Review

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Lactobacillus and Urine Microbiome in Association with Urinary Tract Infections and Bacterial Vaginosis

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The traditional concept of "urine is sterile if urine culture and urinalysis are negative" has been overcome by new approaches using 16S ribosomal ribonucleic acid (rRNA) that demonstrated the presence of urinary microbiota. This mini-review article provides updated information of the human urinary microbiome related to urogenital tract infections (UTIs) and describes *Lactobacillus* in the maintenance of urogenital health and prevention of UTIs. The following keywords were used in combination with "Urinary tract symptoms", "Urogenital symptoms", and "Probiotics" in a search: "Bacterial Vaginosis", "Human Microbiome Project", "Lactobacillus", "Microbiome", and "Urinary Tract Infections." Here, changes in the urinary microbiome and differences in the abundance of *Lactobacillus* were identified in patients with UTI. Further development of key characteristics of urinary microbiomes that utilize 16S rRNA gene sequencing will play a key role in improving our understanding of urinary health diseases, such as UTIs.

Keywords: Bacterial vaginosis; Human Microbiome Project; *Lactobacillus*; Microbiota; Urinary tract infections

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INTRODUCTION

Microbiota is an environment in which pathogenic microorganisms, including bacteria, archaea, protists, fungi, and viruses, exist in various parts of our body, such as the skin, mouth, gastrointestinal tract, and vagina [1]. Microbiome is the genetic materials of this microbiota. Altered microbiota and microbiomes can cause various disorders, including obesity, bowel diseases, and bacterial vaginosis (BV) [2].

The Human Microbiome Project (HMP) is an effort to profile the microbial composition of a healthy population to determine the impact of changes in the microbiota on human health, particularly the composition of nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract. Urine samples were not included as part of HMP because the urinary tract has traditionally been considered as a sterile body niche [3]. However, 16S ribosomal ribonucleic acid (rRNA) sequencing and other technical advancements in the field of molecular biology have overcome the limitations of standard culture-based detection, demonstrating the presence of urinary microbiota [3]. Urinary microbiota is composed of a mixture of bacteria, including Gram-negative and Gram-positive bacteria, fungi, and viruses. An imbalance in the composition of the microbiota in the genitourinary tract must be closely related with urinary tract infections (UTIs) and BV. As the most important bacteria for the prevention of UTIs and BV,

Lactobacillus plays an important role in the host defense mechanism against uropathogens. Lactobacilli are Gram-positive rods, and they are one of the most common microorganisms not only in the healthy vagina but also in the urinary tract. Currently, they are regarded as part of nonpathogenic members of urogenital floras [3-5].

In contrast to many studies that have revealed the characteristics of vagina microbiome after the inclusion of vaginal specimen in HMP, there is only a few studies to date that have applied 16S rRNA sequencing in detecting urinary microbiome of UTIs. As a result, information on microbiomes of the urogenital tract has not been adequately described. This article presents a summary of recent findings on urinary microbiomes via 16S rRNA sequencing, and their relationship to UTIs and urinary health. This article also provides information on changes in *Lactobacillus* in the urinary tract between healthy people and patients with a UTI.

MICROBIOME AND THE URINARY TRACT

1. Human Microbiome Project and the Human Urinary Microbiome

The concept of microbiome has brought revolutionary changes to the concept of environment and development of diseases, not only in the gastrointestinal, oral, and female genital tracts, but also in the urinary tract. HMP, conducted by the National Institutes of Health began in 2008 and ended in 2013. The aim of this project was to understand the human biomes, and to compare and classify the microbiota using sequences of 3,000 genomes from cultured and uncultured bacteria of 300 healthy individuals [6]. The core microbiome was collected from five different sites: nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract [1]. HMP used multiple analytical tools, such as taxonomic profiling using 16S rRNA gene sequences and metagenomics profiling by whole-genomic shotgun sequencing [7]. A phylogenic study largely depends on gene sequencing of the 16S rRNA gene because it exists in all animals, and it is well conserved during evolution. It also differs among species of bacteria and archaea. These regions with different sequences can be used to determine phylogenetic relatedness [3].

2. Gut Microbiome and Urinary Microbiome

Recent progress in the scientific understanding of the human microbiota has revealed that there are significant differences between those of the gut and bladder. The number of gut microbiota is as high as 10^{12} colony forming units (CFU) per gram of feces, whereas that of urine is 10^{2}-10^{5} CFU per ml [8-10]. Moreover, the microbiome of the urinary tract is much less diverse than that of the gut, as only a dozen to dozens of species have been detected, and most urine samples are dominated by one or two bacterial families or genera [8]. The most frequently detected dominant bacteria were *Lactobacillus* and *Gardnerella* from urine samples of 60 patients with urge urinary incontinence (UUI) and 58 cohorts without UUI [8].

3. Sterile Urine Paradigm and Urinary Microbiome

New approaches using 16S rRNA gene sequencing have provided new opportunities to view the traditional concept of a healthy bladder is sterile and urine is sterile if urine culture and urinalysis are negative. Traditionally, UTIs have been defined by the culture of uropathogenic organisms from mid-stream urine of $>$100,000 CFU per ml. However, a standard urine culture can only detect fast-growing aerobic uropathogens, such as uropathogenic *Escherichia coli*, and it cannot detect any slow-growing bacteria, anaerobic bacteria, or those with special nutrient requirements [9]. By contrast, DNA-based diagnostic approaches are more accurate. Nine hypervariable regions (V1-V9) of the 16S rRNA gene can be used for distinguishing various bacteria [3]. A 454 pyrosequencing study of a 26 to 90-year-old healthy population showed that 16S rRNA DNA sequencing identified 94 genera, while only 31 genera were likely to be cultivated by standard culture techniques [11]; this indicated that the remaining two-thirds of the bacteria are not routinely cultured. There are strengths and weaknesses in clinical usefulness between the two diagnostic methods. The general detection rate of bacteria is superior in metagenomics 16S rRNA gene sequencing; however, this method cannot provide quantitative information on deciding the best course of treatment. An enhanced quantitative urine culture protocol has been suggested to overcome the weaknesses of conventional urine culture. Important parts of the methods are increased urine volume, incubation in a 5% CO_2 incubator, incubation for 48 hours, and inclusion of colistin-nalidixic acid agar, in addition to blood and

MacConkey agars [9,12]. Price et al. [12] reported a much higher detection rate compared with the traditional urine culture: the standard urine culture method could detect only 33% of all detected uropathogens via expanded-spectrum enhanced quantitative urine culture, and only 7% of those detected in the non-UTI subjects.

GENDER DIFFERENCES IN THE MICROBIOME

1. Urinary Microbiome and Sexually Transmitted Infections in Men

Healthy female urinary microbiota often include bacteria also identified in the vagina, whereas healthy male urinary microbiota resembles the gut and skin [13]. The urinary microbiome is characterized by a preponderance of *Lactobacillus* in women and *Corynebacterium* in men [14]. Dong et al. [15] compared the microbiomes of 32 paired first-catch urine samples and urethral swab specimens from adult men in a sexually transmitted disease clinic using 16S rRNA polymerase chain reaction (PCR) and deep pyrosequencing. The distribution of the microbiomes of both specimens was remarkably similar, except only the proportions of *Propionibacterium* spp. and *Corynebacterium* [15]. *Neisseria, Streptococcus, Corynebacterium,* and *Ureaplasma* spp. were frequently found in more than 5% of sexually transmitted infections (STI)-positive men compared with *Lactobacillus, Sneathia, Veillonella, Corynebacterium, Provotella, Streptococcus,* and *Ureaplasa* spp. in STI-negative men. *Lactobacillus* spp. were the most frequently found organisms in urine samples in both men and women [16-18]. However, there is a significant inter-subject variability in the urinary microbiome and differences are evident between the groups of STI-positive and STI-negative men [11,17].

Bacterial detection by 16S rDNA sequencing is changing the etiology of STI in men and women. Although *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are the most common pathogenic bacteria, they are commonly not detected in patients with STIs. Even though multiplex PCR for the six most common bacteria is available, and treatment of patients with all negative results can be confusing to clinicians. Manhart et al. [19] suggested that *Leptotrichia/Sneathia* spp. may be associated with nongonococcal urethritis in heterosexual men who are negative for *C. trachomatis, N. gonorrhoeae, Trichomonas vaginalis, Mycoplasma genitalium,* and *Ureaplasma urealyticum*. The results from STI-positive men are compatible with BV-positive women.

2. Urinary Microbiome in Women with Bacterial Vaginosis

Fredricks et al. [20] compared 27 subjects with BV and 46 healthy controls using a bacterium-specific PCR assay of 16S rRNA and fluorescence in situ hybridization of vaginal fluid. They reported that *Atopobium*, *Leptotrichia*, and *Megasphaera* were found in a high percentage of women with BV; however, *Atopobium* was less specific for BV. The causative relationship between these newly detected bacteria and BV in women has not been demonstrated. Although *Gardnerella vaginalis* is present in all specimens from women with BV, it was also found in nearly 60% of women without BV [20]. Contrastingly, Teixeira et al. [21] reported that *G. vaginalis* was observed in 56.7% of women with BV and 17.6% of healthy women. *Lactobacillus* were more frequently detected in healthy women (97.5%) than in women with BV (76.7%). Due to a high detection rate of *G. vaginalis* in asymptomatic women, there is a debate on whether this organism is commensal or pathogen, as well as on the needs of treatment. Although it is clear that *G. vaginalis* and *Atopobium vaginae* are present in high concentrations in grade III BV, *Lactobacillus crispatus* was found in lower concentration in grades II and III BV [22]. After *G. vaginalis* increased and lactobacilli decreased in hypoxic condition, *G. vaginalis* can colonize the vaginal epithelium as a biofilm [23]. *G. vaginalis* predominant biofilms are 5 times more tolerable for hydrogen peroxide and 4 to 8 times more tolerable for lactic acid produced from lactobacilli. In addition to a shift of predominant organisms from lactobacilli to *G. vaginalis*, different virulence factors of *G. vaginalis* can affect occurrence of BV; for example, the production of sialidase A in accordance with the subtypes of *G. vaginalis*. Among *G. vaginalis* found in 87% of healthy women, clade 4 type was found in 79.4% of healthy women, and virulence was detected in low frequency due to the lack of gene coding for sialidase [24]. In contrast, all clade 1 and clade 2, which were more commonly isolated in women with BV, have gene coding for sialidase. Castro et al. [25] also reported that *G. vaginalis* strains from non-BV subjects showed a less virulence

expression with respect to biofilm formation, initial adhesion ability, cytotoxic effect, susceptibility to antibiotics, as well as levels of vaginolysin and sialidase. Treatment is not generally recommended for woman with asymptomatic BV due to *G. vaginalis*. However, benefits of preventive antibiotics treatment for recurrent BV and infection in pregnant woman, along with adjuvant therapy with probiotics, such as lactobacilli, remain to be elucidated.

The degree of diversity of urine microbiota differs in men and women; it is the largest in healthy men, followed by healthy women, and women with acute BV. Diversity was lowest after metronidazole treatment of BV [26].

MICROBIOTA, MYCOBIOME, AND VIROME

1. Urinary Microbiome and Urogenital Tract Infection

Gram negative bacteria are isolated in 75% to 90% of uncomplicated UTIs. Contrastingly, Gram positive bacteria constitute 5% to 15% of all UTI bacteria, and *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Streptococcus agalactiae* are frequently isolated organisms [27]. *Corynebacterium*, *Actinobaculum*, *Gardnerella*, and *Aerococcus* are uncommon Gram-positive bacteria found in the urinary tract, and UTIs caused by Gram-positive organisms usually occur in elderly patients and pregnant women [27].

Fouts et al. [14] suggested a mechanism in which the urinary microbiome in healthy subjects changes into a susceptible microbiome for UTI, comparing a healthy urinary microbiome with asymptomatic bacteriuria in patients with neuropathic bladder (NB) associated with a spinal cord injury. Their results indicated that the presence of *Lactobacillus* decreased over time, and Enterobacteria increased with increasing duration of NB. Finally, Enterobacteria became abundant one year after NB diagnosis, when the urinary microbiome was devoid of *Lactobacillus* [14]. By contrast, *Klebsiella* (males), *Escherichia*, *Enterococcus* (both genders), and *Gardnerella* (females) were significantly more enriched in urine from subjects with NB. *Corynebacterium*, *Staphylococcus*, and *Streptococcus* were more abundant in healthy men and women [14].

2. Urinary Mycobiome and Urogenital Tract Infection

Non-bacterial microbiome, such as fungi, viruses, archaea, and protozoa, remain unknown. Mycobiome refers to the fungal microbiome, and it is an essential part of the human microbiota [28]. Although fungal infections in humans are very common, most are superficial infections, with invasive infections being rare. Because fungi are less abundant compared with bacteria, their communities are less stable and are significantly influenced by environmental conditions [28]. As a consequence, it is unlikely that a simple fungal infection, or a change in the mycobiome results in disease without alterations in the host environment that facilitate the pathology; e.g., immunosuppressed status, altered metabolism, or the tissue microenvironments [28]. *Candida* spp. are the most common pathogens in UTIs; a significantly greater prevalence of *Candida* and *Saccharomyces* was detected in 15.7% of standard culture-negative female patients with chronic pelvic pain syndrome in the flare status [29].

3. Urinary Virome and Urogenital Tract Infection

Virome is the viral microbiota. To date, very little is known about the virome in the genitourinary tract. Santiago-Rodriguez et al. [13] investigated the urinary virome in 10 patients with UTIs and 10 subjects without UTIs. They found approximately 10^7 virus-like particles in the urine, which was lower than those detected in the human saliva. The most identifiable viruses were bacteriophages, and a low-risk herpes virus type detected in 95% of subjects. Interestingly, even though the bacterial microbiome was significantly altered by UTI, the urinary virome was not different. Moreover, the diversity of viral communities was not different between UTI+ and UTI- subjects, as bacterial diversity is higher in urine from UTI- subjects [13].

LACTOBACILLUS AND THE GENITOURINARY TRACT MICROBIOME

L. crispatus and *Lactobacillus iners* are most frequently found in urine of healthy women; *Lactobacillus gasseri* is found less frequently [8,20]. It is hypothesized that the environmental factors of hosts affect the types, numbers, and diversity of urinary and vaginal microbiome. Liu et al. [30] suggested that an abundance of lactobacilli and

composition of other bacteria were changed according to the fasting blood glucose, blood pressure, and blood lipids of participants. They found that urine microbiota were different between patients with diabetes only, diabetes plus hyperlipidemia, diabetes plus hypertension, diabetes plus hypertension, and hyperlipidemia cohorts.

It has been well established that *Lactobacillus* spp. decompose carbohydrates and maintain an acidic intravaginal microflora by generating lactic acid and CO_2, thus preventing vaginal colonization by harmful microorganisms. Furthermore, *Lactobacillus rhamnosus* GR-1 participates in immune activation through the nuclear factor-kappaB pathway, induced by heat-killed *E. coli* cultured in urothelial cells [31]. LGG increases the levels of pro-inflammatory cytokine tumor necrosis factor through increased levels of toll-like receptor-4 in bladder cells [31]. However, the immunologic effects and preventive ability of UTI and BV are different among each lactobacilli. Hutt et al. [32] reported that hydrogen peroxide was produced from 89% of *L. crispatus*, 86% of *Lactobacillus jesenii*, and only 42% of *L. gasseri* strains. Lactic acid production was higher in *L. gasseri* (18.2±2.2 mg/ml), followed by *L. crispatus* (15.6±2.8 mg/ml) and *L. jensenii* (11.6±2.6 mg/ml). *L. crispatus* stains showed the highest antimicrobial activity against *E.coli*, *Candida albicans*, and *Candida glabrat* compared with *L. gasseri* and *L. jensenii*. The antagonistic activity to *G. vaginalis* was not different between the species. Because *L. iners* was detected in women with BV, it does not protect against infections [30].

1. *Lactobacillus* in Bacterial Vaginosis and Urogenital Tract Infections

It is unclear why women with BV are more prone to developing UTI. It is widely accepted that lactobacilli are a dominant vaginal organism in healthy women, whereas women with BV have a more diverse microbiota, mainly Gram-negative anaerobes, such as *Actinobacteria* and *G. vaginalis*. Interestingly, both UTIs and BV are frequently associated with sexual activities and the use of diaphragms. Sumati and Saritha [33] reported that women with BV have an increased risk of developing UTI with an odds ratio of 13.75; pregnancy may also be an important co-risk factor. Yan et al. [34] reported that *L. crispatus* was cultured in 94% of healthy women and 83% of those with BV. Interestingly, a significant difference in the number of lactobacilli was observed, and the mean number of colonies of *L. crispatus* was 10^6 in healthy women and 10^3 in women with BV.

The genus *Lactobacillus* includes more than 130 species, and more than 20 species have been detected in the vagina [18,35]. *Lactobacillus* ferment glycogen produced by vaginal epithelial cells, which produces lactic acid. They inhibit the colonization of pathogenic bacteria; however, this mechanism can be weakened to achieve sufficient inhibitory concentrations under hypoxic conditions [36]. Moreover, lactobacilli have a higher affinity for host cell receptors and can displace adherence of *G. vaginalis* and *N. gonorrhoeae* [37,38]. They can also inhibit uropathogenic *E. coli* and *E. faecalis* [39,40].

CONCLUSIONS

The microbial microbiome and dysbiosis are new approaches for detecting several urologic diseases, including UTIs, urinary incontinence, and bladder cancer. It is evident that *Lactobacillus* and *Corynebacterium* are dominant in urine of healthy subjects, and they can change into pathologic Enterobacteria in patients with NB. A decrease in lactobacilli and an increase in Gram-negative anaerobes occur in patients with BV. *L. crispatus* and *L. iners* are most frequently found in urine of healthy women. Although there are a few studies regarding the microbiomes and viromes in urine, fungal and viral communities seem to have a lower abundance and diversity than bacterial communities. Urinary microbiome studies in the future should be conducted to provide new treatment strategies and prevention modalities through contributing new evidence.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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