REVIEW ARTICLE

Urinary Microbiome

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Abstract

The use of high-throughput sequencing technologies has played a major role in debunking the myth of human urine sterility. With new technologies-16S rRNA amplicon sequencing, metagenomic sequencing- several genera, phyla and species of bacteria resident in the human urinary tract have been discovered and their role in health and disease have been reported. The focus of this review includes classification of microorganisms resident within the urinary tract, techniques used to sample, identify and classify these organisms, and the impact of resident microorganisms on normal and abnormal urinary tract function.

Keywords: Urobiome; Urinary bladder, microbiota

Introduction

Humans are considered holobiontsas a result of the community of microorganisms colonizing the surface, organs and biofluids of the human body[1]. While many of these microorganisms are often regarded as disease-causing microorganisms, research has shown that some resident microorganisms in humans contribute to the health and normal functioning of the body [2]. To classify and investigate the roles these organisms play in the body, an extension of the human genome project called the Human Microbiome Project was created [3]. While the gut, vagina and skin were assessed for resident microorganisms, the urinary tract was excluded [4].

For several decades, urine from a healthy individual was thought to be devoid of microorganisms [5] and therefore, traditional culture-dependent methods were only used in clinical settings to detect the presence of pathogenic bacteria implicated in the development of urinary tract infections[6]. This myth of sterile urine contributed to the exclusion of the urinary tract as a site of microbial colonization in the Human MicrobiomePproject [7]. With the aid of Next-Generation Sequencing technology however, the presence of micro-organisms resident in the urinary tract of a normal individual have been confirmed [8].

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The Microbiome

Although, Whipps et al., gave the first definition of the microbiome as a characteristic community of microorganisms within a well-defined habitat with specific physico-chemical properties [8], several others have come up with definitions that capture the distinct characteristics of the microbiome and its relationship with its habitat. According to ecologists, a microbiome refers to a community of microbes and their relationship with the environment [9]. A genomic definition of the microbiome is the collective genomes of a community of microorganisms or the metagenome of the community microbiota [10]. The microbiome is made up of microbiota, which includes all bacteria, archaea, and other microorganisms of a particular site or organism [11]. The microbiome could be found in plants [12], water-aquatic microbiome, land-terrestrial microbiome [13], animals [14] and humans [15]. The microbiome of a particular organism affects and is affected by its immediate environment [1]. The plant microbiome, also known as phytomicrobiome, plays important roles in general plant health and growth [16]and the microbiome of humans are involved in immune function, and general health and well-being of an individual [17].

The Human Microbiome

About 500-1000 species of bacteria colonize the human body at any point in time [18]. Although a large number of bacterial species have been implicated in the development and transmission of several diseases[19], some of these microorganisms have favorable effects on human health. The human microbiome refers to all the bacteria, archaea, protists and viruses that form an ecological environment in and on the human body [20]. It could also be the total genomes of all the microorganisms residing in the human body [10]. The human microbiome consists of cells (cells of microorganisms) that outnumber the human cells themselves [18].

The Human Microbiome Project (HMP)

In order to estimate the number, types, diversity and function of the microorganisms in the human cells, the Human Microbiome Project (HMP), a continuation of the Human Genome Project, was conceived. This project was also to have the added advantage of providing insights on how the human body has evolved into a supra-organism [18].

The initial attempts of scientists to understand and characterize the micro-organisms resident in human cells involved the use of (16S) small-subunit rRNA sequencing techniques to analyze cultured microbial residents on different sites of the body [21]. The HMP involved analyzing samples from normal healthy volunteers via 16S rRNA gene sequencing techniques [22]. As a result of limitations to 16S rRNA sequencing techniques, such as sequencing errors, short read lengths [23], the results from the sequencing were then compared with available data before being sequenced via metagenomic shotgun sequencing which gave deeper analysis of the microorganisms found in specific sites [24]. A comparison of the microbiota among the different volunteers was also done to find out if humans had a core microbiome common to the vast majority of the human population[12]. The Human Microbiome Project had two phases that spanned 2007 till 2016.

Impact of the Human Microbiome on Health and Physiology

The relationship between human microbiota and human cells is symbiotic and vary from being parasitic to being mutualistic. As hosts to the diverse members of the human microbiota, human cells provide habitat for microorganisms [11]. And some of these, like the firmicutes, in turn, impact human health favorably [25]. On the other hand, resident microorganisms in the human body can be harmful to health as a result of the metabolites they produce[17].

Resident microbiota in the human body also play roles in food digestion, energy generation, normal metabolism, maintenance of mucosal barriers and regulation of the immune system[26].

Anatomical sites of human microbiota

The microorganisms making up the human microbiome are localised in several sites in the human body. These sites include:

The Gut: The majority of the microorganisms making up the human microbiota are found in the gut where they contribute to maintaining normal host physiology[27]. The community of microorganisms and and the combined genetic material of these microorganisms found in the human gut are collectively referred to as the gut microbiome [18]. Bacteriome (e.g*bacteriodes fragilis,Escherica coli*), viriome and mycobiome are the main members of the gut microbiota [27]. An imbalance in the constituents of the gut microbiota, often referred to as dysbiosis, contributes to the dysfunction of host physiology and contributes to a plethora of diseases like: Inflammatory bowel disease, obesity, colorectal cancer among others [28]. Antibiotics, age, diet could result in changes to the gut microbiota composition [29].

The Vagina and Uterus: The healthy vagina and uterus consist of mainly the bacteria *Lactobacillus* which produces antimicrobial substances [30]. A decrease in *Lactobacillus* leads to bacterial vaginosis, an inflammation of the vagina [31]. *L. Iners, Corynebacterium, Anaerococcus, Peptoniphilus* among others are other members of the vagina microbiota [16]. Fluctuating changes in the vaginal microbiome could be as a result of hormonal changes caused by the menstrual cycle or pregnancy [16]

The Skin: The diversity of the skin microbiome is dependent on different factors. For example, oily areas of the skin have resident species of bacteria like *Cutibacteriumanes*which consume lipids [32] while moist areas of the skin like the armpit have species like Corynebacterium [33]. The dryer parts of the skin are rich in *Betaproteobacteria* and *Flavobacteriales*. The resident microbiota play major roles in immune function however an imbalance in the skin microbiome leads to skin infections.

The Oral cavity: The oral cavity, which is the second most diverse microbiome [34], is home to over 700 bacterial species alongside fungi, viruses and protozoa [35] The saliva in the mouth helps maintain a balanced microbiota [31]

The Lungs and Nasal Cavity: Resident microorganisms in the respiratory tract are influenced by not only the distinct sites in the respiratory tract but also by their interaction with both internal and external factors [36]. The nasal cavity contains mostly Actinobacteria, Bacteriodetes, proteobacteria and firmicutes [37]

The Biliary Tract: Firmicutes, Bacteriodetes, Actinobacteria were some of the resident bacteria in the healthy human gall bladder [38]. As a result of the proximity of the liver, biliary tract and the intestines, the resident bacteria in the gut can be passed into the liver via the portal vein [39].

The Urethra and Bladder: Although microbiological culture techniques of screening for bacteria in urine often returns negative in people without urinary tract infections 6, recent use of sequencing methods have been used to confirm the presence of a urinary microbiome.

The Human Urinary Tract

Although urine microbiota often refers to microorganism content of bladder-obtained urine, but depending on the method urine samples are collected, the urinary microbiome may include microorganism species from other parts of the urinary tract like the ureters and urethra [7].

The bladder, a major part of the genitourinary system, is responsible for continence and micturition (i.e storage and release of urine) [40]. The bladder is made up of two major parts: The body and the base [41].The body of the bladder is made up detrusor muscles which are responsible for bladder contraction and urine expulsion [42]. The base of the bladder is formed by the trigone and the beginning of the urethras and is responsible for controlling urine outflow via the autonomic nervous system [43]. An increased number of adrenergic receptors in the neck of the male bladder (found at the base of the trigone) prevent retrograde ejaculation [44].

The Urinary Microbiome

Although several reasons contributed to the urinary microbiome not being a part of the Human Microbiome Project, the long-standing thought that urine is sterile was a major reason 7. With more advanced methods of assessing human microbiome, this notion has been disproved and studies have proven the presence of resident microorganisms in urine [8, 45]. The urinary microbiome consists of the genome of all the resident microorganisms in the urinary tract [46]

Limitations to Study of the Urinary Microbiome and Solutions

The traditional methods of detecting microorganisms in urine and diagnosing UTIs (the standard clinical cultivation procedures) have proven to be an insufficient technique in classifying the different genera and species of microorganisms in urine [47].

The first step in analyzing urinary microbiome is collecting urine samples in such a way as to reduce contamination from other parts of the urogenital tract like the vagina [48]. There are three main methods of urine sampling: Collection of mid-stream clean-catch urine, use of a trans urethral catheter and suprapubic aspiration [49] [5].As a step-up from the traditional culture-dependent methods which detects only about 33% of uropathogens in urine samples, the streamlined Enhanced quantitative urine culture (EQUC) method detects 84% of clinically relevant uropathogens[50].To detect even more elusive urinary microbial species undetected by EQUC and streamlined EQUC, metagenomic sequencing of microbial genome via 16S rRNA amplicon sequencing [51]and/or whole-genome metagenomic shotgun sequencing are applied [52]

Taxonomic Profile of a Healthy Human Urinary Microbiome

A healthy individual without urinary tract infections has low urinary microbiome biomass [53]. With the aid of 16S rRNA sequencing [41] and culture techniques [54], taxonomic profiles that characterize human urinary microbiome have been formed.

A 2011 study discovered 22 different orders and 45 genera of bacteria resident in the female urinary tract. Bacteriodales, Lactobacillales, Clostridales and Bifidobacteriales were the most common order while Lactobacillus, Prevotella and Gardnella which belong to phyla Firmicutes, Bacteriodetes and Actinobacteria were the most common genus [41].

Hrbacek et al. reported that the most common phyla in their 2021 study (which focused on human male urinary microbiota) *were Bacteroidetes, Firmicutes, and Proteobacteria* while the most common phyla were*Prevotella, Veillonella, Streptococcus, Porphyromonas, Campylobacter, PseudomonasStaphylococcus, Ezakiella, Escherichia and Dialister* [55]. Nelson et al. reported that *Streptococcus,Gardnerella, Lactobacillus* and *Veillonella* were the most common genera in adolescent male urinary microbiota[56].

Factors Affecting the Urinary Microbiome

The human urinary microbiome is affected by factors in the urinary tract environment [48]. Urinary oxygen tension is one of these factors. Research has suggested that urinary oxygen tension significantly affects urinary microbiome composition [57.]Another factor affecting urinary microbiome is age. A study by Liu et al. compared urine samples from young and ageing women, they reported a decrease in *Lactobacillus, Bifidobacteria* and an increase *Peptococcus* in the elderly cohort compared to the younger participants[58].

Hormone therapy may also influence microbial composition in the urinary tract. Anglim et al., proved that local estrogen therapy decreased the presence of *Finegoldia Magna* [2], a bacteria species that induces inflammation [3] Infections of the urinary tracts also have a relationship with urinary microbiome. Resident microorganisms increase the risk of urinary tract infections [59]

Evidence for the Relationship between Urinary Microbiome and Urinary Tract Infections

Urinary microbiome dysbiosis, an imbalance in the composition and diversity of urinary microbiota may be involved in the development of some urologic disorders [60]. Urgency urinary incontinence, overactive bladder, neuropathic bladder and recurrent urinary tract infections are thought to be affected by microorganisms in the urinary tract [61]

Although, urgency urinary incontinence is supposed to be as a result of a detrusor neuromuscular dysfunction or abnormal signalling [34], the complexity of the disease makes an imbalance in the urinary microbiome a major causative factor in the development of this disease [62]. In a study to characterise the bacteria responsible for UUI, *Lactobacillus, Gardnerella , Prevotella, Enteronacteriaceae, Staphylococcus, Aerococcus and Bifidobacterum* were found in sequence positive urine samples [63] In a cross-sectional study in Japan to confirm the relationship between the urobiome and overactive bladder, Okamoto et al., discovered that *Faecalibacterium* was more abundant while *Bifidobacterium* was lesser in people with overactive bladder [64]For people suffering from neuropathic bladder, their urobiome is more populated by uropathogens like *Klebsiella, Pseudomonas, Enterococcus* instead of *Lactobacillus*[45] Unfavourable external stimuli to the bladder and environmental stressors adversely affect urinary microbiome, favouring recurrent urinary tract infections [65]. Escherichia coli, a uropathogen is implicated as a cause of recurrent urinary tract infections [66]

Conclusion

The urinary microbiome is a new frontier for urology research with an interesting outlook on potential directions for understanding urinary tract function. With new technologies being developed daily, especially in Next Generation and other sequencing techniques, and the realization that urine is colonized by a community of microorganisms, research has been ongoing which reflects the importance and implications of the urinary microbiota on health.

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