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ORIGINAL ARTICLE

# Female lower urinary tract microbiota do not associate with IC/PBS symptoms: a case-controlled study

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## Abstract

**Introduction and hypothesis** The current etiology of interstitial cystitis/painful bladder syndrome (IC/PBS) is poorly understood and multifactorial. Recent studies suggest the female urinary microbiota (FUM) contribute to IC/PBS symptoms. This study was designed to determine if the FUM, analyzed using mid-stream voided urine samples, differs between IC/PBS patients and controls.

**Methods** This prospective case-controlled study compared the voided FUM of women with symptoms of urinary frequency, urgency, and bladder pain for > 6 months with the voided FUM of healthy female controls without pain. Bacterial identification was performed using 16S rRNA gene sequencing and EQUC, a validated enhanced urine culture approach. Urotype was defined by a genus present at > 50% relative abundance. If no genus was present above this threshold, the urotype was classified as 'mixed.' Group comparisons were performed for urotype and diversity measures.

**Results** A mid-stream voided specimen was collected from 21 IC/PBS patients and 20 asymptomatic controls. The two groups had similar demographics. Urotypes did not differ between cohorts as assessed by either EQUC or 16S rRNA gene sequencing. We detected no significant differences between cohorts by alpha diversity. Cohorts also were not distinct using principle component analysis or hierarchical clustering. Detection by EQUC of bacterial species considered uropathogenic was high in both cohorts, but detection of these uropathogenic species did not differ between groups ($p = 0.10$).

**Conclusions** Enhanced culture and DNA sequencing methods provide evidence that IC/PBS symptoms may not be related to differences in the FUM, at least not its bacterial components. Future larger studies are needed to confirm this preliminary finding.

**Keywords** Interstitial cystitis (IC) · Painful bladder syndrome (PBS) · Microbiome · Urine · Vagina · Genitourinary

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## Introduction

Interstitial cystitis/painful bladder syndrome (IC/PBS) affects nearly 7.9 million US adult women [1]. The AUA and SUFU define IC/PBS as "an unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptoms of more than six weeks duration, in the absence of infection or other identifiable causes" [2].

The etiology of IC/PBS is multifactorial, poorly understood, and assumes that the female urinary tract is sterile in the absence of clinical infection. However, our research team and others have shown that the female urinary tract is not sterile; it possesses communities of microbes called the female urinary microbiota (FUM) [3–6]. Furthermore, the FUM is associated with various lower urinary tract symptoms [7–12]. These findings present a new avenue for studying the etiologies of IC/PBS.

Few groups have reported on the FUM of IC/PBS patients. The few existing studies used various urine collection methods and bacterial detection methods and, in general, had small sample sizes; therefore, the data conflict [13–15]. Our prospective case-controlled study sought to determine if the FUM of women with and without IC/PBS differs, using mid-stream voided urine specimens to avoid pain provocation and using an enhanced culture method to analyze them. Secondarily, we sought to describe the vaginal microbiota of the IC/PBS cohort.

## Materials and methods

### Study design and participant population

Following Institutional Review Board (IRB) approval, we enrolled 41 female patients, 21 with IC/PBS (i.e., IC cohort) and 20 without (i.e., control cohort). Women in the IC cohort experienced symptoms of urinary frequency, urgency, and bladder pain for > 6 months, meeting the AUA IC/PBS definition. A screening questionnaire was provided to candidate control participants from the community: inclusion criteria included English-speaking women between the ages of 21 to 65 years with no history of or current bladder or pelvic pain and in generally stable health. Control participants were excluded if they had a pacemaker or other neurostimulator (gastric/spinal), history of or current urethral stricture, cystitis caused by tuberculosis, radiation therapy or Cytoxan/cyclophosphamide therapy, prior augmentation cystoplasty or cystectomy, systemic autoimmune disorder (such as Crohn's disease, ulcerative colitis, lupus, rheumatoid arthritis, or multiple sclerosis), systemic neuromuscular disease known to affect the lower urinary tract, history of urogenital cancer, current or imminent planned pregnancy/recent delivery < 6 months, current pelvic floor physical therapy, current use of opioid medications, abdominal or pelvic surgery within the last 6 months, and symptoms or diagnosis of UTI within the past 3 months. For both cohorts, basic demographics were collected. The members of the IC cohort completed validated questionnaires including the Beck Anxiety Inventory, Beck Depression Inventory, the Pain Disability Index, the Female Genitourinary Pain Index, the Pain Catastrophizing Scale, the IC Symptom Index Score, and the IC Problem Index Score.

### Sample collection

Midstream voided urine specimens were collected from both cohorts. A vaginal swab specimen was collected from the IC cohort. A portion of each urine sample was placed in a BD Vacutainer® Plus C&S preservative tube for culturing. A separate portion for 16S rRNA gene sequencing was placed at 4 °C for < 4 h following collection; 10% AssayAssure (Sierra Molecular, Incline Village, NV) was added before storage at −80 °C. Puritan Opti-Tranz® Liquid Stuart Swabs were used to collect two aerobic vaginal swab specimens. Each swab was vortexed and diluted in 1 ml phosphate-buffered saline (PBS). One aliquot was used for culture and one was stored for 16S rRNA gene sequencing, as described above.

### Urine culture protocols

A variation of the Expanded Quantitative Urine Culture (EQUC) protocol [6] was used to culture the biological specimens. Because it uses larger urine volumes, multiple growth media and atmospheric conditions, and longer incubation times, EQUC is superior to the standard urine culture method used by most clinical microbiology laboratories [6]. Briefly, 10 μl of urine sample or vaginal swab aliquot was spread quantitatively onto BAP, chocolate, and colistin naladixic acid (CNA) agars (BD BBL™ prepared plated media) and incubated in 5% $CO_2$ at 35 °C for 48 h; onto BAP incubated aerobically at 35 °C for 48 h; and onto CDC anaerobic 5% sheep blood (Anaerobic BAP) agar (BD BBL™ prepared plated media) incubated anaerobically at 35 °C for 48 h. The vaginal swab aliquots were also plated on Thayer-Martin media and incubated in 5% $CO_2$ at 35 °C for 48 h. Each distinct colony morphology was sub-cultured at 48 h to obtain pure culture for microbial identification. Microbial identification was determined using a matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer (MALDI-TOF MS, Bruker Daltonics, Billerica, MA).

### DNA isolation and 16S sequencing

DNA isolation, polymerase chain reaction (PCR) amplification, and 16S rRNA gene sequencing of urine cultures have been described previously [7]. Genomic DNA was extracted from 1 ml of urine or 500 ul of the vaginal swab aliquot, using previously validated protocols developed for the Human Microbiome Project [6, 7, 16]. To isolate genomic DNA from these samples, this protocol includes the addition of mutanolysin and lysozyme to ensure robust lysis of gram-positive and gram-negative species [16].

The hyper-variable region 4 (V4) of the bacterial 16S rRNA gene was amplified via a two-step PCR protocol, as described previously [6, 7]. Extraction negative controls (no urine or swab suspension) and PCR-negative controls (no template) were included to assess the contribution of extraneous DNA from reagents. Ten-microliter aliquots of each reaction mixture were run on a 1% agarose gel. Samples containing a band of approximately 360 bp were considered PCR positive and subjected to further library preparation. Samples with no visible amplified product were considered PCR negative and not processed further. The PCR-positive reaction mixtures were diluted 1:50 and amplified for an additional

ten cycles, using primers encoding the required adapter sequences for Illumina MiSeq sequencing and an 8-nucleotide sample index. The PCR reaction was purified and size selected using Agencourt AMPure XP-PCR magnetic beads (Beckman Coulter, Pasadena, CA). Each sample was quantified using the Qubit fluorometeric system (Thermo-Fisher, Waltham, MA). The samples were pooled, quantified to a standard volume, and placed in the 2 × 250-bp sequencing reagent cartridge, according to the manufacturer's instructions (Illumina, San Diego, CA).

Sample barcodes and sequencing primers were removed using the Illumina proprietary MiSeq post-sequencing software. The mothur program (v1.37.4) was used to process the raw sequences by following the recommended MiSeq standard operating procedure [17]. Briefly, mothur produced 16S contigs by combining the paired end reads based on overlapping nucleotides in the sequence reads; contigs of incorrect length for the V4 region (< 290 bp, > 300 bp) and/or contigs containing ambiguous bases were removed. Chimeric sequences were removed using UCHIME within the mothur package [18]. Subsampling at a depth of 5000 sequences was performed to correct for different sequencing depth of each sample. The sequences were clustered into species-level operational taxonomic units (OTUs) with identity cutoff at 97% [19]. The OTUs were classified using RDP classifier (v2.11) at the genus level [19]. Specimens designated as "undetectable" had < 1000 total sequence reads.

### Statistical analyses

Continuous variables were reported as means with standard deviations (SD); categorical variables were reported as frequencies and percentages. Pearson chi-square tests or Fisher's exact tests and two-sample t-tests or Wilcoxon rank sum tests were used to compare categorical and continuous demographics and culture results, respectively. Data were plotted using R (version 3.4.3). Cluster analysis was performed using hierarchical clustering. Statistical analyses were conducted using SAS software v9.4 (SAS Institute, Cary, NC) or SYSTAT software version 13.1 (SYSTAT Software Inc., Chicago, IL).

## Results

### Demographics

Table 1 displays the demographic characteristics of the two cohorts (IC and control). The entire population had a mean age of 49 ± 13 years and was predominately White/Caucasian (70%), consistent with our clinical patient population. The two groups had similar demographics and percentage of EQUC and 16S sequence-positive specimens ($p > 0.05$ for all comparisons except vaginal parity).

### Description of the lower urinary tract and vaginal microbiomes in IC patients

16S rRNA gene sequencing was performed on all (21) IC voided urine and vaginal swab specimens. The majority of voided urine specimens of IC patients had a *Lactobacillus* (11/21; 53%) or mixed (6/21; 29%) urotype, a measure of bacterial community structure as determined by 16S rRNA gene sequencing (Supplemental Figure 1). For most IC patients, the urotype matched the dominant taxa present in the paired vaginal swab specimen (19/21; 90%) (Supplemental Figure 1). The specimen types (IC vaginal swab, IC urine, control urine) did not significantly differ by several mean alpha diversity measures that report on the richness, evenness, and abundance of community members (Supplemental Figure 2).

Table 2 displays the demographics and validated questionnaire results of the IC cohort stratified by *Lactobacillus* versus non-*Lactobacillus* urotype. IC patients with a *Lactobacillus* urotype were younger ($p = 0.01$) and more likely to be premenopausal ($p = 0.03$) than IC patients with a non-*Lactobacillus* urotype, which were more likely to be postmenopausal ($p = 0.009$); neither the *Lactobacillus* urotype nor the non-*Lactobacillus* urotypes had significantly different mean scores for any of the validated clinical questionnaires.

### Comparison of the lower urinary tract microbiomes of IC and control patients

16S rRNA gene sequencing was performed on voided urines from 19 of 20 control specimens. Consistent with the IC cohort, the control patients had predominately *Lactobacillus* (9/19; 47%) or mixed (6/19; 32%) urotypes by 16S rRNA gene sequencing. Mean alpha diversity measures did not differ between the voided urine specimens of the IC and control cohorts (Supplemental Figure 2). When plotted, principal component analysis did not show visual separation between the cohorts (Fig. 1). A hierarchical cluster analysis of specimens classified at the OTU level from both cohorts is shown in Fig. 2, which does not reveal any clear apparent clustering of specimen types or cohort-specific specimens.

### Detection of uropathogenic bacteria between cohorts

EQUC was performed on all control (20) and 18 of the 21 IC urine specimens. Using EQUC, we detected 39 and 51 unique species in the IC ($N = 18$) and control ($N = 20$) cohorts, respectively. Frequency of detection of *Staphylococcus lugdunensis* (0% IC versus 25% control, $p = 0.04$) and *Streptococcus agalactiae* (i.e., group B *Streptococcus*) (6% IC versus 35%

**Table 1** Demographic and clinical variables for the IC and control cohorts

| Patient and clinical variables | Total cohort ($N = 41$) | IC cohort ($N = 21$) | Control cohort ($N = 20$) | $p$ value |
|---|---|---|---|---|
| Age (years), mean (SD) | 49 (13) | 50 (13) | 48 (12) | 0.36[a] |
| Race: ($N = 40$) | | ($N = 20$) | | |
| White/Caucasian | 29 (71%) | 18 (90%) | 11 (55%) | 0.08[b] |
| Black/African American | 6 (15%) | 1 (5%) | 5 (25%) | |
| NHPI | 0 (0%) | 0 (0%) | 0 (0%) | |
| Asian | 0 (0%) | 0 (0%) | 0 (0%) | |
| Other | 5 (12%) | 1 (5%) | 4 (20%) | |
| Ethnicity: ($N = 40$) | | ($N = 20$) | | |
| Hispanic/Latina | 5 (13%) | 3 (15%) | 2 (10%) | 1.00[b] |
| Not Hispanic/Latina | 35 (87%) | 17 (85%) | 18 (90%) | |
| BMI (kg/m$^2$), mean (SD) | 26.80 (6.40) | 25.52 (5.89) | 28.14 (6.79) | 0.42[a] |
| | | ($N = 20$) | ($N = 18$) | |
| Vaginal parity, median (IQR) ($N = 38$) | 2 (0–2) | 1 (0–2) | 2 (1–4) | **0.04**[c] |
| Menopausal status | | | | |
| Pre-menopausal | 18 (44%) | 10 (48%) | 8 (40%) | 0.81[b] |
| Post-menopausal | 20 (49%) | 10 (48%) | 10 (50%) | |
| Not sure | 3 (7%) | 1 (4%) | 2 (10%) | |
| Marital status | | | | |
| Single | 7 (17%) | 5 (25%) | 2 (10%) | 0.54[b] |
| Married | 29 (71%) | 14 (67%) | 15 (75%) | |
| Divorced | 2 (5%) | 1 (4%) | 1 (5%) | |
| Widowed | 3 (7%) | 1 (4%) | 2 (10%) | |
| Use of hormone replacement therapy | 7 (17%) | 5 (24%) | 2 (10%) | 0.41[b] |
| Smoking | 3 (7%) | 0 (0%) | 3 (15%) | 0.11[b] |
| | | | ($N = 19$) | |
| Alcohol consumption ($N = 40$) | 21 (53%) | 9 (43%) | 12 (63%) | 0.20 |
| | | ($N = 20$) | ($N = 19$) | |
| Use of antimicrobial soap ($N = 39$) | 13 (33%) | 5 (25%) | 8 (42%) | 0.26 |
| Urine dipstick | | | | |
| White blood cells (WBC) | 0 (0%) | 0 (0%) | 0 (0%) | – |
| Nitrites | 0 (0%) | 0 (0%) | 0 (0%) | |
| Red blood cells (RBC) | 0 (0%) | 0 (0%) | 0 (0%) | |
| EQUC positive | | | | |
| Voided urine ($N = 38$) | 37 (97%) | ($N = 18$) 17 (94%) | 20 (100%) | 0.47[b] |
| Vaginal swab (IC cohort only) ($N = 18$) | 18 (100%) | ($N = 18$) 18 (100%) | – | – |
| 16S Sequencing positive | | | | |
| Voided urine ($N = 40$) | 39 (98%) | 20 (95%) | ($N = 19$) 19 (100%) | 1.00[b] |
| Vaginal swab (IC cohort only) ($N = 20$) | 20 (100%) | ($N = 20$) 20 (100%) | – | – |

Chi-square test used unless otherwise indicated. Significant value is shown in bold

*SD* standard deviation, *IQR* interquartile range

[a] Independent t-test

[b] Fisher's exact test

[c] Wilcoxon rank-sum test

control, $p = 0.04$) differed by group (Table 3). Detection of *Escherichia coli* was higher in the IC cohort (28% IC versus 5% control, $p = 0.08$). Detection of bacterial species typically considered uropathogenic was high in both groups (IC = 15/18, 83%; C = 20/20, 100%, $p = 0.10$) (Table 3).

## Discussion

This study failed to detect significant differences in the voided FUM of women with and without IC/PBS. Despite the study limitation of small sample size, our data suggest

**Table 2** Demographic, clinical variables, and symptom questionnaire results for IC patients with *Lactobacillus* and non-*Lactobacillus* urotypes determined by 16S rRNA gene sequencing

| Patient and clinical variables | IC cohort ($N = 21$) | *Lactobacillus* urotype ($N = 11$) | Non-*lactobacillus* urotype ($N = 10$) | *p* value |
|---|---|---|---|---|
| Age (years), mean (SD) | 50 (13) | 43 (13) | 57 (9) | **0.01**[a] |
| Race: ($N = 40$) | ($N = 20$) | ($N = 10$) | | |
| White/Caucasian | 18 (90%) | 9 (90%) | 9 (90%) | 0.74[b] |
| Black/African American | 1 (5%) | 1 (10%) | 0 (0%) | |
| NHPI | 0 (0%) | 0 (0%) | 0 (0%) | |
| Asian | 0 (0%) | 0 (0%) | 0 (0%) | |
| Other | 1 (5%) | 0 (0%) | 1 (10%) | |
| Ethnicity: ($N = 40$) | ($N = 20$) | ($N = 10$) | | |
| Hispanic/Latina | 3 (15%) | 2 (20%) | 1 (10%) | 1.00[b] |
| Not Hispanic/Latina | 17 (85%) | 8 (80%) | 9 (90%) | |
| BMI (kg/m$^2$), mean (SD) | 25.52 (5.89) | 25.33 (5.03) | 25.72 (7.00) | 0.88[a] |
| Vaginal parity, median (IQR) ($N = 38$) | ($N = 20$) | | ($N = 9$) | |
| | 1 (0–2) | 1 (0–2) | 1 (0–2) | 0.84[c] |
| Menopausal status | | | | |
| Pre-menopausal | 10 (48%) | 8 (73%) | 2 (20%) | **0.009**[b] |
| Post-menopausal | 10 (48%) | 2 (18%) | 8 (80%) | |
| Not sure | 1 (4%) | 1 (9%) | 0 (0%) | |
| Validated symptom questionnaires: mean (SD) | | | | |
| Beck Anxiety Inventory | 9 (8) | 10 (7) | 8 (10) | 0.53[a] |
| Becks Depression Inventory | 11 (8) | 12 (6) | 9 (9) | 0.42[a] |
| Pain Disability Index | 29 (15) | 29 (16) | 30 (14) | 0.88[a] |
| Female genitourinary pain index | | | | |
| Pain sub-score | 15 (3) | 15 (3) | 15 (4) | 0.70[a] |
| Urinary sub-score | 7 (3) | 6 (2) | 7 (3) | 0.37[a] |
| Quality of life sub-score | 10 (2) | 10 (1) | 10 (2) | 0.41[a] |
| Total score | 32 (6) | 31 (5) | 32 (7) | 0.74[a] |
| Pain catastrophizing scale | 24 (11) | 27 (10) | 21 (12) | 0.22[a] |
| IC symptom index score | 13 (4) | 12 (4) | 14 (4) | 0.31[a] |
| IC problem index score | 11 (4) | 11 (4) | 12 (4) | 0.73[a] |

Chi-square test used unless otherwise indicated. Significant values are shown in bold

*SD* standard deviation, *IQR* interquartile range

[a] Independent t-test

[b] Fisher's exact test

[c] Wilcoxon rank-sum test

that microbiota of the lower urinary tract may not contribute to the symptoms in women meeting the clinical definition of IC/PBS. This contradicts recent work by others, who have argued for a link between clinical symptoms of IC/PBS and the FUM [13–15]. Abernathy et al. hypothesized a protective role of a more diverse and *Lactobacillus*-dominant microbiome. Catheterized urine of women with IC were found to have fewer OTUs and less likely to contain *Lactobacillus* species, particularly *L. acidophilus*. Furthermore, Abernathy et al. found that the presence of *Lactobacillus* was associated with improved scores on two IC-specific symptom severity indices, suggesting that the urinary microbiome may influence lower urinary tract symptoms. Although our collection techniques differed and our expanded culture technique was additive, these findings were not reproduced in our study nor were the differences in *Lactobacillus* dominance indicative of symptom burden. Siddiqui et al. found that *Lactobacillus* predominance was associated with IC/

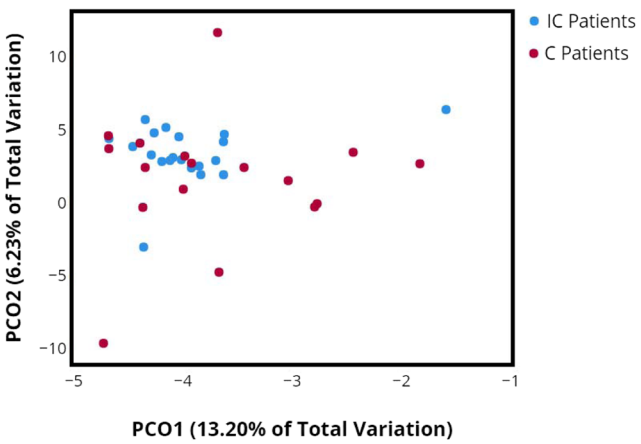


**Fig. 1** Principle component analysis of mid-stream voided urine specimens from IC and control patients. Principle component analysis comparing 16S rRNA gene sequence data among IC patients (21), blue, and control patients (C patients) (19), red

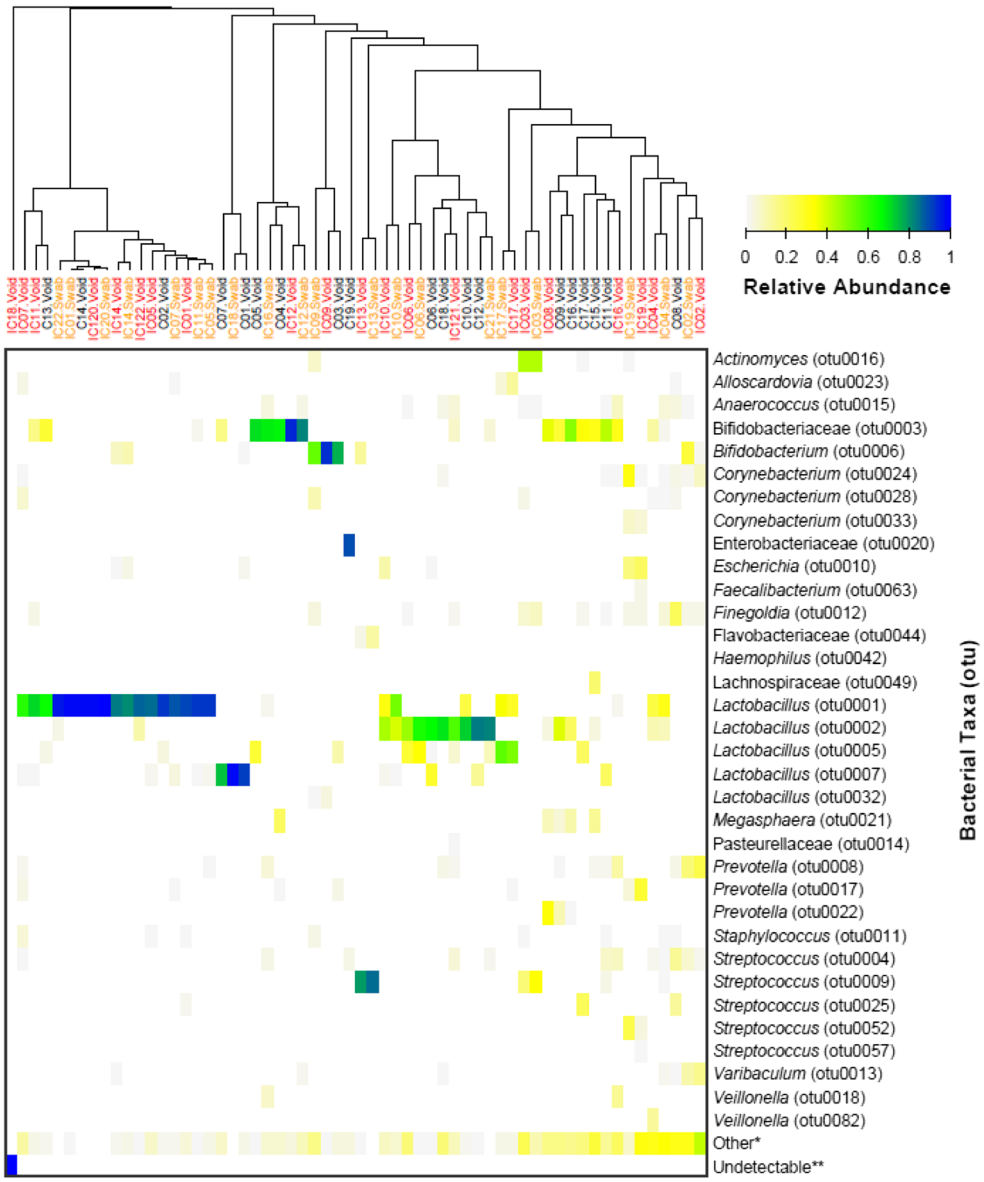


**Fig. 2** Heatmap of relative abundance values for common bacterial OTUs among cohorts and specimen types. Heatmap of the relative abundance of 16S rRNA gene sequence data classified by OTUs. The y-axis lists common bacterial OTU classifications in alphabetical order. The x-axis describes the specimen type and patient cohort of the corresponding sample. Specimens listed in red are mid-stream voided urine specimens from IC patients, orange are vaginal swab specimens from IC patients, and black are mid-stream voided urine specimens from control (C) patients. Data are grouped by hierarchical clustering using the corresponding dendogram

PBS patients (i.e., the opposite trend) using clean-catch voided urine specimens. Finally, Nickel et al. analyzed a larger cohort of IC/PBS participants ($n = 233$) using clean-catch urine during flare vs. non-flare pain states and showed no difference in species composition. However, this study did indicate that the IC group had a higher prevalence of fungi. Fungi were not directly analyzed in our cohort. However, we did detect *Candida* species in both cohorts using EQUC.

Strengths of this study were the use of two complementary identification methods: sequencing and culture as well as the use of vaginal swabs as a comparative specimen to voided urine in the IC cohort. Voided specimens were chosen intentionally so as not to create an IC pain flare by obtaining a catheterized specimen. Furthermore, contemporary urological guidelines do not require a catheterized urine specimen as a part of the diagnostic IC work-up (AUA guideline) and not routinely performed in this patient population because of catheterization discomfort and poor patient compliance [2]. Limitations include the small sample size for both cohorts, lack of vaginal swabs in the controls, and lack of other clinical data in the control group. While cohorts were similar on most characteristics, our sample size precluded subgroup analyses or multivariable models to adjust for any group imbalances due to demographic differences or heterogeneity within PBS. Another limitation is the inability of EQUC to detect fungi, viruses, and some strictly anaerobic and extremely fastidious bacterial species. Although we did not have an a priori sample size estimation, our cohort sizes were similar to those of the Abernethy et al. study, which did show differences in the

**Table 3** List of uropathogenic bacterial taxa identified in mid-stream voided urine specimens of IC and control patients using EQUC

| Uropathogenic bacterial taxa | IC cohort ($N = 18$) | Control cohort ($N = 20$) | $p$ value |
|---|---|---|---|
| *Actinotignum schaalii* | 0 (0%) | 1 (5%) | 1.00[a] |
| *Aerococcus sp.* | 1 (6%) | 5 (25%) | 0.18[a] |
| *Alloscardovia omnicolens* | 3 (17%) | 2 (10%) | 0.65[a] |
| *Candida sp.* | 1 (6%) | 2 (10%) | 1.00[a] |
| *Corynebacterium riegelii* | 0 (0%) | 2 (10%) | 0.49[a] |
| *Enterococcus faecalis* | 9 (50%) | 7 (35%) | 0.35 |
| *Escherichia coli* | 5 (28%) | 1 (5%) | 0.08[a] |
| *Haemophilus influenzae* | 1 (6%) | 0 (0%) | 0.47[a] |
| *Klebsiella pneumoniae* | 0 (0%) | 1 (5%) | 1.00[a] |
| *Neisseria sp.* | 0 (0%) | 1 (5%) | 1.00[a] |
| *Oligella urethralis* | 0 (0%) | 1 (5%) | 1.00[a] |
| *Staphylococcus aureus* | 2 (11%) | 0 (0%) | 0.22[a] |
| *Staphylococcus lugdunensis* | 0 (0%) | 5 (25%) | **0.04[a]** |
| *Streptococcus agalactiae* | 1 (6%) | 7 (35%) | **0.04[a]** |
| *Streptococcus anginosus* | 9 (50%) | 11 (55%) | *0.76* |

Chi-square test used unless otherwise indicated. Significant values are shown in bold

[a] Fisher's exact test

urinary microbiome between groups, so we felt our sample size was adequate for this preliminary analysis. We agree with Nickel et al. that the voided urine does not represent the bladder microbiome, but rather the genitourinary tract. We also recognize that we did not control antibiotic exposure, similar to Abernethy et al. However, we excluded women with active UTI or those who had received antibiotics for any medical reason within the last month prior to urine collection. We also excluded patients that were treated for UTI within the past 3 months.

This case-control study of the FUM in predominantly middle-aged women with IC/PBS compared with controls without pain showed no significant differences in the voided FUM between groups. These findings suggest that microbes may not directly contribute to IC/PBS unlike previously reported literature. Larger scale studies using demographically matched controls and complementary microbial detection techniques similar to ours and assessing multiple urine collection methods (voided and catheterized) would contribute to deeper understanding of the FUM as a potential etiology in IC/PBS.

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## Compliance with ethical standards

**Conflicts of interest** None.

## References

1. Bogart LM, Berry SH, Clemens JQ. Symptoms of interstitial cystitis, painful bladder syndrome and similar diseases in women: a systematic review. J Urol. 2007;177(2):450–6. https://doi.org/10.1016/j.juro.2006.09.032.
2. Hanno PM, Erickson D, Moldwin R, Faraday MM. Diagnosis and treatment of interstitial cystitis/bladder pain syndrome: AUA guideline amendment. J Urol. 2015;193(5):1545–53. https://doi.org/10.1016/j.juro.2015.01.086.
3. Wolfe AJ, Toh E, Shibata N, et al. Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol. 2012;50(4):1376–83. https://doi.org/10.1128/JCM.05852-11.
4. Fouts DE, Pieper R, Szpakowski S, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med. 2012;10:174. https://doi.org/10.1186/1479-5876-10-174.
5. Khasriya R, Sathiananthamoorthy S, Ismail S, et al. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. J Clin Microbiol. 2013;51(7):2054–62. https://doi.org/10.1128/JCM.03314-12.
6. Hilt EE, McKinley K, Pearce MM, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol. 2014;52(3):871–6. https://doi.org/10.1128/JCM.02876-13.
7. Pearce MM, Hilt EE, Rosenfeld AB, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. MBio. 2014;5(4):1283. https://doi.org/10.1128/mBio.01283-14. Accessed Jan 9, 2019.
8. Pearce MM, Zilliox MJ, Rosenfeld AB, et al. The female urinary microbiome in urgency urinary incontinence. Am J Obstet Gynecol. 2015;213(3):11. https://doi.org/10.1016/j.ajog.2015.07.009.
9. Karstens L, Asquith M, Davin S, et al. Does the urinary microbiome play a role in urgency urinary incontinence and its severity? Front Cell Infect Microbiol. 2016;6:78. https://doi.org/10.3389/fcimb.2016.00078. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4961701/.

10. Thomas-White KJ, Hilt EE, Fok C, et al. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J. 2016;27(5):723–33. https://doi.org/10.1007/s00192-015-2847-x.
11. Thomas-White KJ, Gao X, Lin H, et al. Urinary microbes and postoperative urinary tract infection risk in urogynecologic surgical patients. Int Urogynecol J. 2018;29(12):1797–805. https://doi.org/10.1007/s00192-018-3767-3.
12. Fok CS, Gao X, Lin H, et al. Urinary symptoms are associated with certain urinary microbes in urogynecologic surgical patients. Int Urogynecol J. 2018;29(12):1765–71. https://doi.org/10.1007/s00192-018-3732-1.
13. Abernethy MG, Rosenfeld A, White JR, Mueller MG, Lewicky-Gaupp C, Kenton K. Urinary microbiome and cytokine levels in women with interstitial cystitis. Obstet Gynecol. 2017;129(3):500–6. https://doi.org/10.1097/AOG.0000000000001892.
14. Nickel JC, Stephens A, Landis JR, et al. Assessment of the lower urinary tract microbiota during symptom flare in women with urologic chronic pelvic pain syndrome: a MAPP network study. J Urol. 2016;195(2):356–62. https://doi.org/10.1016/j.juro.2015.09.075.
15. Siddiqui H, Lagesen K, Nederbragt AJ, Jeansson SL, Jakobsen KS. Alterations of microbiota in urine from women with interstitial cystitis. BMC Microbiol. 2012;12:205. https://doi.org/10.1186/1471-2180-12-205.
16. Yuan S, Cohen DB, Ravel J, Abdo Z, Forney LJ. Evaluation of methods for the extraction and purification of DNA from the human microbiome. PLoS One. 2012;7(3):e33865. https://doi.org/10.1371/journal.pone.0033865. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0033865.
17. Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75(23):7537–41. https://doi.org/10.1128/AEM.01541-09.
18. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194–200. https://doi.org/10.1093/bioinformatics/btr381. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3150044/.
19. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261–7. https://doi.org/10.1128/AEM.00062-07. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1950982/.

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