Original Research

Urinary Microbiome and Cytokine Levels in Women With Interstitial Cystitis

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OBJECTIVE: To investigate differences in the urinary microbiome and cytokine levels between women with and without interstitial cystitis and to correlate differences with scores on standardized symptom severity scales and depression and anxiety screening tools.

METHODS: Our cross-sectional study compared women presenting to a pelvic floor clinic and diagnosed with interstitial cystitis over a 6-month period with age-matched women in a control group from the same institution. Participants provided a catheterized urine sample and completed symptom severity, quality-of-life, depression, and anxiety screening questionnaires. Urinary microbiomes generated through bacterial ribosomal RNA sequencing and cytokine levels were analyzed using a standard immunoassay. Nonparametric analyses were used for all comparisons.

RESULTS: Participants with interstitial cystitis reported more disability, bothersome urinary symptoms, genitourinary pain, and sexual dysfunction and scored higher on depression and anxiety screens compared with women in the control group. The urine of participants with interstitial cystitis contained fewer distinct operational taxonomic units (2 [median range 2–7, interquartile range 1] compared with 3.5 [median, range 2–22, interquartile range 5.25], P=.015) and was less likely to contain *Lactobacillus acidophilus* (1/14 [7%] compared with 7/18 [39%], P=.05) compared with women in the control group. *L acidophilus* was associated with less severe scores on the Interstitial Cystitis Symptoms Index (1 [median, range 0–17, interquartile range 5] compared with 10 [median, range 0–14, interquartile range 11], P=.005) and the Genitourinary Pain Index (0 [median, range 0–42, interquartile range 22] compared with 22.5 [median, range 0–40, interquartile range 28], P=.03). Participants with interstitial cystitis demonstrated higher levels of macrophage-derived chemokine (13.32 [median, range 8.93–17.05, interquartile range 15.86] compared with 0 [median, range 8.93–22.67, interquartile range 10.35], P=.037) and interleukin-4 (1.95 [median, range 1.31–997, interquartile range 11.84] compared with 1.17 [median, range 0.44–3.26, interquartile range 1.51], P=.029). There was a positive correlation between interleukin-4 and more severe scores on the Interstitial Cystitis Symptoms Index (r=0.406, P=.013). No associations between the presence of lactobacillus species and cytokine levels were observed.

CONCLUSION: The urinary microbiome of participants with interstitial cystitis was less diverse, less likely to contain *Lactobacillus* species, and associated with higher levels of proinflammatory cytokines. It is unknown whether this represents causality and whether the effect of alterations to the urinary microbiome is mediated through an inflammatory response.

(Obstet Gynecol 2017;129:500–6)

DOI: 10.1097/AOG.0000000000001892

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Supported by an American Urogynecologic Society Foundation and International Continence Society–Interstitial Cystitis Research grant to Melinda G. Abernethy, MD.

Presented at the 36th Annual Scientific Meeting of the American Urogynecologic Society, October 13–17, 2015, Seattle, Washington.

Each author has indicated that he or she has met the journal's requirements for authorship.

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Financial Disclosure

Dr. White is the founder and owner of Resphera Biosciences in Baltimore, Maryland. He served as a guidelines methodology consultant to the American Urological Association in 2015. Dr. White performed the species-level taxonomic assignments utilizing Resphera technology. The other authors did not report any potential conflicts of interest.

ISSN: 0029-7844/17

The relationship between alterations to the lactobacilli-dominated vaginal flora and reproductive disorders is well established. Overgrowth of anaerobic and facultative bacteria in the vagina, or bacterial vaginosis, and the increase in proinflammatory cytokines, is implicated as a risk factor

for the development of infectious and obstetric complications.[1–6]

Evidence for protective microbial communities, specifically those of the gastrointestinal tract, has led to investigations of the role played by the human microbiome in health and well-being.[7,8] Such research includes the identification of a biodiverse urinary microbiome that is not isolated by conventional culture.[9] Similar to the influence of microbial communities in the vagina and gastrointestinal tract, alterations to the urinary microbiome may have an effect on the development of lower urinary tract dysfunction.

Characterized by suprapubic pain and urinary frequency, interstitial cystitis is a debilitating condition with an estimated prevalence of 3–8 million U.S. women.[10] The etiology of interstitial cystitis remains elusive, treatment is variable and often unsatisfactory,[11] and affected individuals exhibit higher rates of psychiatric diagnoses and other chronic pain conditions.[12,13] Although an inflammatory component of interstitial cystitis has been suggested, little is known regarding the possible role played by the urinary microbiome and its effect on the immune response.[14,15]

Our primary outcome was to investigate measurable differences in the urinary microbiota and cytokine levels that exist among women with interstitial cystitis compared with healthy women. Furthermore, we sought to determine whether the presence of particular bacteria or cytokines was associated with worsening symptom severity or screening positive for psychiatric comorbidities.

MATERIALS AND METHODS

The institutional review board of Northwestern University approved this cross-sectional study. Female patients, 21 years and older, presenting to the Women's Integrated Pelvic Health Program and diagnosed with interstitial cystitis were approached for participation. The diagnosis of interstitial cystitis was based on symptoms of urinary frequency and suprapubic or bladder pain for at least 6 months. Exclusion criteria included recurrent or current urinary tract infection, urethral strictures, iatrogenic cystitis, bladder augmentation, autoimmune disorder, neuromuscular disease, nonskin cancer, high-grade cervical dysplasia, current pregnancy, or current antibiotic use. Women in a control group were recruited from the same institution among women without genitourinary or pelvic floor complaints, age-matched to within 3 years, and met all exclusion criteria.

After obtaining consent, demographic information was collected and participants were administered validated questionnaires. The Pain Disability Index and Catastrophizing Scale describe the degree to which pain disrupts activities and affects catastrophic thinking, respectively. The Urinary Distress Inventory addresses bother associated with urinary symptoms. Effect on sexual function was obtained with the Female Sexual Function Index. Specific to interstitial cystitis, the Female Genitourinary Pain Index, Interstitial Cystitis Symptom Index, and Interstitial Cystitis Problem Index outlined the degree of experienced symptoms as well as the extent to which they were felt to be problematic. Finally, the Beck Depression and Anxiety Inventories screened for severity of associated symptoms.

A transurethral catheterized urine sample was obtained, transferred to the laboratory, and centrifuged. Supernatant was isolated for cytokine analysis leaving an intact pellet. Both samples were then stored at −80°C.

Bacterial DNA was isolated from the pellet and ribosomal RNA libraries were amplified with polymerase chain reaction. Sequences were generated using Illumina MiSeq technology and filtered for contaminants per standard protocols.[16,17] Using the bioinformatics program QIIME, sequences were aligned against an established database to identify the bacterial families and genera and clustered into bins, or operational taxonomic units, based on similarity.[18] Sequences were also assigned a taxonomic lineage using proprietary Resphera Insight technology.[19] A commercially available immunoassay using antibody detection was used to identify the presence of an established panel of cytokines.[20]

Statistical analysis was completed using SPSS 19. The Wilcoxon rank-sum and Fisher exact tests were used to compare continuous and categorical variables, respectively. Multiple regression analysis was used as appropriate to determine relative associations. Spearman rank correlation was used to describe the relationship between continuous variables. Results were considered significant with a P value of $\leq .05$.

RESULTS

Between November 2013 and April 2014, 40 participants (20 patients, 20 women in the control group) were enrolled. Median age was 34 years (range 21–65 years) and the majority of women were premenopausal (75%) and nulliparous (68%). There were no differences in demographic data between the two groups (Table 1).

Participants diagnosed with interstitial cystitis scored significantly higher on all symptom severity indices (Table 2). Not only did they report higher levels of genitourinary and interstitial cystitis-specific

Table 1. Participant Demographics

Demographic	Patients With Interstitial Cystitis (n=20)	Women in the Control Group (n=20)	*P**
Age (y)	35 (26–46)	33 (28–43)	.968
Race			.314
Caucasian (non-Hispanic)	17 (85)	15 (75)	
African American	3 (15)	1 (5)	
Asian	0	2 (10)	
Other	0	2 (10)	
Premenopausal	15 (75)	16 (80)	.629
Hormone therapy (current)	2 (10)	0	.152
Nulliparous	13 (65)	14 (70)	.637
Cigarette smoker (current)	1 (5)	0	.317

Data are median (interquartile range) or n (%) unless otherwise specified.
* Categorical variables: Kruskal-Wallis or Fisher exact test as appropriate; continuous variables: Wilcoxon rank-sum test.

pain, but also reported a greater degree of disability and catastrophizing related to pain symptoms. They were more likely than healthy women to meet the score criteria for mild anxiety (P=.019) and mild depression (P=.008). Additionally, participants with interstitial cystitis trended toward lower scores on the Female Sexual Function Index, indicating more sexual dysfunction compared with women in the control group (P=.05), and were more likely to have dysfunction related to pain (P=.032). These associations remained significant after controlling for age, menopausal status, and use of hormone therapy or oral contraceptives.

Initial analysis of the sequenced ribosomal RNA amplicons resulted in a normalized data set with 13 patients and 18 women in the control group and identified 54 different operational taxonomic units and 37 different bacterial genera. QIIME output is included in Appendix 1 (available online at http://links.lww.com/AOG/A922), which illustrates the relative abundance of identified genera and operational taxonomic units. Cases were not dominated by a common genus. Sequences specific to *Lactobacillus* species were observed in 5 of 13 (38%) patients and 14 of 18 (78%) women in the control group (P=.06). Participants from whom lactobacilli were isolated in their urine scored lower on the Interstitial Cystitis Symptoms Index (P=.005), Interstitial Cystitis Problem Index (P=.007), and the Female Genitourinary Pain Index (P=.03) (Table 3).

Table 2. Comparison of Symptom Severity, Quality-of-Life, and Psychiatric Comorbidity Screening Scores Between Participants With Interstitial Cystitis and Healthy, Age-Matched Women

Index	Patients With Interstitial Cystitis (n=20)	Women in the Control Group (n=20)	*P**
Pain Disability Index	21 (10–38.50)	0 (0–7.29)	<.001
Pain Catastrophizing Scale	23 (11.50–39)	0 (0–4)	<.001
Urinary Distress Inventory	37.5 (26.04–50)	0	<.001
Female Sexual Function Index	17.3 (9.58–25.65)	28.4 (9.95–33.125)	.05
Female Sexual Function Index–Pain	3.2 (1.2–4.8)	6 (0.5–6)	.032
Female Genitourinary Pain Index	35.5 (24.25–38)	0 (0–1)	<.001
Interstitial Cystitis Symptom Index	11.5 (10.25–14)	1 (0–1.75)	<.001
Interstitial Cystitis Problem Index	11.5 (7–13.75)	0	<.001
Beck Depression Inventory	11 (3–17)	2 (0–4.75)	.003
Mild depression symptoms	12 (60)	3 (15)	.008
Moderate depression symptoms	3 (15)	0	.231
Beck Anxiety Inventory	8.5 (2.25–15.75)	2 (0–6.75)	.021
Mild anxiety symptoms	11 (55)	3 (15)	.019
Moderate anxiety symptoms	5 (25)	1 (5)	.08

Data are median (interquartile range) or n (%) unless otherwise specified.
* Categorical variables: Fisher exact test; continuous variables: Wilcoxon rank-sum test.

Table 3. Comparison of Symptom Severity, Quality-of-Life, and Psychiatric Comorbidity Screening Scores Between Participants With *Lactobacillus* Species Sequences in Urine Compared With Those Without

Index	*Lactobacillus* Species Isolated in Urine (n=19)	*Lactobacillus* Species Not Isolated in Urine (n=12)	*P**
Pain Disability Index	0 (0–6)	16 (0–25)	.101
Pain Catastrophizing Scale	0 (0–18)	13 (2.25–23.25)	.188
Urinary Distress Inventory	0 (0–16.67)	27.08 (6.25–37.5)	.053
Female Sexual Function Index	25.4 (10.7–33.4)	18.5 (8.8–28.1)	.202
Female Sexual Function Index–Pain	5.6 (1.2–6)	3.8 (0.9–5.4)	.166
Female Genitourinary Pain Index	0 (0–22)	22.5 (8–36)	.03
Interstitial Cystitis Symptom Index	1 (0–5)	10 (3–14)	.005
Interstitial Cystitis Problem Index	0 (0–4)	16 (0–20.5)	.007
Beck Depression Inventory	3 (1.2–4.2)	8.5 (0–11.25)	.869
Mild depression symptoms	6 (32)	7 (50)	.472
Moderate depression symptoms	2 (11)	1 (7)	1.0
Beck Anxiety Inventory	2 (0–7)	8.5 (0–15.25)	.324
Mild anxiety symptoms	4 (21)	8 (57)	.066
Moderate anxiety symptoms	2 (11)	3 (21)	.628

Data are median (interquartile range) or n (%) unless otherwise specified.
* Categorical variables: Fisher exact test; continuous variables: Wilcoxon rank-sum test.

The median number of distinct operational taxonomic units found among the urine samples was three (range 20, interquartile range 4). Fewer distinct operational taxonomic units were found among patients compared with women in the control group (2, interquartile range 1 compared with 3.5 interquartile range 5.25, P=.015).

Species-level data were available for 14 patients and 18 women in the control group. Again, no specific taxa with a higher prevalence among patients were identified. However, the lactobacillus genus enrichment in women in the control group was supported with certain species of lactobacillus identified. Specifically, *Lactobacillus acidophilus* was observed in 1 of 14 (7%) patients compared with 7 of 18 (39%) control samples (P=.05, odds ratio [OR] 0.12). An additional lactobacillus assignment (operational taxonomic unit) not distinguished to the species level was found in 0 of 14 patients and 6 of 18 (33%) of women in the control group (P=.023, OR 0.07). Participants with evidence of *L acidophilus* in their urine scored lower on the Interstitial Cystitis Symptoms Index (1.3, interquartile range 1.25) compared with those without (7, interquartile range 12.25, P=.01). Similarly, Female Genitourinary Pain Index scores were lower among patients with *L acidophilus* (0, interquartile range 1.25) compared with those without (17, interquartile range 36, P=.04).

Cytokine analysis identified 25 of the 41 tested cytokines. The panel of included cytokines is presented in Supplemental Digital Content 1. On cytokine analysis, patients with interstitial cystitis demonstrated higher levels of macrophage-derived chemokine (P=.037) and interleukin-4 (IL-4) (P=.029) (Table 4). On bivariate analysis, higher levels of IL-4 correlated with higher scores on the Interstitial Cystitis Symptoms Index (r=0.406, P=.008) and the Pain Disability Index (r=0.302, P=.05). After controlling for the diagnosis of interstitial cystitis, the only significant association that remained was between IL-4 and the Interstitial Cystitis Symptoms Index (P=.013). There was no association between the presence of lactobacillus genera and cytokine levels (Table 5).

DISCUSSION

This investigation of the urinary microbiome demonstrated differences in the urinary microbiome of women with and without a clinical diagnosis of interstitial cystitis. Our findings suggest the possibility of a protective role played by a more diverse, lactobacillus-dominated urinary microbiome because the urine of women with interstitial cystitis was found to have fewer distinct operational taxonomic units and was less likely to contain *Lactobacillus* species, specifically *L acidophilus*, than that of healthy women. Furthermore, the presence of *Lactobacillus* species was associated with improved scores on two interstitial cystitis-specific symptom severity indices, suggesting that the urinary microbiome may influence lower urinary tract symptoms. It is unclear whether these associations could be mediated by an inflammatory component because no differences were found between the presence of *Lactobacillus* species and cytokine levels in the urine.

The appreciation of varied urinary microbiomes among healthy and symptomatic women has been implicated in other analyses.[21,22] Pearce et al[21]

Table 4. Comparison of Cytokine Levels Between Urine Samples of Participants With Interstitial Cystitis and Those of Healthy, Age-Matched Women

Cytokine	Patients With Interstitial Cystitis (n=20)	Women in the Control Group (n=20)	P*
FGF-2	21.53 (11.8–29.23)	19.09 (2.71–29.59)	.463
TGF-α	3.85 (1.84–6.16)	5.03 (2.54–7.60)	.409
G-CSF	7.60 (2.98–25.83)	6.92 (2.69–16.12)	.892
Flt-3L	9.35 (6.96–17.36)	14.69 (8.91–21.40)	.228
GM-CSF	1.81 (1.52–2.31)	2.20 (1.72–2.48)	.386
CX3CL1	47.73 (34.74–68.07)	62.36 (31.10–92.77)	.440
IFNα2	9.03 (4.05–22.50)	6.43 (3.45–11.89)	.323
IFNγ	0 (0–2.67)	0 (0–2.72)	.880
GRO	11.49 (0–21.65)	11.39 (0.53–25.28)	.764
MCP-3	4.55 (0–12.72)	7.10 (0–9.70)	.677
IL-12p40	0 (0–6.43)	0 (0–4.44)	.473
MDC	13.32 (0–15.86)	0 (0–10.35)	.037
PDGF-AA	24.07 (12.07–32.98)	22.25 (7.56–39.96)	.989
PDGF-BB	0 (0–9.54)	2.70 (0–13.53)	.840
IL-15	2.49 (2.21–2.96)	2.49 (1.88–3.11)	.839
sCD40L	0 (0–15.13)	3.59 (0–7.23)	.852
IL-1ra	73.81 (18.01–171.25)	81.57 (45.24–191.00)	.534
IL-4	1.95 (1.35–13.19)	1.17 (0.44–1.95)	.029
IL-7	0 (0–1.92)	0	.300
IL-8	2.80 (0.33–13.49)	4.53 (2.17–12.56)	.342
IP-10	3.47 (0–38.76)	8.98 (0–48.78)	.753
MCP-1	214.50 (114.75–461.50)	296.50 (141.50–522.25)	.507
MIP-1β	1.25 (0.11–1.98)	2.15 (0.33–3.75)	.088
CCL5	5.50 (0–8.04)	6.77 (0–18.73)	.240
VEGF	75.73 (66.19–79.88)	65.29 (53.05–76.57)	.155

FGF, fibroblast growth factor; TGF, transforming growth factor; G-CSF, granulocyte-colony stimulating factor; Flt-3L, FMS-like tyrosine 3 ligand; GM-CSF, granulocyte macrophage colony stimulating factor; CX3CL1, chemokine CSX3C ligand 1 or fractalkine; IFN, interferon; GRO, growth-regulated protein; MCP, monocyte chemotactic protein; IL, interleukin; MDC, macrophage-derived chemokine; PDGF, platelet-derived growth factor; sCD40L, soluble CD40 ligand; IL-1RA, interleukin-1 receptor antagonist; IP-10, interferon γ-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; CCL5, chemokine CC-motif ligand 5; VEGF, vascular endothelial growth factor.

Data are median (interquartile range) unless otherwise specified.

* Wilcoxon rank-sum test.

evaluated the microbiota of catheterized urine samples from 60 women with urgency urinary incontinence compared with healthy women and found that the urgency incontinence group had fewer lactobacillus sequences compared with women in a control group. Specific to bladder pain syndromes, a Norwegian study compared sequences of clean catch urine samples from eight women with interstitial cystitis to healthy women.[23] Although their analysis noted a relative increase in lactobacilli in the urine of women with interstitial cystitis, our study used straight catheterized specimens, which better reflect the urinary microbiome than voided specimens.[9] It is plausible that their collection technique affected the analyzed microbial composition. Furthermore, knowledge regarding the protective benefits of lactobacillus species, specifically *L acidophilus*, in the vaginal microenvironment supports a similar relationship in the lower urinary tract.[24]

In our study, urine samples from participants with interstitial cystitis had less microbial diversity, implicating that a "healthy microbiome" may be a more diverse microbiome. This is substantiated by a recent analysis among women with urgency urinary incontinence in which increased symptom severity was associated with less microbial diversity.[22]

Our finding of relative increases in two proinflammatory cytokines among symptomatic women correlates with other studies supporting the role of urinary cytokines in syndrome development.[14,15] Although these studies did not find an association with macrophage-derived chemokine or IL-4, the number of specific cytokines evaluated in each study was small (seven and three, respectively) and cytokine levels were not correlated with symptom severity. Biologically, macrophage-derived chemokine and IL-4 are active in the inflammatory response. We know that the human microbiota influences varied conditions, including obesity, asthma, irritable bowel syndrome, hypertension, and multiple sclerosis, by regulating the immune inflammatory response, because many of these diseases are

Table 5. Comparison of Cytokine Levels Between Urine Samples Containing Sequences Specific to Lactobacillus Species and Those Without

Cytokine	Lactobacillus Species Isolated in Urine (n=19)	Lactobacillus Species Not Isolated in Urine (n=12)	P*
FGF-2	21.53 (10.83–30.28)	16.48 (10.83–26.08)	.66
TGF-α	4.46 (2.46–7.96)	3.85 (0.91–6.23)	.5
G-CSF	7.43 (4.13–27.45)	6.58 (1.78–11.63)	.196
Flt-3L	14.4 (7.46–22.67)	14.24 (7.32–18.61)	.884
GM-CSF	2.06 (1.7–2.33)	1.77 (1.54–2.61)	.729
CX3CL1fract	65.77 (32.65–95.64)	52.18 (31.24–73.8)	.444
IFNα2	6.43 (5.35–22.88)	8.46 (2.02–14.1)	.688
IFNγ	0 (0–2.67)	0 (0–2.7)	.150
GRO	14.94 (0–27.24)	7.63 (1.6–17.89)	.745
MCP-3	7.78 (0–13.86)	3.89 (0–10.34)	.439
IL-12p40	0 (0–6.43)	0 (0)	.436
MDC	9.39 (0–12.27)	10.35 (0–14.43)	.163
PDGF-AA	25.47 (10.06–40.16)	16.63 (5.83–29.83)	.41
PDGF-BB	3.12 (0–15.54)	0 (0–7.9)	.272
IL-15	2.55 (2.21–3.14)	2.32 (1.88–2.82)	.333
sCD40L	2.84 (0–11.28)	3.73 (0–17.11)	.635
IL-1ra	103 (43.33–219)	66.44 (13.33–165.75)	.216
IL-4	1.62 (0.77–3.06)	1.95 (1.21–3.11)	.596
IL-7	0 (0–2.27)	0 (0)	.333
IL-8	3.01 (1.88–20.21)	4.13 (0–10.78)	.635
IP-10	3.31 (0–51.78)	0 (0–63.42)	.754
MCP-1	237 (129–406)	329 (135.66–607)	.5
MIP-1β	2.01 (0–3.9)	1.39 (0–3.23)	.686
CCL5	6.11 (0–11.83)	6.12 (0–14.64)	.955
VEGF	69.73 (53.05–78.23)	71.43 (53–79.88)	.742

FGF, fibroblast growth factor; TGF, transforming growth factor; G-CSF, granulocyte-colony stimulating factor; Flt3L, FMS-like tyrosine 3 ligand; granulocyte macrophage colony stimulating factor; CX3CL1fract, chemokine CSX3C ligand 1 or fractalkine; IFN, interferon; GRO, growth-regulated protein; MCP, monocyte chemotactic protein; IL, interleukin; MDC, macrophage-derived chemokine; PDGF, platelet-derived growth factor; sCD40L, soluble CD40 ligand; IL-1RA, interleukin-1 receptor antagonist; IP, interferon γ-induced protein; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; CCL5, chemokine CC-motif ligand 5; VEGF, vascular endothelial growth factor.

Data are median (interquartile range) unless otherwise specified.

* Wilcoxon rank-sum test.

linked to a reduction in bacteria that produce chemicals to suppress inflammation.[8] Thus, anti-inflammatory bacteria such as lactobacillus species may be important for sustaining well-being and could play a role in the development and severity of interstitial cystitis.

This study has several limitations. The cross-sectional design prevents distinction of differences as causal or resulting from epiphenomena. Given the use of a convenience sample, we did not establish an a priori sample size and thus our study may have been underpowered to detect differences among some bacterial communities, cytokine levels, and associations with questionnaire data. Furthermore, several of the sequencing reads were not interpretable as a result of incomplete bacterial lysis or primer bias. Such loss of data is inherent in sequencing techniques, and our rate of 80% is in line with, if not improved on, other recent studies.[9,21] Finally, we know that the vaginal microbiome temporarily varies depending on hormonal fluctuations.[25,26] Our single-sample acquisition does not account for changes related to these fluctuations, the effect of transient systemic bacteremia, or prior antibiotic use.

Despite these limitations, we were able to detect a difference in several important and biologically plausible variables. Similar to the effect of vaginal dysbiosis (an imbalance of the microbiota) on pathologic states,[27] it is plausible that a dysbiotic urinary microbiome, mediated through a specific immune response, may be associated with certain urinary symptoms and pelvic floor disorders. Our study highlights the need to fully characterize a healthy urinary microbiome and represents a step toward better understanding of lower urinary tract symptoms and the potential development of more targeted therapies.

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