there is evidence to support the above hypothesis. Golombos et al. [18] have shown that there are more *Bacteroides massiliensis*, and less *Faecalibacterium prausnitzii* and *Eubacterium rectalie* in the gut microbiota of patients with prostate cancer when compared with that of patients with benign prostate. They also suggested that butyrate, an anti-inflammatory micronutrient produced by a range of bacteria including *F. prausnitzii* and *E. rectalie*, can be one of the pathways for the prevention of prostate cancer (Table 1).

In another study, Liss et al. [15] have shown that despite there is overlap of gut bacterial community between patients with and without prostate cancer, there were altered metabolic pathways. The authors have derived a microbiome score from 10 different metabolic pathways and showed statistical significant differences between patients with and without prostate cancer. Although statistically significant, the microbiome score has an area under curve (AUC) of only 0.64, which is suboptimal for a biomarker to look for patients at high risk for prostate cancer. On the other hand, authors suggested that further studies into metabolic pathways of natural B-vitamins may provide a reasonable preventive measure for prostate cancer, echoing the study from Golombos et al. that micronutrient may have a role in the prevention of prostate cancer.

Apart from the looking into the pathogenesis of prostate cancer, the effect of treatment on gut microbiome was evaluated by Sfanos et al. [19], in which they found that men without prostate cancer had a more diverse gut microbiota. The gut microbiota of patients taking oral androgen receptor axis-targeted therapies had enriched steroid/hormone biosynthesis pathway and more abundant *Akkermansia muciniphila*, which is involved in the treatment response to anti-PD-1 immunotherapy in patients with epithelial tumors.

In addition to the gut microbiome, prostate tissue microbiome is also a focus for the study of pathogenesis. Studies have shown that there is also a community of bacteria in the tissue of prostate cancer using NGS [20–22]. They found no significant difference between the composition of microbiome between cancer and benign tissue [21, 22]. However, these studies identified possible associations of specific bacteria with prostate cancer. For example, there is gradual increase in the richness of *Streptococcus* and decrease in the richness of *Staphylococcus* from tumor, peri-tumour to non-tumor tissue [21], and *Pseudomonas* infection is negatively associated with the risk of metastasis [22].

Urinary microbiome was also studied using urine sample after prostatic massage. The two studies showed no significant differences in microbiota composition. However, many species were significantly and differentially abundant among cancer and non-cancer samples. For example, *Veillonella*, *Streptococcus*, and *Bacteroides* were more abundant in patients with prostate cancer, whereas *Faecalibacterium*, *Lactobaccili*, and *Actinectobacter* were less abundant [23]. Conversely, a cluster of pro-inflammatory microbials (e.g., *Streptococcus anginosus, Actinobaculum schaalii and Propionimicrobium lymphophilum*) contained more cancer samples than samples outside this cluster [24].

Bladder cancer

The use of *Bacillus Calmette–Guérin* (BCG, live-attenuated *Mycobacterium bovis*) in the treatment of non-muscle invasive bladder cancer was long before the study of urinary microbiome. Until recently, a preliminary report of the urinary microbiome in eight patients with bladder cancer by Xu et al. from their mini-review revealed that there were differences of urinary microbiome between normal subjects and patients with bladder cancer [25], although they did not include their methodology. They found that *Streptococcus* was significantly elevated in bladder cancer. Two other studies were found in the search of the association of bladder cancer with urinary microbiome. Bučević Popović et al. presented a negative result which showed that the diversity

Table 1 Summary of original articles on prostate cancer

Study	Patients	Sample	Microbial identification	Alpha-diversity	Beta-diversity	Relevant microbials	Functional profiling	Remarks
Liss et al. Eur Urol, 2018 [15]	Patients undergoing transrectal prostate biopsy	Rectal swab	16S rDNA sequencing (V1, V2 regions)	No significant difference	No significant difference	Prostate cancer group—more: genus—*Bacteroides, Streptococcus*	Prostate cancer group: abundance in carbohydrate metabolism pathways lacking natural B-vitamin production	Microbiome score was derived
Golombos et al. Urology, 2018 [18]	Prostate cancer Benign	Fecal swab	Whole-genome Shotgun sequencing	N/A	N/A	Prostate cancer group—more: species—*Bacteroides massiliensis* Benign group—more: species—*Faecalibacterium prausnitzii*, *Eubacterium rectale*	Prostate cancer group: enriched carbohydrate biosynthesis superclass Benign group: enriched for pathways involved in carbohydrate, fatty acid and lipid, and aminoacid biosynthesis and metabolism enriched in energy metabolisms and glycan biosynthesis	
Sfanos et al. Prostate Cancer Prostatic Dis, 2018 [19]	Men with benign prostate or prostate cancers, who have no medicaations or having ADT or oral ATT	Rectal swab	16S rDNA sequencing (V6 region)	Significant difference	Significant difference	Oral ATT group—more: genus—*Ruminococcaceae*, *Lachnospiraceae* species- *Akkermansia muciniphila* Men receiving ADT (vs no ADT)—less: family—*Brevibacteriaceae*, *Erysipelotrichaceae*, *Streptococcaceae*	Oral ATT group (vs no ADT): enriched steroid/hormone biosynthesis, caffeine metabolism and glycosaminoglycan degradatoin	Included quantitative PCR of *Akkermansia muciniphila*
Yow et al. Infect Agent Cancer, 2017 [20]	patients undergone radical prostatectomy Bengin tissue from the same specimen	Prostate tissue	16S rDNA sequencing (V2–V3 and V4 regoin)	N/A	N/A	'Core' community of prostate tissue by V4: *Enterobacteriaceae, Esherichia, Pseudomonadaceae, Comamonadaceae, Ralstonia, Pseudomonas, Acinetobacter* 'Core' community of prostate tissue by V2-3: *Enterobacteriaceae, Streptococcaceae, Staphylococcus, Escherichia, Moraxella, Propionibacterium acnes* and *Streptococcus pseudopneumoniae*	N/A	No comparison made between malignant and benign tissue

Table 1 (continued)

Study	Patients	Sample	Microbial identification	Alpha-diversity	Beta-diversity	Relevant microbials	Functional profiling	Remarks
Cavarretta et al. Eur Urol, 2017 [21]	Patients undergone radical prostatectomy	Prostate tissue	16S rDNA sequencing (V3–V5)	N/A	No significant difference	Tumor tissue—more: family—*Staphylococcaceae* genus—*Staphylococcus* Tumor tissue—less: order—Lactobacillales family—*Streptococcaceae* genus—*Streptococcus*	N/A	
Feng et al. BMC Genomics, 2019 [22]	Patients undergoing radical prostatectomy	Prostate tissue	Whole-genome sequencing	No significant difference	No significant difference	*Escherichia*, *Propionibacterium*, *Acinetobacter*, *Pseudomonas*	N/A	Transcriptome analysis suggested negative association between *Pseudomonas* infection and metastasis
Alanee et al. Prostate, 2018 [23]	patients undergoing transrectal MRI targeted prostate biopsy	rectal swab	16S rDNA sequencing (V3–V5)	No significant difference	No significant difference	Prostate cancer—more: genus—*Bacteroides*	N/A	
		first void urine after prostate massage		No significant difference	No significant difference, but trended to cluster separately	Prostate cancer—more: genus—Veillonella, Streptococcus, Bacteroides Prostate cancer—less genus—Faecalibacterium, Lactobaccili, Actinectobacter		
Shrestha et al. J Urol, 2018 [24]	Patients undergoing prostate biopsy	urine samples after DRE	16S rDNA sequencing (V6)	No significant difference	No significant difference	Prostate cancer—more: *Ureaplasma parvum, Ureaplasma urealyticum* This cluster group of bacterial species contained more cancer samples than those out of cluster: *Streptococcus anginosus, Anaerococcus lactolyticus, Anaerococcus obesiensis, Actinobaculum schaalii, Varibaculum cambriense, Propionimicrobium lymphophilum*	N/A	

and overall urinary microbiome composition were not significantly different between patients with or without bladder cancer [26], whereas Wu and colleagues showed that there is increased richness, but not species diversity, in the bladder cancer group and in those with high risk of disease recurrence and progression according to the EORTC risk stratification [27]. Also, there are significant differences in the beta-diversity between the cancer and non-cancer subjects, as well as those with high risk and low risk of recurrence and progression [27]. A point to note is that Bučević Popović et al. have found that *Streptococcus* was more abundant in normal subjects [26], on the contrary with the preliminary results of Xu et al. Therefore, this signifies the importance of mentioning the method of urine sampling and bacterial identification (Table 2).

Treatment of malignant urological conditions

In recent years, immunotherapy with immune check point inhibitors like the PD-1/PD-L1 inhibitors became a hot topic in the treatment of malignant urological conditions, as it provides a new treatment options with encouraging results with overall survival. However, the response rate of this therapeutic option is suboptimal [28]. Biomarkers like PD-L1 expression and mutational load were suggested to predict the response of immunotherapy [29].

Routy et al. [30] have found that antibiotics given around the initiation of the immunotherapy reduced the effectiveness of the treatment significantly, in which antibiotics is a predictor of resistance to PD-1 blockade in renal cell carcinoma. On the other hand, the response of PD-1 blockade was restored after fecal microbiota transplantation of responder cancer patients into germ-free or antibiotic-treated mice. The authors also noted that *Akkermansia muciniphila* was significantly associated with favorable clinical outcome, and the colonization of *A. muciniphila* in antibiotic-treated mice reversed the non-responsiveness to PD-1 blockade. However, the presence of *A. muciniphila* does not implicate a good response, and a hypothesis suggesting that *A. muciniphila* involves in only one of the multiple steps in the cancer-immunity cycle [31]. This also suggested that gut microbiota is not a perfect biomarker for predicting response to immunotherapy, as variations of the gut microbiota could be associated with other factors, like the histological type of tumor, environmental factors, study populations, or analytical methods [32].

Future studies on the involvement of gut microbiome in immunotherapy and the study of their functional pathway could provide us with more insights into integrating gut microbiome into a composite of biomarker as well as developing an adjuvant therapeutic option for modulating gut microbiome to improve the response rate of immunotherapy.

Limitations

There are common limitations to the studies of microbiome in malignant urological conditions, the studies of which are usually having relatively small sample size, unavailability of a standardized sample collection and lack of longitudinal samples for the confirmation for the cause–effect relationship. Further studies should include a larger sample size with a standardized, longitudinal sampling of specimen from the gut, tissue and urine from human subjects.

Conclusion

There is evidence that the microbiome may have a role in the pathogenesis of malignant urologic conditions. Further large-scale, longitudinal studies are required to identify a causal relationship between cancer and the changes in microbiome. After understanding the pathogenesis, it may provide a foundation to develop biomarkers for diagnosis and risk stratification, novel treatments for cancer and regimes for modulating microbiota to help with treatment responses.

Benign urological conditions

Another large group of urological conditions are the benign conditions, which affect the quality of life (QOL) of the patients. Better understanding of the pathophysiology of these diseases is useful for the treatment, which in turn improves the QOL of the patient.

Urolithiasis

The role of microorganism in the formation of urinary stone is well documented. It is well known that urea-splitting organisms, like *Proteus* species, can increase the pH of urine and, therefore, favor the formation of struvite stones, also known as “infection stones”. Apart from being responsible creating an environment for stone formation, microbials can also create an environment to treat stone disease [33] (Table 3).

Not only the local microbiome affects the formation of urinary stones, but also microbiome from the gut. Recent studies have shown that the imbalance of the gut microbiome (gut dysbiosis) is associated with metabolic diseases such as obesity, hypertension, diabetes mellitus and metabolic syndrome. Coincidentally, these metabolic diseases are also associated with the increased incidence of urinary stones. There were a few attempts in the literature trying to correlate gut dysbiosis and urinary stone formation.

Table 2 Summary of original articles on bladder cancer

Study	Patients	Sample	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Remarks
Xu et al. Am J Clin Exp Urol, 2014 [25]	Urothelial carcinoma patients Healthy individuals	N/A	N/A	N/A	N/A	Bladder cancer—more: genus—*Streptococcus*, *Pseudomonas*, *Anaerococcus*	
Bučević Popović et al. Sci Rep, 2018 [26]	Non-muscle-invasive tumor Control	MSU	16S rDNA sequencing (V4)	No significant difference	No significant difference	Bladder cancer—more: Genera—*Fusobacterium*, *Actinobaculum*, *Facklamia*, *Campylobacter* Species—*Campylobacter hominis*, *Actinobaculum massiliense*, *Jonquetella anthropi* Control—more: Genera—*Veillonella*, *Streptococcus*, *Corynebacterium* Species—*Veillonella dispar*, *Streptococcus cristatus*, *Corynebacterium appendicis*	
Wu et al. Front Cell Infect Microbiol, 2018 [27]	Male patients with bladder cancer Male controls	MSU	16S rDNA sequencing (V4)	Significantly increased bacterial richness in cancer patients	Significantly different bacterial profiles	Bladder cancer—more: genus—*Acinetobacter, Anaerococcus, Rubrobacter, Sphingobacterium, Atopostipes, Geobacillus* Control—more: genus—*Serratia*, *Proteus*, *Roseomonas, Ruminiclostridium-6, Eubacterium-xylanophilum*	Included analysis on high risk of recurrence and high risk of progression

Stern et al. [34] were one of the first groups in trying to characterise the differences between gut microbiome of kidney stone patients with that of non-stone formers. They noted that kidney stone patients had more *Bacteroides* and less *Prevotella* as compared with the control.

From an Indian human study, there were more *Firmicutes and Lachnospiraceae*, and less *Bacteroidetes, Prevotella* and *Eubacterium* in kidney stone patients [35]. The authors explained that the difference in the abundance of *Bacteroidetes* compared with Stern et al. was because of the recruitment of female, inclusion of mixed types of stones and stone formers with other disease-associated complications. The other reason for the difference may lie in sequencing the different variable region of the 16S rRNA.

A more recent study recruited patients from Southern China [36]. There were significant differences in the composition of gut microbiome between kidney stone patients and those healthy controls. The study also showed that there were significantly higher levels of pro-inflammatory bacteria, and a trend toward reduced diversity within samples in those with kidney stones.

All the above studies were great pilot studies in the understanding of the gut microbiome in kidney stone disease. However, there were common limitations, including small sample size and the lack of diet history. Further larger-scale studies with better control of confounding factors are required to characterize whether gut dysbiosis is associated with kidney stone disease.

Oxalate metabolizing bacterial species

As more than 70% of the calcium-containing renal stone is made up with oxalate, the oxalate metabolism is explored in hope to reduce the recurrence rate. Oxalate metabolizing bacterial species (OMBS) in the gut microbiome is one of the focuses in the oxalate metabolism, and they are divided into specialist and generalist OMBS. Generalist OMBS, like *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Clostridium* species, can utilize other compounds as carbon and energy source, whereas specialist OMBS, like *Oxalobacter formigenes*, utilizes oxalate as the sole carbon and energy source.

Oxalobacter formigenes is a Gram-negative, obligate anaerobic bacterium first discovered in 1985 [37]. It was found that *O. formigenes* metabolises ingested oxalate and stimulate oxalate secretion from the colon. Healthy subjects have higher colonization rate than subjects with kidney stones [35]. In subjects with kidney stones, those who are colonized with *O. formigenes* have 70% reduced risk of stone recurrence [38]. Having said that, a recent meta-analysis showed that the role of a specific OMBS may not be that important and having a diverse gut microbiota provides more protection to the prevention of stone disease [39]. With this in mind, it is not surprising that probiotics with preparations including *Oxalobacter*, *Lactobacillus* and/or *Bifidobacterium* species have been disappointing [40].

The use of probiotics for the prevention of oxalate stone formation can be further investigated, and the use of OMBS as a dissolution treatment of formed stone should also be considered.

Urinary tract infection

As urinary tract infection (UTI) involves bacteria, the microbiome would certainly have a role in UTI. Therefore, we would just highlight some of the application of microbiome in UTI. Firstly, not using antibiotic in patients with asymptomatic bacteriuria is justified by the fact that commensals have a protective effect on active UTI [41]. Secondly, NGS can be used to identify the causative pathogen, as well as identify the resistant pattern to antibiotics [42, 43]. Lastly, the use of probiotics is a prevention or treatment of recurrent UTI, either by instilling a single strain into the bladder [44–46] or the whole community by fecal microbiota transplantation [47–49].

Bladder pain syndrome

The etiology of bladder pain syndrome (BPS; or interstitial cystitis or painful bladder syndrome) remains unknown, but by definition, it excludes the presence of infection, which it was traditionally described as chronic sterile bladder inflammation [50, 51] (Table 4).

With the help of the 16S rRNA gene sequencing, it was found that *Lactobacillus* was represented in a higher abundance in the MSU samples in patients with BPS, with a decrease of microbial diversity when compared with healthy women [51]. Another recent study using the National Institute of Health (NIH) Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network showed that the urine samples had less *Corynebacterium* and more *Lactobacillus gasseri* in BPS patients [52]. One of the limitations for the above studies was the use of MSU samples, which may have contamination from the vaginal microbiome. In contrast to the above studies, another study using catheterized urine samples showed lower abundance of Lactobacillus acidophilus, but similarly decreased microbial diversity [53]. Furthermore, they have investigated the pro-inflammatory cytokine levels in the urine and showed that it was higher in patients with BPS [53].

Not only bacteria are studied in this disease, but also fungi and viruses. Nickel and colleagues have shown that there was no difference in the species composition, but a significantly greater prevalence of fungi in those with a flare in symptoms [54]. On the other hand, polyomaviruses and

Table 3 Summary of original articles on urolithiasis

Study	Patients	Sample	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Functional profiling	Remarks
Stern et al. Urolithiasis, 2016 [34]	Kidney stone patients Control	Fecal sample	16S rDNA sequencing (V4 region)	N/A	N/A	Kidney stone patients—more: *Bacteroides* Control—more: *Prevotella*	N/A	
Suryavanshi et al. Sci Rep, 2016 [35]	Symtpomatic kidney stone diseased Healthy control	Fecal samples	16S rDNA sequencing (V3 region)	No significant difference	Significant differences noted	Kidney stone—more: Firmicutes, Proteobacteria, TM7 Kidney stone—less: Bacteroidetes, Cyanobacteria, *Faecalibacterium prausnitzii* Control only: *Prevotella*, *Dialister*	Down regulated in kidney stone disease energy metabolism, glycan synthesis, metabolism of cofactors and vitamins Up-regulated in kidney stone disease lipid metabolism, carbohydrate metabolism, xenobiotic degradation and metabolism	Sensitivity of V1-3 region for O formigenes may be low; the use of Oxalobacter specific 16S rRNA gene primars demonstrated 100% vs 17% colonization of Oxalobacter formigenes
Tang et al. Urolithiasis, 2018 [36]	Nephrolithiasis patients Healthy control	Fecal samples	16S rDNA sequencing (V4 region)	No significant difference	Significant differences noted	Nephrolithiasis—more: Order—*Pseudomonadales*, *Erysipelotrichales* Family—*Moraxellaceae*, *Erysipelotrichaceae* genus—*Alloprevotella, Erysipelatoclostridium, unidentified_Lachnospiraceae, Phascolarctobacterium, Megamonas, Acinetobacter, Escherichia-Shigella, Sutterella* nephrolithiasis—less: genus—*Eubacterium_hallii*_group, *Dorea*, *Ruminiclostridium*_5, *Anaerostipes*, *Fusicatenibacter*, *Subdoligranulum*, *Eubacterium–ruminantium*_group, *Holdemania*, *Dialister*, *Ruminococcus*_1, *Parasutterella*, *Biliphila*	Not statistically different	

Table 4 Summary of original articles on bladder pain syndrome

Study	Patients	Sample collection	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Functional profiling	Remarks
Siddiqui et al. BMC Microbiol, 2012 [51]	Female with interstitial cystitis Healthy female	MSU	16S rDNA sequencing (V1V2 and V6 regions)	Interstitial cystitis significantly lower diversity than healthy females	Significant difference noted	Interstitial cystitis—more: phylum—*Firmicutes* order—*Lactobacillales* genera—*Lactobacillus* Interstitial cystitis—reduced: phylum—*Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria* Interstitial cystitis only: phylum—*Nitrospirae* order—*Sapropsiraceae* genera—*Enterococcus, Atopbium, Proteus, Cronobacter*	N/A	
Nickel et al. J Clin Med, 2019 [52]	Female with interstitial cystitis/bladder pain syndrome (IC/BPS) Controls	MSU	Electrospray ionization—time-of-flight—mass spectrometry	No significant difference	No significant difference	IC/BPS—less: genera—*Corynebacterium* IC/BPS—more: species—*Lactobacillus gasseri* (not statistically significant) IC/BPS only: - *Escherichia coli, Proteus mirabilis, Pseudomonas Aeruginosa, Francisella tularensis, Mycoplasma hyorhinis, helicpbacter hepaticus, Clostridium perfringens, Candida dubliniensis*	N/A	
Abernethy et al. Obstet Gynecol, 2017 [53]	Female with interstitial cystitis Controls	Catheterized urine	rRNA sequencing	N/A	N/A	Interstitial cystitis—less: species—*Lactobacillus acidophilus*	N/A	*1. L acidophilus* was associated with less severe scores on questionnaires 2. Patients with interstitial cystitis demonstrated higher levels of pro-inflammatory cytokines

Table 4 (continued)

Study	Patients	Sample collection	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Functional profiling	Remarks
Braundmeier-Fleming et al. Sci Rep, 2016 [55]	Female with interstitial cystitis Healthy controls	Stool ± vaginal swabs	16S rRNA sequencing (V3–V5 regions)	N/A	No significant difference	Deficient in Interstitial cystitis Pelvic Pain (DIPP) species - *O. splanchnicus, F. prausnitzii, C. aerofaciens, E. sinensis, L. longoviformis*	Significantly different functional pathways: - Fatty acid biosynthesis - Homologous recombination - Nicotinate/nicotinamide metabolism	1. Quantitative PCR used to identify the DIPP species 2. Stool-based interstitial cystitis biomarkers by DIPP species and stool metabolites

Epstein–Barr virus were noted in the bladder samples of patients with BPS, and both were thought to be linked with the pathogenesis of BPS [50].

It was noted that patients with BPS often have other conditions like irritable bowel syndrome, Sjogren's syndrome and mental disorders like depression. Yet, these conditions were shown to have gut dysbiosis. On the other hand, in a study for biomarkers of BPS, authors have found reduced levels of some bacterial species in stool samples from patients with BPS [55]. Whether gut dysbiosis is associated with BPS should be investigated, and results from trans-MAPP II study of Urologic Chronic Pelvic Pain (NCT02898220) are awaited.

Chronic pelvic pain syndrome in men

The etiology of chronic pelvic pain syndrome (CPPS) in men is unknown, and it is a syndrome that is diagnosed by exclusion of infection, anatomic abnormalities, malignant neoplasm, or neurological disorders [50]. Other than being difficult to diagnose, it is also difficult to treat (Table 5).

Although it was shown long ago that microorganisms was isolated from seminal fluid, there is no single causative microbial species that has been identified as the main cause of CPPS [56]. One of the first studies of the microbiota of seminal fluid in patients with CPPS defined the microbial communities in the seminal fluid of patients with CPPS and healthy men by decomplementary activity, and showed that the culprit could be normal commensals that were regarded as contaminants [56]. A more recent study compared the seminal microbiome in men with and without CPPS using 16S rRNA gene sequencing, and they confirmed that seminal fluids were having polymicrobial communities. Furthermore, they demonstrated higher species diversity, as well as less Lactobacilli, in particular *Lactobacillus iners*, in patients with CPPS [57].

Nickel et al. compared the urinary microbiota of healthy controls and patients with CPPS, and they found that the bacteria *Burkholderia cenocepacia*, *Propionibacterium acnes* and *Staphylococcus capitis/caprae* were more prevalent in the initial stream urine specimen in patients with CPPS [58]. In the era of NGS, 16S rRNA gene sequencing showed that the microbiota of mid-stream urine in patients with CPPS has a higher bacterial diversity than in healthy controls, as well as having higher prevalence of anaerobic bacteria [59]. There are also significant differences in the microbiome in different symptom severity and clinical phenotypes, and the authors expected an action of functional pathways by the difference in microbiome [59].

It is known that CPPS is associated with intestinal symptoms [60], and the intestinal symptoms are also associated with gut dysbiosis. Shoskes et al. have shown that the gut microbiome was less diverse and having less *Prevotella*

Table 5 Summary of original articles on chronic pelvic pain syndrome

Study	Patients	Sample collection	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Functional profiling	Remarks
Mändar et al. Int J Urol, 2017 [57]	Male patients consulted for prostatitis coml-paints, fertility status check or prophylactic purposes	Semen by masturbation	16S rDNA sequencing (V6 region)	N/A	N/A	healthy men—more: genus—*Lactobacilli* species—*L. iners*	N/A	Diversity of healthy subjects appeared to be lower (no statistical tests were done)
Nickel et al. J Urol, 2015 [58]	Male patients with CP/CPPS Healthy controls	VB1 (initial stream urine) VB2 (MSU) VB3 (post-prostate massage urine)	Electrospray ionization—time-of-flight—mass spectrometry	N/A	N/A	CP/CPPS—more (in VB1): genus—*Burkholderia, Propionibacterium, Staphylococcus* species—*Burkholderia cenocepacia, Propionibacterium acnes, Staphylococcus capitis/caprae*	N/A	VB2/VB3 showed no significant differences between the two groups
Shoskes et al. Urology, 2016 [59]	Patients with CP/CPPS Controls	MSU	16S rDNA sequencing (V3 and V4 regions)	Statistically higher in CP/CPPS group	Significant difference	CP/CPPS—more: phylum—*Bacteroidetes* order—*Clostridiales, Bacteroidales* genus—*Bacteroides, Blautia, Faecalibacterium, Ruminococcus, Coprococcus* Control—more: genus—*Staphylococcus* order—*Lactobacillales, Bacillales*	Overexpressed in CP/CPPS: sporulation chemotaxis overexpressed in controls: glycolysis phosphotransferase system pyruvate metabolism	
Shoskes et al. J Urol, 2016 [61]	Patients with CP/CPPS Controls	Immersing soiled glove tip after rectal examination in sterile saline	16S rDNA sequencing (V3 and V4 regions)	Statistically lower in CP/CPPS	Significant difference	CP/CPPS—more: genus—*Phyllobacterium* Control—more: phylum—*Verrucomicrobia* genus—*Prevotella, Porphyromonas*	N/A	

(which has an anti-inflammatory role) in patients with CPPS [61]. To fight against gut dysbiosis, several strategy could been considered, like the use of bacterial therapy (live probiotic bacteria) and hydrocolon therapy, which aims to restore a healthy gut microbiota by a gentle and targeted flow of water entering the intestine during the treatment sessions and, therefore, reducing inflammation [60].

The role of the microbiota of partners of patients of CPPS should also be taken into account. A study has shown that the presence of *Gardnerella vaginalis* as a predominant organism in the vaginal microbiota is associated with the presence of leukocytospermia in the male partner [62]. Moreover, another study has revealed that the vaginal, cervical or urethral swab from the partners of patients suffering from CPPS shows the same pathogen isolated in male patients [60]. Further studies in associating the relationship between the genital microbiota of patients with CPPS and their partners are warranted.

Lower urinary tract dysfunction

Lower urinary tract symptom (LUTS) is a board term and describes the different symptoms associated with the dysfunction of the lower urinary tract. LUTS is one of the most commonly encountered complaints in the urology clinic and it affects both male and female patients. Due to the difference in anatomy, male patients usually complain of voiding symptoms, whereas female patients more usually complain of storage symptoms. Other than the difference in anatomy, the male and female urinary microbiota are also noticed to be different, in which females are predominantly by *Lactobacillus* and males predominantly by *Corynebacterium* [9, 63] (Table 6).

Female urinary microbiome and LUTS

Overactive bladder syndrome (OAB) and urinary incontinence (UI) are the most commonly seen lower urinary tract symptoms in female patients. Urinary incontinence can be urgency urinary incontinence (UUI), stress urinary incontinence (SUI) or mixed. Overactive bladder syndrome is also associated with UUI, and studies have been performed to correlate urinary microbiome and OAB or UUI.

Sequence-positive urine samples are shown to be associated with more severe symptoms [64–66], as well as younger age, higher body mass index (BMI), more UUI episodes at baseline, better treatment response and fewer post-treatment UTI risk [65]. Moreover, two specific bacterial species, *Atopobium vaginae* and *Finegoldia magna*, were associated with urinary symptom severity by the overactive bladder questionnaire, suggesting etiology implications of OAB symptoms [66]. The diversity of the urinary microbiome is greater in patients with higher BMI and increased UUI symptoms, and increased diversity was associated with a lower frequency of *Lactobacillus* in hormone-negative women [67].

There are encouraging results from the above studies, but they all contained sequence-negative urine samples. Karstens and colleagues have shown contradicting result with the previous studies, in which lower diversity of urinary microbiome is associated with increased symptom severity in women with UUI [68]. The authors explained the contradicting result by the difference in small sample size, differences in participant populations, differences in urine sample volumes, and preprocessing/filtering techniques used on the data. The difference in result could as well be due to the older subjects. Although having only 19 subjects in this study, it has the advantage of using catheterized urine for urine sampling and a higher urine volume for sequencing, which may be reasons for having all samples sequence positive. This study also reflects that a standard protocol for the collection and extraction of bacterial DNA from urine is important for future studies.

Urgency urinary incontinence is not only a physical disease; it also correlates with the psychological wellbeing of the patients. Wu and colleagues found that the urinary microbiome of OAB patients was having a lower diversity than normal controls, and OAB patients with depression have further reductions in bacterial diversity and richness [69]. Furthermore, they showed that some genera were different between OAB patients with and without anxiety and depression. The authors suggested the existence of a brain-bladder-microbiome axis and suggested further studies on the axis.

The treatment response of oral medications for UUI can be predicted from the pre-treatment urinary microbiome. Thomas-White et al. (from Brubaker's group) showed that apart from the association with the UUI symptoms; urinary microbiome diversity is noted to be higher in patients UUI who do not respond to solifenacin [70].

Despite encouraging results in associating urinary microbiome and OAB and UUI, it seems that there is no association between urinary microbiome and SUI symptoms [67], presumably due to the different pathophysiology.

To sum up, whether the OAB symptoms is associated with higher or lower diversity of urinary microbiome still require further analysis as contradicting results were present. However, through these studies, we can conclude that urinary microbiome is associated with OAB and UUI, and further study is required to determine the association and the clinical implications.

Male urinary microbiome and LUTS

Although the urinary microbiota within the female urinary tract is better studied, the study in male patients is picking

Table 6 Summary of original articles on female lower urinary tract syndrome

Study	Patients	Sample collection	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Remarks
Brubaker et al. Int Urogynecol J, 2014 [64]	Women with moderate to severe UUI	Catheterized urine	Quantitative PCR	N/A	N/A	N/A	Compared sequence-positive and sequence-negative patients
Pearce et al. Am J Obstet Gynecol, 2015 [65]	Women with moderate to severe UUI	Catheterized urine	16S rDNA sequencing (V4 region)	N/A	N/A	UUI *Aerococcus urinae, Gardnerella vaginalis, Lactobacillus gasseri* without LUTS *Lactobacillus crispatus*	Compared sequence-positive and sequence-negative patients
Fok et al. Int Urogynecology J, 2018 [66]	Women undergoing POP/SUI surgery	Vaginal swab perineal swab catheterized urine	16S rDNA sequencing (V4 region)	N/A	N/A	higher OABq symptoms score: *Atopobium vaginae* *Finegoldia magna*	Compared sequence-positive and sequence-negative patients
Thomas-White et al. Am J Obstet Gynecol, 2017 [67]	Women with SUI undergoing surgery	Clean catch or catheterized urine	16S rDNA sequencing (V4 region)	Significant difference	N/A	hormone-positive women—more: Genus—*Lactobacillus, Gardnerella*	
Karstens et al. Front Cell Infect Microbiol, 2016 [68]	Females with daily UUI Controls	Catheterized urine	16S rDNA sequencing (V4 region)	1. No significant differences between groups 2. Reduced in greater symptoms	N/A	UUI—more: Genus—*Sphingomonadales, Chitinophaga, Brevundimonas, Candidatus Planktoluna, Alteromonadaceae, Elizabethkingia, Methylobacterium, Caldicellulosiruptor, Stenotrophomonas* UUI—less: Genus—*Prevotella, Comamonadaceae, Nocardioides, Mycobacterium*	

Table 6 (continued)

Study	Patients	Sample collection	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Remarks
Wu et al. Front Cell Infect Microbiol, 2017[69]	Patients with OAB Controls	Catheterised urine	16S rDNA sequencing (V4 region)	1. Lower in OAB subjects 2. Lower in depression cases	Significant differences present	OAB—more: Phylum—*Actinobacteria* Family—*Bifidobacteriaceae* Genus—*Sneathia, Staphylococcus, Proteus, Helcococcus, Gemella, Mycoplasma, Aerococcus* Control—more: Genus—*Lactococcus, Novosphingobium, Enterococcus, Ureaplasma, Pyramidobacter, Lactobacillus, Anaerococcus, Finegoldia, Campylobacter, Jonquetella, Fusobacterium, Dialister, Prevotella* Anxiety—more Genus—*Sneathia, Porphyromonas, Gallicola, Leptolyngbya, Alicyclobacillus, Helcococcus, Actinobactulum* non-anxiety—more Genus—*Psychrobacter, Collinsella, Methanobrevibacter, Agrobacterium, Crycola, Burkholderia* Non-depression—more Genus—*Clostridium, WAL_1855D, Pedobacter, Actinobaculum*	Negative correlations between the depression score and both bacterial richness and diversity in OAB patients
Thomas-White et al. Int Urogynecology J, 2016 [70]	Women seeking UUI treatment Controls	Catheterized urine	1. 16S rDNA sequencing (V4 region) 2. EQUC	No significant difference	N/A		Unique species isolated was less in the "5 mg-solifenacin responding group" compared to "10 mg-solifenacin-responding group" or "non-responding group"

up. An early attempt in identifying urinary microbiome in male subjects was seen in the study by Nelson et al. [7], by comparing men with sexually transmitted infection (STI) and STI-negative individuals. In finding the association between urinary microbiota and LUTS, Bajic et al. [71] showed that the severity of lower urinary tract syndrome (measured by international prostate symptoms score (IPSS)) is associated with the presence of bacteria in catheterized urine, but not in MSU. However, there is no single species or genera that is associated with the lower urinary tract symptoms. There are a number of limitations for this study. Firstly, although the number of subjects is reasonable, it still seems too little; secondly, the diversity parameters were not included in the analysis; finally, catheterized urine sampling may not be accepted by all patients and this affects the generalizability of the study (Table 7).

Infertility

Although there are studies that focus on association between male infertility and individual bacteria, there are limited studies on the microbiota in the semen. Polymerase chain reaction (PCR) technique was used to identify bacteria in seminal fluid in early studies [72, 73]. In the era of NGS, there are four studies focusing on identifying whether seminal microbiome has association with azoospermia using the 16S rRNA sequencing method (Table 8).

Some studies that grouped participants by sperm quality showed no significant differences in diversity of seminal microbiome [74, 75]. However, individual bacterial identification noted that the sperm quality was negatively associated with *Anaerococcus* [74], and seminal fluid had more *Staphylococcus* and *Lactobacillus*, and less *Prevotella* in participants having normal sperm quality [75]. Chen et al. [76] had semen sample from fertile donors by masturbation (control group) and patients with obstructive or non-obstructive azoospermia by percutaneous epididymal sperm aspiration or testicular sperm extraction (study group) showed that there was significantly higher number of bacteria in control group. The above studies have specimen collected by masturbation, which is a source of contamination from the distal urethra.

To tackle the problem of contamination from distal urethra, Alfano et al. [77] had semen sample by microscopic testicular sperm extraction from patients undergoing unilateral orchidectomy for non-metastatic seminoma (control group) and patients with idiopathic non-obstructive azoospermia (study group) and then sequenced the V3-5 regions. There is an increased number of bacteria in the study group, with predominance of *Acinobacteria* and *Firmicutes*. They also showed that there is decreased bacterial richness and diversity in men with complete germline cell aplasia, which is predominated by *Acinobacteria*, and absence of *Clostridia*, which may reflect an aging process in testes of patients with

Table 7 Summary of original articles on male lower urinary tract syndrome

Study	Patients	Sample collection	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Remarks
Bajic et al. Eur Urol Focus, 2018 [71]	Men undergoing bladder outlet surgery Men undergoing other urology and general surgery procedures	MSU and catheterized urine	1. EQUC 2. 16S rDNA sequencing (region: N/A)	N/A	N/A	No statistically significant differences	Increase in IPSS category was associated with significantly higher odds of detectable bacteria in catheterized urine

Table 8 Summary of original articles on infertility

Study	Patients	Sample	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Functional profiling	Remarks
Hou et al. Fertil Steril, 2013 [74]	Healthy sperm donors asthenozoospermia oligoasthenozoospermia severe oligoasthenozoospermia and azoospermia	Masturbation	16S rDNA sequencing (V1–V2 regions)	No significant difference	N/A	Sperm quality negatively associated with *Anaerococcus*	N/A	
Baud et al. Front Microbiol, 2019 [75]	Healthy men ($n=94$)	Masturbation	16S rDNA sequencing (V1–V2 regions)	Only minor increase in chao1 index in group with abnormal total motility	No significant difference	Abnormal spermiogram—more: genus—*Prevotella* normospermic group—more: genus—*Staphylococcus, Lactobacillus* (sperm morphology)	N/A	
Chen et al. Exp Ther Med, 2018 [76]	Patients with azoospermia Control	Azoospermia: PESA/TESE Control: masturbation	16S rDNA sequencing (V4 region)	N/A	Azoospermia group have higher degree of similarity than control group	Azoospermia group—less: phylum—*Cyanobacteria, Acidobacteria, Gemmatimonadetes, Planctomycetes, Chloroflexi, Crenarchaeota, Armatimonadetes, Elusimicrobia, Nitrospirae, Euryarchaeota, Spirochaetes, Chlorobi, Synergistetes, Chlamydiae, Verrucomicrobia* genus—*Lactobacillus, Prevotella, Proteus, Pseudomonas, Veillonella, Corynebacterium, Rhodococcus, Staphylococcus, Bacillus* Azoospermia group—more: phylum—*Bacteroidetes* genus—*Alicyclobacillus, Amaricoccus, Anaeromyxobacter, Aquicella, Arsenicicoccus, Azospirillum, Chitinimonas, Chlorobaculum, Coprococcus, Desulfovibrio, Dokdonella, Gallionella, Geobacter, Helicobacter, Idiomarina, Kaistia, Kribbella*	Azoospermia group Increased risk of having metabolic, infectious and immune disease Enhanced nucleotide metabolism, metabolism of cofactors and vitamins, glycan biosynthesis and metabolism, enzyme families and metabolism Downregulated xenobiotic biodegradation and metabolism, metabolism of terpenoids and polyketides, lipid metabolism and amino acid metabolism	Azoospermia is divided into non-obstructive and obstructive

Table 8 (continued)

Study	Patients	Sample	Microbial identification	Alpha-diversity	Beta-diversity	Relavant microbials	Functional profiling	Remarks
Alfano et al. Hum Reprod, 2018 [77]	Patients with idiopathic non-obstructive azoospermia (iNOA) Control: Patients undergoing unilateral orchidectomy for non-metastatic seminoma	iNOA: microTESE Control: non-neoplastic tissue from specimen	16S rDNA sequencing (V3–V5 regions)	Higher in iNOA group	Principal component analysis presented, but the statistical comparison not presented	iNOA group—more: phylum—*Actinobacteria* Class—Actinobacteria iNOA group—less: phylum—*Bacteroidetes, Proteobacteria* Class—*Bacteroidi, Flavobacteria, Saprospirae, Bacilli, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*	N/A	

idiopathic non-obstructive azoospermia. However, as studies in other structures have shown that cancer can change the local microbiota, the control group used in this study may not be reliable.

The above studies provide early results on the microbiota of semen and their potential role in azoospermia, although most have small number of subjects. Further larger-scale studies are required to characterize the normal seminal microbiota, to determine whether seminal dysbiosis is associated with male infertility and the function of bacteria in the semen.

Conclusion

Preliminary evidence showed that microbiome has a role in different aspects of urological diseases, including diagnostic, treatment or prognostic. However, the evidence is either weak or inconsistent, which makes the translation into clinical practice difficult. Standardized methodology, such as sample collection and sequencing the same variable region, as well as reporting methods, like including alpha- and beta-diversities, should be developed. After that, further large-scale investigations, like longitudinal studies or combination of the information to develop risks scores for diagnostic tests, should be carried out to translate this knowledge into clinical use, in addition to understand how the microbiome is correlated with urological diseases.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest related to this manuscript.

Human and animal rights statement This article does not contain any studies with human participants or animals performed by any of the authors.

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