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Lactobacillus Composed Urinary Microbiome of Healthy People and Urinary Tract Infection Patients

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Abstract

Lactobacillus has long been known to reconstruct the microbiome of the urogenital organs of healthy people. However, changes in lactobacillus abundance are known occurring in urogenital tract infections (UTI) patients. It is also known that there are Lactobacillus species that have specific genomic characteristics and nutritional requirements that cause them to turn into pathogens. Understanding population characteristics and taxa composition that reconstruct urine microbiome is very important for the diagnosis and treatment of urogenital infections. This review provides an update information of metagenomic sequencing to identify the urinary microbiome profile and the role of lactobacilli in healthy and UTI patients.

1. Introduction

The old paradigm said that a urinary bladder is usually sterile. This paradigm then changes with the discovery of a number of bacterium in the urine of healthy person. Microorganisms in the urinary tract can develop mechanisms to colonize urogenital system. In the case of certain infections, bacteria can reach the kidneys which then enters the bladder. Due to this process, urine can contain bacteria (Frimodt-Møller, 2019). An imbalance in the components of the urinary microbiota is associated with the development of dysbiosis (Kim & Park, 2018).

Microbiota is a collection of microbial taxa that reside in certain environments, including the human body and consists of 10-100 trillion symbiotic microbial cells harbored in each individual, especially in the gut (Ursell et al., 2013). While microbiome is a collection of genomes, genes, and microbial products that exist in certain hosts. Other researchers state that a microbiome includes environmental biotic and abiotic factors, including the microbial genomes, the host genomes, and the environmental factors (Aragón et al., 2018; Schneeweiss et al., 2016; Whiteside et al., 2015).

Human microbiome projects has successfully mapping microbiota in various parts of the body, such as urogenital tract and its relation to health and diseases (Petrova et al., 2017). Unfortunately, a research on urogenital microbiome is still limited. By knowing the microbial genomes, the host genomes, and the environmental factors, the clinical implication for disease management and patient care can be found (Whiteside et al., 2015). This review provides an update information of metagenomic sequencing to identify the urinary microbiome profile and the role of Lactobacillus in healthy and UTI patients.

2. Conventional Culture for Identification

Conventional cultures are usually used to detect aerobic bacteria and fast-growing bacteria such as *Escherichia coli* or *Enterococcus faecalis*. However, this method cannot detect anaerobic, slow growing bacteria and bacteria with unique phenotype. In addition, standard microbiological culture techniques can only identify a fraction of the urinary microbiome taxa (Hasman et al., 2014; Horwitz et al., 2016; Winand et al., 2020).

The identification and characterization of infectious microorganisms are very important for the success of the treatment, the healing process and patient safety. However, not every bacteria can be cultured and available tests cannot detect genetic traits in pathogens that have successfully developed and spread in humans, animals and the environment (Deurenberg et al., 2017). For this reason, appropriate diagnostic methods are needed to help diagnose diseases and treat therapies. Efforts to understand the relationship between the urinary microbiome and the UTI risk play an important role not only in the choice of therapeutic treatments, but also in the development of diagnostic and prognostic tools (Bi et al., 2019; Neugent et al., 2020).

3. Metagenomic Sequencing

As a part of next-generation sequencing (NGS), metagenomic sequencing has led to the development of metagenomics: genetic analysis of the genome directly from samples without carrying out the culture stage (Oulas et al., 2015). This sequencing method can save time for the diagnosis process up to 2 days by reducing the time of culture and incubation. The main steps include DNA purification, sequencing, analysis and comparison with a global database. The analysis result of metagenomic sequencing provides the information about the presence of undetectable bacterial species by conventional culture methods. Metagenomic analysis can conclusively identify a huge number of sequence reads of common uropathogen, as well as fastidious bacteria that associated with infection (Hasman et al., 2014; Moustafa et al., 2018).

Metagenomic sequencing technology is very useful in profiling the microbiome of a number of systems in the human body, including the urinary tract. Researchers have used various platforms for this purpose and compared the results obtained with the NGS results. Amplification with target 16S rRNA gene can detect all organisms detected by urinary culture. A number of taxa identified with Illumina MiSeq targeting V1-V3 regions are not identified by conventional culture methods (Moustafa et al., 2018; Pohl et al., 2020). The 16S rRNA gene has some conserved regions so it is widely used for taxonomic classification by comparing it with the databases (Bukin et al., 2019; Chu & Lowder, 2018; Deurenberg et al., 2017). The use of 16S rRNA gene sequencing in th further development of the urinary microbiome profile will play a major role in understanding of urinary tract diseases, such as UTIs (Kim & Park, 2018).

4. Urinary microbiome of healthy and UTI patients

Several studies have been conducted using different types of urinary samples, such as catheterized and voided urine. The use of different urinary samples has an effect on the 16S microbiome based on the NGS data obtained. The way of collecting urinary specimens from the patient determines the results that will be obtained. Catheterized urinary specimens are better at describing male bladder microbiome than voided urine (Bajic et al., 2018; Pohl et al., 2020).

From a number of studies, it is known that the composition of microbiota in individuals experiencing UTI is different from healthy individuals. Each individual has a different tissue characteristic that ultimately affect the dynamics of microorganisms. Some infections has been known to be correlated with the imbalance of urinary microbiota and the decreased of biodiversity. The 16S rRNA gene sequencing shows that subjects with symptoms of infection have lower bladder diversity than subjects with no symptoms (Horwitz et al., 2016; Kim & Park, 2018; Whiteside et al., 2015). Studies in female patients with overactive bladder (OAB) have also shown a decrease in diversity compared to controls which may have strong implications for the occurrence of OAB (Wu et al., 2017).

The diversity of microbiome sequences of female interstitial cystitis (IC) patients is significantly decreased compared to the healthy female (HF) patients. The dominant phylum, both in the urine of HF and IC, is Firmicutes with percentage of abundance 65% and 93%, respectively. The dominant genus of IC patients is Lactobacillus for 92% of sequences of IC patients while HF is 65%. The 4 genera identified in IC urine that is not found in HF urine are Enterococcus, Atopobium, Proteus and Cronobacter, while 17 genera are only found in HF urine (Schneeweiss et al., 2016; Siddiqui et al., 2012). Studies of urinary microbiome in men are not as much as in women. However, the implementation of the NGS technique shows that the most abundant genus in the urine of healthy men is Lactobacillus and Streptococcus (Bajic et al., 2018; Pohl et al., 2020).

5. Lactobacillus in Healthy Urogenital Women and Men

Lactobacillus has long been known for its role in maintaining the health of female reproductive organs, especially the vagina. Vaginal Lactobacillus provide an effective first line defense against invading pathogenic bacteria in the form of thick biofilm formation (Ventolini, 2015). A study states that when Lactobacillus dominate the vaginal microbiota, women will have more lactic acid as a mechanism of protection (O'Hanlon et al., 2019). However, these bacteria can be detected in normal conditions as well as during vaginal dysbiosis, such as bacterial vaginosis (BV), a condition characterized by an abnormal increase in bacterial diversity and a lack of typical Lactobacillus. This is why the role of Lactobacillus in maintaining vaginal health is still unclear (Petrova et al., 2017).

A number of studies have shown a link between the microorganisms inhabiting the vagina and bladder. They showed similarities not only to commensals, but also to pathogenic microorganisms. Thomas-White et al. have successfully identified 149 strains from catheterized urine samples of 77 healthy women. The strains found include *E. coli*, *Streptococcus anginosus*, *L. iners*, and *L. crispatus*. the results obtained are also in line with the research of Price et al. which suggests that Lactobacillus are the dominant taxa (19%) found in the bladder microbiota (urobiome) of continent women (Gottschick et al., 2017; Price et al., 2019; Thomas-White et al., 2018).

Gottschick et al. conducted a study using healthy male and female subjects as well as women with BV and after receiving metronidazole antibiotic treatment. The high diversity of BV-associated species in male and female urine suggests that urine acts as a reservoir for pathogens and contributes to recurrence (Gottschick et al., 2017).

6. Lactobacillus in UTI

Lactobacillus is the most abundant genera composing the urine of healthy women and men (Schneeweiss et al., 2016; Siddiqui et al., 2012). Although it is difficult to culture, several studies using conventional culture detected the presence of UTI-causing Lactobacillus. Later, it was found to be the etiologic agent for a recurrent UTI (Akter et al., 2018).

Some interesting reports from a number of urinary microbiome researches show that Lactobacillus are as the main taxa of urinary microbiome in patients with UTIs. A research conducted on female patients with IC shows that the microbiome is composed by >90% Lactobacillus. This abundance is greater than Lactobacillus in healthy women, which is 60%. Other studies have also shown that patients with IC have less diversity in the urinary microbiome and are more likely to have Lactobacillus species (Abernethy et al., 2017; Siddiqui et al., 2012).

Other remarkable conclusions should be noticed from the analysis of 16S rRNA gene of 212 catheterized urines of women with mixed urinary incontinence (MUI) and similarly aged controls (Komesu et al., 2018). Lactobacillus predominance does not differ between MUI and controls and some members of the genus Lactobacillus might be associated with urinary symptoms. Similar results are also obtained in metagenomic studies using urine specimens from patients with interstitial cystitis/bladder pain syndrome (IC/BPS) and female control participants. Lactobacilli are the most abundant genus in both cohorts (Bresler et al., 2019; Meriwether et al., 2019; Nickel et al., 2019).

Although it is widely known that Lactobacillus promotes health in the urogenital organs, a number of researches suggest that Lactobacillus is an opportunistic pathogen with some risk factors associated (Rossi et al., 2019). Retrospective analysis conducted in Argentina shows that Lactobacillus has been isolated from patients with bacteremia, meningitis, vaginosis and urinary infection. The most common species of Lactobacillus are *L. rhamnosus*, followed by *L.*

fermentum, *L. paracasei*, *L. oris*, *L. gasseri*, *L. iners*, and *L. salivarius* (Rocca et al., 2018). *Lactobacillus delbrueckii* is found in patients with pyelonephritis and pyuria (Darbro et al., 2009; DuPrey et al., 2012). *Lactobacillus gasseri* is known to be associated with Urgency Urinary Incontinence (UUI) symptoms and *L. crispatus* is in asymptomatic controls (Pearce et al., 2014). Lactobacillus was also identified in newborn girls who were not breastfed and had decreased in immune systems (Divyaswathi & Gourishankar, 2013). These results provide additional evidence of the opportunistic nature of the Lactobacillus. Subsequent imbalance in the newborn urinary microbiome may also result in UTI occurrence (Rossi et al., 2019).

Lactobacillus iners is one of unique Lactobacillus species compared to others. Lactobacillus generally has a genome of 3-4 Mbp in size and niche flexibility, whereas this species has a genome of about 1 Mbp. It also has a complex nutritional requirement that indicates a symbiotic or parasitic nature. The genome of *L. iners* also encodes for inerolysin, a toxin similar to the vaginolysin in *Gardnella vaginalis*. It is likely that this species has variants which in some cases provide vaginal protection and in some cases cause disease and dysbiosis (Macklaim et al., 2013; Petrova et al., 2017). In a study to determine the composition and structure of the vaginal microbiota, it was found that the dominance of *L. iners* was associated with an increased risk factor for *Chlamydia trachomatis* infection. The composition of the vaginal microbiota may indicate an increased host predisposition to acquiring infection, such as STIs. The presence of a specific species or strain of Lactobacillus is a determinant of susceptibility or protection against infection (Van Houdt et al., 2018).

7. Ecological Perspective of Vaginal Microbiota in The Occurence of UTI

Vagina plays a key role and becomes a reservoir for pathogenic bacteria causing UTIs in women (Stapleton, 2016). Metagenomic analysis of gastrointestinal, vaginal and urinary samples show similarity between urinary and vaginal microbome. An interlink between that two has led to the presence of not only commensals but also disease-causing pathogens (Thomas-White et al., 2018).

Women vagina has a dynamic and complex microbiota ecology influenced by pregnancy, hormone level, sexual development, sexual intercourse and personal hygiene factors (DiGiulio et al., 2015; Gajer et al., 2012; Greenbaum et al., 2019; Muhleisen & Herbst-Kralovetz, 2016). Other studies reported that age and menopausal status also contribute to microbial diversity in women vagina. In older women that have more diverse microbiome, dysbiosis may results from decreased in microbial diversity. While in younger women with less diverse microbiome, dysbiosis may results from increased in microbial diversity (Ackerman & Chai, 2019; Curtiss et al., 2018). A deeper understanding of vaginal dynamics has the potential to advance the development of future practices, especially in UTIs treatment.

According to the Walker's drivers and passengers' hypothesis, Lactobacillus has playing role as keystone species in ecosystem so it can promotes vaginal's health (Walker, 1992). This species served as driver taxa that maintains the ecosystem function and stability while the others served as passengers that do not alter its function. However, some vaginal ecosystem consists of a community without any dominant taxa. This condition is what is meant by the rivet hypothesis in which many species give contribution to the ecosystem function and stability (Ehrlich & Walker, 1998; Greenbaum et al., 2019).

According to Karstens et al., decreasing in microbial diversity influenced the severity of UUI symptoms. The diversity of urinary microbiome may have clinical relevance and their change has been linked to occurrence of disease (Karstens et al., 2016). Decreasing in microbiome diversity and richness as well as the higher abundance of Lactobacillus are associated with urinary tract infections including IC and OAB (Horwitz et al., 2016; Siddiqui et al., 2012; Wu et al., 2017). Microbial diversity is one of ecological aspects that should be concerned for assessment of clinical purpose.

Table 1. Summary of studies on urinary microbiome analysis

No	Study	Samples	Notable taxa	Identification technique	Other remarks
1	(Siddiqui et al., 2012)	IC Female patients Healthy Female	Lactobacillus (>90%) Lactobacillus (60%)	454 high throughput sequencing of the 16S variable regions V1V2 and V6	High throughput sequencing analysis of urine microbiota in IC patients is a powerful tool towards a better understanding of this disease
2	(Lewis et al., 2013)	Voided urine of 16 asymptomatic adults	Bacterial diversity was higher in female samples. Firmicutes were the most abundant phylum found in both male and female samples (>50% composition)	454 Pyrosequencing	Age-specific genera: Jonquetella, Parvimonas, Proteiniphilum, and Saccharofermentans. Conventional microbiological methods are inadequate to fully identify around two-thirds of the bacteria identified in this study.
3	(Gottschick et al., 2017)	Healthy women Healthy men Women with BV and after antibiotic treatments	*Lactobacillus crispatus* Not significance *P. amnii*, *S. amni*, *G. vaginalis* and *A. vaginae*	16S gene sequencing V1-V2 region	Antibiotic consumption (women during BV) causes reduced abundance of all species (*G. vaginalis*, *A. vaginae*, and *S. amnii*), except for *L. iners* which actually increases compared to that in healthy urine
4	(Abernethy et al., 2017)	Catheterized urine sample: Women diagnosed with IC	Fewer OTUs was less likely to have *Lactobacillus acidophilus*	16S rRNA sequencing, symptom severity, quality of life, depression, and anxiety screening questionnaires	The presence of Lactobacillus was associated with improved scores on two IC-specific symptom severity indices. Urinary microbiome might influence lower urinary tract symptoms.
5	(Moustafa et al., 2018)	121 urine specimens Women Men	Lactobacillus, Prevotella Enterococcus, Pseudomonas	16S rDNA gene sequencing (Illumina MiSeq) V1-V3 region	A number of taxa identified by NGS were not identified by the culture method

6	(Komesu et al., 2018)	212 Catheterized urines of Women with MUI Similarly aged controls	Lactobacillus predominance did not differ between MUI and controls	16S rRNA gene sequencing V4-6 regions	Some members of the genus Lactobacillus might be associated with urinary symptoms
7	(Thomas-White et al., 2018)	Catheterized urine of 77 women	149 strains; highly similar *Escherichia coli*, *Streptococcus anginosus*, *Lactobacillus iners*, and *Lactobacillus crispatus* in vagina and bladder	Whole-genome metagenomic analysis: Illumina HiSeq 2500	Interlinked female urogenital microbiota that was not only limited to pathogens but was also characteristic of health-associated commensals
8	(Nickel et al., 2019)	Urine specimens of: 181 female IC/BPS 182 female control participants	Overabundance of *Lactobacillus gasseri* in IC/BPS	The Plex-ID molecular diagnostic platform	IC/BPS had a lower prevalence of Corynebacterium compared with control participants
9	(Meriwether et al., 2019)	23 ICBPS 18 non-ICBPS patients	Lactobacilli was the most abundant genus in both cohorts	16S rRNA sequencing using Illumina MiSeq V3-V4 regions	Anaerobic or fastidious predominance was similar between groups
10	(Bresler et al., 2019)	Voided urine of 21 IC/PBS patients 20 asymptomatic controls	Lactobacillus (no significant differences between cohorts)	16S rRNA gene sequencing and EQUC	IC/PBS symptoms might not be related to differences in the FUM
11	(Pramanick et al., 2019)	Vaginal swabs from 199 participants asymptomatic for urogenital infections	*L. iners* (69.4%) in normal *L. iners* (62.1%) in BV	multiplex PCR and species-specific PCR	*L. iners* was a frequent member of normal and dysbiotic microbiota. Lactobacillus abundance and species richness reduced in asymptomatic BV.
12	(Pohl et al., 2020)	Asymptomatic, healthy cohort 6 women & 9 men	Lactobacillus, Streptococcus (higher in woman)	Urine culture 16S sequencing (Illumina)	Urine collection method: voided and catheterized result in different 16S-based NGS data.
13	(Bi et al., 2019)	urine samples from 29 bladder cancer patients and 26 non-cancer patients	A conserved microbiota with four phyla (Firmicutes, Actinobacteria, Proteobacteria and	amplicon-based next-generation sequencing approach	The higher abundance of *A. europaeus* observed in bladder cancer patient samples also suggests that the strain might be indicative of bladder

			Bacteroidetes) five genera of bacteria (Streptococcus, Bifidobacterium, Lactobacillus, Veillonella and Actinomyces) existed in all samples		cancer
14	(Price et al., 2020)	MSU and periurethral swab of adult woman (for over 3 months collections	Lactobacillus	16S rRNA sequencing, EQUC	microbial dynamics related to specific participant-reported factors, particularly menstruation and vaginal intercourse

8. Conclusion

The role of Lactobacillus in maintaining the health of the urogenital tract and causing disease depends on factors related to the host. Changes in the host result in changes to the structure of the microbiome which then cause disease symptoms. The study of the urogenital microbiome should be further developed for the purpose of prevention and appropriate treatment of patients with urogenital disorders, including UTIs.

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