Regional Differences in UTI Microbiome Studies

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Abstract. Urinary Tract Infection (UTI) is a common condition often expressed as a bacterial colonization of the bladder that in severe conditions extends to associated structures in the urinary system. The topic of recurrent UTI and antibiotic resistance has prompted several researchers to focus on the relationship gut and urinary microbiome have with recurrent UTI, searching for potential cures to antibiotic-resistant UTIs from the probiotic approach. This study aimed to find regional differences in microbiome studies, including the taxonomies in microbiomes across regions and their relationships to the local UTI conditions. The study reviewed data and findings of two iconic studies, Choi et al. and Huang et al., to examine how their findings would contribute to future research on probiotic treatments and the role of the gut-bladder axis in UTI. It found out that many identified UTI-causing pathogens are opportunistic species, whose relationships with the host are complicated by weakened immune systems, gut-bladder proximity and genetic plasticity. Additionally, the study pointed out several advantages of NGS technology that provide promising insight into microbiome taxonomies previously unreached by culturing methods. It identified several species as potential UTI-curing probiotics, though their wide applications were cautioned. Further research on UTI microbiome across regions and their effects on the local conditions is still warranted.

Keywords: Urinary Tract Infection (UTI); Probiotic Treatment Methods; NGS Technology; A Bacterial Colonization of the Bladder; Regional Differences in Microbiome Research.

1. Introduction

Urinary Tract Infection (UTI) is one of the most pervasive infections in the world, as half of all women get at least once in their lifetime. [1] It is often seen as a bacterial infection of the bladder and associated structures, although inflammations of the urinary tract as a result of viruses or fungi are possible. [2] A UTI is considered to be recurrent if UTI symptoms, despite medical treatments, keep reemerging within a short time interval; for instance, having 3 UTIs within 12 months is a good indication of recurrent UTI. [3] Recurrent UTI (rUTI) occurs due to either bacterial persistence or bacterial reinfection. Persistent bacteria such as *Escherichia coli*, a gram-negative bacteria species that reside in gut microbiome and invade bladder from gut to urethra, would develop antibiotic-resistant strains that help them survive upon medical treatments. [4] On the other hand, factors such as abnormal anatomical structures, unhealthy diets, or unsanitary environmental conditions may captivate another bacterial intrusion by a new species, subjecting the bladder to repeated attacks. [5]

Previously, the urine liquid in the bladder was assumed to be a sterile environment under normal conditions and would only have bacteria present when a pathogen intrusion occurs. Therefore, the culture-dependent method of extracting urine specimens and growing them on the agar plate is primarily used to indicate infection and possible pathogen species.[6] It is not until recent technological developments such as 16S RNA gene sequencing technology and improvements in PCR that we are able to ascertain the presence of bacteria in the urinary tract beyond playing pathogenic roles.[7] In fact, analogous to gut microbiome, urinary tract possesses a wide variety of bacterial species that together form the urinary microbiome; some species of bacteria previously identified to be harmful in the urinary microbiome also exist in the gut microbiome as a healthy benefactor, hence painting a much more complex picture to the relationship between infection and bacterial presence in the gut and urinary tract (Figure 1).[8]

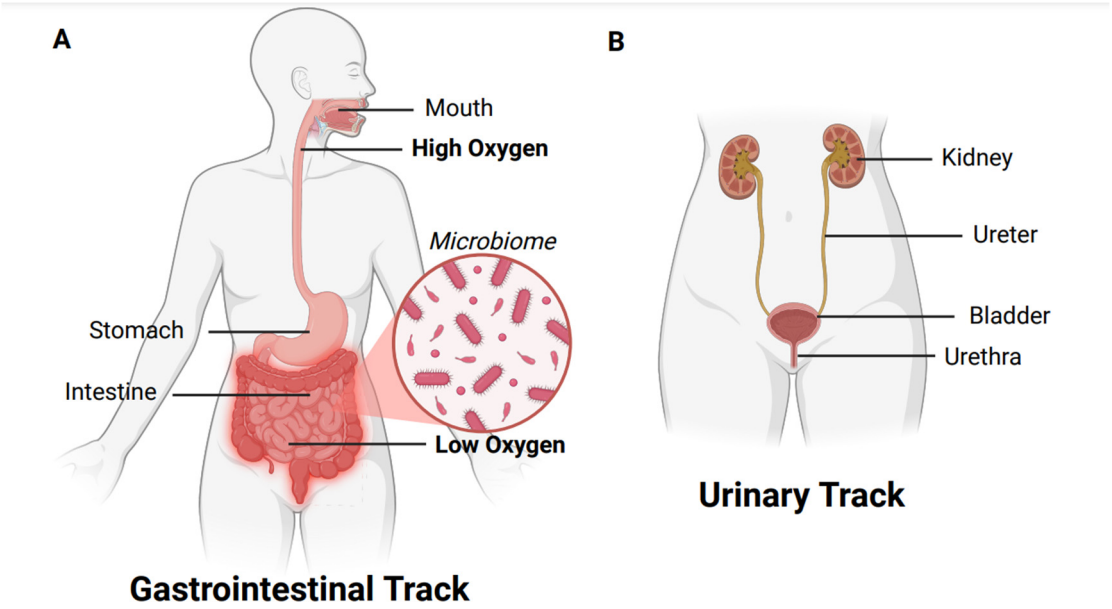

Figure 1. [9] Image A is a figure of the gut, also known as the gastrointestinal tract. The lower gastrointestinal environment is a key influencer of UTI development which is characterized by low levels of oxygen and abundances of nutrients with varying degrees of acidity depending on the specific parts, which welcomes mostly anaerobic bacterial groups such as Firmicutes to colonize the structure. Aiding in crucial functions such as metabolic processes and nutrient absorption, the mutual relationships between the human host and the gut microbiome are extensively studied. [10] Image B represents a female urinary tract. The recently discovered urinary tract microbiome occupies mainly the bladder and urethra, in addition to some parts of the ureter and kidney. Compared to the gut microbiome, it is less diverse due to relatively lower nutrient levels and less stability. The focus of this study, UTI, occurs when pathogens invade from the urethra; the absence of elongated genital structure, which gives a shorter urethra passage, explains the higher likelihood of women getting UTI than men. [2]

In this article, the term "microbiome" is defined as, according to Ursell, Luke K., et al, "the catalog of microbes and their genes." [11] Since the microbiome encompasses all genetic materials present in the environment, it reflects biotic and abiotic aspects that influence the studied environment, providing meaningful information on the medical conditions of a patient. [12] Therefore, in clinical procedures patients' urines and stool samples are collected and grown on culture plates to decide the species present in the sample. Also, researchers recently begin to utilize small RNA segments to determine the taxonomical composition of microbiome, which is a culture-independent process. Notably, the bacterial 16S ribosomal RNA gene is ubiquitous across the phylogenetic tree of Domain Bacteria, and it displays remarkable genetic diversity in different species, serving as a great molecular marker for taxonomy identification and phylogenetics [13]. This sequence is the target for gene sequencing and amplification, and the amplified result would be compared to gene sequences stored in major genetic information database such as Greengenes (Database link: http://greengenes.lbl.gov/) to deduce the taxonomy of the collected sequence. [14] Previous studies that have extracted and analyzed urinary and gut microbiome sequences have provided valuable data and insights on microbiome differences between healthy and UTI patients. Two of them would be analyzed in this paper.

Microbiome taxonomy is determined by many factors; among them the influence of antibiotic treatments and diets are, to the scientists' paramount concern, the most researched. It has been concluded from past animal research that diet is one of the major factors that alters the composition of gut and urinary microbiome, since growth of types of bacteria species corresponds to the type of food ingested in daily intake. [15] On the other hand, understanding how antibiotics alter gut and

urinary microbiome taxonomies has crucial implications in future drug therapies of combating UTI. [16] This paper aims to combine microbiome data from two studies, one in America and one in China respectively and investigate taxonomical compositions of healthy and UTI-present urinary and gut microbiomes, and based on taxonomies the researcher inquiries into the complicated relationship on antibiotics, diets and their effects on microbiome populations.

2. Past Studies & Methodologies:

2.1 Previous Studies

This study would take into account of two similar microbiome researches that investigates the relationship between urinary tract infections and microbiome composition.

One was conducted by Lei Huang, et al. in 2022 China. [17] The research aimed to compare the taxonomy of healthy urinary microbiome and microbiomes with rUTI. It narrowed the population of interest to adult women (age $\geq$ 18), using urine samples taken via midstream collection. In total, only one round of collection occurred, which 67 samples from patients with rUTI and 44 samples of healthy control group were successfully obtained. All patients either displayed more than one symptom 72 hours before the collection or were confirmed to be clinically diagnosed of rUTI in the last 6 months before the enrollment. The researchers amplified the samples before sequencing the samples' 16S rRNA genes. Note that the samples underwent additional testing using DNA next-generation sequencing (NGS), which were proved to be more accurate than standard urine cultures (SUC) in revealing signs of infection in the urine samples. [18]

The Choi, et al. study was a multi-center cohort research in the United States directed by Jennie H. Kwon on behalf of the CDC Prevention Epicenters Program that went through multiple episodes of data collection over 5 months. [19] The longitudinal study is primarily interested in the gut microbiome and the role it plays in the gut-bladder axis, especially the movement of uropathogenic *Escherichia coli.* population from the gut to the urinary microbiome, creating microbiome dysbiosis and resulting in rUTI. [20] Patients must have rUTI caused by antibiotic-resistant organisms (ARO) to be recruited into the study. In total, 125 patients were enrolled; they provided stool and urine samples which the urine samples, unlike Huang et al.'s study, were cultured. The researchers extracted metagenomic DNA from fecal samples using the DNeasy Powersoil Kit, and sequences were trimmed of human contamination and archived in sequence libraries for NGS (Illumina) analysis. Its final analysis incorporated existing datasets of 20 samples on healthy gut microbiomes as control group as well as a data of 15 rUTI and 16 healthy gut and urinary microbiomes. [21]

It is important to emphasize that the previous studies have different subjects of concern: the Choi's article focuses on correlation between gut microbiome dysbiosis and rUTI, so it gathered urine samples to test antibiotics susceptibilities without including them in the taxonomical comparison. Thus, its graphical displays mainly discussed changes in gut microbiome when infected with UTI. [19] In addition, Choi's study acknowledged its limitations that uropathogenic *Escherichia coli.* (UPEC) dominates the majority of its samples; lacking diversity within the selected sample, the study's results could be applied to common scenarios of UTI but may not be generalizable to all populations with UTI, specifically patients infected with rare pathogens (Figure 2).[19] On the other hand, instead of being multi-center and undergoing multiple trials, Huang Lei's research collected all of the samples at a single site, namely Peking University First Hospital, under only one trial.[18] The samples are exclusively urine samples, and the subsequent hierarchical clustering analysis covers all species detected within the urinary microbiome, excluding the gut microbiome out of discussion.[17] Given the drastic differences between the experimental conditions of the two researches, this review would not make direct comparison between the two but instead analyzes them separately and then includes certain parts of data from both studies in the cross-talk analysis.

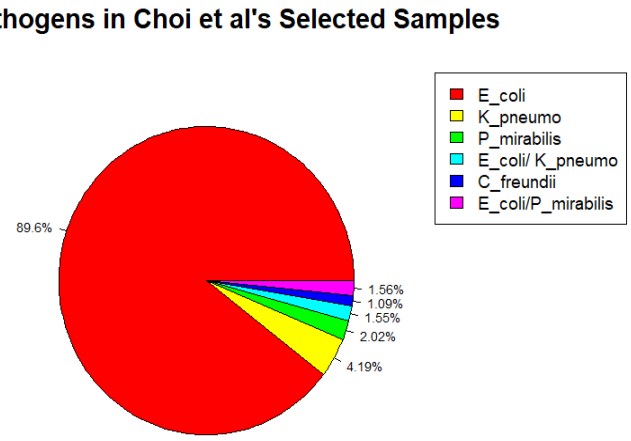

Figure 2. Relevant frequencies of UTI pathogens detected in selected urine samples of the study. Although the significantly high prevalence of UPEC strains helps researchers to investigate general effects of UTIs on gut microbiome, the results of Choi's study are not ideal for predicting special UTI cases that are caused by rare pathogens (meta data obtained from Choi, et al.'s study, graph generated by R).

2.2 Choi et al.'s Findings on Gut Microbiome & UTI

Distinctive differences exist between healthy gut microbiomes and those in UTI conditions. Although overall microbiome richness is slightly higher in healthy microbiomes than UTI microbiomes, it does not vary significantly; instead, species compositions distinguish the microbiomes under UTIs from those healthy ones. Choi's study focused on 11 prevalent intestinal bacterial genera. Comparing UTI and healthy samples, researchers found that 9 of the 11 genera, mostly commensal firmicutes and butyrate producers, thrive in healthy conditions. [19] Their populations decrease drastically in UTI samples, especially evident in commensal genera *Parasutterella*, *Akkermansia*, and *Bilophila* that were noted by researchers as "depleted in intestinal samples of subjects with UTI." [19] In contrast, genera *Flavonifractor* and *Hungatella* increase in richness in UTI microbiomes. [19]

Before the microbiome analysis, researchers proposed that UTI microbiomes have diverse antimicrobial-resistant genes (ARG) compared to healthy microbiomes, but sample analysis indicated that ARG richness does not differ much between these groups. Rather, UTI microbiomes have higher ARG abundances and increasing ARG phenotypic expressions due to UTI-antimicrobial treatment exposure.[19] This led the researchers to investigate relationships between gut microbiome and UTI recurrence.

Within the studied population, patient metadata does not suggest any typical UTI symptom associated with rUTI; rUTI could not be inferred from any clinical characteristics. Nor could it be indicated from microbiome taxonomies, since metagenomic analysis concluded that rUTI microbiomes and non-recurrent UTI microbiomes have no significant taxonomical difference in terms of overall diversity and individual species richness. The most impactful differentiator of microbiome composition is found to be patient IDs, meaning that overall microbiome composition of each selected patient inherently varies from person to person prior to UTI disruptions. [19]

The study did find, however, a possible association between rUTI and antibiotic treatments. The study showed that antibiotic therapy depletes gut microbiome richness, although the extent of depletion depends on the specific antibiotic being used. [19] By days 7 to 14 days in the post-therapy period, the gut microbiome would recover, but this recovery of gut microflora varies distinctively between patients whose urinary tracts were asymptomatically colonized by UPEC with gut *E. coli.* lineages and those without colonization. Fecal samples from UT-colonized patients (Ucol group) revealed significantly enriched *E. coli* and *Paraprevotella xylaniphila* population present in Ucol gut microbiome at 7 to 14 days post-antimicrobial period. Furthermore, Ucol UPEC displays high phenotypic antibiotic resistance, which could be explained by the high ARG abundances in UTI gut

microbiome. Choi and his coresearchers concluded that ARG's phenotypic expressions in the gut are an indication of the crucial role gut microbiome plays in UTI recurrence: gut microbiome is fueling the urinary tract-colonizing pathogens, such as UPEC, with antimicrobial-resistant genes, rendering the eradication of pathogenic colonies within urinary tract via conventional antibiotic treatments extremely difficult. [19]

Within the Ucol group, 54.5% of patients experienced UTI symptomatic recurrence in the follow-up period; these patients had minimally low abundance of species *Bacteroides xylanisolvens*, whereas in those Ucol patients who did not develop a recurrence the Bacteroides population is much higher. Thus, the researchers believed that commensal Bacteroides have high potential in probiotic drug developments, as already corroborated by previous study that their presence in UTI non-recurrent gut microbiome may indicates their salutary role in competing resources with UTI-causing bacteria. [22]

2.3 Huang, et al.'s Findings on Urinary Tract Microbiome & UTI

Taxonomic comparisons of rUTI and asymptomatic urinary microbiota has shown several variations in terms of species diversity and microbiome composition. Calculated alpha indices showed that species richness within rUTI microbiota is lower than asymptomatic ones. If the calculation includes rare species (ex. Chao1 index), whose abundances are close to minimal that they were detected in only one or two trials of metagenomic analysis, the richness gap is even higher. [17] However, there was no statistical difference between distributions of each group, as alpha indices reported the deviations of relative evenness of each sample to be similar in rUTI and control samples. [17]

On the other hand, this does not mean that species abundances between the 2 groups are homogenous; in fact, beta indices, by making a direct intergroup comparison, found that rUTI microbiomes is very different of healthy microbiomes. As specific to genus level, Huang et al. have detected 60 bacterial taxa whose abundances are vastly different under rUTI and asymptomatic conditions. The researchers then identified 11 of them that have the highest LDA scores in their magnitudes: Phylum *Proteobacteria*, order *Burkholderiales*, genus *Ralstonia*, genus *Prevotella*, genus *Dialister* and genus *Corynebacterium* have a considerable increase in their relative abundance in rUTI samples with respect to asymptomatic controls. Vice versa, genus *Lactobacillus*, genus *Gardnerella*, Viridans *Streptococci* and genus *Ezakiella* have significantly high abundance in healthy microbiota relative to those in rUTI conditions. [17] To conclude the UTI and healthy urinary microbiome compositions of selected Chinese females, Huang et al. mapped identified (as well as those unidentified, labelled ambiguious_taxa) taxa found in the samples into an aggregate phylogenetic cladogram. [17]

Genus *Ralstonia* received researchers' attention for further elaboration in this study. The absence of genus *Ralstonia* in asymptomatic urinary microbiome has raised Huang et al.'s interests in the rapid growth of this genus during recurrent pathogenic colonization of the urinary tract. In past research, genus *Ralstonia* has already been associated with infections such as Meningitis; the researchers, on this notion, advocated for further investigation into this bacterial species and whether it may have a pathogenic. [17]

3. Cross-talk Analysis

Two studies provide a valuable insight into the complex roles of bacteria in the urinary tract. The results, at first hand, reject the common misunderstanding that fixated roles of various bacteria species determine which species are pathogens and which are mutualistic. Instead, they suggest that effects of different bacteria population on a typical environment depend on the specific conditions of the body and the local microbiome. A mostly commensal bacteria species that expresses pathogenicity when there's a change in body conditions, generally a weakened immune system, is coined the term "opportunistic species". [23] A previous study that attempted to categorize urinary microbiome bacteria states that the majority of metagenomic sequenced species (59.8%) could be associated with

pathogenicity despite being unlikely to be contagious. [24] However, 22.2% of the detected species remained unclassified, signaling that more sequencing analysis is needed to further investigate those species with unknown properties. [24]

In the studies, Huang et al pointed out *Gardnerella* as a bacterial genus that exemplifies this volatile nature of host-bacterial relationship. Previously, a study that investigated urine microbiota composition of healthy European women showed that *Gardnerella* along with *Lactobacillus* and *Streptococcus* are the most dominant bacterial populations within healthy microbiomes. The study also suggests that the *Gardnerella* and *Lactobacillus* populations contribute to lower urinary tract resilience. [25] Yet, another study reveals an opposite effect of *Gardnerella* that repeated introduction of *Gardnerella*, specifically *Gardnerella vaginalis,* would elicit urothelial exfoliation, exposing the unprotected urothelial cells to colonizing uropathogens. [26] Furthermore, *in vitro* experiment indicates synergy existing between *Gardnerella* and UPEC, which the former aids the latter in recurrent UTI. [24] Given, genus *Gardnerella* prefers healthy conditions of urinary microbiome since Huang et al.'s graphical analysis indeed demonstrates a significant decrease in *Gardnerella* population in rUTI urinary microbiome. [17] Therefore, further research into genus *Gardnerella* and its relationship with the host is warranted, and whether the decrease in *Gardnerella* population is caused by flushing of urines after bladder exfoliation is subject to future investigation. [12]

Besides environmental factors, genome plasticity further complicates the potential of probiotic treatments. As stated in the both studies, the relatively high presence of certain bacterial species/genus promises potential of probiotic treatments via those given taxa; their elevated presence would compete with pathogenic populations for resources, as well as inhibiting crucial binding sites on the urolithiums. [17, 19] *Lactobacillus* specifically receives researchers' attention since its probiotic effects have already been proven in clinical trials. [27] Meanwhile, testing of gut microbiome taxon *Bacteroides* for its probiotic potential is under way. [28] Yet, although probiotic treatments are more advantageous in treating rUTI than antibiotics when considering that probiotics do not enhance antibiotic-resistance of UTI pathogens, pharmacists still reserve the mass application of probiotic treatments in clinical conditions, and antibiotics remain the primary treatment for curing UTI. [2] Pharmacists' primary concern is with the exchange of virulence genes between bacterial species of the microbiome. For example, *Streptococcus thermophilus* has already been applied in dietary products for its salutary effects as well as in probiotic therapies treating inflammation caused by gut diseases. [29] However, researchers hesitate in using the species in UTI probiotics because it has close lineages to UTI-causing species *S. salivarius* and *S. vestibularis*. [30] Despite the fact that virulent genes in *S. thermophilus* are largely absent or inactivated, the species' capability to acquire new genes from other species via horizontal gene transfer mandates pharmacists that more needs to be understood in bacterial interspecies interactions before the mass applications of such probiotics in treating rUTI. [30]

When viewed together, the two studies' results support the gut-bladder axis theory, that the proximity of the gut and urinary tract enables the gut microbiome to influence the defense as well as inflammation of the urinary tract. [30] Previous studies have stated that the gut microbiome may serve as a reservoir of *Escherichia coli*. that provides the urinary tract with a steady supply of intruding pathogens, resulting in recurrent colonization of the urinary tract. [31] Choi et al. corroborated this phenomenon, adding that antibiotics cause microbiome dysbiosis and subsequently increased ARG in the gut, whose phenotype is expressed in the urinary tract, among the antibiotic-enduring bacteria population. [19] Huang et al.'s findings validated that antibiotic treatments are equally destructive to the gut as well as the urinary microbiome, reporting a significant loss of diversity and number in urines that were sampled from antibiotics-using rUTI patients. [17]

Huang et al. also focused on the current phenomenon of antibiotic overuse due to false urine culture results. This raises another concern of contemporary scientists regarding SUC's inaccuracies in contrast to the emerging NGS technology. Huang et al. detected bacteria taxa of extremely low presence that they were previously thought to be non-existent within human urinary tract. [17] The lack of knowledge on low presence population is attributed to the low sensitivity of SUC, which

inclines to reflect more abundant taxa on the culture and leave out slow-growing, anaerobic bacteria. [17], [32] Considering that certain gut bacteria possessing virulence factors tend to migrate to the urinary tract with notable examples such as UPEC and *Klebsiella pneumoniae*, researchers in recent years began to assess the viability of NGS replacing SUC in UTI diagnosis. In a systematic review of notable UTI microbiomes (gut-bladder axis) studies, Szlachta-McGinn et al. concluded that NGS demonstrated consistently higher bacteria detection rates in urine samples via various extraction methods over SUC. [33] Its strong genotypic detection enables it to map microbiome diversity, rendering NGS a competent tool for microbiome analysis.

However, its high sensitivity does not guarantee it being more successful than SUC in terms of disease diagnosis; NGS only presents the genotypes of urinary tract microbiomes, but it does not reveal phenotypic expressions of those detected genes.[33] In other words, it could not distinguish between genetic materials from dead cells and materials in a vibrant pathogenic bacterium. NGS, therefore, could not thoroughly reflect the intricate situations occurring in the urinary tract: NGS diagnosis could still contribute to antibiotic overuse when antibiotics are assigned to an asymptomatic person whose urinary tract possesses virulent bacteria genes, even though there is no inflammation as a result of those genes. [17, 32, 33]

Conclusions regarding regional microbiome taxonomical differences due to diets are difficult to make: as already indicated by Choi et al., human microbiomes do not differ significantly from one to another, and slight to moderate variations depend on the individual body conditions (obesity, age, sex, etc.), which is not necessarily correlated with the region of inhabitance. [19] Moreover, taxonomical differences in microbiomes across various nations have already been conducted by Pesoa et al. Pesoa et al. discovered significant taxonomical differences between UK, US, and Argentina; however, the differences are primarily a result of different obesity rates present within each country. [34] It was the obesity proportion of a country that contrasts with another country, not simply because of the differences in nationalities. Therefore, healthier diets indeed lead to a decreasing chance of UTI, but it has minimal relation to regions. [34], [35] When assessing the microbiomes of Chinese and American patients, this study also did not find any significant differences since the bacterial genera detected are largely the same, and few additional genera found in Huang et al.'s study could be attributed to the high sensitivity of NGS technology.

4. Conclusion

Choi et al. and Huang et al. exemplify two primary methods for contemporary researchers to investigate differences in rUTI and healthy microbiomes. While Choi et al. studied the gut-bladder relationship and effects of recurrent antibiotic usages on microbiome diversity through extracting, culturing and testing urine samples for multiple trials, Huang et al. analyzed rUTI and asymptomatic microbiomes by mapping out their general composition detected by NGS technology. When it comes to population differences between UTI and healthy microbiomes, Choi et al.'s study focused specifically on 11 taxa, and for urinary microbiomes UPEC was the main testing object. But Huang et al.'s research analyzed the changes of as many bacterial taxa detected; NGS technology, viewed from the 2 studies, is proven to be more sensitive than SUC by sensing bacteria taxa with low abundance. Both studies reached the consensus that the relationship between UTI and microbiome is more convoluted than previously thought; symptoms could not simply be attributed to whether virulent factors exist within the urinary tract but it also depends on the conditions of the environment, whether the environment allows opportunistic bacteria population to adapt pathogenic roles via uncontrolled proliferation or genetic transformation.

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