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ORIGINAL ARTICLE

# Urinary symptoms are associated with certain urinary microbes in urogynecologic surgical patients

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## Abstract

**Introduction and hypothesis** Persistent and de novo symptoms decrease satisfaction after urogynecologic surgery. We investigated whether the preoperative bladder microbiome is associated with urinary symptoms prior to and after urogynecologic surgery.

**Methods** One hundred twenty-six participants contributed responses to the validated OABq symptom questionnaire. Catheterized (bladder) urine samples and vaginal and perineal swabs were collected immediately preoperatively. Bacterial DNA in the urine samples and swabs was sequenced and classified.

**Results** Preoperative 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 preoperative urinary symptom severity in women undergoing POP/SUI surgery. The reservoir for *Atopobium vaginae* may be adjacent pelvic floor niches. This observation should be validated in a larger cohort to determine whether there is a microbiologic etiology for certain preoperative urinary symptoms.

**Keywords** Microbiome · Urinary incontinence · Bladder · Urinary symptoms

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## Introduction

Surgery is highly effective for treatment of certain urogynecologic conditions, including stress urinary incontinence (SUI) and pelvic organ prolapse (POP) [1]. Unfortunately, persistent or de novo urinary symptoms may occur, decreasing patient satisfaction [2, 3]. There is little evidence to predict the occurrence or severity of bothersome postoperative urinary symptoms. In addition to urinary symptoms, transient events occur, including postoperative urinary tract infection (UTI), which affects at least 7–24% of women in the first 6 weeks after their urogynecologic surgery, despite prophylactic antibiotics and other preventive measures [4–6].

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 postoperative UTIs [7, 8], urgency urinary incontinence [9–11], and response to overactive bladder treatment [12]. They also have identified associations between some bacterial species and the lack of symptoms or protection against post-instrumentation UTI [10, 11]. 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 preoperative microbiomes of the bladder urine, vagina, and perineum and symptoms in women prior to and after urogynecologic surgery.

## Materials and methods

### Study design

Recruitment and initial characterization of the main study cohort were previously described [7]. Briefly, after institutional review board approval, we approached women undergoing POP/SUI surgery at Loyola University Medical Center. Preoperatively and again 3 months after surgery, participants self-completed the validated Pelvic Floor Distress Inventory (PFDI) [13, 14] and overactive bladder questionnaire (OAB-q) [15], with higher scores indicating greater symptom severity. Clinical data, such as age, BMI, medical comorbidities, type of POP/SUI surgery, and hormone status, were 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 [16, 17]. 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 [10]. 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 [10, 18, 19]. 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 -negative species [19]. 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 (5000 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 [10, 18]. 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% 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 eight-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 were performed in duplicate (technical replicates), and samples were classified as either sequence-positive or -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 [20]. 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 [21]. To correct for different sequencing depths 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 [22]. Representative OTUs were selected on the basis of abundance and further classified using BLCA [23] at the species level. Student's t-test was applied to examine the differences in age, symptom scores, and other continuous variables in clinical and demographic data between sequence-positive and -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 -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 of the bacterial abundance among the bladder, vaginal, and perineal microbiome.

For the linear regression analysis, we included only participants who (1) completed the PFDI and OABq at baseline and 3 months, (2) had a complete preoperative sample set (urine, vaginal swab, and perineal swab), and (3) whose bladder urine samples successfully underwent sequencing (i.e., sequence-positive). The associations between bacteria and the OABq Symptom Severity (OABqSS) score of the OAB-q were identified using linear 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 among three pelvic floor niches using Spearman's rank correlation.

## Results

### Patient characterization

Table 1 displays the demographics for the 126 women who met the inclusion criteria for this analysis; urine samples were sequence positive in 55 women and sequence negative in the remaining 71. 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 [7]. The mean preoperative 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 scores were similar (sequence positive 15.6 vs. sequence negative 15.2, $p = 0.89$). There was no difference in the PFDI subscale scores pre- or postoperatively between the sequence-positive and -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 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 on 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 two specific

**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%) | Caucasian = 61 (86%) | 0.49 |
| | African American = 3(6%) | African American = 3(4%) | |
| | Latina =1 (2%) | Latina =1 (1%) | |
| | Other = 1 (2%) | Asian = 4 (6%) | |
| | | Other = 2 (3%) | |
| POP/UI surgery | POP = 21 (38%) | POP = 27 (38%) | 0.98 |
| | UI = 14 (26%) | UI = 19 (27%) | |
| | POP/UI = 20 (36%) | POP/UI = 25 (35%) | |
| 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%) | Premenopausal = 18 (25%) | 0.61 |
| | Postmenopausal on hormones = 7 (13%) | Postmenopausal on hormones = 12 (17%) | |
| | Postmenopausal no hormones = 30 (55%) | Postmenopausal no hormones = 41 (58%) | |
| 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) | Preop = 99.10 (±52) | 0.37 |
| | Postop = 28.85 (±33) | Postop = 27.93 (±27) | 0.86 |
| | Difference = −78.67 (±51) | Difference = 71.18 (±54) | 0.43 |
| POPDI | Preop = 113.36 (±71) | Preop =103.51 (±69) | 0.43 |
| | Postop = 26.76 (±32) | Postop =24.76 (±28) | 0.71 |
| | Difference = −86.59 (±64) | Difference = −78.67 (±68) | 0.50 |
| CRADI | Preop = 83.62 (±85) | Preop = 103.51 (±71) | 0.73 |
| | Postop = 32.16 (±43) | Postop =31.15 (±35) | 0.89 |
| | Difference = −44.54 (±73) | Difference = −47.61 (±62) | 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] $p < 0.05$ is considered significant; significant value is bolded

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 ($p < 0.001$) and a weaker correlation between bladder urine and the vagina ($p = 0.036$) and a borderline significant correlation between the bladder urine and perineum ($p = 0.054$). We did not detect significant correlations for *F. magna* among the bladder urine, vagina, and perineum. Neither age nor hormonal status influenced these associations.

## 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 preoperative urinary symptom 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

**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 was identified using linear regression with the bacterial abundance as the independent variable and OABqSS scores as the dependent variable. 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] $p < 0.05$ is considered significant; significant values are bolded

along with *Gardnerella vaginalis* in establishing an adherent biofilm thought to be responsible for some BV treatment failures [24]. It has also has been implicated in maternal sepsis and pre-term birth [25–27]. 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 perimenopausal women with and without urgency urinary incontinence [10, 11]. 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 [28–30]. 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 a reservoir for this emerging uropathogen. The second microbe of interest, *F. magna,* is an opportunistic human pathogen that normally colonizes the 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 [31]. Prior to our study, it had not been associated with lower urinary tract symptoms.

**Table 3** Correlation of two bacterial species in the 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 among three pelvic floor niches was tested with Spearman's rank correlation. The correlation coefficient, rho, ranges from −1 to 1 corresponding to negative and positive correlation, respectively

[b] $p < 0.05$ is considered significant; significant $p$ values are bolded

The prevailing definition of a clinical uropathogen label is dichotomous, suggesting that a microbe is either always or never pathogenic. However, the pathogenicity clearly depends on the host response, including the health of the local biologic community. For organisms that have associations with urinary symptoms (e.g., *A. vaginae*), we hypothesize that they make 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 add to the emerging evidence that demonstrates that bacteria exist in the bladders of healthy women and women with lower urinary tract symptoms [8–12, 16, 18, 32–35], including patients undergoing urogynecologic surgery [8, 16]. With appropriate testing techniques, especially in women with higher levels of urinary symptom severity, the presence in either the vagina or 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 perioperative 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 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 perioperative 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 such as surgery.

Our study had many strengths, including longitudinal, perioperative symptom assessment with a validated questionnaire and rigorous attention to avoidance of bacterial contamination and sequencing errors, which are essential when working with low biomass samples, such as urine. In contrast, we did not collect postoperative 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 perioperative urinary symptoms in women undergoing urogynecologic surgery. Preoperative knowledge of an individual woman's urinary microbial community, including the presence of *A. vaginae,* may allow refinements to counseling regarding postoperative symptom resolution.

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### Compliance with ethical standards

**Conflicts of interest** L. Brubaker—Editorial Stipends: Journal of American Medical Association, Female Pelvic Medicine and Reconstructive Surgery, Up To Date; Research Funding: NIH; Board Stipend: American Board of Obstetrics and Gynecology.

A. J. Wolfe—Investigator Initiated Studies: Astellas Scientific and Medical Affairs, Inc.; Kimberly Clarke Corp.

E.R. Mueller—Astellas-Advisory Board, Boston Scientific-Advisory Board.

The remaining authors claim no conflicts of interest.

**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; *BMI*, Body mass index; *DNA*, Deoxyribonucleic acid; *PCR*, Polymerase chain reaction; *OTU*, Operational taxonomic unit; *SUI*, Stress urinary incontinence

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