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Korean urobiome platform (KUROM) study for acute uncomplicated sporadic versus recurrent cystitis in women: Clinical significance

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Purpose: To investigate urine microbiome differences among healthy women, women with recurrent uncomplicated cystitis (rUC), and those with sporadic/single uncomplicated cystitis (sUC) to challenge traditional beliefs about origins of these infections.

Materials and Methods: Patients who underwent both conventional urine culture and next-generation sequencing (NGS) of urine were retrospectively reviewed. Symptom-free women with normal urinalysis results as a control group were also studied. Samples were collected via transurethral catheterization.

Results: In the control group, urine microbiome was detected on NGS in 83.3%, with *Lactobacillus* and *Prevotella* being the most abundant genera. The sensitivity of urine NGS was significantly higher than that of conventional urine culture in both the sUC group (91.2% vs. 32.4%) and the rUC group (82.4% vs. 16.4%). In urine NGS results, *Enterobacterales, Prevotella,* and *Escherichia/Shigella* were additionally found in the sUC group, while the recurrent urinary tract infection (rUTI)/rUC group exhibited the presence of *Lactobacillus, Prevotella, Enterobacterales, Escherichia/Shigella,* and *Propionibacterium*. Moreover, distinct patterns of urine NGS were observed based on menopausal status and ingestion of antibiotics or probiotics prior to NGS test sampling.

Conclusions: Urine microbiomes in control, sUC, and rUTI/rUC groups exhibited distinct characteristics. Notably, sUC and rUC might represent entirely separate pathological processes, given their distinct urine microbiomes. Consequently, the use of urine NGS might be essential to enhancing sensitivity compared to conventional urine culture in both sUC and rUTI/rUC groups.

Keywords: 16S rRNA next-generation sequencing; Cystitis; Microbiome; Urinary tract infections

INTRODUCTION

Ahmed et al. [1] have reported that over 92% of bacteria causing urinary tract infections (UTIs) are resistant to at least one common antibiotic. In 2021, the World Health Organization recognized antimicrobial resistance as a top

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global public health threat. Cystitis can be categorized as either sporadic/single uncomplicated cystitis (sUC) or recurrent urinary tract infection (rUTI)/recurrent uncomplicated cystitis (rUC), each with unique diagnostic and management challenges. sUC often presents suddenly with symptoms such as dysuria, frequency, urgency, and hematuria without a recent history of similar infections. Conversely, rUC is marked by frequent UTIs and chronic discomfort due to recurring episodes. It is verified by positive urine cultures and typical symptoms. While sUC could be managed with short-term antimicrobial therapy, rUTI/rUC requires more complex treatment strategies due to frequent relapses and potential complications, highlighting the need for a deeper understanding of its mechanisms [2].

Advancements in next-generation sequencing (NGS) technologies have dramatically enhanced our ability to study complex microbial communities in the human body [3,4]. In the context of UTIs, NGS is a powerful tool for analyzing urinary microbiota, offering detailed insights into the diversity and composition of microbial species across different stages of cystitis [5]. By comparing microbial profiles of patients with acute and recurrent cystitis, we can identify key differences that help explain the persistence of symptoms and guide the development of targeted treatments.

Previously, our research group uncovered significant differences in urine microbiome between sUC and rUTI/rUC patients in a pilot study [6]. However, that pilot study had certain limitations, including the absence of a control group and a limited number of patients in the rUTI/rUC group, which hindered the generalizability of our findings. Therefore, in this study, we gathered data from a more extensive patient cohort over an extended duration to explore variations in urine microbiome among healthy controls, sUC patients, and rUTI/rUC patients. Additionally, we examined the influence of menopause and antibiotic usage on urine microbiome.

MATERIALS AND METHODS

1. Patients and study protocol

From March 2020 to January 2023, data were gathered from individuals at Soonchunhyang University Bucheon Hospital who displayed typical cystitis symptoms, focusing on those diagnosed with either sUC or rUTI/rUC. Eligible patients were 20 years or older and underwent urinalysis, conventional urine culture, and urine NGS. We excluded patients with anatomical abnormalities such as stones, pregnancy, indwelling catheters, or diaphragm use. Classification of rUC and sUC was based on medical records, including those from external hospitals when applicable. According to the European Association of Urology (EAU) guidelines, an acute episode of cystitis is identified by the sudden appearance of symptoms like dysuria, frequency, urgency, and/or hematuria [7]. Patients were categorized as sUC if they had an isolated episode or infrequent episodes not meeting recurrence criteria, and as rUTI/rUC if they experienced two or more episodes within six months or three or more episodes within a year. Urine samples were collected during an acute episode before treatment initiation. However, as our hospital is a tertiary referral center, many patients had already received antibiotics from other healthcare facilities before presenting to our institution. Thus, those who had taken antibiotics at another hospital before urine sample collection at our hospital were classified as the antibiotic-taking group.

In addition, data from a control group without cystitis symptoms, abnormal urinalysis results, and those undergoing urine NGS for health checkups were collected. In total, we included 42 control subjects, 34 sUC patients, and 488 rUTI/rUC patients who met the criteria. The study protocol received approval from the Institutional Review Board (IRB) of Soonchunhyang University Bucheon Hospital (approval number: 2023-10-001) and adhered to the ethical guidelines of the World Medical Association Declaration of Helsinki. The written informed consent was waived by the IRB due to the retrospective nature of the study.

2. Conventional urine culture

We collected urine samples from patients suspected of having cystitis using a transurethral catheter. A small volume of well-mixed urine (0.001 mL) was inoculated onto both blood agar and MacConkey agar plates (Asan Medical). These plates were subsequently incubated overnight in a 5% CO_2 incubator at a temperature ranging from 35℃ to 37℃. Following the incubation period, the culture plates were examined, and bacterial growth equal to or exceeding 10^3 colony-forming units per milliliter (CFU/mL) was considered indicative of significant bacteriuria. Bacterial identification was carried out using matrix-assisted laser desorption ionization-time of flight mass spectrometry (ASTA MicroIDSys).

3. Sample collection, transport, and DNA extraction

After collection, urine samples of 50 mL were promptly frozen at -20℃ until further processing. DNA extraction was performed using the Chemagic DNA Stool Kit (PerkinElmer Inc.), following the manufacturer's guidelines with necessary pretreatment and modifications. The urine samples were centrifuged at 3,000 rpm for 15 minutes, and the resulting

Table 1. Baseline characteristics of patients

	Total (n=540)	Control (n=18)	Acute uncomplicated cystitis (n=34)	rUTI/recurrent cystitis (n=488)	p-value
Age (y)	53.27±14.51	49.22±14.49	49.35±16.85	53.69±14.31	<0.001
Menopause	330 (61.1)	10 (55.6)	17 (50.0)	303 (62.1)	0.333
Prior antibiotic use	291 (53.9)	0 (0.0)	25 (73.5)	266 (54.5)	<0.001
Prior pre/probiotics use	56 (10.4)	0 (0.0)	1 (2.9)	55 (11.3)	0.133

Values are presented as mean±standard deviation or number (%).
rUTI, recurrent urinary tract infection.

urinary pellets were washed twice with a 10-fold volume of phosphate-buffered saline. These washed pellets were subsequently suspended in 700 μL of lysis buffer and added to a silica bead tube. To disrupt cells, the bead-suspension mixture was vigorously vortexed for 5 minutes. After centrifugation, the supernatant underwent thermal disruption, proteinase K digestion, and bead-binding and elution steps, following the manufacturer's instructions. The concentration of DNA was quantified fluorometrically using the Qubit® 3.0 Fluorometer (Thermo Fisher Scientific) and the Qubit™ dsDNA HS Assay Kit.

4. 16S rRNA amplicon sequencing and bioinformatics analysis

The urine NGS test was conducted at Green Cross Laboratories through Green Cross Genome, as in our previous study [6]. Processed DNA was used to create 16S libraries with the NEXTflex 16S V4 Amplicon-Seq kit from Bioo Scientific. The resulting library underwent sequencing using the Illumina MiSeq Reagent Kit v2 (500 cycles), following the manufacturer's instructions. For the analysis of the 16S sequence data, we employed QIIME 2. The data, after demultiplexing and trimming of primer sequences, underwent quality filtration and denoising with DADA2 [8,9]. Amplicon sequence variants (ASVs) with fewer than 10 reads or those found in only one sample were removed. Taxonomy was assigned to each ASV using naive Bayes machine-learning taxonomy classifiers within q2-feature-classifier against the National Center for Biotechnology Information (NCBI) RefSeq database. Taxonomic weight assembly was conducted using q2-clawback [10,11].

5. Statistical analysis

Continuous baseline characteristics were presented as mean±standard deviation and compared using Student's t-test or the Kruskal–Wallis test. Categorical characteristics were presented as counts and percentages and compared between groups using the chi-squared test. Statistical significance was defined as $p<0.05$. All statistical analyses were conducted using R version 4.3.1 (R Foundation for Statistical Computing).

Table 2. Comparison of sensitivity of conventional urine cultures and urine NGS in acute uncomplicated cystitis and recurrent cystitis

	No. of patients	Conventional urine culture (+)	Urine NGS (+)
Control	18 (100.0)	0 (0.0)	15 (83.3)
Acute episode of sUC	34 (100.0)	11 (32.4)	31 (91.2)
rUTI/rUC	488 (100.0)	80 (16.4)	402 (82.4)
Total	522 (100.0)	91 (17.4)	433 (83.0)

Values are presented as number (%).
NGS, next-generation sequencing; sUC, sporadic/single uncomplicated cystitis; rUTI, recurrent urinary tract infection; rUC, recurrent uncomplicated cystitis.

RESULTS

1. Baseline characteristics

Patient characteristics are summarized in Table 1. All patients were females, with a mean age of 53.27±14.51 years. The mean age of the rUTI/rUC group was slightly higher at 53.69 years compared to the sUC group (49.35 years) and the control group (49.22 years). There were no significant differences in diabetes mellitus between groups. The rUTI/rUC group had a slightly higher rate of menopause (62.1%) than the other two groups, although the difference was not clinically significant. Two hundred ninety-one (53.9%) patients had taken antibiotics or probiotics prior to their visit to our hospital.

2. Comparison of sensitivity of conventional urine culture and urine NGS

Subsequently, we conducted a sensitivity comparison between conventional urine culture and urine NGS. A summary of sensitivity data for each group can be found in Table 2. In the control group, urine culture yielded no bacterial detections, while urine NGS returned positive results for 15 patients (83.3%). In the sUC group, positivity rates for urine

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Table 3. Common bacteria identified in urine culture

Category	Total (n=91)	Acute episode of sUC (n=11)	rUTI/rUC (n=80)	p-value
Candida glabrata	1 (1.1)	0 (0.0)	1 (1.3)	0.756
Citrobacter koseri	1 (1.1)	0 (0.0)	1 (1.3)	
Enterococcus faecalis	4 (4.4)	0 (0.0)	4 (5.0)[a]	
Escherichia coli	69 (75.8)	9 (81.8)[a]	60 (75.0)[a]	
Klebsiella pneumoniae	9 (9.9)	1 (9.1)[a]	8 (10.0)[a]	
Proteus mirabilis	2 (2.2)	1 (9.1)[a]	1 (1.3)	
Pseudomonas aeruginosa	2 (2.2)	0 (0.0)	2 (2.5)	
Staphylococcus epidermidis	1 (1.1)	0 (0.0)	1 (1.3)	
Staphylococcus saprophyticus	1 (1.1)	0 (0.0)	1 (1.3)	
Streptococcus agalactiae	1 (1.1)	0 (0.0)	1 (1.3)	

Values are presented as number (%).
p-value indicates difference in the overall population, not in individual groups.
sUC, sporadic/single uncomplicated cystitis; rUTI, recurrent urinary tract infection; rUC, recurrent uncomplicated cystitis.
[a]:Bacteria frequently found in each group are marked.

culture and urine NGS were 32.4% and 91.2%, respectively. In the rUTI/rUC group, positivity rates for urine culture and urine NGS were 16.4% and 82.4%, respectively, which were slightly lower than those in the sUC group.

3. Common bacteria identified in urine culture

Subsequently, we verified bacterial strains identified in 91 patients (11 with sUC, 80 with rUTI/rUC) with positive urine culture results (Table 3). In the sUC group, *Escherichia coli* was the predominant strain. It was detected in 9 patients (81.8%), with *Klebsiella pneumoniae* and *Proteus mirabilis* each found in 1 patient (9.1%). In the rUTI/rUC group, *E. coli* was the most frequently identified bacterium (60 patients, 75.0%), followed by *K. pneumoniae* (8 patients, 10.0%) and *Enterococcus faecalis* (4 patients, 5.0%).

4. Genus detected in over 10% of urine NGS

We conducted an analysis to identify the genera frequently detected in urine NGS within the three study groups (Table 4). A total of 751 genera were found in over 10% of urine NGS samples from 522 patients. In the control group, 24 genera were detected, with the most prevalent being *Lactobacillus* (11 individuals, 45.8%) and *Prevotella* (2 individuals, 8.3%). The sUC group exhibited a total of 40 detected genera, with *Enterobacterales* (10 patients, 25.0%) being the most common, followed by *Prevotella* (6 patients, 15.0%), *Escherichia/Shigella* (4 patients, 10.0%), and *Propionibacterium* (3 patients, 7.5%). In contrast, the rUTI/rUC group showed a relatively diverse range of detected genera. *Lactobacillus* (102 patients, 14.8%) was the most prevalent, followed by *Prevotella* (68 patients, 9.9%), *Enterobacterales* (59 patients, 8.6%), *Escherichia/Shigella* (57 patients, 8.3%), and *Propionibacterium* (50 patients, 7.3%). In summary, five bacterial genera exhibited statistically significant differences between the cystitis group and the control group: *Enterobacterales*, *E. coli*, *Lactobacillus*, *Prevotella*, and *Propionibacterium*.

5. Effect of menopause on NGS results

We proceeded to examine how urine NGS results varied based on menopausal status in both sUC and rUTI/rUC groups (Table 5). In the sUC group, we observed that *Escherichia/Shigella*, *Gardnerella*, *Lactobacillus*, *Prevotella*, and *Streptococcus* were the predominant genera detected before menopause. However, following menopause, proportions of *Enterobacterales* and *Propionibacterium* showed notable increases. In the rUTI/rUC group, we found that detection rates of *Gardnerella* and *Lactobacillus* were relatively high before menopause. After menopause, detection rates of *Anaerococcus*, *Enterobacterales*, *Escherichia/Shigella*, and *Prevotella* showed noticeable increases.

6. Effect of taking antibiotics and probiotics on rUTI/recurrent cystitis NGS results

Lastly, we analyzed effects of prior probiotic or antibiotic usage before hospital visits on urine NGS results (Table 6). In the sUC group, only one patient had taken probiotics. Thus, our analysis was focused on the effect of antibiotics. When antibiotics were administered, detection rates of *Enterobacterales*, *Escherichia/Shigella*, and *Gardnerella* showed significant decreases, whereas detection rates of *Prevotella* and *Propionibacterium* were notably higher.

Within the rUTI/rUC group, antibiotic usage was associated with marked reductions of detection rates of *Entero-*

Table 4. Genus detected in over 10% of urine NGS

Category	Total (n=751)	Control (n=24)	Acute episode of sUC (n=40)	rUTI/rUC (n=687)	p-value
Acidaminococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	0.006
Acinetobacter	5 (0.7)	0 (0.0)	0 (0.0)	5 (0.7)	
Actinobaculum	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Actinomyces	5 (0.7)	0 (0.0)	0 (0.0)	5 (0.7)	
Alcaligenaceae	1 (0.1)	1 (4.2)	0 (0.0)	0 (0.0)	
Alcaligenes	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.3)	
Alloscardovia	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Amycolatopsis	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Anaerococcus	27 (3.6)	1 (4.2)	0 (0.0)	26 (3.8)	
Anaerosphaera	4 (0.5)	0 (0.0)	0 (0.0)	4 (0.6)	
Atopobium	13 (1.7)	1 (4.2)	1 (2.5)	11 (1.6)	
Bacillus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Bacteroides	31 (4.1)	0 (0.0)	1 (2.5)	30 (4.4)	
Bifidobacterium	14 (1.9)	0 (0.0)	1 (2.5)	13 (1.9)	
Blastococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Bradyrhizobium	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Campylobacter	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Chryseobacterium	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Citrobacter	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Cloacibacterium	3 (0.4)	0 (0.0)	0 (0.0)	3 (0.4)	
Clostridium sensu stricto	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Clostridium XIVa	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Corynebacterium	21 (2.8)	0 (0.0)	0 (0.0)	21 (3.1)	
Cutibacterium	1 (0.1)	0 (0.0)	1 (2.5)	0 (0.0)	
Dermacoccus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Dolosigranulum	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Enhydrobacter	7 (0.9)	0 (0.0)	1 (2.5)	6 (0.9)	
Enterobacterales	69 (9.2)	0 (0.0)	10 (25.0)[a]	59 (8.6)[a]	
Enterococcus	11 (1.5)	1 (4.2)	1 (2.5)	9 (1.3)	
Eremococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Escherichia/Shigella	62 (8.3)	1 (4.2)	4 (10.0)[a]	57 (8.3)[a]	
Ezakiella	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Facklamia	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Faecalibacterium	11 (1.5)	0 (0.0)	0 (0.0)	11 (1.6)	
Finegoldia	11 (1.5)	0 (0.0)	0 (0.0)	11 (1.6)	
Fusobacterium	3 (0.4)	0 (0.0)	0 (0.0)	3 (0.4)	
Gardnerella	36 (4.8)	0 (0.0)	2 (5.0)[a]	34 (4.9)	
Gemella	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.3)	
Gracilibacter	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Haemophilus	4 (0.5)	0 (0.0)	1 (2.5)	3 (0.4)	
Janthinobacterium	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.3)	
Lactobacillales	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Lactobacillus	115 (15.3)	11 (45.8)[a]	2 (5.0)[a]	102 (14.8)[a]	
Lactococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Lishizhenia	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Megamonas	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.3)	
Megasphaera	3 (0.4)	0 (0.0)	0 (0.0)	3 (0.4)	
Micromonospora	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Millisia	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	

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Table 4. Continued

Category	Total (n=751)	Control (n=24)	Acute episode of sUC (n=40)	rUTI/rUC (n=687)	p-value
Mobiluncus	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.3)	
Murdochiella	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Mycobacterium	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Neisseria	3 (0.4)	0 (0.0)	0 (0.0)	3 (0.4)	
Nocardiopsis	1 (0.1)	1 (4.2)	0 (0.0)	0 (0.0)	
Novosphingobium	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Paracoccus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Pediococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Peptoniphilus	14 (1.9)	0 (0.0)	0 (0.0)	14 (2.0)	
Peptostreptococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Porphyromonas	4 (0.5)	0 (0.0)	0 (0.0)	4 (0.6)	
Prevotella	76 (10.1)	2 (8.3)[a]	6 (15.0)[a]	68 (9.9)[a]	
Propionibacterium	54 (7.2)	1 (4.2)	3 (7.5)[a]	50 (7.3)[a]	
Propionimicrobium	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Proteus	5 (0.7)	0 (0.0)	1 (2.5)	4 (0.6)	
Pseudomonas	26 (3.5)	1 (4.2)	1 (2.5)	24 (3.5)	
Ralstonia	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Rhodobacteraceae	1 (0.1)	1 (4.2)	0 (0.0)	0 (0.0)	
Roseburia	2 (0.3)	0 (0.0)	0 (0.0)	2 (0.3)	
Rothia	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Ruminococcaceae	1 (0.1)	1 (4.2)	0 (0.0)	0 (0.0)	
Saccharopolyspora	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Salmonella	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Sneathia	2 (0.3)	1 (4.2)	0 (0.0)	1 (0.1)	
Sphingobacteria	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Sphingomonas	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Staphylococcus	14 (1.9)	0 (0.0)	1 (2.5)	13 (1.9)	
Streptococcus	21 (2.8)	0 (0.0)	2 (5.0)[a]	19 (2.8)	
Streptophyta	3 (0.4)	0 (0.0)	0 (0.0)	3 (0.4)	
Tannerella	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Thalassobaculum	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Turicibacter	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Ureaplasma	8 (1.1)	0 (0.0)	1 (2.5)	7 (1.0)	
Vagococcus	1 (0.1)	0 (0.0)	0 (0.0)	1 (0.1)	
Varibaculum	4 (0.5)	0 (0.0)	0 (0.0)	4 (0.6)	
Veillonella	5 (0.7)	0 (0.0)	0 (0.0)	5 (0.7)	

Values are presented as number (%).
p-value indicates difference in the overall population, not in individual groups.
NGS, next-generation sequencing; sUC, sporadic/single uncomplicated cystitis; rUTI, recurrent urinary tract infection; rUC, recurrent uncomplicated cystitis.
[a]:Bacteria frequently found in each group are marked.

bacterales and *Escherichia/Shigella*, alongside a significant increase in the detection rate of *Lactobacillus*. Detection rates of *Bifidobacterium* and *Lactobacillus* were higher while detection rates of *Enterobacterales* and *Escherichia/Shigella* were lower in rUTI/rUC patients who had taken probiotics than in those who had not taken probiotics.

DISCUSSION

In a prior pilot study, we observed the effectiveness of urine NGS in cystitis patients and discerned differences in urine microbiome profiles between sUC and rUTI/rUC [6]. However, this earlier pilot study had limitations, including the absence of data from a control group and the failure to

Table 5. Effects of menopause on NGS results

Category	Menopause (-)	Menopause (+)	p-value
Acute uncomplicated cystitis	(n=22)	(n=18)	<0.001
Atopobium	0 (0.0)	1 (5.6)	
Bacteroides	0 (0.0)	1 (5.6)	
Bifidobacterium	0 (0.0)	1 (5.6)	
Cutibacterium	0 (0.0)	1 (5.6)	
Enhydrobacter	1 (4.5)	0 (0.0)	
Enterobacterales	4 (18.2)[a]	6 (33.3)[a]	
Enterococcus	1 (4.5)	0 (0.0)	
Escherichia/Shigella	3 (13.6)[a]	1 (5.6)	
Gardnerella	2 (9.1)[a]	0 (0.0)	
Haemophilus	0 (0.0)	1 (5.6)	
Lactobacillus	2 (9.1)[a]	0 (0.0)	
Prevotella	4 (18.2)[a]	2 (11.1)[a]	
Propionibacterium	1 (4.5)	2 (11.1)[a]	
Proteus	0 (0.0)	1 (5.6)	
Pseudomonas	1 (4.5)	0 (0.0)	
Staphylococcus	0 (0.0)	1 (5.6)	
Streptococcus	2 (9.1)[a]	0 (0.0)	
Ureaplasma	1 (4.5)	0 (0.0)	
rUTI/rUC	(n=270)	(n=417)	<0.001
Acidaminococcus	1 (0.4)	0 (0.0)	
Acinetobacter	2 (0.7)	3 (0.7)	
Actinobaculum	0 (0.0)	1 (0.2)	
Actinomyces	1 (0.4)	4 (1.0)	
Alcaligenes	0 (0.0)	2 (0.5)	
Alloscardovia	0 (0.0)	1 (0.2)	
Amycolatopsis	0 (0.0)	1 (0.2)	
Anaerococcus	5 (1.9)	21 (5.0)[a]	
Anaerosphaera	0 (0.0)	4 (1.0)	
Atopobium	4 (1.5)	7 (1.7)	
Bacillus	1 (0.4)	0 (0.0)	
Bacteroides	13 (4.8)	17 (4.1)	
Bifidobacterium	8 (3.0)	5 (1.2)	
Blastococcus	1 (0.4)	0 (0.0)	
Bradyrhizobium	0 (0.0)	1 (0.2)	
Campylobacter	1 (0.4)	0 (0.0)	
Chryseobacterium	0 (0.0)	1 (0.2)	
Citrobacter	1 (0.4)	0 (0.0)	
Cloacibacterium	2 (0.7)	1 (0.2)	
Clostridium sensu stricto	0 (0.0)	1 (0.2)	
Clostridium XIVa	0 (0.0)	1 (0.2)	
Corynebacterium	7 (2.6)	14 (3.4)	
Dermacoccus	0 (0.0)	1 (0.2)	
Dolosigranulum	1 (0.4)	0 (0.0)	
Enhydrobacter	2 (0.7)	4 (1.0)	
Enterobacterales	16 (5.9)[a]	43 (10.3)[a]	
Enterococcus	4 (1.5)	5 (1.2)	
Eremococcus	0 (0.0)	1 (0.2)	
Escherichia/Shigella	12 (4.4)	45 (10.8)[a]	

Table 5. Continued 1

Category	Menopause (-)	Menopause (+)	p-value
Ezakiella	0 (0.0)	1 (0.2)	
Facklamia	1 (0.4)	0 (0.0)	
Faecalibacterium	6 (2.2)	5 (1.2)	
Finegoldia	2 (0.7)	9 (2.2)	
Fusobacterium	0 (0.0)	3 (0.7)	
Gardnerella	19 (7.0)[a]	15 (3.6)	
Gemella	0 (0.0)	2 (0.5)	
Gracilibacter	0 (0.0)	1 (0.2)	
Haemophilus	0 (0.0)	3 (0.7)	
Janthinobacterium	0 (0.0)	2 (0.5)	
Lactobacillales	0 (0.0)	1 (0.2)	
Lactobacillus	72 (26.7)[a]	30 (7.2)[a]	
Lactococcus	0 (0.0)	1 (0.2)	
Lishizhenia	1 (0.4)	0 (0.0)	
Megamonas	0 (0.0)	2 (0.5)	
Megasphaera	1 (0.4)	2 (0.5)	
Micromonospora	1 (0.4)	0 (0.0)	
Millisia	0 (0.0)	1 (0.2)	
Mobiluncus	1 (0.4)	1 (0.2)	
Murdochiella	1 (0.4)	0 (0.0)	
Mycobacterium	1 (0.4)	0 (0.0)	
Neisseria	2 (0.7)	1 (0.2)	
Novosphingobium	0 (0.0)	1 (0.2)	
Paracoccus	0 (0.0)	1 (0.2)	
Pediococcus	1 (0.4)	0 (0.0)	
Peptoniphilus	2 (0.7)	12 (2.9)	
Peptostreptococcus	0 (0.0)	1 (0.2)	
Porphyromonas	1 (0.4)	3 (0.7)	
Prevotella	21 (7.8)[a]	47 (11.3)[a]	
Propionibacterium	18 (6.7)[a]	32 (7.7)[a]	
Propionimicrobium	0 (0.0)	1 (0.2)	
Proteus	1 (0.4)	3 (0.7)	
Pseudomonas	8 (3.0)	16 (3.8)	
Ralstonia	0 (0.0)	1 (0.2)	
Roseburia	1 (0.4)	1 (0.2)	
Rothia	0 (0.0)	1 (0.2)	
Saccharopolyspora	1 (0.4)	0 (0.0)	
Salmonella	0 (0.0)	1 (0.2)	
Sneathia	0 (0.0)	1 (0.2)	
Sphingobacteria	0 (0.0)	1 (0.2)	
Sphingomonas	1 (0.4)	0 (0.0)	
Staphylococcus	6 (2.2)	7 (1.7)	
Streptococcus	7 (2.6)	12 (2.9)	
Streptophyta	3 (1.1)	0 (0.0)	
Tannerella	0 (0.0)	1 (0.2)	
Thalassobaculum	0 (0.0)	1 (0.2)	
Turicibacter	0 (0.0)	1 (0.2)	
Ureaplasma	5 (1.9)	2 (0.5)	
Vagococcus	1 (0.4)	0 (0.0)	

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Table 5. Continued 2

Category	Menopause (-)	Menopause (+)	p-value
Varibaculum	1 (0.4)	3 (0.7)	
Veillonella	2 (0.7)	3 (0.7)	

Values are presented as number (%).
p-value indicates difference in the overall population, not in individual groups.
NGS, next-generation sequencing; rUTI, recurrent urinary tract infection; rUC, recurrent uncomplicated cystitis.
[a]:Bacteria frequently found in each group are marked.

account for various confounding factors such as administration of antibiotics or probiotics before NGS. Consequently, the present study sought to address these shortcomings by collecting a more extensive dataset from sUC and rUTI/rUC patients. It also included data from a control group. We meticulously analyzed urine microbiomes of the cystitis patient cohort and investigated effects of menopause, antibiotics, and probiotics on these microbiomes.

First, our study aimed to characterize urine microbiome in a population of normal women. Contrary to the long-standing belief that urine is sterile, we found that NGS yielded positive results in 83.3% of the control group. This discovery is particularly significant as it provides insight into the normal microbiome within the bladder collected through a transurethral catheter. Knowledge about vaginal and urinary tract microbiota in normal women is limited. In our study, the genera predominantly detected in control group included *Lactobacillus* and *Prevotella*. These findings were consistent with previous reports [12-15]. However, *Streptococcus* was not detected in our control group, although it has been frequently reported in other studies involving normal women. Both *Lactobacillus* and *Streptococcus* are lactic acid bacteria naturally present in the urinary system. They are known for having protective effects against pathogens [16,17].

Second, our study demonstrated that urine NGS was superior to conventional urine culture in detecting pathogens in both sUC and rUTI/rUC patients. In a pilot study conducted by our research team, the urine NGS positivity rate was 72.7% in the sUC group and 67.7% in the rUTI/rUC group. In this current study, these rates increased to 91.2% and 82.4%, respectively [6]. In contrast, the positive rate of urine culture during the same period remained relatively stable, at approximately 32%–36% in the sUC group and 9.3%–16.3% in the rUTI/rUC group. Urine represents a low biomass sample. Techniques employed in urine NGS, such as pretreatment and extraction, are of paramount importance compared to other samples such as stool [18,19]. The predominant bacteria detected in the sUC group included *Enterobacterales*, *Escherichia/Shigella*, and *Propionibacterium*, consistent with previous reports. Other studies that performed urine NGS on sUC patients also reported the detection of *Gardnerella*, *Candida*, and *Trichomonas*. However, it was worth noting that these studies analyzed mid-stream urine, potentially leading to contamination by urethral or vaginal contents. In our study, unlike previous reports, *Prevotella* was detected in 15.0% of the sUC group. Given that *Prevotella* is typically associated with the gut or vagina, this finding suggests that alterations in the urine microbiome through the gut-bladder axis or vaginal-bladder axis may represent another pathogenesis of sUC [20-22]. Additionally, we observed a reduction in *Lactobacillus* in the sUC group compared to that in both control and rUTI/rUC groups. This decrease in *Lactobacillus* might be a consequence of increased pathogenic bacteria, potentially diminishing the protective effect and contributing to the exacerbation of sUC.

Meanwhile, the most frequently reported species in the rUTI/rUC group were *Propionibacterium*, *Enterobacterales*, and *Escherichia/Shigella*, showing no significant differences in the frequency of detection from our previous pilot study. Research involving urine NGS in patients with recurrent cystitis is relatively scarce. For instance, Huang et al. [23] have performed urine NGS for 90 recurrent UTI patients using midstream voided urine samples and detected causative bacteria such as *Ralstonia*, *Prevotella*, *Dialister*, and *Corynebacterium*. Additionally, urine NGS results from patients with recurrent UTIs due to vesicoureteral reflux have shown increases of *Dorea* and *Escherichia* but decreases of *Prevotella* and *Lactobacillus* [24]. In the past, *E. faecalis* was reported as the primary cause of rUTI/rUC group [25]. In our study, *E. faecalis* was significantly more common in the rUTI/rUC group based on conventional urine culture. However, this significance was not observed in the NGS test. Consequently, there appears to be a discrepancy between conventional culture and NGS, suggesting that a more comprehensive bioinformatics analysis may be necessary.

Third, we conducted an in-depth investigation into effects of menopause, antibiotics, and probiotics on urine NGS. In the sUC group, the proportion of *Escherichia/Shigella* and genera associated with vaginitis (*Gardnerella*, *Prevotella*) was higher before menopause, whereas the proportion of *Enterobacterales* and *Propionibacterium* was increased after menopause. Conversely, in the rUTI/rUC group, *Lactobacillus* decreased after menopause while the proportion of *Enterobacterales* and *Escherichia/Shigella* increased after menopause. Sekito et al. [26] have also found that vaginal microbiota of postmenopausal women with rUC differs

Table 6. Effects of taking antibiotics and probiotics on urine NGS results

Category	Total	Antibiotics (-)	Antibiotics (+)	p-value	Probiotics (-)	Probiotics (+)	p-value
Acute uncomplicated cystitis	(n=39)[a]	(n=11)	(n=28)	0.533	-	-	-
Atopobium	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Bacteroides	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Bifidobacterium	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Cutibacterium	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Enhydrobacter	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Enterobacterales	10 (25.6)	4 (36.4)[b]	6 (21.4)[b]		-	-	
Enterococcus	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Escherichia/Shigella	3 (7.7)	2 (18.2)[b]	1 (3.6)		-	-	
Gardnerella	2 (5.1)	2 (18.2)[b]	0 (0.0)		-	-	
Haemophilus	1 (2.6)	1 (9.1)[b]	0 (0.0)		-	-	
Lactobacillus	2 (5.1)	1 (9.1)[b]	1 (3.6)		-	-	
Prevotella	6 (15.4)	1 (9.1)[b]	5 (17.9)[b]		-	-	
Propionibacterium	3 (7.7)	0 (0.0)	3 (10.7)[b]		-	-	
Proteus	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Pseudomonas	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Staphylococcus	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
Streptococcus	2 (5.1)	0 (0.0)	2 (7.1)		-	-	
Ureaplasma	1 (2.6)	0 (0.0)	1 (3.6)		-	-	
rUTI/rUC	(n=687)	(n=323)	(n=364)	0.730	(n=608)	(n=79)	0.003
Acidaminococcus	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Acinetobacter	5 (0.7)	4 (1.2)	1 (0.3)		3 (0.5)	2 (2.5)	
Actinobaculum	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Actinomyces	5 (0.7)	2 (0.6)	3 (0.8)		5 (0.8)	0 (0.0)	
Alcaligenes	2 (0.3)	0 (0.0)	2 (0.5)		2 (0.3)	0 (0.0)	
Alloscardovia	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Amycolatopsis	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Anaerococcus	26 (3.8)	14 (4.3)	12 (3.3)		24 (3.9)	2 (2.5)	
Anaerosphaera	4 (0.6)	3 (0.9)	1 (0.3)		4 (0.7)	0 (0.0)	
Atopobium	11 (1.6)	5 (1.5)	6 (1.6)		10 (1.6)	1 (1.3)	
Bacillus	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Bacteroides	30 (4.4)	14 (4.3)	16 (4.4)		28 (4.6)	2 (2.5)	
Bifidobacterium	13 (1.9)	6 (1.9)	7 (1.9)		9 (1.5)	4 (5.1)[b]	
Blastococcus	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Bradyrhizobium	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Campylobacter	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Chryseobacterium	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Citrobacter	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Cloacibacterium	3 (0.4)	0 (0.0)	3 (0.8)		2 (0.3)	1 (1.3)	
Clostridium sensu stricto	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Clostridium XIVa	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Corynebacterium	21 (3.1)	9 (2.8)	12 (3.3)		20 (3.3)	1 (1.3)	
Dermacoccus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Dolosigranulum	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Enhydrobacter	6 (0.9)	3 (0.9)	3 (0.8)		5 (0.8)	1 (1.3)	
Enterobacterales	59 (8.6)	31 (9.6)[b]	28 (7.7)[b]		54 (8.9)[b]	5 (6.3)[b]	
Enterococcus	9 (1.3)	5 (1.5)	4 (1.1)		8 (1.3)	1 (1.3)	
Eremococcus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Escherichia/Shigella	57 (8.3)	35 (10.8)[b]	22 (6.0)[b]		56 (9.2)[b]	1 (1.3)	
Ezakiella	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	

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Table 6. Continued 1

Category	Total	Antibiotics (-)	Antibiotics (+)	p-value	Probiotics (-)	Probiotics (+)	p-value
Facklamia	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Faecalibacterium	11 (1.6)	5 (1.5)	6 (1.6)		10 (1.6)	1 (1.3)	
Finegoldia	11 (1.6)	5 (1.5)	6 (1.6)		11 (1.8)	0 (0.0)	
Fusobacterium	3 (0.4)	2 (0.6)	1 (0.3)		2 (0.3)	1 (1.3)	
Gardnerella	34 (4.9)	16 (5.0)	18 (4.9)		29 (4.8)	5 (6.3)[b]	
Gemella	2 (0.3)	0 (0.0)	2 (0.5)		2 (0.3)	0 (0.0)	
Gracilibacter	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Haemophilus	3 (0.4)	1 (0.3)	2 (0.5)		3 (0.5)	0 (0.0)	
Janthinobacterium	2 (0.3)	0 (0.0)	2 (0.5)		2 (0.3)	0 (0.0)	
Lactobacillales	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Lactobacillus	102 (14.8)	43 (13.3)[b]	59 (16.2)[b]		78 (12.8)[b]	24 (30.4)[b]	
Lactococcus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Lishizhenia	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Megamonas	2 (0.3)	2 (0.6)	0 (0.0)		2 (0.3)	0 (0.0)	
Megasphaera	3 (0.4)	1 (0.3)	2 (0.5)		3 (0.5)	0 (0.0)	
Micromonospora	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Millisia	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Mobiluncus	2 (0.3)	0 (0.0)	2 (0.5)		1 (0.2)	1 (1.3)	
Murdochiella	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Mycobacterium	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Neisseria	3 (0.4)	2 (0.6)	1 (0.3)		2 (0.3)	1 (1.3)	
Novosphingobium	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Paracoccus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Pediococcus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Peptoniphilus	14 (2.0)	5 (1.5)	9 (2.5)		13 (2.1)	1 (1.3)	
Peptostreptococcus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Porphyromonas	4 (0.6)	2 (0.6)	2 (0.5)		4 (0.7)	0 (0.0)	
Prevotella	68 (9.9)	34 (10.5)[b]	34 (9.3)[b]		64 (10.5)[b]	4 (5.1)[b]	
Propionibacterium	50 (7.3)	22 (6.8)[b]	28 (7.7)[b]		48 (7.9)	2 (2.5)	
Propionimicrobium	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Proteus	4 (0.6)	3 (0.9)	1 (0.3)		4 (0.7)	0 (0.0)	
Pseudomonas	24 (3.5)	9 (2.8)	15 (4.1)		23 (3.8)	1 (1.3)	
Ralstonia	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Roseburia	2 (0.3)	1 (0.3)	1 (0.3)		1 (0.2)	1 (1.3)	
Rothia	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Saccharopolyspora	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	
Salmonella	1 (0.1)	0 (0.0)	1 (0.3)		0 (0.0)	1 (1.3)	
Sneathia	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Sphingobacteria	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Sphingomonas	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Staphylococcus	13 (1.9)	6 (1.9)	7 (1.9)		11 (1.8)	2 (2.5)	
Streptococcus	19 (2.8)	9 (2.8)	10 (2.7)		16 (2.6)	3 (3.8)	
Streptophyta	3 (0.4)	2 (0.6)	1 (0.3)		3 (0.5)	0 (0.0)	
Tannerella	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Thalassobaculum	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Turicibacter	1 (0.1)	1 (0.3)	0 (0.0)		1 (0.2)	0 (0.0)	
Ureaplasma	7 (1.0)	3 (0.9)	4 (1.1)		6 (1.0)	1 (1.3)	
Vagococcus	1 (0.1)	0 (0.0)	1 (0.3)		1 (0.2)	0 (0.0)	

Table 6. Continued 2

Category	Total	Antibiotics (-)	Antibiotics (+)	p-value	Probiotics (-)	Probiotics (+)	p-value
Varibaculum	4 (0.6)	1 (0.3)	3 (0.8)		2 (0.3)	2 (2.5)	
Veillonella	5 (0.7)	4 (1.2)	1 (0.3)		4 (0.7)	1 (1.3)	

Values are presented as number (%).
p-value indicates difference in the overall population, not in individual groups.
NGS, next-generation sequencing; rUTI, recurrent urinary tract infection; rUC, recurrent uncomplicated cystitis.
[a]:One patient was excluded from the analysis because it was uncertain whether she had taken antibiotics before the NGS test.
[b]:Bacteria frequently found in each group are marked.

significantly from that of healthy controls and those with uncomplicated cystitis mainly due to a lack of *Lactobacillus* and a dominance of *Enterobacteriaceae*. In this vein, vaginal administration of *Lactobacillus crispatus*-containing suppositories might prevent rUC by restoring the balance of vaginal microbiota and reducing pathogenic bacteria virulence. Vaginal and urinary microbiomes can be significantly influenced by menopause and estrogen levels [27,28]. In contrast, the impact of taking antibiotics on urine NGS patterns was not as substantial as anticipated. This could be attributed to the fact that, unlike conventional urine culture, urine NGS can confirm the presence of bacteria even if they are not viable [29]. Given that a majority of rUTI/rUC patients visiting tertiary hospitals have previously taken antibiotics, this aspect of NGS can offer a significant advantage in achieving accurate diagnoses. However, it was worth noting that after taking antibiotics, *Enterobacterales* and *Escherichia/Shigella*, the primary causative bacteria of cystitis, exhibited a somewhat decreasing pattern, warranting caution when interpreting NGS test results. Lastly, in patients taking probiotics, the proportion of *Escherichia/Shigella* was significantly decreased from 9.2% to 1.3%, which appeared to be associated with an increase of *Lactobacillus* from 12.8% to 30.4%.

While our study marks progress beyond previous pilot studies, it has several limitations. First, our use of 16S ribosomal sequencing, although informative, is less precise than shotgun sequencing. Second, the lack of antibiotic resistance analysis restricts our ability to provide specific clinical recommendations for antibiotic selection. Third, our reliance on existing reports limited our capability to perform a more thorough bioinformatics analysis. Fourth, we used only 0.001 mL of urine for culture, which, while standard, might have a low sensitivity for detecting low-abundance or non-traditionally cultured pathogens. Lastly, the small number of control subjects necessitates further data collection to better understand the normal bladder microbiome.

CONCLUSIONS

In conclusion, although research on urobiome is still limited, our NGS data suggest a new mechanism through which the urinary microbiome affects disease states of sUC and rUTI/rUC. Given the reduced efficacy of standard antibiotics due to the emergence of multidrug-resistant urinary pathogens, our study provides important insights for developing microbiome-based treatments in South Korea where antibiotic resistance is widespread. We anticipate that this foundational study will be instrumental in distinguishing disease environments in the context of antibiotic resistance development.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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