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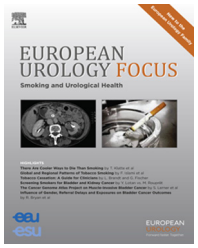

Review – Incontinence

The Urinary Tract Microbiome in Health and Disease

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Abstract

Context: The urinary tract, previously considered a sterile body niche, has emerged as the host of an array of bacteria in healthy individuals, revolutionizing the urology research field.
Objective: To review the literature on microbiome implications in the urinary tract and the usefulness of probiotics/prebiotics and diet as treatment for urologic disorders.
Evidence acquisition: A systematic review was conducted using PubMed and Medline from inception until July 2016. The initial search identified 1419 studies and 89 were included in this systematic review.
Evidence synthesis: Specific bacterial communities have been found in the healthy urinary tract. Changes in this microbiome have been observed in certain urologic disorders such as urinary incontinence, urologic cancers, interstitial cystitis, neurogenic bladder dysfunction, sexually transmitted infections, and chronic prostatitis/chronic pelvic pain syndrome. The role of probiotics, prebiotics, and diet as treatment or preventive agents for urologic disorders requires further investigation.
Conclusions: There is a microbiome associated with the healthy urinary tract that can change in urologic disorders. This represents a propitious context to identify new diagnostic, prognostic, and predictive microbiome-based biomarkers that could be used in clinical urology practice. In addition, probiotics, prebiotics, and diet modifications appear to represent an opportunity to regulate the urinary microbiome.
Patient summary: We review the urinary microbiome of healthy individuals and its changes in relation to urinary disorders. The question to resolve is how we can modulate the microbiome to improve urinary tract health.
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1. Introduction

It is well known that even in a healthy state the body hosts a variety of microorganisms such as bacteria, fungi, viruses, and protozoa. In fact, the body houses approximately ten times more microbial cells than human cells. However, although microorganism residents in the human body have evolved with man, the relationship is not always perfect [1]. The term microbiota refers to microbes living inside and on an individual, while the term microbiome denotes the

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collection of genomes, genes, and products of the microbes present in a particular host [2–4].

The Human Microbiome Project (HMP) was established in 2008 with the aim of developing a comprehensive characterization of the human microbiome and analysis of its role in human health and disease. Initially the HMP did not include investigation of the bladder microbiome. One of the reasons for this was that 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 [5]. Moreover, the bladder and urine have long been considered sterile in healthy individuals because of technical difficulties in characterizing the full spectrum of urinary bacterial species using standard microbiological methods. Advances in molecular biology techniques and culture methods have allowed definition of a specific microbiome associated with several body sites previously believed to be sterile, including the urinary tract (UT) [6–10]. The recent identification of a specific microbiome in the UT may have important implications in the maintenance of health and/or the development of certain diseases [10–15]. However, it is difficult to establish a strict relation between the microbiome and health and disease without considering that the human microbiome can change during the life cycle and seasonally, or with environmental changes (infection, treatments, diet, hormone state, or lifestyle) [1,16]. Therefore, these findings opened an emerging research field to explore, especially in the urology context, in terms of future design of treatments/drugs targeting specific microorganisms of the UT. In the present review, we summarize the main recent publications regarding the urinary microbiome (UM) with the aim of evaluating future needs in the field and the option of using probiotics, prebiotics, and diet as a treatment for urinary diseases.

2. Evidence acquisition

A systematic literature search was performed using PubMed and Medline databases from inception until July 2016. Papers written in English were selected following the Preferred Reporting Items for Systematic Reviews and Meta-Aaalyses (PRISMA) methodology. A flowchart of the systematic search process is shown in Figure 1. The following keywords were included in this systematic review: "*microbiome, microbiome and bacteriuria*" in combination with "*urinary tract, urinary incontinence, urinary tract infection, cancer, urothelial cancer, bladder cancer, prostate cancer, neurogenic bladder dysfunction, interstitial cystitis, urolithiasis*" and/or "*probiotics, prebiotics, diet, cranberry, pomegranate*". The initial search identified 1419 studies. Only 89 were selected for inclusion in the review.

3. Evidence synthesis

Selected papers were published between 1991 and 2016. Information regarding the UM in healthy individuals was extracted from 11 articles (Table 1). Six articles were

Table 1 – Microbiome composition of urine among healthy individuals

Study population	Main bacterial taxa	Sample collection	Technique used	Ref.
Healthy men aged ~18 yr (n = 9)	*Lactobacillus, Corynebacterium, Escherichia*, and *Streptococcus*	FC urine	16S rRNA GS	[22]
Healthy men (n = 22) age ≥18 yr, median 28 yr	*Lactobacillus, Sneathia, Veillonella, Corynebacterium, Prevotella, Streptococcus, Ureaplasma, Mycoplasma, Anaerococcus, Atopobium, Aerococcus, Staphylococcus, Gemella, Enterococcus*, and *Finegoldia*	FC urine	16S rRNA GS	[23]
Healthy females aged 27–67 yr (n = 8)	*Lactobacillus, Prevotella, Gardnerella, Peptoniphilus, Dialister, Finegoldia, Anaerococcus, Allisonella, Streptococcus*, and *Staphylococcus*	CC MSU	16S rRNA GS	[24]
Healthy males aged 24–50 yr (n = 11) Healthy females aged 22–51 yr (n = 15)	*Lactobacillus, Klebsiella, Corynebacterium, Staphylococcus, Streptococcus, Aerococcus, Gardnerella, Prevotella, Escherichia*, and *Enterococcus*	MSU	16S rRNA GS	[25]
Healthy males aged 14–17 yr (n = 18)	*Corynebacterium, Lactobacillus, Staphylococcus, Gardnerella, Streptococcus, Anaerococcus, Veillonella, Prevotella*, and *Escherichia*	FC urine	16S rRNA GS	[26]
Healthy women (n = 12) age NA	*Lactobacillus, Actinobaculum, Aerococcus, Anaerococcus, Atopobium, Burkholderia, Corynebacterium, Gardnerella, Prevotella, Ralstonia, Sneathia, Staphylococcus, Streptococcus*, and *Veillonella*	CC MSU, SPA, and TUC	16S rRNA GS	[17]
Healthy men aged 39–86 yr (n = 6) Healthy woman aged 26–90 yr (n = 10)	Male and female samples: Firmicutes; female samples: Actinobacteria, Bacteroidetes	CC MSU	16S rRNA GS	[18]
Healthy women (n = 24) age NA	*Lactobacillus, Corynebacterium, Streptococcus, Actinomyces, Staphylococcus, Aerococcus, Gardnerella, Bifidobacterium*, and *Actinobaculum*	TUC	16S rRNA GS and/or EUCT	[21]
Healthy women aged 35–65 yr (n = 58)	*Lactobacillus, Gardnerella, Corynebacterium*, Enterobacteriaceae, *Anaerococcus, Bifidobacterium, Streptococcus, Staphylococcus, Sneathia, Peptoniphilus, Atopobium, Rhodanobacter, Trueperella, Alloscardovia*, and *Veillonella*	TUC	16S rRNA GS and/or EQUC	[19]
Healthy women aged 35–65 yr (n = 60)	*Lactobacillus, Gardnerella, Staphylococcus, Streptococcus, Enterococcus, Bifidobacterium, Atopobium*, and Enterobacteriaceae	TUC	16S rRNA GS and/or EQUC	[20]
Healthy women (n = 10)	*Anoxybacillus, Lactobacillus, Prevotella, Gardnerella, Arthrobacter, Escherichia*, and *Shigella*	TUC	16S rRNA GS	[27]

NA = not available; EUCT = enhanced urine culture technique; EQUC = expanded quantitative urine culture; GS = gene sequencing; FC = first catch; CC = clean catch; MSU = midstream urine; SPA = suprapubic aspirate; TUC = transurethral catheter.

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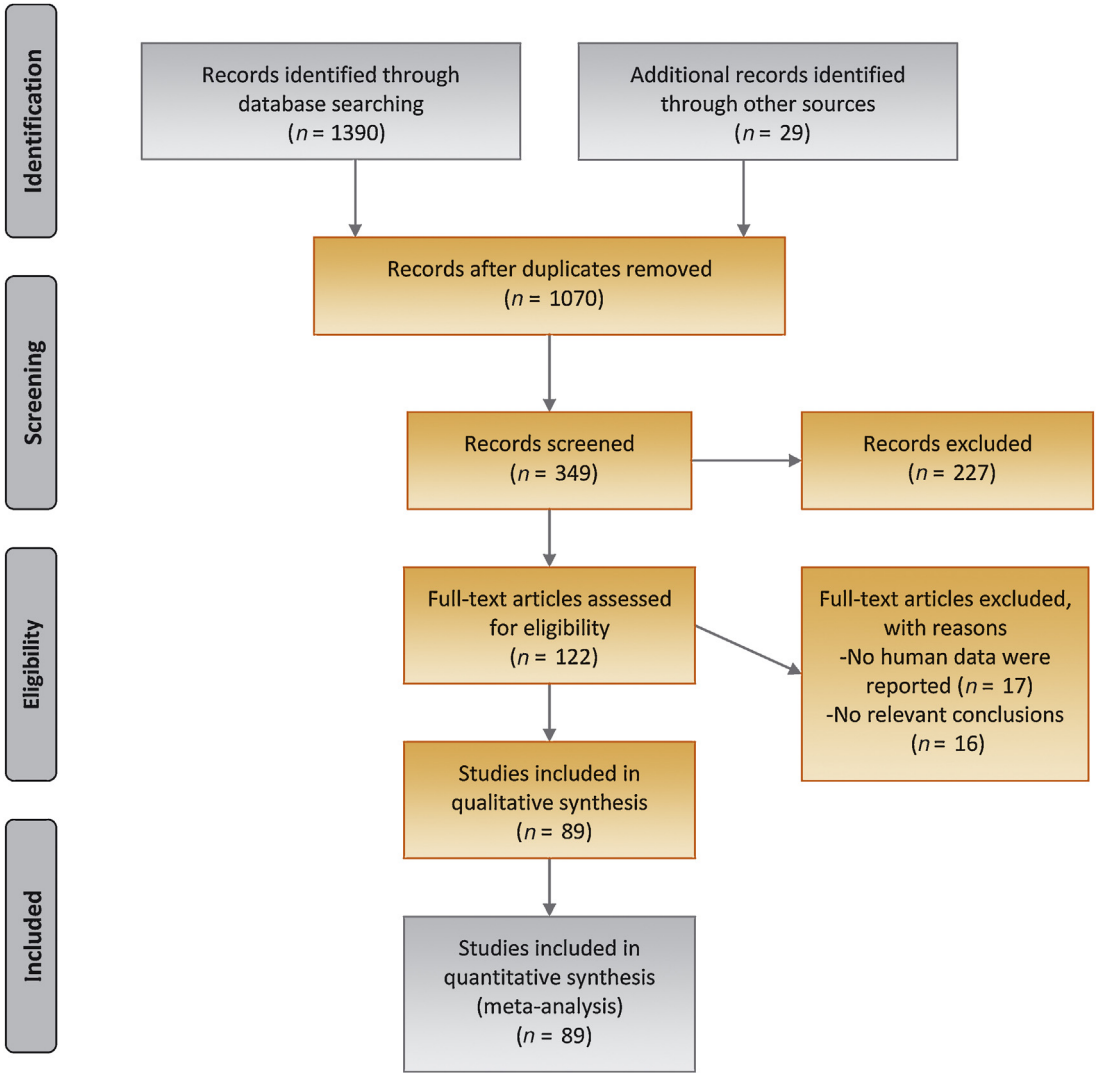

Fig. 1 – Flow chart of the study selection process according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement.

focused on the microbiome in urinary incontinence (Table 2); six on the microbiome in urologic cancer; five on the microbiome in other urinary diseases (Table 3); and 20 on prebiotics, probiotics, or diet as a treatment for urinary diseases (Table 4). The remaining manuscripts cited were used for general information and discussion.

3.1. *Microbiome associated with the urinary tract*

Traditionally, the study of urinary bacterial communities mainly included standard urine cultures, which had significant limitations for detection of the full spectrum of urinary bacterial species (slow-growing bacteria that die in the presence of oxygen). Advances in new approaches such as 16S rRNA sequencing and enhanced or expanded quantitative urine culture (EQUC) have led to rapid progress in UM knowledge [17–21]. EQUC allows isolation of bacteria from 80% of urine samples collected via transurethral catheter, for most of which the result according to standard urine culture was "no growth" [19]. This technique combines a wide variety of culture media, aerobic and anaerobic conditions, and different growth temperatures. The UM reported for healthy individuals is summarized in Table 1 [17–27]. It is important to consider variations in the bacterial genera described for the UM in the different studies because of differences in the sex of patients, sample size selection, and the urine collection methods and techniques used to study the UM. Nevertheless, in general *Lactobacillus* and *Streptococcus* have been the genera most frequently reported for the UM, and were present in all studies published to date. Both genera are lactic acid bacteria intimately associated with several body tissues, including the urogenital tract, where they play a protective role against pathogens [28]. Other bacterial genera such as *Alloscardovia*, *Burkholderia*, *Jonquetella*, *Klebsiella*, *Saccharofermentans*, *Rhodanobacter*, and *Veillonella* were found in the UM less frequently (Supplementary Table 1). The results detailed in Table 1 highlight that the urine collection method (clean-catch midstream urine, first-void urine, suprapubic aspiration, or intermittent transurethral

Table 2 – Urine microbiome in UUI and SUI patients

Study aim	Inclusion criteria	Sample collection	Technique used	Main conclusions	Main urotypes (sequencing)	Ref.
Relationship of bacterial DNA to UUI and UTI risk and symptoms after instrumentation. (n = 155)	♀ with moderate to severe UUI included in clinical trial for UUI treatment (anticholinergic vs botulinum toxin A) without clinical evidence of UTI.	Cystoscopic injections	qPCR	38,5% bacterial DNA + > Daily UUIE in qPCR+. < UTI risk in the qPCR+	NA	[38]
UM of ♀ with UUI (n = 60) vs without UUI (n = 58)	♀ with/without UUI. Inclusion criteria for both cohorts: non-current or recurrent UTI, no antibiotic exposure (≥4 wk), no immunologic deficiency, no neurologic disease affecting UT, no pelvic malignancy, no POP greater than POP-Q stage II and no pregnancy	TUC	16S rRNA GS and EQUC	Significant difference in bacterial frequency and abundance: > *Gardnerella* and < *Lactobacillus* in UUI	Non-UUI: *Lactobacillus* (60%), *Gardnerella* (12%), others (20%) UUI: *Lactobacillus* (43%), *Gardnerella* (26%), others (17%)	[19]
UM characterization in ♀ treated for UUI to establish associations with urinary symptoms, responses to treatment and UTI risk (n = 182)	♀ with moderate to severe UUI, treated with onabotulinum toxin A or anticholinergic medication/ without clinical UTI	TUC	16S rRNA GS	51.1% sequence +. ≠* bacterial abundance. Sequence +: > daily UUIE +++ response to treatment, < UTI.	*Lactobacillus* (45%), *Gardnerella* (17%), others (25%)	[36]
Possible relationship between UUI UM and response to UUI treatment (74 UUI and 60 non-UUI)	UUI with ♀ with solifenacin treatment/ unaffected ♀ inclusion criteria = Pearce et al., 2014.	TUC	16S rRNA GS and EQUC	> UM diversity and abundance in UUI ♀ +++ response to solifenacin in UUI ♀/ < bacterial abundance; < diversity	Non-UUI: *Lactobacillus* (61%), *Gardnerella* (15%), others (15%) UUI: *Lactobacillus* (40%), *Gardnerella* (22%), others (12%)	[20]
UM of ♀ with/without UUI (N = 20; 10 UUI and 10 Non-UUI).	♀ with UUI/without UUI. Inclusion criteria for both groups: non-current UTI, non antibiotic exposure (≥1 month), non history of pelvic irradiation or bladder cancer, non prior UUI surgery, non history of SUI (>1 week), non neurological disease affecting UT, non symptomatic POP.	TUC	16S rRNA GS	Significant difference in RA of 14 BOTUs for UUI vs non-UUI; in UUI♀ < microbial diversity →> UUI symptom severity	NA	[27]
UM of ♀ with SUI (n = 197)	♀ with SUI; inclusion criteria: SUI symptoms (≥3 mo), postvoid residual <150 ml, non UTI; clinical assessment of urethral mobility; desire for SUI surgery; positive stress urinary test; and qualifying MESA questionnaire	CC (n = 174) TUC (n = 23)	16S rRNA GS	Increased diversity → UUI symptoms, hormonal status, and BMI; UM not → SUI symptoms	*Lactobacillus* (46–37%), *Gardnerella* (18–14%), diverse (12–5.1%)	[35]

♀ = woman; UM = urinary microbiome; NA = not available; UUI = urgency urinary incontinence; SUI = stress urinary incontinence; UTI = urinary tract infection; POP = pelvic organ prolapse; POP-Q = POP quantification; GS = gene sequencing; TUC = transurethral catheterization; CC = clean catch; UT = urinary tract; UUIE = UUI episodes; EQUC = expanded quantitative urine culture; qPCR = quantitative polymerase chain reaction; MESA = Medical, Epidemiologic, and Social Aspects of Aging; BMI = body mass index; RA = relative abundance; BOTU = bacterial operational taxonomic unit; ≠* Non significant differences; > = greater; < = lower; +++ = better response; → = associated with.

catheterization) and the technique used to characterize the UM can determine the microbial diversity detected. In this context, Wolfe et al [17] compared different urine collection methods to discern bacteria present in the bladder and concluded that the best methods are supra-pubic aspiration and transurethral catheterization, since these techniques minimize vulvovaginal contamination [17]. Comparisons revealed that 16S rRNA sequencing and EQUC detected similar but not identical microbiome profiles for catheterized females [19,21]. The *Trueperella* genus was only found in bacterial cultures, whereas some bacterial genera such as *Atopobium* were only detected via sequencing. A possible explanation for these differences is that certain bacterial genera do not grow under EQUC conditions, and this culture technique can also promote the growth of some bacterial groups under-represented in the urine samples [19]. The limitation of 16S rRNA sequencing methodology is that it does not differentiate between living, dead, and ruptured bacteria [29]. However, a sensible hypothesis is that all bacteria detected via

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Table 3 – Urinary microbiome in urinary diseases

Control individuals	Main bacteria taxa found in controls	Patients	Main bacteria taxa found in patients	Sample collection	Technique used	Ref.
Men without STI (n = 9)	*Lactobacillus, Corynebacterium, Streptococcus*, and *Escherichia*	Men with STI (n = 10)	*Dialister, Gemella, Streptococcus, Sneathia, Prevotella, Aerococcus, Veillonella*, and *Atopobium*	FC urine	16S rRNA GS	[22]
NA	NA	Women with IC (n = 8)	*Lactobacillus, Gardnerella, Corynebacterium, Prevotella, Ureaplasma, Enterococcus, Peptoniphilus, Anaerococcus, Staphylococcus, Finegoldia, Streptococcus, Dialister, Atopobium, Proteus*, and *Cronobacter*	CC MSU	16S rRNA GS	[49]
Healthy males (n = 11) Healthy females (n = 15)	↑ *Lactobacillus, Klebsiella*, ↑ *Corynebacterium*, ↑ *Staphylococcus*, ↑ *Streptococcus, Aerococcus, Gardnerella, Prevotella, Escherichia*, and *Enterococcus*	Men with NBD (n = 13) Women with NBD (n = 14)	*Lactobacillus*, ↑ *Klebsiella, Corynebacterium, Staphylococcus, Streptococcus, Aerococcus, Gardnerella, Prevotella*, ↑ *Escherichia*, and ↑ *Enterococcus*	MSU, ICT, Foley catheter	16S rRNA GS	[25]
NA	NA	Patients with IC (n = 233)	*Bifidobacterium, Lactobacillus, Propiniobacterium, Corynebacterium, Streptococcus, Staphylococcus, Corynebacterium*, and *Finegoldia*	Initial stream and MSU	Ibis T-5000 Universal Biosensor	[53]
Asymptomatic men or men with only LUTS (n = 25)	5 bacterial taxa were over-represented in controls over cases (eg, Bacillus class)	Men with CP/CPPS (n = 25)	17 bacterial taxa were over-represented in patients over controls (eg, Clostridia and Bacteroidia classes)	MSU	16S rRNA GS	[54]

NA = not available; STI = sexually transmitted infection; LUTS = lower urinary tract symptoms; IC = interstitial cystitis; NBD = neurogenic bladder dysfunction; CP/CPPS = chronic prostatitis/chronic pelvic pain syndrome; GS = gene sequencing; FC = first catch; CC = clean catch; MSU = midstream urine; ICT = intermittent catheterization; ↑ = predominant versus the comparison group.

Table 4 – Probiotics, prebiotics, and diet modifications used in urologic disorders

Probiotics/prebiotics/diet modification	Urinary diseases treated	Administration	Preventive effect against urinary disease	Refs.
Antimicrobial therapy and *Lactobacillus* suppositories	UTI	Vaginal	Yes	[59]
Lactobacillus rhamnosus GR-1 and *Lactobacillus reuteri* B-54	UTI	Vaginal	Yes	[60]
Lactobacillus GG and cranberry-lingonberry juice	UTI	Oral	No	[80]
L. rhamnosus GG	UTI in premature babies	Oral	Reduction in UTIs (nonsignificant differences)	[62]
Lactobacillus drinks and berry juice	UTI	Oral	Yes	[81]
L. rhamnosus GR-1 and *Lactobacillus fermentum* RC-14	UTI	Oral	Yes	[63]
Cranberries	UTI	Oral	No	[86]
Cranberry juice capsule	UTI after surgery	Oral	Yes	[83]
D-Mannose	UTI	Oral	Yes	[87]
Bacillus Calmette-Guérin immunotherapy	Bladder cancer	Intravesical	Yes (noninvasive [stage 0] or minimally invasive [stage 1] bladder cancer)	[44]
Lactobacillus casei	Bladder cancer	Oral	Yes (primary multiple tumors and recurrent single tumors)	[68,69]
L. casei strain Shirota	Bladder cancer	Oral	Yes	[70]
Lactic acid bacteria [a]	Urolithiasis	Oral	Yes	[71]
L. casei and *Bifidobacterium breve*	Urolithiasis	Oral	No	[72]
Oxalabacter formigenes	Urolithiasis	Oral	Yes	[76]
Commercially available probiotic	Urolithiasis	Oral	Yes	[77]
O. formigenes	Urolithiasis	Oral	No	[78]
Supplemental calcium	Urolithiasis	Oral	Yes	[88]
Diet low in sodium and animal protein	Urolithiasis	Oral	Yes	[89]

UTIs = urinary tract infections.
[a] *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus brevis, Streptococcus thermophilus, Bifidobacterium infantis.*

high-throughput sequencing, including dead bacteria, were alive in the human body at some point, and thus contributed to the human microbiome. Therefore, identification of bacterial communities (alive or dead) via sequencing allows us to obtain additional information that cannot be gleaned via culture methods.

Although the number of studies is limited, some authors have observed UM variations between gender and age

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groups [18,25]. Using 16S rRNA sequencing of clean-catch midstream urine from male and female patients of a wide age range, Lewis et al [18] found that *Jonquetella*, *Proteiniphilum*, *Saccharofermentans*, and *Parvimonas* genera were only in the UT of individuals aged >70 yr. However, UTs in this age group also contained other bacterial genera that are commonly present in the UT independent, of age [18]. Regarding UM differences between the sexes, Fouts et al [25] used 16S rRNA sequencing of clean-catch midstream urine and concluded that the UM was characterized by a preponderance of Lactobacillales species in women and *Corynebacterium* in men. The UM results in relation to age and sex are not surprising, and could be related to differences in urinary metabolites, voiding habits, and hygiene among between children, adults, and the elderly, as well as differences in anatomic structures, hormones types/levels, and histology [30] between the sexes. Therefore, it would be interesting to study the UM in the transgender community before and after surgery to understand how these factors influence the individual UM on the basis of gender differences. Knowledge regarding the dynamics of the human microbiome, especially age-related changes, is limited, even for the vagina and gut. Several studies have revealed variations in the vaginal microbiome between women of reproductive age and adolescents girls before the onset of menstruation [31] and post-menopausal women [32,33]. Such age-related microbiome changes have also been found in other systems; for example, changes in the Firmicutes/Bacteroidetes ratio for the gut microbiome have been associated with age [34].

In the present review, we conclude that the study design and methodology used in different studies were quite heterogeneous, complicating overall integration of the microbiome changes observed. Larger sample sizes and standardization of the methods used to characterize the UM will allow significant progress in this field.

3.2. The UM in health and disease

In the context of the HMP, several studies have found an association between specific disease states with variations in cutaneous, gastric, colon, and gut microbiomes [1]. Some studies have observed changes in the UM among patients with urologic disorders such as urinary incontinence (UI), bladder and prostate cancer, neurogenic bladder dysfunction (NBD), interstitial cystitis (IC), sexually transmitted infections (STIs), and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). The main investigations carried out are described in more detail below.

3.2.1. UM changes in UI

UI is an extremely common complaint worldwide and can be classified as urgency UI (UUI), stress UI (SUI), or mixed UI (MUI). Six studies have described a possible role for the UM in UUI and SUI (Table 2). Pearce et al [19] and Karstens et al [27] investigated bacterial communities in the UT of healthy volunteers and women with UUI for urine samples collected using transurethral catheterization, and studied the UM using 16S rRNA sequencing with EQUC and 16S rRNA sequencing only, respectively. They observed an altered microbiome in the cohort of adult women with UUI [19,27]. Pearce et al [19] reported that patients with UUI had a higher *Gardnerella* and lower *Lactobacillus* load compared to non-UUI microbiomes [19]. Karstens et al [27] found differences in the relative abundance of 14 bacterial operational taxonomic units between UUI and non-UUI patients, as well as greater UUI symptom severity among UUI patients with lower microbial diversity. Another study that used 16S rRNA sequencing for catheterized or voided urine revealed that in patients with SUI, the UM was not associated with SUI symptoms [35]. UUI is a poorly understood heterogeneous urinary condition with symptoms that can overlap urinary infection symptoms, and is usually attributed to abnormal neuromuscular signaling and/or functioning [36]. SUI is a complex and usually multifactorial condition that involves denervation, muscle degeneration and apoptosis, chronic muscle atrophy, fibrosis, and connective tissue disorders [37]. Therefore, it is not unusual to think that UM changes may contribute to UUI symptoms, but not to SUI symptoms.

Other studies have investigated clinical associations between UM and different UUI treatments. Brubaker et al [38] analyzed bacterial DNA in catheterized urine from women with UUI in the ABC trial using quantitative polymerase chain reaction. They concluded that the UM contributed to UUI episodes (UUIEs), symptom severity, and post-treatment UTI risk. [38]. In the same ABC trial context, Pearce et al [36] used 16S rRNA sequencing to identify bacterial communities in women with UUI who received different therapies (anticholinergic or onabotulinum toxin A). No differences in sequence profiles were observed between the two treatment cohorts. However, the authors found a lower number of *Lactobacillus* sequences in women who experienced a post-treatment UTI compared to those without UTI [36]. They concluded that urinary bacterial DNA was associated with treatment response and concurred with the findings of Brubaker et al [38] regarding the implications of bacterial DNA in UUIEs and post-treatment UTI. Thomas-White and collaborators [20] investigated whether UM characteristics are related to a clinically relevant treatment response to solifenacin, an orally administered medication for UUI. They combined 16S rRNA sequencing and EQUC and collected urine via transurethral catheterization. The response to solifenacin was better in women with lower bacterial abundance and bacterial diversity. The authors concluded that an individual's UM could be related to UUI status and treatment response. Therefore, if there is a microbial signature associated with treatment response, it would be interesting to be able to phenotype the UM of UUI-affected women before treatment decisions.

In summary, the studies published to date have demonstrated a clear role of the UM in UUI and in the response to UUI treatment. Future studies in UUI patients would be very helpful in determining whether differences in the UM between sexes and age groups could be associated with susceptibility to UUI and in efforts to improve treatments for this condition.

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3.2.2. The UM and urologic cancer

Numerous studies have focused on the relationship between the host microbiome and cancer susceptibility in systems other than the UT [39]. There is clear evidence of such an association in colorectal adenoma and adenocarcinoma and in gastric, colorectal, and hepatobiliary cancer, for which bacteria can influence cancer risk by interfering with β-catenin signaling [39,40], among other mechanisms. Bacteria can also modulate cancer risk through the metabolism and generation of carcinogenic chemicals (eg, nitrosamines and acetaldehyde) [41]. A role of the UM in certain cancers of the UT has not yet been elucidated. However, in urothelial bladder cancer, the most common urologic cancer, tobacco smoke and occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons have been recognized as the most important risk factors [42]. Infectious risk factors associated with squamous cell bladder cancer, such as *Schistosoma haematobium* infestation, also induce endogenous synthesis of *N*-nitrosamines and oxygen radicals [43]. These compounds are excreted to the renal system, where they have a carcinogenic effect [42]. Xu et al [41] reported on a preliminary study of the UM involving a small number of patients with urothelial cell carcinoma (UCC). They found changes in the microbiome community of UCC patients in comparison to healthy individuals. These patients showed enrichment of *Streptococcus* in urine; however, large-scale studies are required to confirm these results [41]. Prospective studies are needed to disentangle the association between UCC development and UM dysbiosis, as well as the possible role of these bacterial communities in the metabolism of carcinogenic compounds present in the UT [5]. Beneficial associations have also been established between bladder cancer and the attenuated *Mycobacterium tuberculosis* administered in the bacillus Calmette-Guérin (BCG) vaccine. This vaccine has been the most effective therapy for intermediate-risk and high-risk non–muscle-invasive bladder tumors for nearly four decades [44]. However, the mechanism of action of BCG vaccine in preventing cancer recurrence and progression is not fully known yet [41,45]. Current evidence suggests that the bacteria in the BCG vaccine administered into the bladder provoke an inflammatory reaction that induces an antitumor immune response, which has a crucial role in the therapeutic antitumor effect [45]. In this regard, the possible role of BCG in promoting or diminishing the indigenous bladder microbiome is not clear, or whether bladder bacterial communities could interact with the innate immune system to either reduce or increase inflammation [46]. Further research addressing these issues to improve the efficacy of BCG or establish new treatment targets in bladder cancer would be useful.

A recent study demonstrated significant variations in microbial populations in prostatic secretions, voided urine, and seminal fluid from patients diagnosed with prostate cancer or benign prostatic hyperplasia [47]. Comparative denaturing gradient gel electrophoresis sequencing and phylogenetic analysis of prostatic secretions revealed that men with prostate cancer showed an increase in Bacteroidetes, Alphaproteobacteria, Firmicutes, Lachnospiraceae, *Propionicimonas*, *Sphingomonas*, and *Ochrobactrum* loads. The prostate cancer group also had a higher number of *Escherichia coli* in seminal fluid and prostatic secretion and a lower number in urine samples. Moreover, *Enterococcus* numbers were higher in the seminal fluid of patients with prostate cancer, but no significant differences were observed for urine and prostatic secretion samples [47]. Therefore, the authors suggested a possible role for the microbiome in the pathobiology of prostate cancer. Nonetheless, it might be hypothesized that the UM in prostate cancer patients is not a causative factor but rather a reaction to the effects of diagnostic procedures (transrectal biopsy) and treatment (radiotherapy or hormones). In fact, it has been demonstrated that radiotherapy affects the gut microbiome in patients with gynecologic cancer [48]. A reduction in the abundance of bacterial communities was observed in fecal samples from patients treated with radiotherapy compared to healthy individuals. Moreover, this dramatic change in gut microbiome during radiation therapy was associated with acute diarrhea [48]. The effect of such treatments on the UM has not been studied to date. Further studies are necessary to elucidate the relationship between dysbiosis in the UT due to the cancer treatments and the risk of certain urologic disorders.

3.2.3. UM changes in other urinary diseases

Several studies investigated the UM in relation to IC, NBD, STIs, and CP/CPPS (Table 3).

High-throughput sequencing analysis of the UM for clean-catch midstream urine from women with IC showed clear differences in the taxonomic composition, richness, and diversity compared to the microbial profile for asymptomatic healthy individuals [49]. A significant increase in abundance of *Lactobacillus* genus and a decrease in overall richness and ecological diversity were found in IC urine samples. *Lactobacillus* has generally been associated with the vaginal microflora, where it maintains an acidic environment that plays a protective role against infections [50,51]. Nevertheless, some studies have indicated that specific *Lactobacillus* species such as *L. delbrueckii* and *L. gasseri* could be associated with UTI and UUI, respectively [19,52]. Nickel et al [53] compared the microbiome of the lower UT between female patients with IC who reported a symptom flare and those who did not report a flare. The authors analyzed microorganisms (bacteria and fungi) present in initial stream and midstream urine samples from 228 IC patients using Ibis T-5000 Universal Biosensor system technology. They found more than 80 different microorganism species (more than 30 genera) in the urine samples. Although no significant differences in species composition were found between flare and non-flare cases, patients with symptom flare had higher levels of fungal species such as *Candida* and *Saccharomyces* [53].

UM variations were also observed using 16S rRNA sequencing analysis for male and female patients with normal bladder function compared to NBD patients [25]. Urine samples from healthy control bladders had significant enrichment in *Lactobacillus* and *Corynebacterium*

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genera, whereas other bacterial genera such as *Klebsiella, Enterococcus*, and *Escherichia* were predominant in NBD urine. Nelson and co-workers [22] used 16S rRNA sequencing to analyze the bacterial composition for initial stream urine samples from healthy males compared to males with STIs. They proposed that bacterial communities in the male urogenital tract might impact the risk of STIs. The UM among men with STIs was clearly dominated by bacteria genera that do not grow under standard culture conditions, such as *Sneathia*, *Gemella*, *Aerococcus*, *Anaerococcus*, *Prevotella*, and *Veillonella* [22]. A study using 16S rRNA sequencing on midstream urine showed that the UM from patients with CP/CPPS exhibited higher bacterial diversity and enrichment in Clostridia class compared to control samples. These variations were also related to certain severity and clinical phenotypes, as well as functional metabolism pathway perturbations [54].

The findings from all of these studies suggest that certain urinary diseases could be directly or indirectly associated with the UM. These results raise new questions about possible cause-and-effect relations. Therefore, further prospective investigations are necessary to determine whether microbial changes could be relevant in the development of these urinary system disorders and to develop new microbiome-based biomarkers to provide information in relation to diagnosis, disease severity, or treatment response.

3.3. Role of probiotics, prebiotics, and diet in urologic diseases

Therapeutic use of probiotic microorganisms as treatment for different diseases is a controversial field [55,56]. Application of probiotics has been used to modify the intestinal microbiome. Probiotics such as fecal transplants have allowed manipulation of intestinal microbial communities. These changes have been associated with suppression of pathogens, differentiation or fortification of intestinal barrier stimulation, immunomodulation, and epithelial cell proliferation [57]. Various clinical trials have been also performed to study the role of certain beneficial strains in urogenital infections, bladder cancer, and renal stone formation (Table 4).

The most popular treatment for UTIs is antibacterial therapy. However, use of broad-spectrum antibiotics can negatively affect beneficial bacterial flora in the host and consequential selective overgrowth of pathogenic bacteria. Long-term use of antibiotics leads to bacterial resistance in up to 50% of cases for specific antimicrobials [58]. Thus, probiotics have emerged as an alternative or adjuvant therapy for prevention and treatment of UTIs. A beneficial effect in the management of UTIs has been demonstrated for different *Lactobacillus* strains such as *L. rhamnosus* GR1, *L. fermentum* RC-14, and *L. reuteri* B-54 [59–64]. The antibacterial activity of *Lactobacillus* strains relies mostly on lactic acid excreted into the environment on metabolism of carbohydrates in the glycosaminoglycan layer of the vaginal epithelium. The lactic acid causes the pH to drop substantially (pH $\leq$4.5) and leads to an unfavorable microenvironment for the majority of pathogenic bacteria [65]. Moreover, *Lactobacillus* species produce additional antibacterial metabolites, including hydrogen peroxide and bacteriocin [66,67]. Two preliminary studies evaluated the prophylaxis effect of an oral *Lactobacillus casei* preparation in 138 patients with superficial transitional cell carcinoma of the bladder. The results indicated that *L. casei* strain Shirota could be effective for prevention and treatment of non–muscle-invasive bladder tumors [68,69]. A clinical trial study in 180 patients from Japan also demonstrated that habitual intake of lactic acid bacteria reduced the risk of bladder cancer [70]. Different lactic acid bacteria have also been used to treat other urinary diseases such as urolithiasis, with conflicting results [71,72]. Several studies revealed an inverse relationship between intestinal colonization with *Oxalobacter formigenes* and the development of calcium oxalate stones [73,74]. These bacteria are essential for degradation of dietary oxalate in the human body. A study in 247 adult patients with recurrent calcium oxalate stones found that colonization with *O. formigenes* was associated with a 70% reduction in urolithiasis risk [75]. Gastrointestinal recolonization with *O. formigenes* represents a valid treatment; however, studies exploring this strategy are contradictory. A study published in 2002 showed that single oral ingestion of *O. formigenes* HC1 (5×10^{10} colony-forming units) by adult volunteers was enough to reduce urinary oxalate excretion [76]. Okombo and Liebman [77] demonstrated that consumption of an oral commercial probiotic by 11 healthy volunteers during 4 wk significantly decreased oxalate absorption. However, oral administration of Oxabact (*O. formigenes*) in 42 patients with primary hyperoxaluria did not result in significant changes in urine and plasma oxalate levels [78]. A study using high-throughput sequencing technology and EQUC demonstrated that kidney stones are associated with a microbiome that includes Enterobacteriaceae species such as the uropathogenic bacterium *E. coli* [79]. A murine model in which mice were inoculated with glyoxalate and *E. coli* showed an increase in kidney calcium oxalate deposits and in the innate immune response compared to mice inoculated with only sodium glyoxalate. Therefore, the authors proposed that bacteria present in calcium oxalate deposits may contribute to calcium oxalate renal disease.

Dietary factors can also affect the risk of contracting urinary diseases by altering the properties of the urogenital bacterial flora. It has been hypothesized that cranberry juice and fermented milk products reduce the incidence of recurrent UTIs [80–83]. Cranberry juice contains compounds such as proanthocyanidins and D-mannose with bacterial antiadhesion activity against uropathogenic *E. coli* bacteria that reduces the pathogen's ability to remain in the UT [84,85]. A 2012 Cochrane review evaluated the effectiveness of cranberries in UTI prevention in susceptible populations including women and children with recurrent UTIs [86]. The review included different randomized controlled trials involving a total of 4473 participants who used cranberry juice/concentrate; cranberry tablets/capsules only; cranberry juice and tablets; or cranberry capsules and tablets. The authors concluded that cranberry juice was less effective in preventing UTIs than previously indicated; nonetheless, there were no statistically significant differences. A pilot

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study revealed that D-mannose (one component of berries) administered to patients with acute cystitis or a history of recurrent UTIs was effective in reducing UTI incidence in a 6-mo period [87]. Moreover, use of D-mannose in concentrations up to 20% had no side effects on human metabolism [87]. Other studies have demonstrated that certain diets can influence the risk of stone formation. Supplemental calcium intake was positively associated with risk of urolithiasis in a study that included more than 90 000 women [88]. Conversely, a diet low in sodium and animal protein induced changes in the urinary environment that decreased the risk of urolithiasis [89]. In conclusion, dietary habits that may change the microbiome may be important factors associated with urologic pathol'ogies. Therefore, dietary modifications could be a first step in preventing certain urinary disorders.

3.4. Discussion

It was not long ago that bacteria were only considered in urology as pathogenic agents causing infections or organisms used for treatment of superficial bladder cancer or UTI. The identification and characterization of a specific microbiome related to the healthy UT, as well as its role in certain urinary diseases, has raised many new questions in urology. Should UM screening be performed before treatment as part of patient management in urology? Could artificial modifications of the UM result in lower risk or better control of disease? Are actual treatments affecting the UM? What effect could the use of probiotics, drugs, or diet modifications have on the UM? UT diseases remain a problem affecting the quality of life of many individuals. Options used by urologists include surgery and pharmaceutical therapy, and sometimes probiotics. If gut microbiome transplants can improve health in some gut diseases, would UM transplantation be feasible and useful? Would it help to transplant the microbiome from a young woman to an old woman with UI? Microbiome-based biomarkers might represent new diagnostic and prognostic factors for certain urologic disorders. However, there is still much work to do to be able to translate the UM knowledge to urologic practice: (1) harmonize standard operating procedures in UM studies; (2) characterize the specific microbiome associated with different UT locations (bladder, urethra, etc.); (3) identify physiological factors that could modify urinary microbial communities, including individual genetic characteristics; (4) analyze whether the UM could be used as a biomarker with diagnostic value, as well as predictive and prognostic treatment response value; and (5) determine the role of UM dysregulation as risk factor for certain urologic disorders and the possible interaction of these communities with other known risk factors. Therefore, knowledge regarding the UM may play a very important role in the future to improve the diagnosis, the treatment and prevention of UT diseases.

4. Conclusions

There is evidence that the healthy UT has a microbiome that can change in urologic disorders. However, conclusions on the bacterial genera associated with each condition are hampered by lack of harmonization in the methodology (sample collection and bacterial analysis) used by researchers. Nonetheless, results published to date open the opportunity to further study new diagnostic, prognostic, and predictive microbiome-based biomarkers that could be used in clinical urology practice. In addition, despite previous controversy regarding the use of probiotics, prebiotics, and diet modifications as treatment for urologic disorders, there are increasing signs that it may be possible use them as a first step in regulating the UM to reduce the risk of or as a treatment for certain urinary diseases.

Author contributions: María F. Lara had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Aragón, Herrera-Imbroda, Lara.
Acquisition of data: Aragón, Lara, Castillo.
Analysis and interpretation of data: Aragón, Lara.
Drafting of the manuscript: Aragón, Lara, Queipo-Ortuño, Gómez-Millán, Herrera-Imbroda.
Critical revision of the manuscript for important intellectual content: Aragón, Lara, Queipo-Ortuño, Gómez-Millán, Herrera-Imbroda, Sequeira-García del Moral, Yucel.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.euf.2016.11.001.

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