The Urobiome and Its Role in Overactive Bladder

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Urine is no longer considered to be sterile. After the existence of the microbiome was revealed through metagenomic analysis using next-generation sequencing, the relationship between characteristics of the microbiome and diseases have been studied and published in various journals. A microbiome exists in the urinary tract and is associated with urinary tract infection, malignancy of the genitourinary tract, and lower urinary tract symptoms. Based on the urine sampling method, sampling site, culture method, and sex, the characteristics of the microbiome vary. Most of the Lactobacillus species are identified mainly in women, and various other species are identified in men. These microorganisms can cause or prevent various diseases. Variations in the microbiome are seen in those with and without disease, and an asymptomatic status does not indicate the absence of microbes. This microbiome has been implicated in a variety of lower urinary tract symptoms and diseases, in particular, overactive bladder. The microbiome differs between patients with urgency and urge urinary incontinence and healthy individuals. There are many aspects of the microbiome yet to be studied in relation to other lower urinary tract symptoms.

Keywords: Microbiota; Overactive bladder; Biomarker

INTRODUCTION

The term microbiota was first described by Lederberg and McCray, and ‘microbiome’ refers to the entire habitat, including the microorganisms such as bacteria, archaea, lower and higher eukaryotes, viruses, and their genome and surrounding environment [1]. Through next-generation sequencing (NGS) [2], in addition to the pathological strains previously confirmed through culture tests, many normal flora (microbiota) were identified. These microbial environments keep humans healthy, prevent diseases, and play a role in homeostasis. Changes in these environments cause diseases by disrupting homeostasis. Microbiomes are commonly found in the skin, placenta, mammary glands, seminal fluids, uterus, ovarian follicles, lung, saliva, oral mucosa, conjunctiva, biliary tract, and gastrointestinal tract system [3,4]. In 2015, Kogan et al. [5] announced that human urine was not sterile. Many researchers have confirmed the existence of microbiota in the human urinary tract through metagenomics (DNA-dependent) and metaculturomics (culture-dependent) technologies. Previously, it was believed that urine is sterile and that uropathogens invaded and caused urinary tract infections (UTIs). However, as the existence of the microbiome in urine-the urobiome-was confirmed, a new paradigm for the pathogenesis of diseases was suggested. The urobiome plays a role in preventing infections caused by pathogens, forms a barrier, and is necessary to maintain a healthy...
urinary tract; it has helped us gain a deeper understanding of various diseases. Currently, the microbiome is being studied in various fields of urology, such as UTI, interstitial cystitis, and genitourinary tract malignancy.

In addition to UTIs, the notion that the microbiome is associated with various urinary tract diseases is gaining popularity, and its relationship with various lower urinary tract symptoms is being studied [6,7]. A precise understanding of the urobiome is necessary to understand and treat urinary diseases in relation to the microbiome.

Among the disease groups with lower urinary tract symptoms, there are diseases whose prevalence is increasing owing to aging of the population, and the representative disease among them is overactive bladder (OAB). In the absence of an obvious infection, overactive bladder syndrome is characterized by urinary urgency, with or without urgency urinary incontinence, and is usually accompanied by frequency and nocturia [8]. The cause of OAB remains unknown. However, obesity, caffeine intake, constipation, diabetes, poor mobility, and chronic pelvic pain may be risk factors [9,10]. The diagnosis is mainly based on the signs and symptoms after UTI or neurological deficit has been excluded. It has a prevalence of 9%–43% in women and 7%–27% in men, and the prevalence tends to increase with age. Originally, detrusor overactivity (DO) was believed to be the major pathophysiological cause of OAB. DO can either be ‘myogenic,’ where the detrusor muscle contracts due to autonomic stimulation, or ‘neurogenic,’ where detrusor contraction is induced by the urgency signal released by the central nervous system. Recently, as the importance of the urothelium/suburothelium has been emphasized; different approaches and analyses for pathophysiology have been developed. In terms of the urothelium/suburothelium, various elements are being studied, including the urinary microbiota or urobiome. Peyronnet et al. [11] reported the pathophysiological factors affecting OAB are metabolic syndrome, affective disorder, sex hormone deficiency, urinary microbiota, gastrointestinal functional disorder, and subclinical autonomic nervous system dysfunction. In this regard, we aimed to investigate the urobiome and its relevance in OAB.

WHAT IS UROBIOME?

The urobiome refers to the microbiome of the urinary tract [12]. Since there was a strong assumption that urine is sterile in healthy people, the microbiome of urine was not included in the initial Human Genome Project. However, as the microbiomes of other organs have been attracting attention, and various microbiomes have been identified in various environments since then, there is an increase in the research regarding the microbiome of the urinary tract and its involvement in urologic diseases. However, studies show different results depending on the study conditions, and there are some difficulties in matching these conditions for comparisons between studies. There are several criteria to be considered when conducting research, which are: (1) collection method, (2) sex, (3) age, and (4) specific diseases of each organ of the urinary tract.

METHODS USED FOR URINARY MICROBIOME ANALYSIS

Microorganism detection methods can be divided into culture-based and sequencing-based methods. Traditionally, microorganisms have been identified by a standard urine culture method using MacConkey agar or blood agar. Rapid-growing and aerobic microorganisms were mainly identified. However, there is a disadvantage to culture-based methods as anaerobic and slow-growing microorganisms cannot be cultured.

The existence of the microbiome was confirmed by metagenomic analysis using a method called NGS [13]. It is a sequencing-based method that identifies bacteria through DNA sequences rather than the traditional method of cultivating bacteria. It is divided into 2 types, amplicon sequencing using ‘marker genes’ such as 16S rRNA subunit with 9 hypervariable regions, and shotgun sequencing to analyze the entire microbiome sequence [14]. The disadvantage of the sequencing methods is that the viability of the microbes cannot be confirmed. An enhanced quantitative urine culture (EQUC), as a traditional culture-based method, can identify bacteria that were previously difficult to identify and compensate for such shortcomings [15]. An EQUC is performed by incubating the sample that was cultured using blood, chocolate, and colistin-nalidixic acid agar for 48 hours at 35°C. A disadvantage of EQUC is that it identifies a broad range of bacteria, requiring interpretation of the results between patients and healthy people. Therefore, it is recommended that 16S rRNA gene sequencing and EQUC be performed simultaneously.

PROBLEMS WITH URINE COLLECTION

The existence of a microbiome in urine has been established
by NGS and has been drawing attention from researchers. The detection of the diversity of the microbiome is impacted by various factors, one often mentioned is the sample collection method [16]. Since the microbiome is greatly influenced by the surrounding environment, different results are achieved depending on where the sample used for the study was collected. Pohl et al. [17] reported that a different microbiome was identified in the urine that passed through both the bladder and urethra through self-voiding compared with the sample that bypassed the urethra through the catheter. Wolfe and Brubaker [18], 'Is it self voiding?' 'Is it midstream?' 'Is it Catheterization?' 'Is it a blade puncture?' Different results were obtained depending on the differences in the urinary tract through which the sample urine passed [18] (Fig. 1). In another study, there was no difference in the microbiome results between the first and mid streams [19]. This means that the microbiome in the urethral environment and the microbiome of the bladder are different, and whether the microbiome of the urethra is included impacts the results. Regarding the upper ureter, its microbiome is affected by changes in the microbial environment at the kidney level. Studies have shown that a decrease in estimated glomerular filtration rate reduces microbiome diversity [20]. Although the microbiome differs from individual to individual, depending on odor, method of sample collection, and sex, there is one important thing in common: there is a difference between asymptomatic and symptomatic patients. It is important to remember that an asymptomatic status does not indicate the absence of disease.

DIFFERENCES IN THE URINARY MICROBIOME BETWEEN MEN AND WOMEN

The male and female reproductive microbiomes are different [21]. In a recent study, it was confirmed that the microbiome found in the genital area of men and women was different before sexual intercourse, but was similar after sexual intercourse [22]. The urine microbiome also differs between men and women [17,23]. There are several studies that analyzed urine samples collected from healthy men and women using various methods, such as clean catch urine, transurethral catheter urine, suprapubic puncture, midstream urine, and first catch urine; the common discovery was that Lactobacillus was mainly identified in women, despite the difference in the sampling methods. However, in men, a heterogeneous result was obtained based on the sampling method (Table 1).

AGE

There is a controversy about age as a factor influencing the microbiome, there are reports of a lack of relevance [24] and correlation [3,25,26]. When checked if microbiome is directly affected by age, it was found that there was no significant age-related change; however, there was a difference between the young and old population [27,28]. Rather than considering age as a factor, the change in microbiome can be considered to be a secondary change associated with aging. Various changes in the body as a result of aging can cause changes in the environmental aspect of the microbiota, which affects microbiome diversity and commensal microorganisms, which can be seen not only in people with diseases but also in healthy people. In women, hormonal changes occur because of aging; therefore, menopause is an important criterion for change in the microbiome. It is evident that environmental changes in the vaginal microbiome caused by female hormones cause changes in the urobiome. According to several studies, Lactobacillus is abundant in the urine of premenopausal women, and less so in postmenopausal women. In addition, Mobiluncus spp. were found abundantly in postmenopausal women [24].

Fig. 1. Differences in urine collection methods in relation to the urobiome (invasiveness and contamination). In the case of suprapubic puncture, only the microbiome of the bladder was identified, and the invasiveness was the highest. Conversely, self-voiding methods (first catch urine, midstream urine, and clean catch urine) are less invasive but are contaminated with microbiota from the urethral flora.
### Table 1. Strains identified in the urobiome according to sex and urine collection method

| Sex   | Study                        | No. of patients | Urine collection method | Urobiome                                                                                       |
|-------|------------------------------|-----------------|--------------------------|------------------------------------------------------------------------------------------------|
| Female | Siddiqui et al. [65] (2011)  | 8               | CCU                      | Lactobacillus, Prevotella, Gardnerella, Peptoniphilus, Dialister, Finegoldia, Anaerococcus, Allstonella, Streptococcus, Staphylococcus |
|       | Wolfe et al. [66] (2012)     | 12              | CCU, TUC, SPA            | Lactobacillus, Actinobaculum, Aerococcus, Anaerococcus, Atopobium, Burkholderia, Corynebacterium, Gardnerella, Prevotella, Ralstonia, Sneathia, Staphylococcus, Streptococcus, Veillonella |
|       | Fouts et al. [14] (2012)     | 15              | MSU                      | Lactobacillus, Corynebacterium, Staphylococcus, Streptococcus, Prevotella                      |
|       | Lewis et al. [67] (2013)     | 10              | CCU                      | Firmicutes, Actinobacteria, Bacteroidetes                                                     |
|       | Hilt et al. [68] (2014)      | 24              | TUC                      | Lactobacillus, Corynebacterium, Streptococcus, Actinomyces, Staphylococcus, Aerococcus, Gardnerella, Bifidobacterium, Actinobaculum |
|       | Pearce et al. [29] (2014)    | 58              | TUC                      | Lactobacillus, Gardnerella, Corynebacterium, Enterobacteriaceae, Anaerococcus, Bifidobacterium, Streptococcus, Staphylococcus, Sneathia, Peptoniphilus, Atopobium, Rhizobacter, Trueperella, Alloscardovia, Veillonella |
|       | Karstens et al. [56] (2016)  | 10              | TUC                      | Anoxybacillus, Lactobacillus, Prevotella, Gardnerella, Arthrobacter, Escherichia, Shigella    |
|       | Thomas-White et al. [30] (2016) | 60             | TUC                      | Lactobacillus, Gardnerella, Staphylococcus, Streptococcus, Enterococcus, Bifidobacterium, Atopobium, Enterobacteriaceae |
|       | Wu et al. [69] (2017)        | 25              | TUC                      | Lactobacillaceae, Prevotellaceae, Enterobacteriaceae, Veillonellaceae, Tissierellaceae, Bifidobacteriales |
|       | Gottschick et al. [70] (2017) | 49             | MSU                      | Lactobacillus crispatus                                                                     |
|       | Abernethy et al. [71] (2017) | 20              | TUC                      | Lactobacillus acidophilus                                                                   |
|       | Wang et al. [72] (2017)      | 21              | MSU                      | Lactobacillus, Variibaculum, Porphyromonas, Prevotella, Bacteroides                          |
|       | Rani et al. [73] (2017)      | 5               | MSU                      | Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes                                    |
|       | Komesu et al. [28] (2018)    | 84              | TUC                      | Lactobacillus, Gardnerella, Tepidimonas, Prevotella                                         |
|       | Meriwehther et al. [74] (2019) | 18             | MSU                      | Lactobacillus, Prevotella                                                                   |
|       | Bresler et al. [75] (2019)   | 20              | MSU                      | Lactobacillus                                                                               |
|       | Liu et al. [76] (2020)       | 3               | TUC                      | Gardnerella, Pontibacter, Sphingomonas, Prevotella, Propionibacterium                        |
| Male   | Fouts et al. [14] (2012)     | 11              | MSU                      | Corynebacterium, Staphylococcus, Streptococcus, Lactobacillus, Gardnerella, Veillonella      |
|       | Nelson et al. [77] (2012)    | 18              | FCU                      | Corynebacterium, Lactobacillus, Staphylococcus, Gardnerella, Streptococcus, Anaerococcus, Veillonella, Prevotella, Escherichia |
|       | Lewis et al. [67] (2013)     | 6               | CCU                      | Firmicutes                                                                                  |
|       | Rani et al. [73] (2017)      | 3               | MSU                      | Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes                                    |
|       | Wu et al. [78] (2018)        | 18              | MSU                      | Escherichia-Shigella, Staphylococcus, Streptococcus, Aeromonas, Acinetobacter, Bacteroides, Lactobacillus |
|       | Bučević Popović et al. [79] (2018) | 19         | MSU                      | Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria                                    |
|       | Kassiri et al. [80] (2019)   | 10              | TUC                      | Staphylococcus, Variibaculum, Peptoniphilus, Actinobaculum                                  |
|       | Xie et al. [81] (2020)       | 21              | NA                       | Acinetobacter, Prevotella, Oscillospira, Parabacteroides, Fusobacterium                      |
|       | Liu et al. [76] (2020)       | 9               | TUC                      | Gardnerella, Pontibacter, Sphingomonas, Prevotella, Propionibacterium                        |

*Lactobacillus* was mainly identified in women, despite the difference in the sampling methods.

CCU, clean catch urine; TUC, transurethral catheter; SPA, suprapubic aspirate; MSU, midstream urine; FCU, first catch urine; NA, not available.
DISEASE-RELATED CHANGES IN THE URINARY MICROBIOME IN WOMEN IN RELATION TO CHANGES IN THE GUT MICROBIOME

Some urologic diseases in women are a result of the imbalance in the urobiome, such as: OAB syndrome, urge urinary incontinence (UUI), interstitial cystitis/bladder pain syndrome, asymptomatic bacteriuria, and neurogenic bladder dysfunction. Although there is no clear mechanism or association yet, many studies have shown a link between the disease and the microbiome [14,25,29-33]. Lactobacillus, which was identified as an important contributor in these diseases, changes the vaginal flora while also changing the gut microbiome through oral probiotics and causes diseases such as diarrhea in the gastrointestinal tract. These changes play a role in the prevention of UTIs. A recent study also reported that changes in the gut microbiota affect OAB and daily urgency [34]. There was a difference in the gut microbiota between the group with OAB symptoms and urgency and the asymptomatic group. It is common to consider the urinary microbiome to be an ascending infection of the gut. The urinary microbiome was found to be 62.5% similar to the gut microbiome and 32% similar to the vaginal microbiome; therefore, they are closely related to each other [16,35-37].

URINARY BIOMARKERS AND MICROBIOME IN OVERACTIVE BLADDER

In OAB syndrome, various urine biomarkers were extensively studied to distinguish phenotypes along with the diagnosis [3,4,38,39]. A representative example is nerve growth factor (NGF) and brain-derived neurotrophic factor, a neuropeptide that is produced in the urothelium or smooth muscle cells is widely used as a marker of lower urinary tract dysfunction. According to Suh et al. [40], urinary NGF increases in patients with OAB, hence is considered an important biomarker for the treatment of OAB. Another biomarker is microRNA in genomics. miRNAs have also been used as biomarkers for urothelial carcinoma in the urological field [41,42]. Some microRNAs, such as miR-103a-3p, miR-10a-5p, and miR-199a-3p, have been used as urinary biomarkers of DO or underactivity [43].

The interdependence between antimicrobial peptides (AMPs), specifically beta-adhesive AMP, and the microbiota increased the risk of UTI after surgery [44]. In addition, levels of prostaglandin E_{2} (PGE_{2}), an inflammatory marker also increases in OAB [45]. PGE_{2} is also associated with bladder capacity, and its levels are significantly decreased after administration of intravesicular botulinum toxin A injection [46]. Furthermore, high levels of PEG; are observed in UUI and is considered as a potential target for future treatment. Urinary adenosine triphosphate (ATP) is also involved in contraction and relaxation of the detrusor muscle, and in patients with OAB, it may be overexpressed or its concentration in urine may change [47,48]. Urinary b3-adrenoceptor (B3-AR) is involved in detrusor relaxation, and the Trp64Arg polymorphism in B3-AR is closely related to DO [49]. The urinary microbiome shows characteristics specific to the disease, a large number of specific strains are identified in diseases such as UUI across various samples. As a result, it can be used as a useful biomarker of urine if it is further standardized and researched based on a large number of subjects (Table 2).

CORRELATION BETWEEN OVERACTIVE BLADDER SYMPTOMS AND MICROBIOME

In the absence of a UTI, the signs and symptoms (urgency complaints) are diagnosed as a representative bladder storage disorder. However, as the urothelium/suburothelium is known to play an important role in pathophysiology of OAB, studies about the correlation between the symptoms and microbiome of OAB are being conducted. According to Peyronnet et al. [11], OAB phenotypes include metabolic syndrome, affective disorder, sex hormone deficiency, functional gastrointestinal disorder, autonomic nervous system dysfunction, and ‘urinary microbiota’ depending on the pathophysiological factors. It is also predicted that brain-bladder-microbiota amblyopia exists as the gut microbiota forms the brain-gut-microbiota axis.

However, it has not yet shown any significant results for each symptom, and the relationship with the microbiome has been revealed in some symptoms. In general, symptoms of OAB include frequency, urgency, nocturia, and urge incontinence. The role of the microbiome in each can be summarized as shown in Table 2. Studies have shown that there is a difference in the microbiome of patients diagnosed with OAB, regardless of the symptoms. According to Curtiss et al. [50], there is a study showing that Proteus was significantly abundant in patients with OAB compared to healthy people, and at the same time, Lactobacillus was less prevalent (Table 3).
Table 2. Urinary biomarkers of overactive bladder

| Type         | Urinary biomarker in OAB | Source                        | Function                                                                                                  | Levels                        |
|--------------|--------------------------|-------------------------------|-----------------------------------------------------------------------------------------------------------|-------------------------------|
| Neurotrophin | NGF                      | Urothelium and smooth muscle cell | Induce bladder overactivity                                                                               | Increased in OAB              |
|              | BDNF                     |                               |                                                                                                           |                               |
| Genomics     | miRNA                    | RNA                           | Biomarker for bladder dysfunction and storage symptoms after surgery                                     | Marker for fibrosis and BOO   |
|              |                           |                               | Distinguish between detrusor overactivity and detrusor underactivity                                       |                               |
|              |                           |                               | Related to bladder outlet obstruction relief moment and bladder fibrotic pathway                           |                               |
|              |                           |                               | For clinical application, it is difficult to obtain tissue because it is invasive                         |                               |
|              | Urinary b3-adrenoceptor (B3-AR) |                               | Genetic variation in B3-AR lead to impaired detrusor relaxation                                           |                               |
| Compound     | Urinary ATP              | Urothelium                    | Bladder stretch or inflammation transduce bladder information into suburothelial nerve fibers           | Increased in OAB              |
| Immunity     | Antimicrobial peptide    | Host innate immunity          | Related to UTI risk                                                                                      | Increased in OAB              |
|              | Prostaglandin E<sub>2</sub> | Inflammatory marker          | Not clear in OAB but changes in detrusor state                                                           | Increased in OAB decreased in DU|
| Microorganism| Urinary microbiota       | Normal flora or gut or vagina | Closely related UUI, SUI, UTI, IC/BPS or CP/CPSS                                                        | Closely related to UUI        |

NGF, nerve growth factor; BDNF, brain-derived neutrophic factor; OAB, overactive bladder; miRNA, micro ribonucleic acid; BOO, bladder outlet obstruction; ATP, adenosine triphosphate; UTI, urinary tract infection; DU, detrusor underactivity; UUI, urge urinary incontinence; SUI, stress urinary incontinence; IC/BPS, interstitial cystitis/bladder pain syndrome; CP/CPPS, chronic prostatitis/chronic pelvic pain syndrome.

**Frequency**

There are negligible number of papers that mention the correlation between frequency and the microbiome. However, when examining each symptom cluster using EQUC in patients UTI, the presence of *Lactobacillus* or *Klebsiella* among the symptom clusters with frequent symptoms were significantly prevalent compared to the asymptomatic group [51]. We concluded that more clear research is needed in this area.

**Nocturia**

Although the correlation between nocturia symptoms and the microbiome is unknown, Holland et al. [52] reported that nocturia was related to the International Prostate Symptom Score (IPSS). *Lachnospiraceae Blautia* in urinary operational taxonomy units has bothersome symptoms, is associated with severity, has protective effects in IPSS, and is also associated with nocturia.

**Urgency**

A study compared urgency with Overactive Bladder Symptom Score using the gut microbiome and not urine microbiome. In the OAB group, bacterial diversity was low, the *Bifidobacterium* group was low, and the *Faecali* bacterium group was abundant.

**Urinary incontinence**

Among the symptoms associated with OAB, urinary incontinence is the most studied and is the best-known symptom of microbiome imbalance [53]. Although incontinence and, in particular, UUI, are not essential symptoms of OAB, various studies have considered their relationship with DO [25,29,30,33,34,54-59].

Pearce et al. [29] compared patients with and without UUI symptoms and confirmed a difference in prevalence of *Gardnerella* and *Lactobacillus*. In the UUI group, presence of *Gardnerella* was high and *Lactobacillus* was low. In the same study, *Lactobacillus* spp. levels varied in the control group, *Lactobacillus crispatus* was high while *Lactobacillus gasseri* was low. In UUI, vaginal estrogen therapy in postmenopausal women resulted in a decrease in microbial diversity, a change in *Lactoba*
cillus levels in urine, and a change in UUI [60].

In addition to these strains, *E. coli* and some *Gardnella vaginalis* strains, classified as uropathogenic microorganisms, induce the release of calcium ions or ATP from the urothelial epithelium and are involved in the contraction of bladder smooth muscle cells, causing incontinence. In contrast, *Lactobacillus* has been reported to inhibit this process [61]. Furthermore, in patients with refractory UUI with recurrent UTI, diverse microbiota exists, but if colonization continues, the disease persists, so appropriate intervention is necessary [62].

There are studies on the role of the urobiome not only with respect to the presence or absence of UUI symptoms, but also to symptom severity. Karstens et al. [56] cultured bladder microbiomes of healthy individuals and individuals with UUI. The study identified 14 strains and established that there are differences in the relative abundance in healthy people and patients with UUI, and that the microbiome in individuals with UUI differed according to severity.

**LIMITATION OF OAB RESEARCH USING THE MICROBIOME**

There are several problems in studying the association between the symptoms of OAB, pathophysiology, and microbiome:

1. It can be concluded that there is some association, but in some cases, there is uncertainty regarding the predominance of a specific microbiome in the diseased and nondiseased individuals, for example: *Lactobacillus* predominance does not differ between adult women with mixed urinary incontinence and age-matched asymptomatic women, but some members of the genus *Lactobacillus* might be associated with urinary symptoms [28].

2. In many cases, the collected data are not suitable for studying the urobiome and data on a specific microbiome are insufficient or absent. Public databases are inadequate for studies of the urobiome and its relationship with bladder health and diseases because these databases lack urobiome-specific genomes [63]. This is due to the fact that the dominant microbiome is different under various environmental conditions. However, since there is a lack of standardization, several factors, such as the method and timing of sample collection, sex, and comorbidities, must be considered. Only when the research is conducted by considering all these aspects will it be possible to derive more reliable results on the relevance of a specific microbiome with respect to diseases or symptoms.

3. It is necessary to standardize the sample, especially in men, and the urethral environment must be considered. Men with more severe urinary symptoms are more likely to have detectable bladder bacteria than those with few severe or no symptoms. Voided urine does not adequately characterize the male bladder urobiome and catheterized urine should be used instead [64].

4. Studies with large sample sizes are scarce. More extensive and large-scale data is needed on samples of healthy and diseased individuals.

| Symptom            | Study                          | Year | Microbial strains                                                                 |
|--------------------|--------------------------------|------|-----------------------------------------------------------------------------------|
| Frequency          | Burnett et al. [51]            | 2021 | Few related studies in UTI, *Lactobacillus* or *Klebsiella* increased culture group has 'frequency' symptoms |
| Nocturia           | Holland et al. [52]            | 2020 | Urinary OUT related with *Lachnospiraceae Blautia* (protective correlation [negative correlation with symptom]) |
| Urgency            | Okamoto et al. [34]            | 2021 | *Bifidobacterium* group was low, and the *Faecalibacterium* bacterium group was abundant in gut microbiome (not urobiome) |
| Urge incontinence  | Pearce et al. [29]             | 2014 | Increased *Gardenerella* and decreased *Lactobacillus* in UUI                      |
|                    | Thomas-White et al. [60]       | 2020 | After vaginal estrogen therapy, decreased diversity and increased *Lactobacillus* |
|                    | Abbasian et al. [61]           | 2019 | *Lactobacillus* inhibit ATP and calcium ion release from urothelium and inhibit UUI decreased *Lactobacillus*, cannot inhibit bladder contraction, and UUI happen |
|                    | Karstens et al. [56]           | 2016 | 14 Bacterial species identification in healthy and UUI decreased diversity has effects to symptom severity |

UTI, urinary tract infection; UUI, urge urinary incontinence; ATP, adenosine triphosphate.
CONCLUSIONS

The discovery of the microbiome using NGS has completely changed the disease paradigm. Urine is not considered sterile anymore. In situations where association between various diseases of the urinary tract and the role of the urothelium are emerging, various clinical aspects of OAB have been shown closely related to the urobiome. Various approaches and considerations, such as the gut microbiome and vaginal flora, are required to study the relationship between the urobiome and OAB. However, since the size of the study and the sample size was not large enough, a clearer and more accurate analysis is needed through a larger-scale and harmonized research method.

AUTHOR CONTRIBUTION STATEMENT

· Conceptualization: CH
· Data curation: BSR
· Formal analysis: BSR, CH
· Funding acquisition: BSR, CH
· Methodology: CH
· Project administration: CH
· Visualization: BSR
· Writing - original draft: BSR
· Writing - review & editing: BSR, CH

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REFERENCES

1. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. Microbiome 2015;3:31.
2. Park ST, Kim J. Trends in next-generation sequencing and a new era for whole genome sequencing. Int Neurourol J 2016;20(Suppl 2):S76-83.
3. Drake MJ, Morris N, Apostolidis A, Rahnama’i MS, Marchesi JR. The urinary microbiome and its contribution to lower urinary tract symptoms; ICI-RS 2015. Neurourol Urodyn 2017;36:850-3.
4. Aragón IM, Herrera-Imbroda B, Queipo-Ortuño MI, Castillo E, Del Moral JS, Gómez-Millán J, et al. The urinary tract microbiome in health and disease. Eur Urol Focus 2018;4:128-38.
5. Kogan MI, Naboka YL, Ibishev KS, Gudima IA, Naber KG. Human urine is not sterile - shift of paradigm. Urol Int 2015;94:445-52.
6. Kim A, Ahn J, Choi WS, Park HK, Kim S, Paick SH, et al. What is the cause of recurrent urinary tract infection? Contemporary microscopic concepts of pathophysiology. Int Neurourol J 2021;25:192-201.
7. Kim MS, Jung SI. The urinary tract microbiome in male genitourinary diseases: focusing on benign prostate hyperplasia and lower urinary tract symptoms. Int Neurourol J 2021;25:3-11.
8. Drake MJ. Do we need a new definition of the overactive bladder syndrome? ICI-RS 2013. Neurourol Urodyn 2014;33:622-4.
9. Al-Shaiji TF, Radomski SB. Relationship between body mass index and overactive bladder in women and correlations with urodynamic evaluation. Int Neurourol J 2012;16:126-31.
10. Uzun H, Yilmaz A, Kemik A, Zorba OU, Kalkan M. Association of insulin resistance with overactive bladder in female patients. Int Neurourol J 2012;16:181-6.
11. Peyronnet B, Mironska E, Chapple C, Cardozo L, Oelke M, Dmochowski R, et al. A comprehensive review of overactive bladder pathophysiology: on the way to tailored treatment. Eur Urol 2019;75:988-1000.
12. Shoemaker R, Kim J. Urobiome: an outlook on the metagenome of urological diseases. Investig Clin Urol 2021;62:611-22.
13. Wolfe AJ, Brubaker L. “Sterile urine” and the presence of bacteria. Eur Urol 2015;68:173-4.
14. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med 2012;10:174.
15. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, et al. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. J Clin Microbiol 2016;54:1216-22.
16. Perez-Carrasco V, Soriano-Lerma A, Soriano M, Gutiérrez-Fernández J, García-Salcedo JA. Urinary microbiome: yin and yang of the urinary tract. Front Cell Infect Microbiol 2021;11:617002.
17. Pohl HG, Groah SL, Pérez-Losada M, Ljungberg I, Sprague BM, Chandal N, et al. The urine microbiome of healthy men and women differs by urine collection method. Int Neurourol J 2020;24:41-51.
18. Wolfe AJ, Brubaker L. Urobiome updates: advances in urinary microbiome research. Nat Rev Urol 2019;16:73-4.
19. Ozer MS, Yildiz HA, Incir C, Deger MD, Bozkurt O, Ergor G, et al.
Urinary microbiota; which non-invasive urine collection method should we use? Int J Clin Pract 2021;75:e14193.
20. Kramer H, Kuffel G, Thomas-White K, Wolfe AJ, Vellanki K, Leehey DJ, et al. Diversity of the midstream urine microbiome in adults with chronic kidney disease. Int Urol Nephrol 2018;50:1123-30.
21. Koedooder R, Mackens S, Budding A, Fares D, Blockeel C, Laven J, et al. Identification and evaluation of the microbiome in the female and male reproductive tracts. Hum Reprod Update 2019;25:298–325.
22. Ghorbani M, Torres AR, Duncan G, Colwell R, Daddani M, Mc Cord B. The genital microbiome and its potential for detecting sexual assault. Forensic Sci Int Genet 2021;51:102432.
23. Lee SJ. Commentary on “the urine microbiome of healthy men and women differs by urine collection method”. Int Neurourol J 2020;24:182-4.
24. Curtiss N, Balachandran A, Kriska L, Peppiatt-Wildman C, Wildman S, Duckett J. Age, menopausal status and the bladder microbiome. Eur J Obstet Gynecol Reprod Biol 2018;228:126-9.
25. Pearce MM, Zilliox MJ, Rosenfeld AB, Richter HE, Nager CW, et al. The female urinary microbiome in urgency urinary incontinence. Am J Obstet Gynecol 2015;213:347.e1-11.
26. Thomas-White KJ, Gao X, Lin H, Fok CS, Ghanayem K, Mueller ER, et al. Urinary microbes and postoperative urinary tract infection risk in urogynecologic surgical patients. Int Urogynecol J 2018;29:1797-805.
27. Liu F, Ling Z, Xiao Y, Yang Q, Zheng L, Jiang P, et al. Characterization of the urinary microbiota of elderly women and the effects of type 2 diabetes and urinary tract infections on the microbiota. Oncotarget 2017;8:100678-90.
28. Komesu YM, Richter HE, Carper B, Dinwiddie DL, Lukasz ES, Siddiqui NY, et al. The urinary microbiome in women with mixed urinary incontinence compared to similarly aged controls. Int Urogynecol J 2018;29:1785-95.
29. Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. mBio 2014;5:e01283-14.
30. Thomas-White KJ, Hilt EE, Fok C, Pearce MM, Mueller ER, Kliethermes S, et al. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J 2016;27:723-33.
31. Siddiqui H, Lagesen K, Nederbragt AJ, Jeansson SL, Jakobsen KS. Alterations of microbiota in urine from women with interstitial cystitis. BMC Microbiol 2012;12:205.
32. Neugent ML, Hulyalkar NV, Nguyen VH, Zimmern PE, De Nisco NJ. Advances in understanding the human urinary microbiome and its potential role in urinary tract infection. mBio 2020;11:e00218-20.
33. Price TK, Lin H, Gao X, Thomas-White KJ, Hilt EE, Mueller ER, et al. Bladder bacterial diversity differs in continent and incontinent women: a cross-sectional study. Am J Obstet Gynecol 2020;223:729.e1-10.
34. Okamoto T, Hatakeyama S, Imai A, Yamamoto H, Yoneyama T, Mori K, et al. Altered gut microbiome associated with overactive bladder and daily urinary urgency. World J Urol 2021;39:847-53.
35. Modena BD, Milam R, Harrison F, Cheeseman JA, Abecassis MM, Friedewald JJ, et al. Changes in urinary microbiome populations correlate in kidney transplants with interstitial fibrosis and tubular atrophy documented in early surveillance biopsies. Am J Transplant 2017;17:712-23.
36. Diop K, Dufour JC, Levasseur A, Fenollar F. Exhaustive repertoire of human vaginal microbiota. Hum Microbiome 2019;11:100051.
37. Morand A, Cornu F, Dufour JC, Tsinaratos M, Lagier JC, Raoult D. Human bacterial repertoire of the urinary tract: a potential paradigm shift. J Clin Microbiol 2019;5:e00675-18.
38. Antunes-Lopes T, Cruz F. Urinary biomarkers in overactive bladder: revisiting the evidence in 2019. Eur Urol Focus 2019;5:329-36.
39. Suh YS, Ko KJ, Kim TH, Lee HS, Sung HH, Cho WJ, et al. Potential biomarkers for diagnosis of overactive bladder patients: urinary nerve growth factor, prostaglandin E2, and adenosine triphosphate. Int Neurourol J 2017;21:171-7.
40. Suh YS, Ko KJ, Kim TH, Lee HS, Sung HH, Cho WJ, et al. Urinary nerve growth factor as a potential biomarker of treatment outcomes in overactive bladder patients. Int Neurourol J 2017;21:270-81.
41. Sapre N, Macintyre G, Clarkson M, Naeem H, Cmero M, Kowlczyk A, et al. A urinary microRNA signature can predict the presence of bladder urothelial carcinoma in patients undergoing surveillance. Br J Cancer 2016;114:454-62.
42. Yun SJ, Jeong P, Kang HW, Kim YH, Kim EA, Yan C, et al. Urinary microRNAs of prostate cancer: virus-encoded hsv1-miR-H18 and hsv2-miR-H9-5p could be valuable diagnostic markers. Int Neurourol J 2015;19:74-84.
43. Monastyrskaya K, Burkhard FC. Urinary biomarkers for bladder outlet obstruction. Curr Bladder Dysfunt Rep 2017;12:129-37.
44. Nienhouse V, Gao X, Dong Q, Nelson DE, Toh E, McKinley K, et al. Interplay between bladder microbiota and urinary antimicrobial peptides: mechanisms for human urinary tract infection risk and symptom severity. PLoS One 2014;9:e114185.
45. Kim JC, Park EY, Hong SH, Seo SI, Park YH, Hwang TK. Changes...
of urinary nerve growth factor and prostaglandins in male patients with overactive bladder symptom. Int J Urol 2005;12:875-80.
46. Chuang YC, Yoshimura N, Huang CC, Wu M, Chiang PH, Chancellor MB. Intravesical botulinum toxin A administration inhibits COX-2 and EP4 expression and suppresses bladder hyperactivity in cyclophosphamide-induced cystitis in rats. Eur Urol 2009;56:159-66.
47. Ford AP. In pursuit of P2X3 antagonists: novel therapeutics for chronic pain and afferent sensitization. Purinergic Signal 2012;8:3-26.
48. Harvey RA, Skennerton DE, Newgreen D, Fry CH. The contractile potency of adenosine triphosphate and ecto-adenosine triphosphatase activity in guinea pig detrusor and detrusor from patients with a stable, unstable or obstructed bladder. J Urol 2002;168:1235-9.
49. Qu HC, Zhang W, Liu YL, Wang P. Association between polymorphism of β3-adrenoceptor gene and overactive bladder: a meta-analysis. Genet Mol Res 2015;14:2495-501.
50. Curtiss N, Balachandran A, Kraska L, Peppiatt-Wildman C, Wildman S, Duckett J. A case controlled study examining the bladder microbiome in women with Overactive Bladder (OAB) and healthy controls. Eur J Obstet Gynecol Reprod Biol 2017;214:31-5.
51. Burnett LA, Hochstedler BR, Weldon K, Wolfe AJ, Brubaker L. Recurrent urinary tract infection: association of clinical profiles with urobiome composition in women. Neurourol Urodyn 2021;40:1479-89.
52. Holland B, Karr M, Delfino K, Dynda D, El-Zawahry A, Braund ME, Fleming A, et al. The effect of the urinary and faecal microbiota on lower urinary tract symptoms measured by the International Prostate Symptom Score: analysis utilising next-generation sequencing. BJU Int 2020;125:905-10.
53. Angelini KJ. An integrative review of current research on the role of the female urinary microbiota in overactive bladder symptoms. Urol Nurs 2017;37:94-100.
54. Govender Y, Gabriel I, Minassian V, Fichorova R. The current evidence on the association between the urinary microbiome and urinary incontinence in women. Front Cell Infect Microbiol 2019;9:133.
55. Brubaker L, Wolfe AJ. Microbiota in 2016: associating infection and incontinence with the female urinary microbiota. Nat Rev Urol 2017;14:72-4.
56. Karstens L, Asquith M, Davin S, Stauffer P, Fair D, Gregory WT, et al. Does the urinary microbiome play a role in urgency urinary incontinence and its severity? Front Cell Infect Microbiol 2016;6:78.
57. Ke QS, Lee CL, Kuo HC. Recurrent urinary tract infection in women and overactive bladder - is there a relationship? Tzu Chi Med J 2021;33:13-21.
58. Richter HE, Carnes MU, Komesu YM, Lukacz ES, Arya L, Bradley M, et al. Association between the urogenital microbiome and surgical treatment response in women undergoing midurethral sling operation for mixed urinary incontinence. Am J Obstet Gynecol 2022;226:93.e1-93.e15.
59. Siddiqui H, Lagesen K, Nederbragt AJ, Eri LM, Jeansson SL, Jakobsen KS. Pathogens in urine from a female patient with overactive bladder syndrome detected by culture-independent high throughput sequencing: a case report. Open Microbiol J 2014;8:148-53.
60. Thomas-White K, Taege S, Limeira R, Brincat C, Joyce C, Hilt EE, et al. Vaginal estrogen therapy is associated with increased Lactobacillus in the urine of postmenopausal women with overactive bladder symptoms. Am J Obstet Gynecol 2020;223:727.e1-11.
61. Abbasian B, Shair A, O’Gorman DB, Pena-Diaz AM, Brennan L, Engelbrecht K, et al. Potential role of extracellular ATP released by bacteria in bladder infection and contractility. mSphere 2019;4:e00439-19.
62. Chen Z, Phan MD, Bates LJ, Peters KM, Munkerjee C, Moore KH, et al. The urinary microbiome in patients with refractory urge incontinence and recurrent urinary tract infection. Int Urogynecol J 2018;29:1775-82.
63. Thomas-White K, Forster SC, Kumar N, Van Kuiken M, Putonti C, Stores MD, et al. Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. Nat Commun 2018;9:1557.
64. Bajic P, Van Kuiken ME, Burge BK, Kirshenbaum EJ, Joyce CJ, Wolfe AJ, et al. Male bladder microbiome relates to lower urinary tract symptoms. Eur Urol Focus 2020;6:376-82.
65. Siddiqui H, Nederbragt AJ, Lagesen K, Jeansson SL, Jakobsen KS. Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. BMC Microbiol 2011;11:244.
66. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol 2012;50:1376-83.
67. Lewis DA, Brown R, Williams J, White P, Jacobson SK, Marchesi JR, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. Front Cell Infect Microbiol 2013;3:41.
68. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol 2014;52:871-6.
69. Wu P, Chen Y, Zhao J, Zhang G, Chen J, Wang J, et al. Urinary microbiome and psychological factors in women with overactive
bladder. Front Cell Infect Microbiol 2017;7:488.
70. Gottschick C, Deng ZL, Vital M, Masur C, Abels C, Pieper DH, et al. The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. Microbiome 2017;5:99.
71. Abernethy MG, Rosenfeld A, White JR, Mueller MG, Lewicky-Gaupp C, Kenton K. Urinary microbiome and cytokine levels in women with interstitial cystitis. Obstet Gynecol 2017;129:500-6.
72. Wang H, Altemus J, Niazi F, Green H, Calhoun BC, Sturgis C, et al. Breast tissue, oral and urinary microbiomes in breast cancer. Oncotarget 2017;8:88122-38.
73. Rani A, Ranjan R, McGee HS, Andropolis KE, Panchal DV, Hajjiri Z, et al. Urinary microbiome of kidney transplant patients reveals dysbiosis with potential for antibiotic resistance. Transl Res 2017; 181:59-70.
74. Meriwether KV, Lei Z, Singh R, Gaskins J, Hobson DTG, Jala V. The vaginal and urinary microbiomes in premenopausal women with interstitial cystitis/bladder pain syndrome as compared to unaffected controls: a pilot cross-sectional study. Front Cell Infect Microbiol 2019;9:92.
75. Bresler L, Price TK, Hilt EE, Joyce C, Fitzgerald CM, Wolfe AJ, Fe-
76. Liu F, Zhang N, Jiang P, Zhai Q, Li C, Yu D, et al. Characteristics of the urinary microbiome in kidney stone patients with hypertension. J Transl Med 2020;18:130.
77. Nelson DE, Dong Q, Van der Pol B, Toh E, Fan B, Katz BP, et al. Bacterial communities of the coronal sulcus and distal urethra of adolescent males. PLoS One 2012;7:e36298.
78. Wu P, Zhang G, Zhao J, Chen J, Chen Y, Huang W, et al. Profiling the urinary microbiota in male patients with bladder cancer in china. Front Cell Infect Microbiol 2018;8:167.
79. Bučević Popović V, Šitum M, Chow CT, Chan LS, Roje B, Terzić J. The urinary microbiome associated with bladder cancer. Sci Rep 2018;8:12157.
80. Kassiri B, Shrestha E, Kasprenski M, Antonescu C, Florea LD, Sfanoš KS, et al. A prospective study of the urinary and gastrointestinal microbiome in prepubertal males. Urology 2019;131:204-10.
81. Xie J, Huang JS, Huang XJ, Peng JM, Yu Z, Yuan YQ, et al. Profiling the urinary microbiome in men with calcium-based kidney stones. BMC Microbiol 2020;20:41.