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Urinary Symptoms are Associated With Certain Urinary Microbes in Urogynecologic Surgical Patients

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Abstract

OBJECTIVES: Persistent and *de novo* symptoms decrease satisfaction after urogynecologic surgery. We investigated whether the pre-operative bladder microbiome is associated with urinary symptoms prior to and after urogynecological surgery.

METHODS: 126 participants contributed responses to the validated OABq symptom questionnaire. Catheterized (bladder) urine samples and vaginal and perineal swabs were collected immediately pre-operatively. Bacterial DNA in the urine samples and swabs was sequenced and classified.

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RESULTS: Pre-operative symptom severity was significantly worse in sequence-positive patients. Higher OABq Symptom Severity (OABqSS) scores (more symptomatic) were associated with higher abundance in bladder urine of two bacterial species: *Atopobium vaginae* and *Finegoldia magna*. The presence of *Atopobium vaginae* in bladder urine also was correlated with its presence in either the vagina or perineum.

CONCLUSIONS: Two specific bacterial species detected in bladder urine, *Atopobium vaginae* and *Finegoldia magna*, are associated with pre-operative urinary symptoms severity in women undergoing POP/SUI surgery. The reservoir for *Atopobium vaginae* may be an adjacent pelvic floor niches. This observations should be validated in a larger cohort to determine whether there is a microbiological etiology for certain pre-operative urinary symptoms.

BRIEF SUMMARY:

In women undergoing POP/SUI surgery, two bacterial species detected in bladder urine, *Atopobium vaginae* and *Fingoldia magna*, are associated with pre-operative urinary symptoms severity.

Keywords

Microbiome; Urinary Incontinence; Bladder; Urinary Symptoms

INTRODUCTION:

Surgery is highly effective for treatment of certain urogynecologic conditions, including stress urinary incontinence (SUI) and pelvic organ prolapse (POP). Unfortunately, persistent or *de novo* urinary symptoms may occur, decreasing patient satisfaction, . There is little evidence to predict the occurrence or severity of bothersome post-operative urinary symptoms. In addition to urinary symptoms, transient events occur, including post-operative urinary tract infection (UTI), which affects at least 7–24% of women in the first six weeks after their urogynecology surgery, despite prophylactic antibiotics and other preventive measures–.

Until recently, the bladder was considered sterile. However, enhanced urine culture and culture-independent methods have revealed the presence of live microbes (bladder urine microbiome) in urine obtained directly from the urinary bladders of adult women. These studies have revealed associations between these bacteria and post-operative UTIs, , urgency urinary incontinence– and response to overactive bladder treatment. They also have identified associations between some bacterial species and the lack of symptoms or protection against post-instrumentation UTI, . These results suggest that the female urinary bladder contains microbes that can influence urinary symptoms.

In this analysis, we used culture-independent 16S rRNA gene sequencing to characterize associations between the pre-operative microbiomes of bladder urine, vagina and perineum and symptoms in women prior to and after urogynecological surgery.

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MATERIALS AND METHODS

Study design

Recruitment and initial characterization of the main study cohort was previously described. Briefly, after institutional review board approval, we approached women undergoing POP/SUI surgery at Loyola University Medical Center. Pre-operatively and again 3 months after surgery, participants self-completed the validated Pelvic Floor Distress Inventory (PFDI), and overactive bladder questionnaire (OAB-q), with higher scores indicating greater symptom severity. Clinical data, such as age, BMI, medical co-morbidities, type of POP/SUI surgery and hormone status was extracted from the electronic medical record. Hormone status was clinically defined and categorized as pre-menopausal, post-menopausal on hormones, or post-menopausal not on hormones.

Following induction of anesthesia and prior to systemic antibiotic administration, swabs were collected from the vagina (approximately 3 cm distal to the hymen) and perineum, and a catheterized urine sample was collected via the urinary catheter placed as part of the clinical surgical protocol. We used catheterized urine because previous studies showed that it was the proper collection for bladder urine, . A portion of this urine sample was sent for standard clinical urine culture, and a portion was stored at −80C in 10% AssayAssure (Sierra Molecular, Incline Village, NV) prior to DNA isolation for sequencing. Each swab was suspended in 1 ml of PBS and the suspension was stored at −80°C in 10% AssayAssure prior to DNA isolation for sequencing.

DNA isolation from urine

DNA isolation, polymerase chain reaction (PCR) amplification, and 16S rRNA gene sequencing of urine cultures has been described previously. To avoid contamination, isolation of DNA was performed in a laminar flow hood. Genomic DNA was extracted from 1 ml of urine or 1 ml of PBS swab suspension, using previously validated protocols developed for the Human Microbiome Project, , . To isolate genomic DNA from urine samples and swabs, this protocol includes the addition of the peptidoglycan degrading enzymes, mutanolysin and lysozyme, that ensure robust lysis of Gram-positive and Gram-negative species. Briefly, 1 ml of urine was centrifuged at 13,500 rpm for 10 min, and the resulting pellet was resuspended in 200 μl of filter-sterilized buffer consisting of 20 mM Tris-Cl (pH 8), 2 mM EDTA, 1.2% Triton X-100, and 20 μg/ml lysozyme and supplemented with 30 μl of filter-sterilized mutanolysin (5,000 U/ml; Sigma-Aldrich, St. Louis, MO). The mixture was incubated for 1 h at 37°C, and the lysates were processed through the DNeasy blood and tissue kit (Qiagen, Valencia, CA), according to the manufacturer's protocol. The DNA was eluted into 50 μl of buffer AE, pH 8.0, and stored at −20° C.

Hyper-variable region 4 (V4) of the bacterial 16S rRNA gene, commonly used in human microbiome studies, was amplified via a two-step PCR protocol, as described previously, . Briefly, in the first amplification, the V4 region was amplified using Illumina MiSeq modified universal primers 515F and 806R. Extraction negative controls (no urine) 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%

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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 10 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).

Because bladder urine samples typically contain small amounts of bacteria (i.e., low biomass), amplification and sequencing was performed in duplicate (technical replicates) and samples were classified as either sequence-positive or sequence-negative. A sequence-positive sample was one in which DNA was amplified from both replicas and, if present, the dominant taxon (representing >50% of sequences from the sample) was the same in both replicas. For each sequence-positive sample, both replicas were used for further analysis. Because they usually contain high biomass, vaginal and perineal swabs were sequenced a second time only if the first attempt was negative.

Data analysis

The Illumina proprietary MiSeq post-sequencing software was used to preprocess sequences by removing primers and sample indices. Raw sequences were processed using the open-source program mothur (v1.37.4) by following its recommended MiSeq pipeline with minor alterations. Briefly, mothur combined the paired end reads and removed contigs (overlapping sequence data) of incorrect length (<290 bp, >300 bp) and/or contigs containing ambiguous bases. Chimeric sequences were detected and removed using UCHIME within the mothur suite. To correct for different sequencing depth of each sample, subsampling at a depth of 5000 total reads was performed. The sequence reads were clustered into species-level operational taxonomic units (OTUs) with identity similarity cutoff at 0.97. Representative OTUs were selected on the basis of abundance and further classified using BLCA at the species level. Student's t-test was applied to examine the differences in age, symptoms scores and other continuous variables in clinical and demographic data between sequence-positive and sequence-negative patients. The Chi-squared test was applied to examine hormone status and other categorical variables in clinical and demographic data between sequence-positive and sequence-negative patients. Linear regression was applied to identify the association between bacterial abundance and symptom scores. Spearman's rank correlation was applied to examine the association between the bacterial abundance amongst bladder, vaginal, and perineal microbiome.

For the linear regression analysis, we included only participants who *(i)* completed the PFDI and OABq at baseline and 3 months, *(ii)* had a complete pre-operative sample set (urine, vaginal swab and perineal swab) and *(iii)* whose bladder urine samples successfully underwent sequencing (i.e., sequence-positive). The associations between bacteria and OABq Symptom Severity (OABqSS) score of the OAB-q were identified using linear

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regression with bacterial abundance as the independent variable and OABqSS scores as the dependent variables. The positive coefficient values display the increase in OABqSS scores related to a 1% increase in bacterial abundance. We also explored the linear regression model using age and hormonal status as confounding factors. In addition, we assessed the correlation for bacterial abundance amongst three pelvic floor niches using Spearman's rank correlation.

RESULTS

Patient characterization

Table 1 displays the demographics for the 126 women who met inclusion criteria for this analysis; urine samples were sequence positive in 55 women and sequence negative in the remaining 71 women. There was no significant difference in demographics between the two sequence groups. Most participants (88%) were white; the mean age of participants was 59 years. The demographics of this group did not differ significantly from the larger, previously described study group. The mean pre-operative OABq symptom score was significanty higher in women with sequence-positive urine samples (sequence-positive women 40.5 vs. sequence-negative 32.1, p=0.03); the mean post-surgery OABq symptom score were similar (sequence positive 15.6 vs. sequence negative 15.2, p=0.89). There was no difference in the PFDI subscale scores pre-operatively or post-operatively between the sequence positive and sequence negative groups.

Association of microbes with urinary symptoms

The most abundant genus in bladder urine was *Lactobacillus* with a median abundance of 30.34%, followed by *Corynebacterium* (10.08%), *Gardnerella* (6.06%), *Staphylococcus* (5.57%) and *Enterobacter* (4.67%) (Supplemental Figure 1A). In the women with sequence positive urine samples, all of the vaginal swabs were positive. The most abundant genus in the vaginal swabs was *Lactobacillus* with a median abundance of 25.71%, followed by *Corynebacterium* (10.98%), *Anaerococcus* (9.68%), *Peptoniphilus* (5.94%) and *Gardnerella* (5.34%) (Supplemental Figure 1B). Fifty-two of the perineal swabs were sequence positive In the women with sequence positive urine samples. The most abundant genus in the perineal swabs was *Lactobacillus* with a median abundance of 55.29%, followed by *Gardnerella* (12.77%), *Prevotella* (3.81%), *Anaerococcus* (3.47%) and *Corynebacterium* (2.89%) (Supplemental Figure 1C).

Linear regression analysis identified an association between higher OABq symptoms scores and 2 specific bacterial species in the urine: *Atopobium vaginae* (p=0.006) and *Finegoldia magna* (p=0.008) (Table 2). We also observed correlations for abundances of *A. vaginae* in adjacent pelvic floor niches (Table 3): a strong correlation between *A. vaginae* abundances in the vagina and perineum (p-value <0.001); a weaker correlation between bladder urine and vagina (p=0.036) and a borderline significant correlation between bladder urine and perineum (p=0.054). We did not detect significant correlations for *F. magna* between bladder urine, vagina, and perineum. Neither age nor hormonal status influenced these associations.

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DISCUSSION:

In women undergoing urogynecologic surgery for SUI and/or POP, two specific bacterial species in urine, *A. vaginae* and *F. magna*, are associated with pre-operative urinary symptoms severity. This novel finding may provide additional insight into the etiology of certain urinary symptoms.

Most clinicians are not familiar with these two bacterial species; neither is detected on standard urine cultures. Both are fastidious anaerobic members of the Gram-positive phylum Actinobacteria. *A. vaginae* is associated with bacterial vaginosis; evidence exists that it plays a major role along with *Gardnerella vaginalis* in establishing an adherent biofilm thought to be responsible for some BV treatment failures. It has also has been implicated in maternal sepsis and pre-term birth–. Here, we have identified an association between *A. vaginae* and increased severity of overactive bladder symptoms. Previously, we used 16S rRNA gene sequencing to detect the genus *Atopobium* in catheterized urine obtained from peri-menopausal women with and without urgency urinary incontinence, . This raises the question: do all *A. vaginae* cause lower urinary tract symptoms. The answer may be no, as *A. vaginae* is commonly found in the vaginas of women with no bacterial vaginosis symptoms–. Thus, it is possible that some *A. vaginae* isolates are causative agents of disease, while others are not. Alternatively, the same isolate might cause symptoms in one woman but not in another. Correlations between abundances of *A. vaginae* in adjacent pelvic floor niches suggest that the vagina and/or perineum might act as reservoirs for this emerging uropathogen. The second microbe of interest, *F. magna*, is an opportunistic human pathogen that normally colonies skin and mucous membranes; it has been associated with vaginoses, as well as wound infections, soft tissue and non-puerperal abscesses, bone and prosthetic joint infections, septic arthritis and occasionally infectious endocarditis. Prior to our study, it had not been associated with lower urinary tract symptoms.

The prevailing definition of a clinical uropathogen label is dichotomous, suggesting that an microbe is either always or never pathogenic. Yet pathogenicity clearly depends on the host response, including the health of the local biological community. For organisms that have associations with urinary symptoms (e.g. *A. vaginae*), we hypothesize that they have some contribution to a urinary dysbioses associated with clinical symptoms. We hypothesize that some of these previously under-detected bacteria could be pathogenic in certain settings, and somewhat dependent on functions within a specific urinary microbiome, for example the status of protective mechanisms, such as co-presence of *Lactobacillus*.

The novel findings of this analysis adds to the emerging evidence that demonstrates that bacteria exist in the bladders of healthy women and women with lower urinary tract symptoms–, , , –, including patients undergoing urogynecologic surgery, . With appropriate testing techniques, especially in women with higher levels of urinary symptom severity, the presence either in the vagina or in the bladder urine of microbes such as *A. vaginae*, or even the overall diversity of the bladder microbiota, could be determined prior to surgery, potentially improving pre-surgical counseling and peri-operative symptom control. Although these two species were not associated with symptoms in the overall cohort at 3 months after surgery, we believe that our findings warrant replication and futher analysis to determine if

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certain individuals or subgroups may have a microbial basis for persistent or *de novo* OAB symptoms. At this time, we do not recommend changes to the current clinical use of peri-operative antibiotics. However, future studies may provide refinements to the current strategy. Such studies would be complemented by an understanding of microbial restoration mechanisms after disruptive events like surgery.

Our study had many strengths, including longitudinal, peri-operative symptom assessment with a validated questionnaire and rigorous attention to avoidance of bacterial contamination and sequencing errors, which is essential when working with low biomass samples, such as urine. In contrast, we did not collect post-operative samples, which would have allowed us to describe the composition and characteristics of the post-surgical microbiota. Despite these limitations, we hope our findings can inform the design of future studies that seek to reduce the presence of bothersome urinary symptoms in women undergoing urogynecologic surgery.

CONCLUSIONS:

Detection of urinary bacteria is correlated with peri-operative urinary symptoms in women undergoing urogynecologic surgery. Pre-operative knowledge of an individual woman's urinary microbial community, including the presence of *A. vaginae*, may allow refinements to counseling regarding post-operative symptom resolution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

OABq	Overactive Bladder questionnaire
UDI	Urinary Distress Inventory
POPDI	Pelvic Organ Prolapse Distress Inventory
CRADI	Colorectal Anal Distress Inventory (CRADI)
UTI	Urinary Tract Infection
POP	Pelvic Organ Prolapse
UI	Urinary Incontinence

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BMI	Body Mass Index
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
OTU	Operational Taxonomic Unit
SUI	Stress Urinary Incontinence

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Table 1.

Baseline Participant Demographic and Clinical Characteristics

Variable[a]	Sequence Positive (N = 55)	Sequence Negative (N = 71)	p-value
AGE	57 years (30 –85)	60 years (35–86)	0..22
BMI	28 kg/m2 (21–44)	27 kg/m2 (16–70)	0.25
RACE	Caucasian= 50 (91%) African American = 3(6%) Latina =1 (2%) Other = 1 (2%)	Caucasian= 61 (86%) African American = 3(4%) Latina =1 (1%) Asian = 4 (6%) Other = 2 (3%)	0.49
POP/UI surgery	POP = 21 (38%) UI = 14 (26%) POP/UI = 20 (36%)	POP = 27 (38%) UI = 19 (27%) POP/UI = 25 (35%)	0.98
Diabetes	0	3 (4%)	0.12
HTN	19 (35%)	17 (24%)	0.19
Coronary Artery Disease	4 (7%)	5 (7%)	0.96
Smoking	2 (4%)	0	0.11
Hormone Status	Premenopausal = 18 (33%) Postmenopausal on hormones = 7 (13%) Postmenopausal no hormones = 30 (55%)	Premenopausal = 18 (25%) Postmenopausal on hormones = 12 (17%) Postmenopausal no hormones = 41 (58%)	0.61
OABq SS preop	40.5 (10–95)	32.1 (0–82.5)	**0.03**
OABq SS postop	15.6 (0–77.5)	15.2 (0–85)	0.89
OABq SS difference	−24.8 (−87.5–62.5)	−16.9 (−75–37.5)	0.06
UDI	Preop = 107.52 (±51) Postop = 28.85 (±33) Difference = −78.67 (±51)	Preop = 99.10 (±52) Postop = 27.93 (±27) Difference = 71.18 (±54)	0.37 0.86 0.43
POPDI	Preop = 113.36 (±71) Postop = 26.76 (±32) Difference = −86.59 (±64)	Preop =103.51 (±69) Postop =24.76 (±28) Difference = −78.67 (±68)	0.43 0.71 0.50
CRADI	Preop = 83.62 (±85) Postop = 32.16 (±43) Difference = −44.54 (±73)	Preop = 103.51 (±71) Postop =31.15 (±35) Difference = −47.61 (±62)	0.73 0.89 0.80

[a]Abbrevations: BMI, body mass index; HTN, hypertension; OABqSS, OABq Symptom Severity; UDI, Urinary Distress Index; POPDI, Pelvic Organ Prolapse Distress Inventory; CRADI, Colorectal Anal Distress Inventory.

[b]p-values < 0.05 are considered significant; significant value is bolded.

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Table 2.

Associations of two urinary bacterial species with pre-surgery OABqSS values

Species	Mean Relative Abundance Within the Sample (%)	Coefficient value[a]	P-value[b]
A. vaginae	1.07	0.015	**0.006**
F. magna	1.32	0.030	**0.008**

[a]The association between the bacteria and OABqSS scores were identified using linear regression with the bacterial abundance as the independent variable and OABqSS scores as the dependent variables. The positive coefficient values display the increase in OABqSS scores related to a 1% increase in bacterial abundance. We also explored the linear regression model using age and hormonal status as confounding factors (similar results).

[b]p-values < 0.05 are considered significant; significant values are bolded.

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Table 3.

Correlation of two bacterial species in bladder urine, vagina, and perineum

Body Sites	species	rho[a]	p-value[b]
urine vs vagina	*A. vaginae*	0.332	**0.036**
	F. magna	0.248	0.123
urine vs perineum	*A. vaginae*	0.307	**0.054** [b]
	F. magna	−0.104	0.527
vagina vs perineum	*A. vaginae*	0.809	**<0.0001**
	F. magna	0.194	0.230

[a]The correlation for each bacterial abundance amongst three pelvic floor niches were tested with Spearman's rank correlation. The correlation coefficient, rho, ranges from −1 to 1 corresponding to negative and positive correlation, respectively.

[b]p-values < 0.05 are considered significant; significant p-values are bolded.