Running Title: Urinary Microbiome and Genitourinary System

Urinary Microbiome and its Correlation with Disorders of the Genitourinary System

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A B S T R A C T

Purpose: Until recently, the urine of healthy individuals was assumed to be sterile. However, improvement of bacterial detection methods has debunked this assumption. Recent studies have shown that the bladder contains microbiomes, which are not detectable under standard conditions. In this review, we aimed to present an overview of the published literature regarding the relationship between urinary microbiota and functional disorders of the genitourinary system.

Methods: We searched Medline, PubMed, Embase, The Cochrane library and Scopus to identify RCTs published, with MeSH and free keywords including microbiota, bladder pain syndrome, prostatitis, kidney stone disease, and bladder cancer until September 2020. Randomized controlled trials investigating microbiome and lower urinary tract symptoms were included. Non-randomized trials, cross-over trials and pooled studies were excluded. The articles were critically appraised by two reviewers.

Conclusion: The urine microbiome is a newly introduced concept, which has attracted the attention of medical researchers. Since its recent introduction, researchers have conducted many fruitful studies on this phenomenon, changing our perspective toward the role of bacteria in the urinary tract and our perception of the genitourinary system health.

Patient Summary: A deeper understanding of the urinary microbiome can help us to develop more efficient methods for restoring the microbiota to a healthy composition and providing symptom relief. Modification of the urinary microbiome without antibiotic use can be a possible venue for future research.

Keywords

Microbiome; Urinary Microbiota; Urine Culture, Enhanced Quantitative Urine Culture, 16s Rrna Sequencing, Viruses and LUTS, Bacteriophages and LUTS, Fungi and LUTS, Lower Urinary Tract Symptoms, Urinary Tract Infection, Overactive Bladder Syndrome, Urinary Incontinence, Bladder Pain Syndrome, Detrusor Underactivity, Chronic Prostatitis, Pelvic Pain, Kidney Stones, Bladder Cancer

Introduction

The lower urinary tract consists of the bladder and the urethra, which contain smooth and striated muscles, supported by other muscles and ligaments. The urothelium is described as the inner lining of the bladder, with a barrier function. According to the International Continence Society (ICS), lower urinary tract symptoms (LUTS) can be divided into storage, voiding, and post-micturition symptoms. In male patients, LUTS are usually attributed to benign prostatic enlargement and bladder outlet obstruction, whereas in female patients, it is predominantly associated with overactive bladder syndrome (OAB). [1-3]

In recent years, with the application of novel urinalysis methods, urine is no longer considered to be sterile, as a certain microbiome was detected in healthy, asymptomatic individuals. This finding provided a new horizon for detecting the causes and treatments of LUTS. [4, 5] An integral part of the microbiome is the microbiota, which refers to the assemblage of living microorganisms in a defined environment. [7] The term "microbiome" can be used to represent microorganisms (i.e., bacteria, archaea, lower and higher eukaryotes, and viruses), their habitats, their genomes, and their surrounding environmental conditions. [8]

The Human Microbiome Project (HMP) investigated the microbiota communities in the human body. However, they mostly focused on organs, such as the skin, mouth, genital tract, eyes, gut, and blood. Also, the correlation of urinary microbiome with LUTS has been the subject of investigation in many studies.[6] In this study, we aimed to present an overview of the published literature on the relationship between urinary microbiota and different disorders of the genitourinary system, including LUTS, bladder pain syndrome (BPS), prostatitis, kidney stone disease, and bladder cancer (Table 1).

Until recently, the urine of healthy individuals was assumed to be sterile. The sterility of healthy urine was thought to be maintained by host factors, such as physical barriers between the source of pathogenic bacteria and the urinary tract, the ongoing flow of urine through the urinary tract, and complete bladder emptying during voiding. These factors, along with antibodies, proteins, and other factors, which destroy or restrict the ability of microbes to grow, should prevent infection.[7] The uroepithelial defense is the main mechanism, preventing bacterial growth and infection. Since the epithelial cell lining releases Tamm-Horsfall protein, lactoferrin, and lipocalin, in addition to constitutive and inducible antimicrobial peptides and cathelicidin, the urinary tract can prevent the attachment of transient bacteria under healthy conditions. [8, 9] The frequent absence of bacterial growth in

the urine cultures of asymptomatic individuals, as well as diagnostic settings, has led to the assumption of urine sterility. However, recent studies, using new techniques, have introduced bacterial markers in voided urine, which was found to be sterile, based on conventional laboratory cultures.[8, 9] Therefore, the urine is now established as unsterile. Overall, the differences of urinary microbiota between fit, ambulatory individuals and patients with LUTS may propose an etiological principle for numerous bladder disorders. [10]

Methods and Material:

Data Sources and Searches

We searched Medline (PubMed), Embase, The Cochrane library, Web of Science, Scopus and ProQuest to identify RCTs published, with MeSH, Emtree and free keywords including microbiota, lower urinary tract symptoms, bladder pain syndrome, prostatitis, kidney stone, bladder cancer, viruses, Bacteriophages, Fungi, until September 2020. Randomized controlled trials investigating microbiome and lower urinary tract symptoms were included. The complete search strategy is attached in appendix 1.

Study Selection

Two reviewers screened titles and abstracts to identify the relevant studies. Disagreements between two reviewrs were resolved through discussion or by the assistance of a third reviewer. The selected abstracts were then included for the full-text screening.

Data Extraction

We extracted the demographic characteristics of the articles including name of the author, publishing year, sample collection, analysis technique, and assosiated microbiom.

Risk of Bias Assessment

Two reviewers independently selected the evidence. In case of disagreement, a third reviewer re-evaluated the documents to solve the discrepancies.

Data Presentation

The data found, are presented in the results section and ordered in subheadings starting with what is known on microbiome, the different species and their relationship to LUTS, followed by what is known on different urological conditions that cause LUTS and their relation to the urinary microbiome.

Results:

We initially identified 4004 records using the search strategy, of which 2464 articles remained after the removal of duplications. Also, 2339 articles did not meet the inclusion criteria and were removed after screening for eligibility by reading the titles and abstracts, 125 fulltexts were assessed further by the exclusion of another 111 papers. Finally, a total of 13 studies were included in this systematic review. Figure 1 shows the details of the study selection process. Table 1, showes the characteristics of the included studies.

Microbiome

The term "microbiota" refers to a collection or community of microbes. The term "microbiome" refers to the entire collection of genes found in all microbes.[11, 12] Despite the common misuse of these two terms, they carry distinct meanings, highlighting their careful use. Microbial populations are present in the human body and reside in different organs, such as the gastrointestinal tract, skin, upper respiratory tract, and genital tract.[11] There are different elements contributing to the shape characteristics of the microbiome communities in the body, including age, gender, hormonal status, body mass index (BMI), diet, environment, host genetics, and early microbial exposure. [7, 13-15]

Moreover, the beneficial or pathogenic nature of bacterial populations is a relative concept, depending on the individual's microbiome characteristics. [16, 17] Generally, maintenance of a normal microbiome is thought to be essential in the intestines and probably the bladder. [18] Also, it may have an impact on the risk of infection, caused by pathogenic organisms, as well as the occurrence of immune-mediated diseases and carcinogenesis. [19]

Detection of Urinary Bacteria

From the 1950's until the present day, standard urine culture (SUC) has been the routine protocol for identifying pathogenic bacteria in the urine of LUTS patients. [20, 21] Commonly, SUC is performed in a clinical laboratory by plating 1 µL of urine onto blood and MacConkey agar plates and incubating aerobically at 35˚C for 24 hours. Since the introduction of the original method, SUC has been applied as the standard diagnostic tool for identifying urinary tract infections (UTIs), although some investigators have reported several disadvantages of this method.[22, 23]

Laboratories generally rely on standard urine culture protocols for detecting frequent, quickly growing aerobic uropathogens, particularly uropathogenic *Escherichia coli* (UPEC).[24] Also,

UTIs may be caused by other Gram-negative bacteria, such as *Pseudomonas aeruginosa*, several Enterobacteriaceae species (e.g., common *Proteus* and *Klebsiella* species), several Gram-positive bacteria (e.g., *Staphylococcus saprophyticus* and *Enterococcus faecalis*), and certain fungi (e.g., *Candida* species).[25, 26]

Moreover, many other microorganisms have been classified as emerging uropathogens. [27, 28] These microorganisms have been discovered with high colony counts in patients with UTI symptoms and/or acute cystitis. Nevertheless, these methods cannot detect the presence of certain bacteria, such as anaerobic bacteria, bacteria embedded within biofilms, commensals or symbionts, and genera, such as *Corynebacterium, Lactobacillus,* and *Ureaplasma* [5, 17]. Also, they cannot effectively detect urinary microbiota members, including many uropathogens. This necessitates the use of enhanced urine culture techniques, which are better correlated with methods, such as DNA sequencing and culture-independent assays (e.g., diagnostic PCR assay).

To document the female urinary microbiota more specifically, many researchers have used the next generation sequencing (NGS) technique of 16S rDNA. In this regard, a previous study used NGS to examine the urine samples of subjects, collected by transurethral catheterization and suprapubic aspiration. The results indicated the presence of a resident bladder microbiome rather than a vulvovaginal contamination in the collected samples. [29]

Detection of Urinary Microbiota

Culture-independent methods, particularly 16S rRNA gene sequencing, were primarily used in HMP [17, 30] to elucidate the composition of microbial communities in different microbial niches of the human body. In addition, NGS was employed in many studies to describe the microbiota in urine, collected from the bladder of individuals with and without LUTS. [31-33] To fully comprehend the results of studies on urinary microbiome, we must consider the following collection techniques: A) collection of midstream voided urine, either with or without a clean catch, after cleaning the skin; B) sampling up the urethra or using transurethral catheterization; and C) suprapubic aspiration of urine.[34]

Although sequencing is a sensitive method, it cannot quantify the detected organisms or determine whether the bacteria are alive or dead. On the other hand, this method provides information about the presence of DNA in microbes, which requires non-standard culture conditions and cannot be detected otherwise. Enhanced quantitative urine culture (EQUC) protocols have been expanded to overcome this limitation.

Bacteriophages and LUTS

In addition to bacteria, viruses and eukaryotic microorganisms (i.e., archaea and fungi) are also included in microbial communities colonizing the body, with continuous and intricate interactions with one another and the surrounding human niche. Bacterial viruses (bacteriophages) play a significant role in the microbial community dynamics.[35, 36] Extensive research into the heterogeneity of phages, residing in the bladder, can provide important information about changes in the microbial community of the bladder.[37-40] Also, similar investigations have been conducted on the vaginal microbiota.[41]

Moreover, *Lactobacillus* phages seem to contribute to structural changes in the vaginal microbial community and improve bacterial vaginosis. [42] The abundance of lysogenic phages in the bladder shows that phages may be possible contributors to the stability of urinary microbiota. In addition, changes in the phage populations of bacterial strains, isolated from patients without OAB symptoms, suggest that phages can contribute to urinary health; however, the results are not of great statistical importance. [43]

Actinomycetaceae phages have been found in strains, isolated from women with OAB. Moreover, in a previous study, a *Varibaculum cambriense* strain was collected from a female patient with stress urinary incontinence. On the other hand, Actinomycetaceae phages were not detected in any *Actinomyces* strains from the asymptomatic controls. *In vitro* studies have documented how phages can develop intrinsic lytic activities for bacteria, collected from patients with UTI due to a spinal cord injury. In addition, resistance optimization is achievable through immediate adaptation of bacteriophages. [44]

Additionally, phages can degrade uropathogenic *E. coli* biofilms. [45] Also, use of phages for the treatment of UTI has been reported ;[46] therefore, they may be regarded as the best option for the prevention and treatment of UTI. [47] Nonetheless, there are certain negative aspects to phages. One major disadvantage is secondary infection, which refers to virion interactions with already phage-infected bacteria, resulting in the so-called “superinfection exclusion”. [48] Matching phages are commonly collected from phage banks, which are assemblages of already characterized phage isolates. However, it should be noted that *in vitro* phage activity is not consistently predictive of *in vivo* therapeutic efficacy (i.e., immune responses). [49]

Overall, phages have many beneficial properties, and phage therapy has limited side effects. The presence of phages with significant sequence similarity in the microbiota of women suggests the presence of a fundamental phage community within the bladder. In addition,

oscillation of phage populations in women with and without OAB symptoms shows that phages can improve urinary health. [43]

Viruses and LUTS

Hantaviruses, which have been recently introduced as common viruses in Europe, [50] can cause severe Hantavirus diseases, with a mortality rate of approximately 15%. These viruses, which belong to the Bunyaviridae family and contribute to the development of kidney failure, cause a group of clinically similar diseases, known as Hemorrhagic Fever with Renal Syndrome (HFRS). HFRS is mainly caused by some Old-World Hantaviruses, including *Hantaan orthohantavirus*, *Seoul orthohantavirus*, *Dobrava-Belgrade orthohantavirus*, and *Puumala orthohantavirus*. These viruses are associated with renal dysfunction, hemorrhage, fever, thrombocytopenia, acute renal insufficiency, abdominal pain, and occasional acute pancreatitis. The diagnosis of Hantavirus infections is based on immunofluorescence assays using virus-infected cells, enzyme immunoassays, and Western blot analysis with nucleocapsid recombinant proteins. [51]

During 2001-2003, almost 200 clinically apparent hantavirus infections were registered annually in Germany. [50] Neutralization assays have indicated almost exclusively human infections, caused by *Puumala* and *Dobrava* Hantaviruses and rarely by *Tula* Hantavirus. However, further explorations are needed to develop a more precise concept regarding the distribution of Hantaviruses in Germany and to calculate the risk for the human population. Human papillomavirus infection, causing condyloma acuminate, is another example of viral infection and consecutive LUTS. It is also recognized as a rarely viral infectious cause of acute urinary retention in women. [50] Urethral condyloma can be effectively treated by local excision for early improvement of voiding function. [52]

Herpes zoster can involve the spinal cord and anterior horn cells, causing various neurological disorders. Involvement of the sacral center, leading to bladder dysfunction and urological changes, has been reported to be unusual, and its acute type is mainly reported in men. This finding was reported for the first time in 1890. [53] However, a limited number of cases (~150) has been reported over the years. Another virus, correlated with urinary tract symptoms, is the human T-cell leukemia virus-type 1 (HTLV-1). Nevertheless, the majority of patients infected with this virus are carriers, despite having multiple urinary symptoms of OAB, which are common in HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). In this regard, a previous study concluded that some immunological and viral

factors, as well as proviral profiles, can be found in patients with HAM/TSP. Another conclusion was that HTLV-1 overactive bladder patients are still able to down regulate their inflammatory immune response. [54]

Fungi and LUTS

The fungal microbiota have been rarely studied in the literature. Mycobiome is a lesser-known, but significant aspect of the human microbial ecosystem. Generally, the pathogenic nature of such organisms is vivid. Aberrant fungal infections of the urinary tract may be life-threatening due to limitations of culture studies, low suspicion of fungal involvement, and absence of preventative and therapeutic options.[55] *Candida* species are the most ubiquitously found urinary pathogens. Nevertheless, common invasive fungal species, such as *Cryptococcus*, *Aspergillus*, *Mucoraceae*, *Histoplasma*, *Blastomyces*, and *Coccidioides*, can also infect the urinary tract. These fungi can attach to the urothelium to form mixed biofilms with bacteria or grow in soluble microcolonies in the urine, without any direct contact with the urothelium.

There is a possibility that fungi remain quiescent in the bladder wall cells, as can be seen in the intracellular bacterial colonies of *E. coli*. [56] Today, the most recurrent nosocomial fungal infection is the urinary tract candidiasis, which results in the development of UTI. Meanwhile, proliferation of *Candida* species in urinary tract candidiasis may lead to the development of antifungal-resistant *Candida* species. *Candida albicans* is the dominant species, responsible for urinary tract candidiasis.

To prevent, control, and treat urinary tract candidiasis, it is essential to monitor *Candida* species, accounting for UTIs. [57] With an optimistic view and a better understanding of the function of fungi in UTIs, we can exercise targeted manipulation of mycobiome for therapeutic applications, as shown in other organ systems. *Saccharomyces boulardii* is currently under trial as a probiotic for the treatment of diarrheal diseases. [58-60]

Urinary Tract Infection (UTI)

UTI, as an acute urinary disease, refers to the presence or predominance of uropathogenic microbes within the bladder, the urethra, or the upper urinary tracts (i.e., kidneys and ureters). Gram-negative bacteria, including *E. coli* and *K. pneumoniae*, are the most common uropathogenic bacteria, causing UTI.[61] Recent reviews have demonstrated the importance of screening methods and diagnostic tools for uncomplicated UTI.[62] These reviews concentrated on the relationship between exposure to organisms and human diseases, in

addition to the host response. According to 16S rRNA gene sequencing, light microscopy, and bacterial culture, bacterial DNA inhabits the bladder of adult women with or without UTI.[29]

Researchers have conducted comprehensive studies to incubate *E. coli* and *E. faecalis* with *Lactobacillus*, which is a well-represented member of the genitourinary system microbiome, containing human bladder epithelial cells. Evidence suggests a decrease in the uropathogen adherence via incubation with *Lactobacillus*; this decline seems to be correlated with a lower pH. Finally, these findings may be attributed to some factors, such as decreased uropathogen adherence in a more acidic environment or higher activities of antimicrobial peptides (AMPs) as products of the innate immune system, promoted by *Lactobacillus*.[63, 64]

Microbiome and OAB

Urinary urgency is an epicentric symptom of OAB, characterized by an abrupt and compelling desire to void.[3] Although this symptom is underreported and undertreated, it has significant effects on one's quality of life, sleep, sexual function, and mental health.[65] This so-called "hidden condition" is also a diagnosis of exclusion for ruling out other symptoms. Urine culture is used to rule out infections in patients with OAB symptoms. It is possible that microbes, inhabiting the lower urinary tract (urinary microbiota), influence the OAB symptoms. [4]

Following the discovery of urinary microbiome, 16S RNA gene sequencing has been used to determine if bacteria, not discovered previously in the urinary tract, can plainly or circuitously contribute to or result from OAB.[13] This method, along with EQUC, has been used to compare urine catheterization between women with and without urgency urinary incontinence (UUI). The results suggest the possibility of significant contrasts in the urinary microbiome of women with and without UUI, which can have significant effects on the prevention, diagnosis, and treatment of UUI.

In another study, more *Lactobacillus* species were found in individuals without UUI, compared to cohorts with UUI, whereas in UUI patients, more *Gardnerella* species were found.[13] According to these results, we can conclude that *Lactobacillus* is a member of the normal vaginal flora, with distinct functions in the bladder. A similar scenario might be also true for *Gardnerella*, which was detected more frequently in UUI patients. Also, an increased sequence abundance was found in the UUI cohort, compared to the non-UUI cohort. Since *Gardnerella* was detected in the urine of women without UUI, the mere presence of

Gardnerella might not represent a dysbiotic environment.[13] Moreover, bacterial genera, including *Actinobaculum, Actinomyces, Aerococcus, Arthrobacter, Corynebacterium, Oligella, Staphylococcus,* and *Streptococcus,* had higher frequencies in the UUI cohort. *L. gasseri* was found to be related to UUI, whereas *L. crispatus* was detected in women without LUTS. [13]

A study using 16S rRNA gene sequencing of catheter specimens of urine, collected from women with UUI, without treatment, showed that more than one-half of the samples tested positive for bacterial DNA, and the positive results were associated with younger age, higher BMI, more severe UUI, better response to treatment, and lower susceptibility to UTI.[32] Also, *Lactobacillus* species were detected in urine samples, and *Gardnerella* was the second most frequently detected genus.[32] These results suggest that some bacteria may have protective effects on the bladder, as they do so in other human biological niches.

In previous studies, the results of 16S rRNA gene sequencing of the urine samples of OAB patients and the controls indicated a diversity in the classification of detected bacteria. Seven genera, including *Proteus* and *Aerococcus,* were increased, whereas 13 genera, including *Lactobacillus* and *Prevotella,* were decreased in OAB patients, compared to the controls. [4, 66] Other researchers have proposed that an increase in the severity of OAB symptoms is associated with the reduction of microbial miscellany. [33] On the other hand, another study demonstrated a relationship between the OAB symptoms and pyuria. Also, notable urinary urgency was correlated with pyuria and epithelial cell shedding. In this study, routine urine cultures could not distinguish OAB patients from the controls.[67]

In a more recent study, researchers examined the urinary microbiome of a female OAB patient via high-throughput pyrosequencing of 16S rDNA in successive urine samples, which were collected within a one-year interval. In the first sample, the standard urine culture was positive for *Streptococcus*. Accordingly, the patient received an antibiotic treatment, and one year later, the standard urine culture was found to be negative; however, the urinary symptoms were still reported. The presence of fastidious and anaerobic bacteria was detected by 16S rDNA sequencing of urine samples, with insignificant variations in microbial diversity over one year. Therefore, the relatively sustained microbiome and persistence of urinary symptoms, regardless of antibiotic treatment, indicated a possible association between OAB and urinary microbiota. [68]

On the other hand, a later publication concluded that UUI patients, who responded to treatment, were more likely to have less diverse bacteria in their urine, and a relationship was

proposed between urinary microbiota and response to incontinence medications.[69] Overall, based on the results of available studies, it may be suggested that *Lactobacillus* plays a preventive role against UUI. These findings can have significant implications for the future diagnosis, treatment, and prevention of UUI.

Microbiome and Bladder Pain Syndrome (BPS)/Interstitial Cystitis (IC)

BPS or IC is described as "the complaint of suprapubic pain, related to bladder filling accompanied by other symptoms, such as increased daytime and nighttime frequency in the absence of urinary infection or other obvious pathologies of the lower urinary tract".[1] The BPS/IC symptoms are reminiscent of infection, with UTI often accompanying this condition. Therefore, in clinical practice, the diagnostic process is often prone to explicit falsification, and an inaccurate diagnosis can lead to chronic pain syndrome and treatment resistance. [70]

In this regard, a previous study examined the assumption that BPS/IC has no relationship with bacterial colonization. For this purpose, 16S rDNA sequencing was performed on clean-catch urine samples, and the urinary microbiota of BPS/IC patients were characterized and compared with the urine samples of healthy women. Although no specific pathogen was detected for BPS/IC, the results demonstrated a major contrast in the bacterial composition of the two groups. [71] In this study, the composition of urinary microbiota changed, the microbial diversity decreased, and more *Lactobacillus* species were found in female patients with BPS/IC, compared to healthy women.[72, 73] Also, in BPS/IC women, fewer genera were detected, compared to the healthy controls (31 vs. 45). Overall, *Lactobacillus* was the most commonly detected genus (92% of sequences in BPS/IC patients vs. 57% of sequences in healthy females), followed by *Gardnerella* (2%) and *Corynebacterium* (2%).

In another study, the frequency of genus *Corynebacterium* decreased in patients, compared to the controls. [74] BPS/IC may be associated with a specific microbiome of the genitourinary system. Moreover, in a study on premenopausal women with BPS/IC and the healthy controls, *Lactobacilli,* with anaerobic or fastidious dominance, was observed in both cohorts. In other words, premenopausal women with BPS/IC were not significantly different from those without BPS/IC in terms of urinary and vaginal microbiomes, although their socioeconomic characteristics and pelvic floor functions had deteriorated. [75]

A more recent study examined the urinary microbiota of female patients, suffering from urologic chronic pelvic pain syndrome, with symptom flares and standard negative urine cultures. Two urine samples were collected from all subjects, including an initial stream urine

(VB1) sample and a clean-catch midstream urine (VB2) sample. These samples were observed using molecular methods, which focused on bacterial and fungal DNA. At the genus level, *Lactobacillus* decreased in both groups, followed by *Staphylococcus* in VB1 and *Propionibacterium* in VB2 samples. The significant prevalence of fungi (*Candida* and *Saccharomyces*) was reported in BPS/IC women with symptom flares, compared to those who did not experience any symptom exacerbation; this result suggests the involvement of these fungi in the pathogenesis of a subtype of BSP/IC, associated with symptom flares. [73]

Recent developments suggest that microbiome can be of great significance in BPS/IC, as the role of certain *Lactobacillus* species in the healthy bladder milieu is now more evident than ever. [76] However, in another study, a hypothesis was made as to how BPS/IC symptoms could not be possibly related to variations in the urinary microbiome.[77] These results indicate the need for further research to reach a better understanding of this matter.

Microbiome and Chronic Prostatitis (CP)/ Male Chronic Pelvic Pain Syndrome (CPPS)

CP/CPPS refers to chronic pain and discomfort in the pelvic area, commonly correlated with LUTS, sexual dysfunction, and psychosocial problems. It commonly lasts for a minimum of 3-6 months, with significant impacts on the person's quality of life and financial burden. [78] A study using 16S rRNA sequencing showed that the total bacterial diversity was considerably higher in the CP/CPPS group, compared to the controls. Many bacterial classes (e.g., Clostridiales and Bacteroidetes) were overrepresented in CP/CPPS patients, whereas other bacterial classes, such as Bacilli, were underrepresented. Also, the anaerobic bacteria were much more common in CP/CPPS patients, compared to the controls. This group of bacteria is related to potential pathogens, which are not often cultured or treated in regular clinical practice. In addition, a greater phylogenetic diversity was reported in CP/CPPS patients [79]. The most remarkable difference between the groups was related to the *Lactobacilli* count, especially *Lactobacillus iners*, which was higher in healthy men, compared to prostatitis patients. [79]

In another study on 30 patients, urine and fecal samples were collected before and after transrectal biopsy of the prostate. DNA was extracted from the urine after prostate massage and before and after prostate biopsy. DNA was also extracted from fecal samples before biopsy. *Lactobacillus* and *Staphylococcus* bacteria were observed in the urinary microbial profiles before biopsy. The count of *Lactobacillus* decreased, whereas increased levels of

Prevotella bacteria were detected in the urinary microbial profiles after biopsy. The results showed that *Bacteroides* bacteria were predominant in the fecal samples. [80]

Microbiome and Detrusor Underactivity (DU) / Underactive Bladder Syndrome

DU is among the most common conditions causing LUTS. It is associated with a variety of interactions between the brain and the bladder at different levels, resulting in diminished voiding efficiency and bladder acontractility.[81] It is described as failure to reach complete bladder emptying in a normal time span and/or extended duration of bladder emptying, caused by a contraction of reduced strength and/or duration.[82] Based on our literature review, no study has yet focused on the relationship between DU and urinary tract microbiome; therefore, further research is warranted in this area.

Microbiome and Urolithiasis

The presence of microbiome in patients with kidney stones has been detected by examining the content of stone microflora, using PCR and standard microbiological methods.[83] Calcium oxalate is a major element in the composition of kidney stones. Accordingly, urinary oxalate is considered a risk factor for kidney stones. This microorganism, along with the intestinal microbiome, contributes to the pathogenesis of kidney stones. *Oxalobacter formigenes* is a Gram-negative bacterium, which decreases oxalates in the gut, leading to the reduction of oxalate excretion.[84] There is a correlation between urinary microbiome and formation of struvite stones, as urease production, involved in struvite stone formation, is the sole function of bacteria in urinary stone disease (USD).[85] On the other hand, recent studies have shown that some bacteria, including *S. epidermidis*, *E. cloacae*, *E. coli*, and *L. gasseri*, are associated with stones of a non-struvite composition. These bacterial species in the urine can adhere to stones or contribute to the formation of stones. [86]

Moreover, a significant bacterial diversity, including *Enterobacteriaceae, Gardnerella*, and *Lactobacillus* species, has been detected in a recent study, using NGS sequencing technologies.[87] In this study, the murine kidney calcium oxalate deposits were induced by *E. coli*, and the kidneys were transurethrally inoculated with uropathogenic *E. coli*. The assumption that renal calcium oxalate deposits can be an amendable risk factor for kidney and urinary tract infections should not be dismissed, as bacteria may be adjacent to calcium oxalate deposits and induce positive effects on calcium oxalate renal disease. [87] It is also suggested to consider the kidney stone microbiome in predicting the recursive behavior of kidney stones; however, this suggestion must be further examined. [64]

Bacteriotherapy, which can be used to eliminate oxalates and reduce the risk of USD, has shown limited success. Similarly, oxalate-decreasing probiotics do not yield favorable results. Common shifts in the gut microbiota and a diverse microbial network, associated with oxalate metabolism, may play a role in the onset of USD. However, it is important to consider the correlation between the gut microbiota and USD rather than solely focusing on a specific functional microbial species. We can develop a more successful bacteriotherapy by adjusting it to target a broad range of bacteria rather than only a few selected species. [88]

Microbiome and Urothelial Carcinoma

Some findings support the hypothesis that members of the genitourinary microbiota are causative factors or cofactors in genitourinary cancers. So far, the information related to gastrointestinal microbes is mainly used for identifying cancer treatment responses. It must be noted that therapeutic options for genitourinary cancers may be affected by the human-associated microbiota. Urothelial carcinoma is the most common type of cancer in the urinary tract, and the bladder is the most frequently affected area.[89] Infections caused by *Schistosoma haematobium* are associated with the development of squamous cell carcinoma of the bladder due to chronic inflammation. In this regard, a previous study suggested that microbiota are possible contributors to the pathogenesis of several chronic diseases, such as cancer. In both men and women, the Firmicutes were found, while actinomycetes, including *Mycobacteria* and *Bacteroidetes,* were only found in women. [9] Nevertheless, the relationship between UTI and urothelial carcinoma has not been established yet.

The Bacillus Calmette–Guerin (BCG) vaccine, which prevents tuberculosis, has been used to prevent the recurrence of bladder cancer. [5] This vaccine is identified as a *Mycobacterium,* and therefore, it is an actinomycete. Controversial studies have proposed the same potential for *L. casei*, as a bacterium from the phylum Firmicutes in the urinary microbiota.[5] Microbiota, primarily composed of actinomycetes, may account for the lower occurrence of bladder cancer in women, as actinomycetes can have preventive effects, similar to BCG, which is known to influence the treatment and prevention of bladder cancer relapse; therefore, we must reconsider the prevention and risk factors of bladder cancer.[90,91]

In this regard, a microbiome study was conducted on urine samples collected from six healthy subjects and eight urothelial carcinoma patients, using the 454 sequencing technology. A total of 329 genera were documented. *Acinetobacter* was the most frequent

genus, while *Streptococcus, Pseudomonas, Finegoldia, Gardnerella, Anaerococcus, Escherichia,* and *Enterococcus* were the most abundant genera in certain specimens.

In the mentioned study, there was almost no *Streptococcus* species in the majority of healthy samples, with the exception of one outlier. On the other hand, the *Streptococcus* count increased in five out of eight cancer patients. *Pseudomonas* or *Anaerococcus* was the most dominant genus in two out of three cancer patients, with a low abundance of *Streptococcus*; these results suggest that urothelial carcinoma may be correlated with the altered microbiota of the urinary tract.[92] However, the association of UTI with urothelial carcinoma is yet to be attested. Accumulating evidence suggests that indigenous microbiota in the urinary tract may play a significant role in the tumorigenesis of urothelial carcinoma, similar to other tumors.[93]

Additionally, another study examined the possible urinary microbial community, which might be correlated with bladder cancer. The mid-stream urine samples were collected from 31 male patients with bladder cancer and 18 non-neoplastic controls. DNA was extracted from the urine pellet specimens and processed for high-throughput 16S rRNA amplicon sequencing at V4 region in an Illumina MiSeq system. The results indicated the increase of some bacterial genera (e.g., *Acinetobacter, Anaerococcus,* and *Sphingobacterium*) and reduction of some other genera (e.g., *Serratia, Proteus,* and *Roseomonas*) in the cancer group, compared to the non-cancer group. The enrichment of *Herbaspirillum, Porphyrobacter,* and *Bacteroides* was detected in cancer patients, with potential recurrence and progression of the disease, which indicates that these genera are potential biomarkers for risk stratification.[93]

Moreover, the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUS) demonstrated the enrichment of several functional pathways in the cancer group, including *S. aureus* infection, glycerolipid metabolism, and retinol metabolism. However, this study could not show whether changes in the microbiome contribute to cancer or vice versa. A more comprehensive understanding of the role of microbiome in the progression and evolution of bladder cancer can help us investigate new therapeutic options and biomarkers. [93]

Microbiome and Prostate Cancer

A previous study assessed the possible correlation between bacteria and prostate cancer by investigating the type of microbiota in the expressed prostatic secretions (EPS) of patients

with prostate cancer and benign prostatic hyperplasia (BPH). The results showed a significant increase in *Bacteroidetes*, *Alphaproteobacteria*, *Firmicutes*, *Lachnospiraceae*, *Propionicimonas*, *Sphingomonas*, and *Ochrobactrum*, whereas *Eubacterium* and *Defluviicoccus* decreased in the prostate cancer group, compared to the BPH group. Also, *E. coli* decreased significantly in the urine of the prostate cancer group, despite an increase in the EPS and seminal fluid. On the other hand, the *Enterococci* count considerably increased in the seminal fluid, with limited alterations in the urine and EPS. [95]

Discussion

Today, it is generally accepted that the urine of healthy individuals is not sterile. Evidence shows that the bladder contains microbiomes, which are not detectable under standard conditions. The benefits or pathogenic roots of microbiomes are dependent on their characteristics. Various studies have been published in recent years to determine the relationship between microbiome and LUTS. In these studies, urine was collected using a transurethral catheter to elude the urine from bacterial contamination by external tissues. To determine the resident microbes, bacterial 16S rRNA was amplified using PCR assays. Also, NGS sequencing was performed in an Illumina MiSeq system. In all studies concentrating on the relationship between microbiome and OAB, the *Lactobacillus* count increased in OAB patients, compared to subjects without OAB. [4, 29, 32, 69]

So far, no study has examined the relationship between the urinary tract microbiome and DU. The urinary microbiome is a novel concept, which has attracted the attention of medical researchers. Since its introduction, many research teams, by conducting multiple fruitful studies, have changed our perspective toward the role of bacteria in the urinary tract and our perception of the genitourinary system health.

Implications for Future Research

Many questions remain to be answered in this area. We need to determine how microorganisms interact with one another or with the host. We also need to specify the role of non-bacterial microbes and their stability in the urinary microbiota. Moreover, it is important to know whether the urinary microbiota changes throughout the individual's life, whether it responds to diet, and whether it is resilient or not. Also, we must characterize the actual role of urinary microbiota and determine if it interacts with the urothelium. Finally, it is important

to understand whether modifications of the urinary microbiota can improve the prevention of LUTS.

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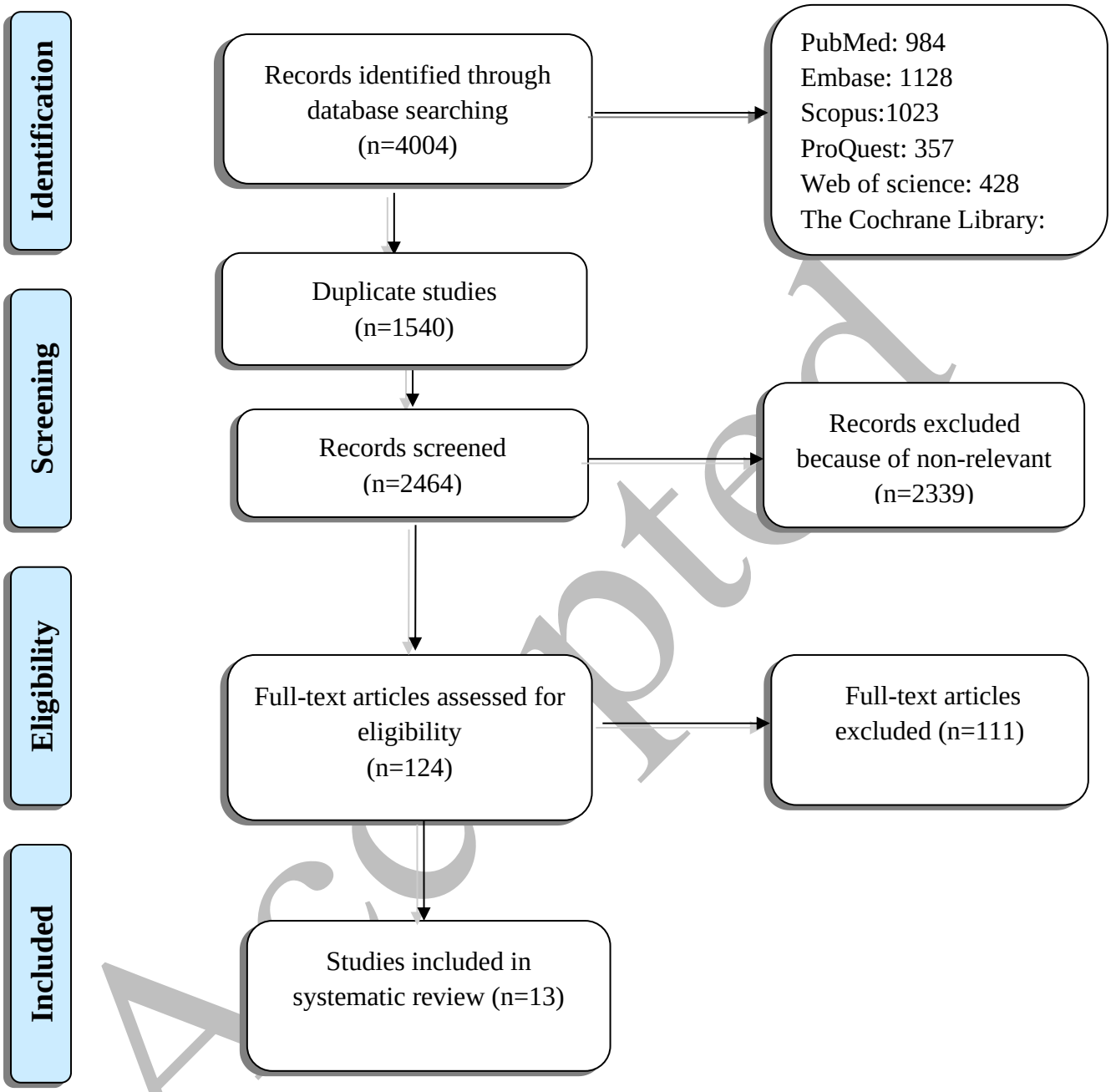
Figure 1. Search and selection process of systematic review.



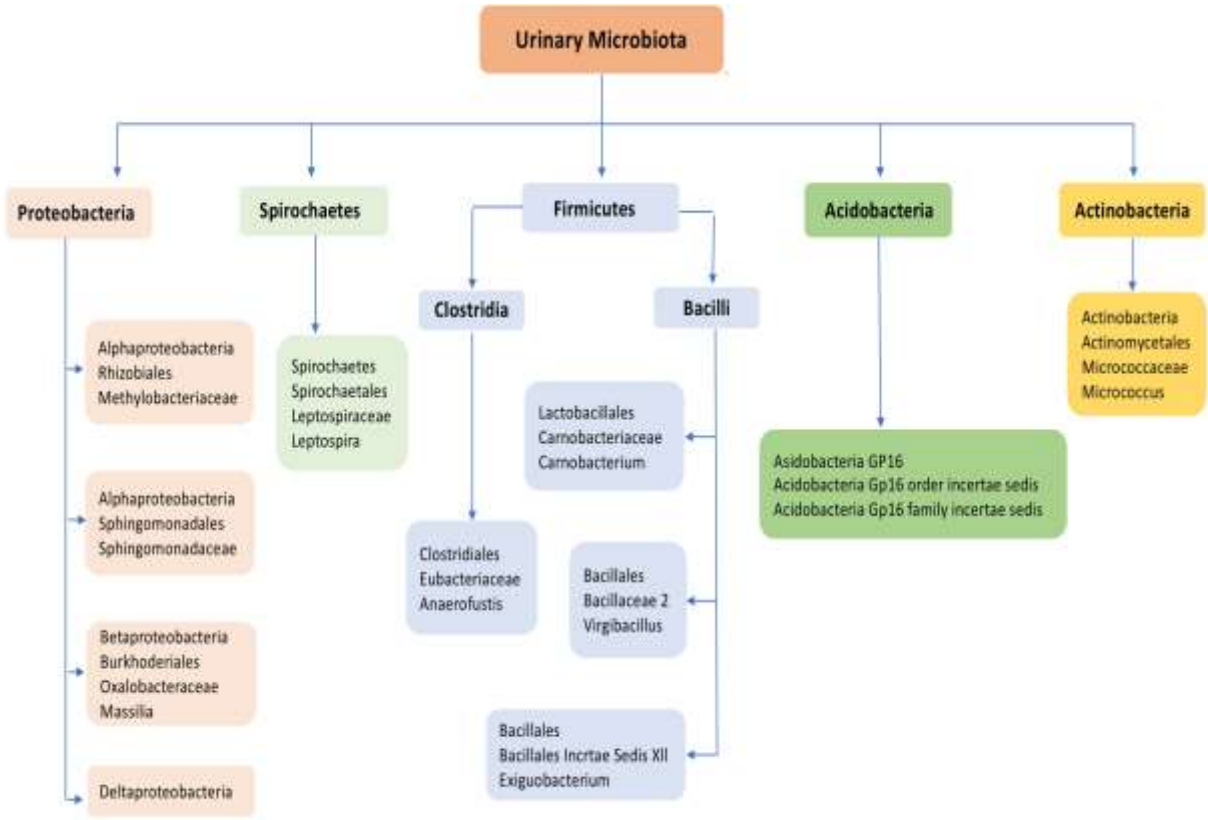


Figure 2: The urinary microbiota

Appendix 1: Search Strategy of Embase

#1
('lower urinary tract symptom'/exp OR 'lutd':ti,ab OR 'luts':ti,ab OR 'lower urinary tract disease':ti,ab OR 'lower urinary tract diseases':ti,ab OR 'lower urinary tract disorder':ti,ab OR 'lower urinary tract disorders':ti,ab OR 'lower urinary tract dysfunction':ti,ab OR 'lower urinary tract symptom':ti,ab OR 'lower urinary tract symptoms':ti,ab OR 'urinary tract infection'/exp OR 'uti (urinary tract infection)':ti,ab OR 'genitourinary tract infection':ti,ab OR 'lower urinary tract infection':ti,ab OR 'tractus urogenitalis infection':ti,ab OR 'urinary infection':ti,ab OR 'urinary tract infection':ti,ab OR 'urinary tract infections':ti,ab OR 'urine infection':ti,ab OR 'urine tract infection':ti,ab OR 'urologic infection':ti,ab OR 'urological infection':ti,ab OR 'overactive bladder syndrome':ti,ab OR 'urine incontinence'/exp OR 'bladder incontinence':ti,ab OR 'incontinence, urine':ti,ab OR 'incontinentia urinae':ti,ab OR 'urinary incontinence':ti,ab OR 'urinary leakage':ti,ab OR 'urine bladder incontinence':ti,ab OR 'urine incontinence':ti,ab OR 'urine leakage':ti,ab OR 'wetting, urine':ti,ab OR 'bladder pain syndrome'/exp OR 'bladder pain syndrome':ti,ab OR 'hypotonic bladder'/exp OR 'atonic bladder':ti,ab OR 'atonic urinary bladder':ti,ab OR 'bladder atonia':ti,ab OR 'bladder atony':ti,ab OR 'bladder hypotonia':ti,ab OR 'detrusor underactivity':ti,ab OR 'flaccid bladder':ti,ab OR 'hypotonic bladder':ti,ab OR 'hypotonic urinary bladder':ti,ab OR 'underactive bladder':ti,ab OR 'underactive detrusor':ti,ab OR 'underactive detrusor function':ti,ab OR 'underactive urinary bladder':ti,ab OR 'urinary bladder, underactive':ti,ab OR 'prostatitis'/exp OR 'prostate infection':ti,ab OR 'prostatitis':ti,ab OR 'pelvic pain'/exp OR 'pelvic pain':ti,ab OR 'pelvis pain':ti,ab OR 'nephrolithiasis'/exp OR 'calculosis, kidney':ti,ab OR 'calculus, kidney':ti,ab OR 'familial nephrolithiasis':ti,ab OR 'kidney calculi':ti,ab OR 'kidney calculosis':ti,ab OR 'kidney calculus':ti,ab OR 'kidney calix stone':ti,ab OR 'kidney calyx stone':ti,ab OR 'kidney lithiasis':ti,ab OR 'kidney pelvis stone':ti,ab OR 'kidney stone':ti,ab OR 'kidney stone passage':ti,ab OR 'kidney stone, pelvis':ti,ab OR 'kidney stones':ti,ab OR 'nephrolith':ti,ab OR 'nephrolith passage':ti,ab OR 'nephrolithiasis':ti,ab OR 'renal calculogenesis':ti,ab OR 'renal calculosis':ti,ab OR 'renal calculus':ti,ab OR 'renal lithiasis':ti,ab OR 'renal pelvis stone':ti,ab OR 'renal stone':ti,ab OR 'renolithiasis':ti,ab OR 'stone, kidney':ti,ab OR 'bladder cancer'/exp OR 'bladder cancer':ti,ab OR 'urinary bladder cancer':ti,ab OR 'urine bladder cancer':ti,ab OR 'vesical cancer':ti,ab) AND ('microbiome'/exp OR 'microbiome':ti,ab OR 'microbiomes':ti,ab OR microbiota:ti,ab) 984
#2

('lower urinary tract symptom'/exp OR 'lutd':ti,ab OR 'luts':ti,ab OR 'lower urinary tract disease':ti,ab OR 'lower urinary tract diseases':ti,ab OR 'lower urinary tract disorder':ti,ab OR 'lower urinary tract disorders':ti,ab OR 'lower urinary tract dysfunction':ti,ab OR 'lower urinary tract symptom':ti,ab OR 'lower urinary tract symptoms':ti,ab) AND ('virus'/exp OR 'virus':ti,ab OR 'viruses':ti,ab OR 'bacteriophage'/exp OR 'bacterial virus':ti,ab OR 'bacteriophage':ti,ab OR 'bacteriophages':ti,ab OR 'fungus'/exp OR 'fungi':ti,ab OR 'fungus':ti,ab OR '16s rrna sequencing':ti,ab) 144