GYNECOLOGY

Association between the urogenital microbiome and surgical treatment response in women undergoing midurethral sling operation for mixed urinary incontinence

Holly E. Richter, PhD, MD; Megan U. Carnes, PhD; Yuko M. Komesu, MD; Emily S. Lukacz, MD; Lily Arya, MD; Megan Bradley, MD; Rebecca G. Rogers, MD; Vivian W. Sung, MD; Nazema Y. Siddiqui, MD; Benjamin Carper, MS; Donna Mazloomdoost, MD; Darryl Dinwiddie, PhD; Marie G. Gantz, MD; On behalf of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Pelvic Floor Disorders Network

BACKGROUND: The urogenital microbiome is associated with urgency and mixed urinary incontinence symptoms and differential treatment responses to pharmacotherapy for urgency urinary incontinence.

OBJECTIVE: This study aimed to describe whether the preoperative urinary and vaginal microbiomes were associated with surgical treatment responses at 12 months after a midurethral sling operation in women with mixed urinary incontinence.

STUDY DESIGN: This cohort study compared the preoperative microbiome compositions of urine and vaginal samples from a subset of women undergoing a midurethral sling operation in the Effects of Surgical Treatment Enhanced With Exercise for Mixed Urinary Incontinence trial (NCT01959347) and compared the microbiota in women who were surgical responders vs surgical nonresponders. Twelve-month objective response was defined as a $\geq$70% reduction from baseline urinary incontinence episodes on a 3-day diary. Subjective response was defined as a change from baseline in the Urogenital Distress Inventory scores. Bacterial abundance and beta diversity were assessed using 16S ribosomal RNA sequencing. The primary differential abundance analysis described predominant bacterial operational taxonomic units associated with responders vs nonresponders using unadjusted and age-adjusted linear models.

RESULTS: Objective nonresponders (n=28) compared with responders (n=72) were older (58.5±10.7 vs 51.6±10.2 years) and more likely postmenopausal without hormone use (odds ratio, 6.4; 95% confidence interval, 1.8–22.6). Vaginal and urinary microbiota beta diversities were associated with age ($P<.05$) for both responders and nonresponders. Overall, predominant operational taxonomic units (genera) were *Lactobacillus*, *Gardnerella*, *Tepidimonas*, *Escherichia*, *Streptococcus*, and *Prevotella*. Operational taxonomic units from baseline urine samples were not significantly associated (P threshold=.05) with surgical treatment responses. A greater abundance of baseline vaginal *Lactobacillus* was associated with an objective response ($P=.04$) and *Prevotella* with an objective nonresponse ($P=.01$). Adjusting for age, only a greater abundance of baseline vaginal *Prevotella* was associated with an objective nonresponse ($P=.01$). Moreover, less abundant vaginal operational taxonomic units were associated with objective and subjective responses and persistent urinary incontinence symptoms ($P<.05$).

CONCLUSION: Women meeting a 70% reduction of urinary incontinence treatment episodes (objective responders) had greater vaginal *Lactobacillus* at the time of the surgical procedure; however, controlling for age diminished this association. Women not meeting a 70% reduction of urinary incontinence episodes 1 year after a midurethral sling operation had greater vaginal *Prevotella* at the time of the midurethral sling operation. Further research is needed to determine whether therapy altering the vaginal microbiome may impact surgical treatment responses in women with mixed urinary incontinence.

Key words: midurethral sling procedure, mixed urinary incontinence, surgical outcomes, urinary microbiome, vaginal microbiome

Introduction

The urogenital microbiome is associated with lower urinary tract health, although the relationship is complicated. Although some investigators have found that urgency urinary incontinence (UUI) symptoms are associated with the urinary microbiome,[1–3] a more complex relationship has been found in women with mixed urinary incontinence (MUI).[4] Specifically in women <51 years of age, urinary microbiome community types with fewer *Lactobacillus* ($\leq$61% of genera) was associated with MUI, whereas a higher proportion of controls had high *Lactobacillus* communities (89% *Lactobacillus*).[4]

Recently, we demonstrated that the composition of the urinary microbiome is correlated with the vaginal microbiome in women with MUI.[5] This complements previous genome-level work showing that vaginal and bladder microbiota are functionally distinct from gastrointestinal microbiota but similar to each other.[6] The interrelatedness of the vaginal and urinary microbiomes may offer unique opportunities to optimize lower urinary tract health and surgical outcomes.[5–7] For example, vaginal therapies could have beneficial downstream effects on the urinary microenvironment and surgical outcomes. Alternatively, just as differences in the urinary microenvironment are associated with differential treatment responses to medication for UUI,[2] the vaginal microenvironment at the time of

Cite this article as: Richter HE, Carnes MU, Komesu YM, et al. Association between the urogenital microbiome and surgical treatment response in women undergoing midurethral sling operation for mixed urinary incontinence. Am J Obstet Gynecol 2021;XX:x.ex–x.ex.

0002-9378/$36.00

https://doi.org/10.1016/j.ajog.2021.07.008

AJOG at a Glance

Why was this study conducted?

This study aimed to evaluate whether preoperative urinary or vaginal microbiomes are associated with surgical treatment responses at 12 months in women with mixed urinary incontinence (MUI) undergoing a midurethral sling placement.

Key findings

Older and postmenopausal women without hormone replacement more commonly were surgical nonresponders. A greater abundance of preoperative vaginal *Lactobacillus* was associated with improved objective surgical response (decreased urinary incontinence episodes); this effect diminished after adjusting for age. A greater abundance of preoperative vaginal *Prevotella* was associated with worsened objective surgical responses even after adjusting for age. Urinary microbiota were not associated with surgical treatment responses.

What does this add to what is known?

Baseline vaginal microbiome findings were associated with surgical treatment responses to midurethral slings in women with MUI.

the midurethral sling operation in women with MUI could be related to differential treatment responses. This is plausible given that surgical outcomes following pancreatic and colon operations may be associated with specific gut microbiome compositions and targeting of the gut microbiome has been suggested as a means of improving surgical outcomes.[8−10]

This was a planned secondary analysis in a subset of women participating in the effect of behavioral and pelvic floor muscle therapy combined with surgery vs surgery alone on incontinence symptoms among women with mixed urinary incontinence (Effects of Surgical Treatment Enhanced With Exercise for Mixed Urinary Incontinence [ESTEEM]) trial (NCT01959347).[11] This study aimed to describe preoperative urinary and vaginal bacterial taxa associated with 12-month responses after a midurethral sling operation in women with MUI. It was hypothesized that the relative abundance of *Lactobacillus* among preoperative urinary or vaginal microbiota would be associated with surgical treatment responses.

Materials and Methods

Study population

After institutional review board approval and written informed consent were obtained, catheterized urine and vaginal bacterial samples were collected from a subset of women (126 of 480) with MUI enrolled in the ESTEEM trial at 1 time point, before surgery. As previously described, ESTEEM participants were randomized in a 1:1 ratio to either midurethral sling operation or midurethral sling operation plus perioperative behavioral and pelvic floor muscle therapies.[12] Baseline and follow-up lower urinary tract symptoms were obtained using the 19-item Urogenital Distress Inventory (UDI),[13] a validated questionnaire measuring symptom severity, and 3-day bladder diary characterizing incontinence episodes. Here, 2 subjective measures and 1 objective measure of response were evaluated. The binary subjective response was defined as a reduction in the UDI score from baseline to 12 months meeting the minimal clinically important difference (MCID) threshold (−26.1 points).[12] The second subjective measure was a change from baseline in the UDI score (Δ UDI) at 12 months. For participants who underwent additional urinary incontinence (UI) treatment before 12 months, Δ UDI was determined using the participant's UDI score from the last questionnaire completed before the initiation of additional treatments. Binary objective response to treatment was defined as a ≥70% reduction from baseline in UI episodes (UIEs) on the 3-day diary at 12 months and no additional treatment, as previously described.[14]

Sample processing

Sample processing has previously been described.[4,15] Briefly, after DNA extraction, the 16S rRNA variable regions (V4−V6) were amplified using polymerase chain reaction primers 515F and 1114R and sequenced with 300 bp paired-reads on the Illumina MiSeq (Illumina, Inc, San Diego, CA). Sample duplicates in 10% of the samples were performed, and negative controls were included for quality control. Operational taxonomic unit (OTU) counts were generated using the Illumina BaseSpace 16S Metagenomics App (version 1.01) for sequencing data from urine and vaginal samples. The pipeline classified OTUs to the genus level using the Ribosomal Database Project Classifier[16] and a curated version of the Greengenes database.

Statistical analyses

Clinical and demographic differences between surgical responders and nonresponders were assessed using unadjusted general linear and logistic models and Wilcoxon rank-sum tests implemented in Statistical Analysis System software (version 9.4; SAS Institute Inc, Cary, NC). OTU count data were normalized using total sum scaling to calculate relative abundances. To evaluate beta diversity differences associated with clinical and demographic characteristics and surgical treatment responses, Bray-Curtis dissimilarity metrics were calculated for urinary and vaginal sequencing data and analyzed using permutational multivariate analysis of variance (ANOVA).[17,18] These analyses were implemented in R (version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria) using the vegdist and adonis functions in the vegan package (version 2.5-6).[19] Beta diversity was examined for each clinical and demographic variable independently. Multivariate models were used to identify covariates to be included in subsequent differential abundance analyses.

The primary differential abundance analysis focused on characterizing the most prevalent genera, defined as being the predominant OTU in urine or vaginal samples. For this analysis, only the OTUs present in 3 or more subjects were considered. The differential abundance of the predominant genera (n=6) was compared between treatment responders and nonresponders, using the normalized and arcsine square-root–transformed OTU data with unadjusted and adjusted generalized linear models (GLMs) implemented with MaAsLin[20] with a significance threshold of $P<.05$. The GLM implemented in MaAsLin was also used to evaluate the relationship between differential OTUs and participant characteristics selected as model covariates.

Moreover, the differential abundance analysis was expanded to include an analysis of less abundant OTUs, defined as the presence in at least 10% of the samples and a minimum abundance (normalized OTU count) of 0.0001. False discovery rate (FDR) adjustment was used as a multiple testing correction using the Benjamini-Hochberg procedure.[21] For discovery purposes, an FDR-corrected P value threshold of .10 was applied. Differential OTUs were annotated using Medical Subject Headings (https://www.nlm.nih.gov/mesh/meshhome.html, annotated files created 7-20).

An exploratory analysis was performed to characterize whether the relationships between genera and surgical treatment responses were more closely associated with the stress or urgency components of UI. The relative abundance of 4 OTUs of interest were compared among women who had persistent stress urinary incontinence (SUI), UUI, MUI, or none (defined as reporting only SUI, only UUI, both, or neither in the 12-month diary) using unadjusted and adjusted ANOVA and the Tukey Honest Significant Difference tests. All analyses and plots were performed in R (version 3.6.0) unless otherwise noted.

Results

Of 126 women with MUI who contributed urine and vaginal samples, 100 provided objective treatment response data, and 104 provided subjective response data at 12 months. Nearly all participants (93%) met the MCID criteria for the reduction from baseline UDI score at 12 months. Therefore, because of the small number of nonresponders, this binary subjective outcome was not analyzed further, and the change in UDI (Δ UDI) score was assessed as the subjective outcome.

Compared with responders (n=72), objective nonresponders (n=28) were older and more likely to be postmenopausal not on hormones. Other clinical and demographic variables are noted in Table 1. Mean UIEs at 12 months were higher in nonresponders by 2.9 incontinence episodes per day (95% confidence interval [CI], 2.0–3.8). Responders had a greater reduction in leaks from baseline with a mean difference of 2.2 incontinence episodes per day (95% CI, 0.8–3.6). Objective responders had greater improvements in UDI score at 12 months overall and by UI subtype (Table 2).

Significant differences in microbiome composition (beta diversity) were found in vaginal specimens concerning objective response, along with age and menopausal status (all $P<.05$). Significant differences in microbiome composition were found in urine specimens concerning age ($P=.001$) (Table 3). When age, menopausal status, and hormonal status were analyzed together, only age remained significant ($P<.05$). Therefore, only age was included in the adjusted models.

Six predominant OTUs, defined as the most abundant OTU in 3 or more samples, were identified, 4 of which were predominant in both urine and the vagina (*Lactobacillus*, *Prevotella*, *Gardnerella*, and *Streptococcus*) and 2 were predominant in urine only (*Escherichia* and *Tepidimonas*). There was no significant (all P-values $>.05$) association of urinary OTUs with objective and subjective (Δ UDI) surgical treatment responses (data not shown).

In the unadjusted models, both vaginal *Lactobacillus* and *Prevotella* were associated with objective treatment responses. After controlling for age, *Prevotella* remained the only predominant vaginal OTU associated with objective treatment responses, and no genus was associated with subjective responses (Table 4, Figure 1). A lower abundance of *Lactobacillus* was associated with older age, and *Lactobacillus* was inversely associated with *Prevotella* (Figure 2).

Differences in microbiome composition beyond the most predominant OTUs were investigated, hypothesizing that less abundant genera may also influence response to the treatment. None of the 274 less abundant OTUs present in urine were associated with surgical treatment responses (data not shown). However, 16 of the 133 less abundant vaginal OTUs were significantly associated with objective responses (FDR-corrected $P<.10$). Notably, 4 were significantly (FDR-corrected $P<.10$) associated with subjective treatment responses, and 2 (*Campylobacter* and *Facklamia*) were associated with both outcomes (Supplemental Tables 1 and 2). These less abundant OTUs were all inversely correlated with *Lactobacillus* (Figure 3) and were all increased in women with worse responses to treatment (eg, objective nonresponders).

The relationship between vaginal OTUs and incontinence resolution or persistence at 12 months based on incontinence subtypes (SUI, UUI, MUI), irrespective of responder status, was also explored. Because of small numbers, subjects with SUI-only persistence (n=3) were not included in the analysis. ANOVAs were performed comparing selected vaginal OTUs in those subjects with neither SUI nor UUI episodes (n=57) and those with MUI (n=11) and UUI only (n=26) at 12 months. No association between incontinence subtype persistence and vaginal *Lactobacillus* or *Prevotella* was seen (Supplemental Figure, Supplemental Table 3). Less abundant vaginal OTUs associated with both objective and subjective responses, *Campylobacter* and *Facklamia,* were more abundant in the persistent MUI and UUI-only groups compared with the group without SUI or UUI (Supplemental Table 3). However, these OTUs did not distinguish persistent MUI from UUI alone.

TABLE 1
Baseline characteristics of HMS-ESTEEM participants with 12-month objective response data

Variable	Category	Total (N=100)	Objective nonresponders (n=28)	Objective responders (n=72)	Unadjusted mean or median difference or odds ratio (95% CI)
Age (y)		53.5 (10.8)	58.5 (10.7)	51.6 (10.2)	6.9 (2.2–11.6)
Body mass index		32.3 (7.2)	34.2 (7.2)	31.6 (7.1)	2.6 (−0.6 to 5.8)
Race	White	71 (71.0)	16 (57.1)	55 (76.4)	Ref
	>1 race	1 (1.0)	1 (3.6)	0 (0.0)	—[a]
	Asian	2 (2.0)	1 (3.6)	1 (1.4)	3.4 (0.2–60.3)
	Black or African American	12 (12.0)	5 (17.9)	7 (9.7)	2.5 (0.7–8.9)
	American Indian or Alaskan Native	1 (1.0)	0 (0.0)	1 (1.4)	—[a]
	Other	13 (13.0)	5 (17.9)	8 (11.1)	2.1 (0.6–7.6)
Ethnicity	Not Hispanic or not Latina	72 (72.0)	18 (64.3)	54 (75.0)	Ref
	Hispanic or Latina	26 (26.0)	10 (35.7)	16 (22.2)	1.9 (0.7–4.9)
	Unknown or not Reported	2 (2.0)	0 (0.0)	2 (2.8)	—[a]
Smoking status	Never smoked	58 (58.0)	16 (57.1)	42 (58.3)	Ref
	Quit smoking ≥6 mo ago	29 (29.0)	9 (32.1)	20 (27.8)	1.2 (0.4–3.2)
	Quit smoking <6 mo ago	2 (2.0)	0 (0.0)	2 (2.8)	—[a]
	Currently smoking	11 (11.0)	3 (10.7)	8 (11.1)	1.0 (0.2–4.3)
Current UTI symptoms		2 (2.0)	0 (0.0)	2 (2.8)	—[a]
Vaginal deliveries, median (min–max)		2 (0–8)	2 (0–6)	2 (0–8)	0 (−1 to 1)
Menopause and hormone status	Premenopausal	31 (31.0)	4 (14.3)	27 (37.5)	Ref
	Postmenopausal with hormones	19 (19.0)	4 (14.3)	15 (20.8)	1.8 (0.4–8.4)
	Postmenopausal without hormones	33 (33.0)	16 (57.1)	17 (23.6)	6.4 (1.8–22.6)
	Not sure	17 (17.0)	4 (14.3)	13 (18.1)	2.1 (0.4–9.8)

Data are presented as mean (standard deviation) or number (percentage), unless otherwise indicated.

CI, confidence interval; *ESTEEM*, Effects of Surgical Treatment Enhanced With Exercise for Mixed Urinary Incontinence; *HMS*, Human Microbiome Study; *max*, maximum; *min*, minimum; *Ref*, reference; *UTI*, urinary tract infection.

[a] Could not be calculated because of small sample size.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

TABLE 2
Outcomes of HMS-ESTEEM participants with 12-month objective response data

Variable	Total (N=100)	Objective nonresponders (n=28)	Objective responders (n=72)	Unadjusted mean difference (95% CI)
Daily UIEs from a 3-d diary				
Total UIEs				
Baseline	5.2 (3.0)	5.7 (3.2)	5.0 (2.9)	0.7 (−0.7−2.1)
12 mo	1.0 (1.8)	3.1 (2.3)	0.2 (0.4)	2.9 (2.0−3.8)
Change from baseline to 12 mo	−4.2 (3.0)	−2.6 (3.2)	−4.8 (2.8)	2.2 (0.8−3.6)
Stress UIEs				
Baseline	2.3 (1.7)	2.1 (1.7)	2.4 (1.6)	−0.3 (−1.1 to 0.5)
12 mo	0.2 (0.7)	0.7 (1.2)	0.0 (0.1)	0.7 (0.2−1.1)
Change from baseline to 12 mo	−2.1 (1.8)	−1.3 (2.0)	−2.4 (1.6)	1.0 (0.2−1.9)
Urge UIEs				
Baseline	2.4 (2.4)	3.1 (2.7)	2.2 (2.3)	0.9 (−0.3 to 2.0)
12 mo	0.6 (1.4)	1.9 (2.0)	0.1 (0.4)	1.8 (1.0−2.6)
Change from baseline to 12 mo	−1.8 (2.3)	−1.1 (2.4)	−2.1 (2.3)	0.9 (−0.1 to 2.0)
Urogenital Distress Inventory				
Total score				
Baseline	179.2 (42.4)	181.3 (39.3)	178.4 (43.7)	2.9 (−15.5 to 21.2)
12 mo	46.3 (60.2)	118.0 (60.3)	19.5 (31.5)	98.5 (73.7−123.3)
Change from baseline to 12 mo	−132.9 (69.0)	−63.3 (55.3)	−159.0 (54.0)	95.6 (70.7−120.6)
Stress subscore				
Baseline	86.0 (17.4)	83.3 (18.5)	87.0 (17.1)	−3.7 (−11.9 to 4.5)
12 mo	19.5 (28.5)	48.8 (31.7)	8.6 (17.5)	40.2 (27.1−53.3)
Change from baseline to 12 mo	−66.5 (33.5)	−34.6 (33.6)	−78.5 (24.5)	43.9 (29.5−58.3)
Irritative subscore				
Baseline	66.9 (18.6)	72.0 (19.0)	65.0 (18.2)	7.0 (−1.6 to 15.5)
12 mo	20.0 (27.2)	53.3 (27.0)	7.6 (13.2)	45.7 (34.7−56.8)
Change from baseline to 12 mo	−46.9 (28.1)	−18.7 (24.7)	−57.5 (21.3)	38.8 (27.9−49.6)

Data are presented as mean (standard deviation), unless otherwise indicated.

CI, confidence interval; *ESTEEM*, Effects of Surgical Treatment Enhanced With Exercise for Mixed Urinary Incontinence; *HMS*, Human Microbiome Study; *UIE*, urinary incontinence episode.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

Comment

Principal findings

This planned secondary analysis of the ESTEEM trial characterized the urogenital microbiome of women undergoing a midurethral sling operation for MUI. A greater abundance of vaginal *Prevotella*, more commonly found in nonresponders, was associated with a lack of objective treatment response, even after adjusting for age. Less abundant vaginal OTUs were also associated with objective and subjective treatment responses. The urinary microbiome was not associated with surgical treatment responses.

Results

This study found that 2 predominant OTUs in the vagina, *Lactobacillus* and *Prevotella*, were associated with objective treatment responses. *Lactobacillus* was associated with women attaining a ≥70% improvement in UIEs, although this association did not remain after adjusting for age. A greater abundance of vaginal *Prevotella* was associated with women failing to meet this threshold of UIE improvement, and this relationship remained significant ($P<.05$) after adjusting for age. Interestingly, although a relationship existed between predominant vaginal OTUs and surgical treatment responses, a relationship between the urinary microbiome and responder status was not noted. Exploratory analyses also revealed a relationship between less abundant vaginal OTUs concerning

TABLE 3
Microbiome beta diversity analysis results (global association)

Variable	Urine		Vagina	
	R^2	*P* value	R^2	*P* value
Age	0.04	.001[a]	0.06	.001[a]
Body mass index	0.01	.15	0.01	.30
Race	0.01	.83	0.01	.67
Ethnicity	0.01	.80	0.01	.64
Current smoking	0.01	.10	0.01	.23
Current UTI symptoms	0.004	.96	0.01	.73
Ever pregnant	0.01	.46	0.01	.60
Menopause and hormone status	0.03	.08	0.05	.01[a]
Oral or patch hormone use	0.01	.72	0.01	.14
Vaginal cream or tablet hormone use	0.01	.76	0.01	.46
Any hormone use	0.01	.47	0.01	.19
Δ UDI at 12 mo	0.02	.09	0.02	.07
Objective response	0.01	.39	0.02	.03[a]

Beta diversity analyses were conducted using the permutational multivariate analysis of variance implemented in adonis with a significance threshold of $P<.05$. Results shown are from unadjusted analyses of 1 demographic or clinical variable at a time.

UDI, Urogenital Distress Inventory; *UTI*, urinary tract infection.

[a] Results met the significance threshold of $P<.05$.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

objective and subjective outcomes and persistent and recurrent UI symptoms at 12 months.

Clinical implications

Taken together, the study findings suggested a framework in which the vaginal microbiome may modulate surgical treatment responses following a mid-urethral sling operation.

Considerable evidence exists surrounding the protective nature of *Lactobacillus* in the stabilization of the vaginal environment. There is a greater abundance of *Lactobacillus* in well-estrogenized premenopausal women than in atrophic postmenopausal women. *Lactobacillus* plays a role in many lower urinary tract disorders, including urinary tract infections (UTIs), and in the protection from certain vaginal infections.[22,23] *Prevotella,* a gram-negative anaerobe, is a common vaginal commensal bacteria, but its

TABLE 4
Associations of predominant OTUs in the vagina with objective and subjective responses at 12 months

Vagina	Predominant in n (%) of samples	Objective response (27 nonresponders/70 responders)		Subjective response (n=101)	
		P value of an unadjusted model	*P* value of an age-adjusted model	*P* value of an unadjusted model	*P* value of an age-adjusted model
Lactobacillus	78 (63)	.04[a]	.25	.22	.69
Prevotella	11 (10)	.01[a]	.01[a]	.91	.99
Gardnerella	12 (11)	.97	.65	.22	.41
Streptococcus	6 (5)	.97	.26	.32	.92

For each sample, the predominant OTU was defined as the OTU with the highest relative abundance. This analysis included OTUs that were predominant in $\geq$3 samples in either the urine or vagina. *P* values were calculated using unadjusted and age-adjusted generalized linear models implemented with MaAsLin, testing for the association of normalized and transformed OTU data and each outcome. *Tepidimonas* was filtered from the vaginal OTU analysis because it did not meet relative abundance thresholds (present in at least 10% of the samples with a minimum abundance [normalized OTU count] of 0.0001).

OTU, operational taxonomic unit.

[a] Results met the significance threshold of $P<.05$.

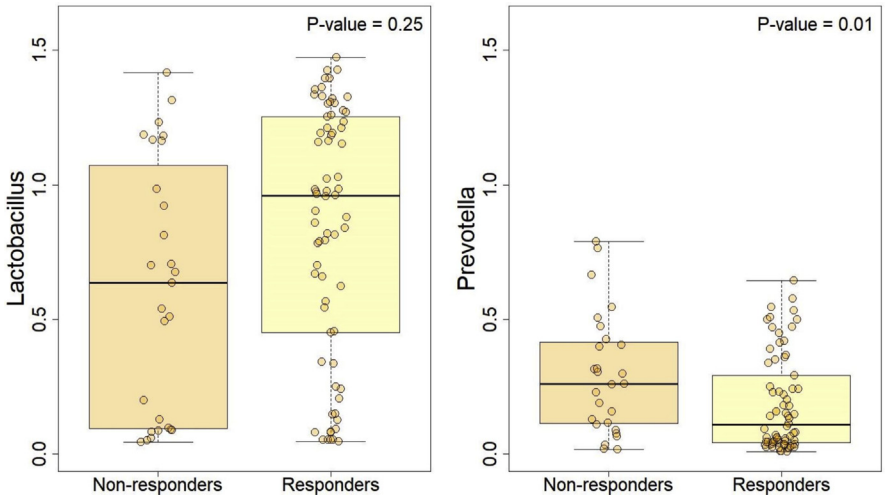
Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

association with bacterial vaginosis (especially *Prevotella bivia*) suggests a role in vaginal dysbiosis.[24] It is plausible that an increase in pathogenic bacteria (either predominant or less abundant genera) in women with MUI could create a more inflammatory milieu, modulating the response to surgical intervention for women with MUI similar to its impact on other genitourinary conditions, such as UTIs. A potential conceptual model is that, as age increases, the associated decrease in *Lactobacillus* (especially in the absence of estrogen replacement) results in dysbiosis associated with suboptimal response to surgical treatment. However, it is important to note that in this analysis, one cannot attribute treatment responses to a single genus or increased pathogenic genera as a whole. Further research is necessary to show whether modulation of the vaginal microbiome, either by probiotics or vaginal estrogen therapy, would be associated with improved surgical outcomes.[25]

Because the vaginal and urinary microbiota are related as previously demonstrated, one might presume that dysbiosis in the vagina would be reflected in the urinary microenvironment.[4–6] Differences were identified in the vaginal microbiome that were associated with surgical treatment responses, but associations were not identified in the urinary microbiome. The lack of a relationship between the urinary microbiome and responder status in the current study may be because of the relatively small numbers of nonresponders or the fact that the bacterial community in the urine is somewhat isolated from the connective tissue environment of the midurethral sling.

Moreover, it is possible that technical factors in urinary microbiome assessment in this study limited the sensitivity of our analysis to find associations between urinary microbiota and surgical treatment response. Compared with the vaginal environment, which is considered to be a "high biomass" environment, the urinary bladder is "low biomass" with fewer bacteria overall. With fewer bacteria, and the known variability among individual samples, it may be harder to discern statistical differences among groups when using low biomass samples.[26] Alternatively, it may be that our findings hold true and vaginal microbiota are associated with outcomes after midurethral sling, whereas urinary microbiota are not; however, these urinary results are a surrogate for a different interaction. It is possible that higher *Prevotella* exists in

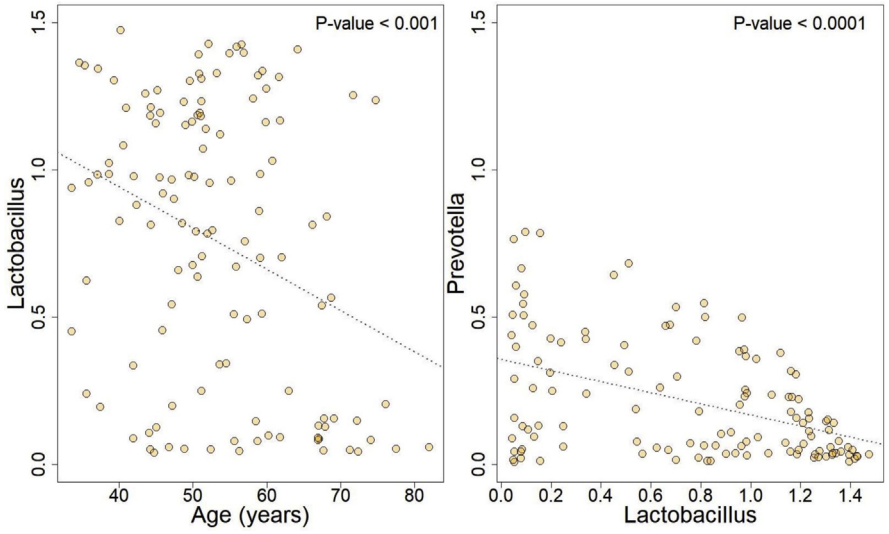
FIGURE 1
Associations of *Lactobacillus* and *Prevotella* with objective response at 12 months



P values were calculated using an age-adjusted generalized linear model testing for the association of normalized and transformed *Lactobacillus* and *Prevotella* counts and objective response (n=97).

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

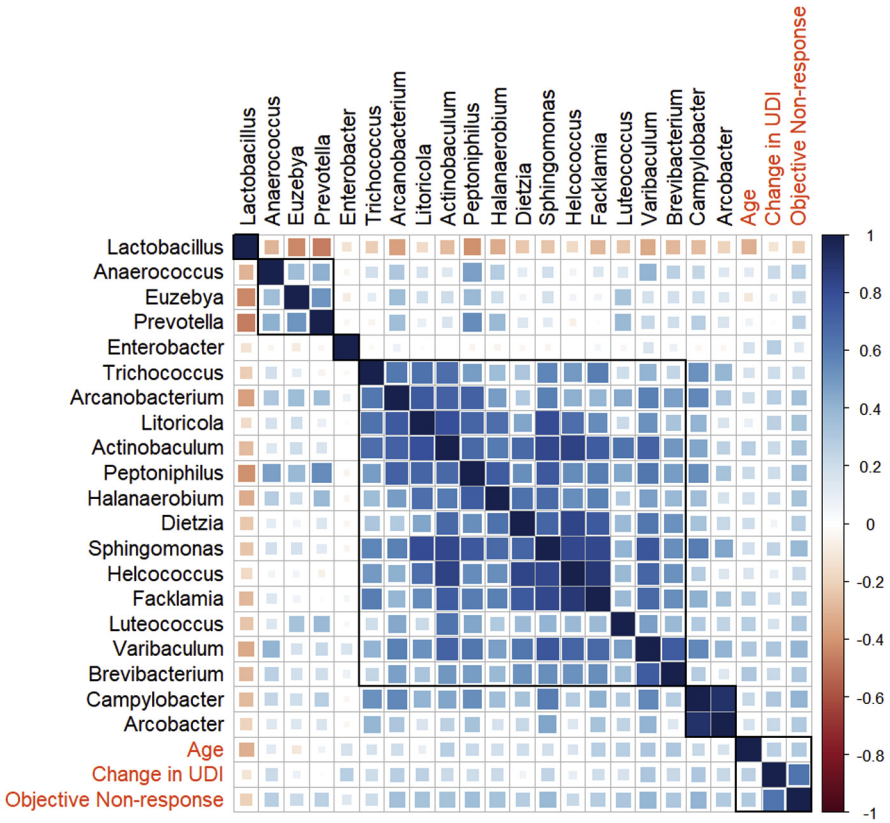
FIGURE 2
Association of vaginal *Lactobacillus* with age and vaginal *Prevotella*



P values were calculated using a generalized linear model testing for the association of normalized and transformed vaginal *Lactobacillus* operational taxonomic unit counts with age and with vaginal *Prevotella*.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

FIGURE 3
Correlation matrix of genera associated with response to treatment and age



The correlation matrix shows the relationship of the most significant genera associated with objective nonresponse and change in UDI in the vagina, including predominant (*Lactobacillus* and *Prevotella*) (Table 4) and less abundant (Supplemental Tables 1 and 2) OTUs. Moreover, the correlation matrix shows the negative correlation between *Lactobacillus* and other genera, the interrelatedness of the individual OTUs, and their correlation with age and response to treatment.

OTU, operational taxonomic unit; *UDI*, Urogenital Distress Inventory.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

the vagina in the setting of decreased *Lactobacillus*; in the bladder, a different anaerobic genus may increase when *Lactobacillus* is decreased. In both cases, there is a shift to a more anaerobic environment but with different genera predominant in the vagina and bladder. Larger studies using both expanded culture and genomic techniques may be required to sort out the differential relationship of urinary and vaginal microbiota concerning surgical responder status.

In exploratory, hypothesis-generating analyses, it was noted that several of the less abundant OTUs were related to objective treatment response (eg, *Sphingomonas*, *Campylobacter*, *Varibaculum*, *Arcobacter*, and others). Interestingly, 2 OTUs, *Campylobacter* and *Facklamia*, were increased in both objective and subjective nonresponders. *Campylobacter* is a gram-negative spiral zoonotic bacterium and a major cause of human gastroenteritis worldwide. It has also been found to cause infections in the reproductive and lower urinary tracts and is associated with pulmonary and brain infections.[27] *Facklamia* are gram-positive facultative anaerobic cocci that have been isolated from the vagina, urine, and cerebrospinal fluid. Although the genus may be a part of the normal flora in the female genital tract, it has been associated with the development of chorioamnionitis.[28,29] *Prevotella*, *Campylobacter*, and *Facklamia* have all been found in the urinary microbiome associated with bladder cancer.[30] As less abundant OTUs can suffer from sequencing biases in compositional data, these data should be interpreted with some caution despite using standard data normalization and transformation techniques.

Surgical treatment response decreased with age, as did *Lactobacillus* abundance. Whether *Lactobacillus* is a determinant or marker of improved surgical treatment response cannot be ascertained in this study because of its close association with age and because of the nature of the study that included only baseline OTU information. Similarly, it cannot be stated that *Prevotella,* or *Campylobacter* and *Facklamia* among the less abundant genera, are determinants of treatment nonresponse, only that they are noted to be associated with surgical treatment responses.

Research implications

There are abundant opportunities for continued evaluation of surgical outcomes associated with the genitourinary microbiome. Future work will be required to explore whether increasing *Lactobacillus* abundance with vaginal supplementation or manipulation will improve surgical outcomes and whether this would affect the abundance of OTUs associated with poor surgical outcomes. In addition, with advancements in microbiome research, future studies will likely include repeated analyses, newer databases, and newer bioinformatic approaches, which could yield further insight into these publicly available files.

Strengths and limitations

The study strengths included the use of well-established standardized genetic assessment methodology to a novel population looking at clinically important outcomes. This study population was well characterized with validated questionnaires to define subjective responder status and with bladder diaries to characterize objective responder status. A standardized protocol was utilized across sites for the collection of

samples and processing performed at 1 core laboratory, reducing technical artifacts. Study weaknesses included its relatively smaller subset of women recruited from its larger main trial as all participants were asked to participate until the sample size of this study was achieved.[4,5] However, we maximized our ability to find associations by looking at subjective responses to treatment as a continuous trait for both the urine and vaginal samples. The study was limited by the utilization of the retired Greengenes database; however, this analysis was a standard approach at trial design and was applied to all samples, limiting bias in the comparison of surgical responders and nonresponders. In addition, analyses of rare OTUs can be biased because of differences in sampling, so caution should be used when interpreting the association of specific, less abundant OTUs and these results should be considered exploratory. Finally, the definition of the objective outcome precludes a definitive conclusion regarding which component of MUI is responsible for nonresponder status. The attributes of the study population are of value to the research community with sequencing files that are now publicly available at https://www.ncbi.nim.nih.gov/bioproject/PRJNA703967/.

Conclusions

The genera *Lactobacillus* and *Prevotella* were associated with objective treatment responses, and after controlling for age, 1 predominant OTU (*Prevotella*) and other less abundant OTUs were associated with surgical treatment nonresponses in women undergoing a midurethral sling operation for MUI. Further research is needed to corroborate the current findings and to further explore potential clinical applications in the setting of a surgical procedure for pelvic floor disorders.

Take-Home Message

In women with MUI, urinary microbiota were not associated with outcomes after a midurethral sling operation. However, vaginal microbiota at the time of the surgery were associated with subjective and objective treatment responses 1 year later. Those with greater vaginal *Prevotella*, which was correlated with decreased *Lactobacillus*, had less reduction of incontinence. Research is needed to determine whether altering the vaginal microbiome affects surgical outcomes. ■

Acknowledgments

The authors would like to thank the research coordinators and all those who made this work possible, including the following:

- The University of Alabama at Birmingham: R. Edward Varner, MD; Robert L. Holley, MD; David R. Ellington, MD; Isuzu Meyer, MD; Alison Parden, MD; Alicia Ballard, MD; Velria Willis, BSN; Nancy Saxon, BSN; and Kathy Carter, BSN.
- Brown University: Deborah Myers, MD; Charles Rardin, MD; Brittany Star Hampton, MD; Cassandra Carberry, MD; Kyle Wohlrab, MD; Ann Meers, BSN; and Katheryn Rhodes, BA.
- The Cleveland Clinic Foundation: Matthew Barber, MD; Marie Paraiso, MD; Eric Jelovsek, MD; Cecile Unger, MD; Audra Hill, MD; Ly Pung, RN; Kathleen Dastoli, RN; Annette Graham, RN; and Maryori Edington.
- Duke University: Cindy Amundsen, MD; Anthony Visco, MD; Alison Weidner, MD; Amie Kawasaki, MD; Shantae McLean; Nicole Longoria; and Akira Hayes.
- University of California, San Diego: Marianna Alperin, MD; Michael Albo, MD; Laura Aughinbaugh, RNP; Joann Columbo; Cindy Furey, PT; Sherella Johnson; Charles Nager, MD; and Patsy Riley, RN.
- Kaiser Permanente San Diego: Shawn Menefee, MD; Jasmine Tan-Kim, MD; Keisha Dyer, MD; Gouri Diwadkar, MD; Karl Luber, MD; Lynn Hall, RNP; Gisselle Zazueta-Damian; and Linda MacKinnon.
- Kaiser Permanente Bellflower: John N. Nguyen, MD; Sharon Jakus-Walkman, MD; Azadeh Rezvan, MD; Christina Liao, MD; Arty Patel, PT; Mary Simmons, PT; Mercedes Cardona; and Nancy Flores.
- The University of New Mexico: Gena Dunivan, MD; Peter Jeppson, MD; Sara Cichowski, MD; Karen Taylor, BA; Cassandra Castaneda, BA; Julia Middendorf, BSN; Susan Tigert, BA, BS; Kurt Schwalm, BS; and Amy Overby, BS, CG, MB, PA(ASCP)CM.
- University of Pennsylvania: Heidi Harvie, MD; Uduak Andy, MD; Lorraine Flick; and Michelle Kinglee.
- University of Pittsburgh: Pamela Moalli, PhD, MD; Michael Bonidie; Gary Sutkin, MD; Jonathan Shepherd, MD; Christopher Chermansky, MD; Judy Gruss; Karen Mislanovich; and Lori Geraci.
- RTI International: Dennis Wallace, PhD; Carolyn Huitema, MS; and Michael Ham, BS.

References

1. 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. e01283–14.
2. Thomas-White KJ, Hilt EE, Fok C, et al. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J 2016;27:723–33.
3. Brubaker L, Nager CW, Richter HE, et al. Urinary bacteria in adult women with urgency urinary incontinence. Int Urogynecol J 2014;25:1179–84.
4. Komesu YM, Richter HE, Carper B, et al. The urinary microbiome in women with mixed urinary incontinence compared to similarly aged controls. Int Urogynecol J 2018;29:1785–95.
5. Komesu YM, Dinwiddie DL, Richter HE, et al. Defining the relationship between vaginal and urinary microbiomes. Am J Obstet Gynecol 2020;222:154.e1–10.
6. Thomas-White K, Forster SC, Kumar N, et al. Culturing of female bladder bacteria reveals an interconnected urogenital microbiota. Nat Commun 2018;9:1557.
7. Lewis AL, Gilbert NM. Roles of the vagina and the vaginal microbiota in urinary tract infection: evidence from clinical correlations and experimental models. GMS Infect Dis 2020;8:Doc02.
8. Schmitt FCF, Brenner T, Uhle F, et al. Gut microbiome patterns correlate with higher postoperative complication rates after pancreatic surgery. BMC Microbiol 2019;19:42.
9. Gaines S, Shao C, Hyman N, Alverdy JC. Gut microbiome influences on anastomotic leak and recurrence rates following colorectal cancer surgery. Br J Surg 2018;105:e131–41.
10. Kinross JM, Markar S, Karthikesalingam A, et al. A meta-analysis of probiotic and synbiotic use in elective surgery: does nutrition modulation of the gut microbiome improve clinical outcome? JPEN J Parenter Enteral Nutr 2013;37:243–53.

11. Sung VW, Borello-France D, Newman DK, et al. Effect of behavioral and pelvic floor muscle therapy combined with surgery vs surgery alone on incontinence symptoms among women with mixed urinary incontinence: the ESTEEM randomized clinical trial. JAMA 2019;322:1066–76.
12. Sung VW, Borello-France D, Dunivan G, et al. Methods for a multicenter randomized trial for mixed urinary incontinence: rationale and patient-centeredness of the ESTEEM trial. Int Urogynecol J 2016;27:1479–90.
13. Shumaker SA, Wyman JF, Uebersax JS, McClish D, Fantl JA. Health-related quality of life measures for women with urinary incontinence: the Incontinence Impact Questionnaire and the urogenital Distress Inventory. Continence Program in Women (CPW) Research Group. Qual Life Res 1994;3:291–306.
14. Sung VW, Richter HE, Moalli P, et al. Characteristics associated with treatment failure 1 year after midurethral sling in women with mixed urinary incontinence. Obstet Gynecol 2020;136:482–91.
15. Komesu YM, Richter HE, Dinwiddie DL, et al. Methodology for a vaginal and urinary microbiome study in women with mixed urinary incontinence. Int Urogynecol J 2017;28:711–20.
16. 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;73:5261–7.
17. Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 1957;27:325–49.
18. Warton DI, Wright ST, Wang Y. Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol Evol 2012;3:89–101.
19. Oksanen J, Guillaume Blanchet F, Friendly M, et al. vegan: community Ecology Package. R package version 2.5-6. 2019. Available at: https://CRAN.R-project.org/package=vegan. Accessed January 29, 2020.
20. Morgan XC, Tickle TL, Sokol H, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol 2012;13:R79.
21. Benjamini Y, Hochberg Y. Controlling the false delivery rate: a practical and powerful approach to multiple testing. JR Stat Soc B 1995;57:289–300.
22. Gliniewicz K, Schneider GM, Ridenhour BJ, et al. Comparison of the vaginal microbiomes of premenopausal and postmenopausal women. Front Microbiol 2019;10:193.
23. Govender Y, Gabriel I, Minassian V, Fichorova R. The current evidence on the association Between the urinary microbiome and urinary incontinence in women. Front Cell Infect Microbiol 2019;9:133.
24. Randis TM, Ratner AJ. Gardnerella and Prevotella: co-conspirators in the pathogenesis of bacterial vaginosis. J Infect Dis 2019;220:1085–8.
25. Thomas-White K, Taege S, Limeira R, et al. Vaginal estrogen therapy is associated with increased Lactobacillus in the urine of postmenopausal women with overactive bladder symptoms. Am J Obstet Gynecol 2020;223:727.e1–11.
26. Karstens L, Asquith M, Caruso V, et al. Community profiling of the urinary microbiota: considerations for low-biomass samples. Nat Rev Urol 2018;15:735–49.
27. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of Campylobacter infection. Clin Microbiol Rev 2015;28:687–720.
28. Parvataneni KC, Iyer S, Khatib R, Saravolatz LD. Facklamia species and Streptococcus pneumoniae meningitis: a case report and review of the literature. Open Forum Infect Dis 2015;2:ofv029.
29. LaClaire L, Facklam R. Antimicrobial susceptibilities and clinical sources of Facklamia species. Antimicrob Agents Chemother 2000;44:2130–2.
30. Popovic VB, Situm M, Chow CET, et al. The urinary microbiome associated with bladder cancer. Nat Sci Rep 2018;8:12157.

Author and article information

From the Department of Obstetrics and Gynecology, The University of Alabama at Birmingham, Birmingham, AL (Dr Richter); Biostatistics and Epidemiology Division, RTI International, Research Triangle Park, NC (Dr Carnes, Mr Carper and Dr Gantz); Department of Obstetrics and Gynecology, University of New Mexico Health Sciences Center, Albuquerque, NM (Dr Komesu); Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Diego, San Diego, CA (Dr Lukacz); Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA (Dr Arya); Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Pittsburgh, PA (Dr Bradley); Department of Obstetrics and Gynecology, Dell Medical School, The University of Texas at Austin, Austin, TX (Dr Rogers); Department of Obstetrics and Gynecology, Alpert Medical School of Brown University, Providence, RI (Dr Sung); Department of Obstetrics and Gynecology, Duke University, Durham, NC (Dr Siddiqui); Gynecologic Health and Disease Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD (Dr Mazloomdoost); Department of Pediatrics, University of New Mexico Health Sciences Center, Albuquerque, NM (Dr Dinwiddie); and Clinical Translational Science Center, University of New Mexico Health Sciences, Albuquerque, NM (Dr Dinwiddie).

Received April 27, 2021; revised July 6, 2021; accepted July 14, 2021.

H.E.R. received research funding from Renovia, Allergan, the National Institute on Aging, and the NICHD; received royalties from UptoDate; received travel reimbursement related to editor duties from the *American Journal of Obstetrics & Gynecology* (AJOG) and the *International Urogynecology Journal* (IUJ); is a board member of the Worldwide Fistula Fund; and is a member for the Data and Safety Monitoring Board, BlueWind, and Symposia Medicus CME speaker. Y.M.K. received research funding from Cook Myosite. R.G.G. received royalties from UpToDate, the American Board of Obstetrics and Gynecology, the IUJ, the AJOG, and the American College of Obstetricians and Gynecologists. E.S.L. received research funding from Axonics, Boston Scientific, and Cogentix/Uroplasty; received royalties from UptoDate; and is an advisory board member of Pathnostics and Urovant. N.Y.S. received a research grant from Medtronic, royalties from UptoDate, and grant number K23-DK110417 from the National Institute of Diabetes and Digestive and Kidney Diseases).

This study was funded by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD; grant numbers HD04121, HD06931, FP1810/3RG40, HD054214, HD069010, HD069006, HD06913, and HD041267) and the National Institutes of Health Office of Research on Women's Health. M.U.C., B.C., and M.G.G. are directly responsible for data integrity and analysis fidelity.

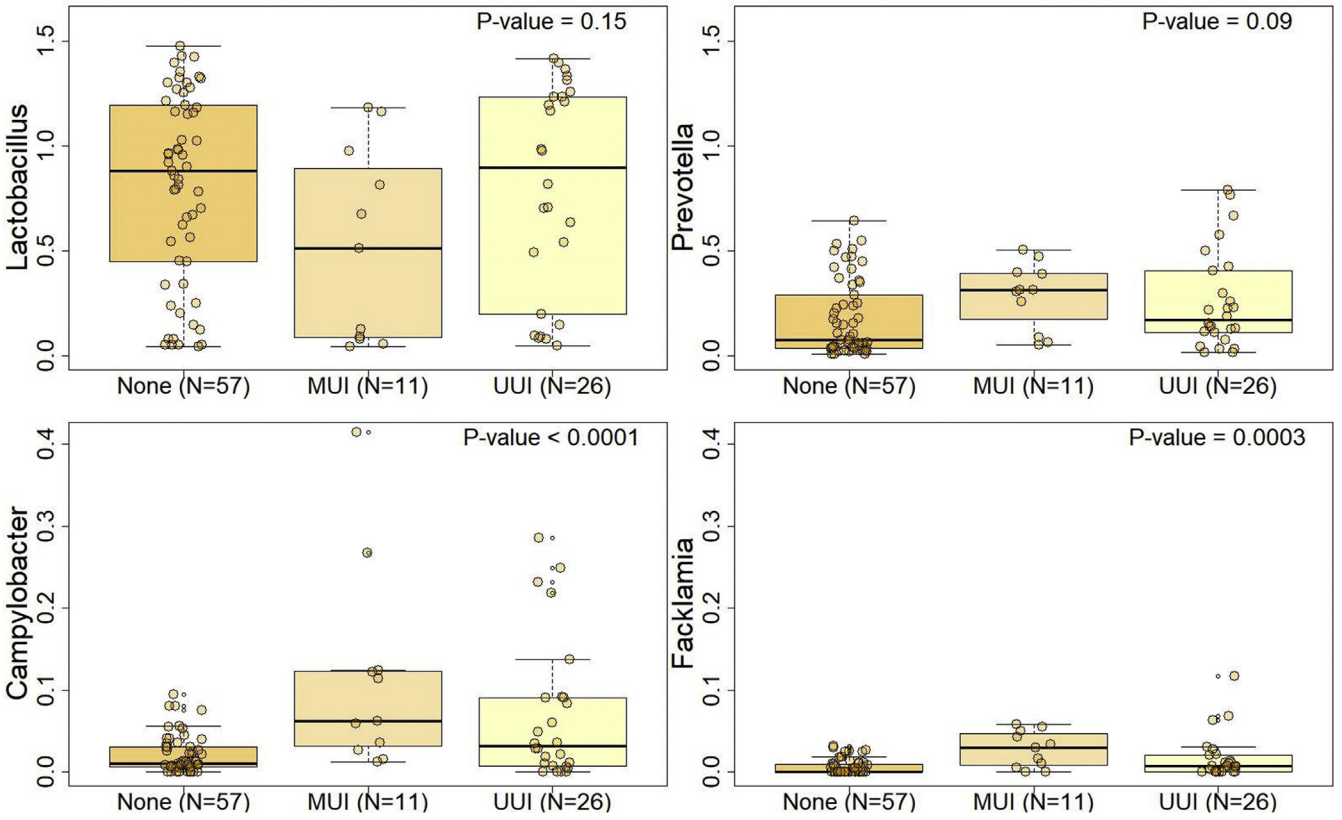
This study is registered on ClinicalTrials.gov (NCT01959347; available at http://www.clinical trials).

This study was presented at the annual virtual meeting of the American Urogynecologic Society, October 6—9, 2020, and at the virtual meeting of the International Continence Society, November 19—22, 2020, receiving the "Best Presentation Award."

Corresponding author: Holly E. Richter, PhD, MD. hrichter@uabmc.edu

SUPPLEMENTAL FIGURE

Associations of vaginal OTUs with persistent mixed or urgency incontinence on 3-day diary at 12 months



The plot shows normalized and transformed OTU data by UI subtype, comparing persistent MUI and UUI to resolved UI. Subytpes were defined using 3-day diary data on UI episodes at 12 months. *P* values are from age-adjusted analysis of variance (Supplemental Table 3).

MUI, mixed urinary incontinence; *OTU*, operational taxonomic unit; *UI*, urinary incontinence; *UUI*, urgency urinary incontinence.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

SUPPLEMENTAL TABLE 1

Associations of vaginal OTUs with objective responses (27 nonresponders/70 responders)

OTU	*P* value (FDR-corrected) unadjusted model	*P* value (FDR-corrected) age-adjusted model	MeSH scope note
Sphingomonas[a]	.0003	.006	A genus of gram-negative, aerobic, rod-shaped bacteria characterized by an outer membrane that contains glycosphingolipids but lacks lipopolysaccharide. They have the ability to degrade a broad range of substituted aromatic compounds.
Campylobacter[a]	.0003	.007	A genus of bacteria found in the reproductive organs, intestinal tract, and oral cavity of animals and humans. Some species are pathogenic.
Varibaculum[a]	.0003	.009	—
Arcobacter[a]	.0005	.009	A genus of gram-negative, aerotolerant, spiral-shaped bacteria isolated from water and associated with diarrhea in humans and animals.
Luteococcus[a]	.0004	.009	—
Litoricola[a]	.008	.02	—
Actinobaculum[a]	.001	.03	—
Peptoniphilus[a]	.007	.05	—
Halanaerobium[a]	.01	.05	—
Anaerococcus[a]	.008	.05	—
Helcococcus[a]	.008	.05	—
Arcanobacterium[a]	.008	.05	A genus of facultatively anaerobic, gram-positive bacteria in the family *Actinomycetaceae*, order *Actinomycetales*. They are obligate parasites of the pharynx in humans and farm animals.
Facklamia[a]	.008	.08	—
Dietzia[a]	.02	.08	—
Euzebya[a]	.09	.08	—
Brevibacterium[a]	.01	.09	A gram-positive organism found in dairy products, fresh and salt waters, marine organisms, insects, and decaying organic matter.
Macrococcus	.26	.11	—
Corynebacterium	.02	.12	A genus of the asporogenous bacteria that is widely distributed in nature. Its organisms appear as straight to slightly curved rods and are known to be human and animal parasites and pathogens.
Desulfovibrio	.06	.12	A genus of gram-negative, anaerobic, rod-shaped bacteria capable of reducing sulfur compounds to hydrogen sulfide. Organisms are isolated from anaerobic mud of fresh and salt water, animal intestines, manure, and feces.
Trichococcus	.06	.12	—
Negativicoccus	.09	.12	—

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021. *(continued)*

SUPPLEMENTAL TABLE 1

Associations of vaginal OTUs with objective responses (27 nonresponders/70 responders) *(continued)*

OTU	*P* value (FDR-corrected) unadjusted model	*P* value (FDR-corrected) age-adjusted model	MeSH scope note
Porphyromonas	.04	.14	A genus of gram-negative, anaerobic, nonsporeforming, nonmotile rods or coccobacilli. Organisms in this genus had originally been classified as members of the *Bacteroides* genus but overwhelming biochemical and chemical findings indicated the need to separate them from other Bacteroides species, and hence, this new genus was created.
Enterococcus	.03	.16	A genus of gram-positive, coccoid bacteria consisting of organisms causing variable hemolysis that are normal flora of the intestinal tract. Previously thought to be a member of the genus *Streptococcus*, it is now recognized as a separate genus.
Actinopolymorpha	.05	.22	—
Brachybacterium	.13	.24	—
Saccharopolyspora	.15	.25	A genus of gram-positive bacteria whose spores are round to oval and covered by a sheath.

FDR-corrected *P* values were calculated using unadjusted and age-adjusted generalized linear models implemented with MaAsLin, testing for the association of normalized and transformed vaginal OTU data and objective response. Results shown include any OTU with a corrected *P* value of $<.25$ in the age-adjusted model. MeSH scope notes in blanks indicate no known information for these genera.

FDR, false discovery rate; *MeSH*, Medical Subject Headings; *OTU*, operational taxonomic unit.

[a] OTUs with an FDR-corrected $P<.10$. All significant OTUs had increased relative abundances in nonresponders compared with responders.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

SUPPLEMENTAL TABLE 2

Associations of vaginal OTUs with subjective responses (Δ UDI)

Vaginal OTU	*P* value (FDR-corrected) unadjusted model	*P* value (FDR-corrected) age-adjusted model	MeSH scope note
Campylobacter[a]	.03	.06	A genus of bacteria found in the reproductive organs, intestinal tract, and oral cavity of animals and humans. Some species are pathogenic.
Facklamia[a]	.03	.08	—
Enterobacter[a]	.04	.08	Gram-negative gas-producing rods found in feces of humans and other animals, sewage, soil, water, and dairy products.
Trichococcus[a]	.06	.08	—
Arcobacter	.04	.10	—
Enterococcus	.04	.10	A genus of gram-positive, coccoid bacteria consisting of organisms causing variable hemolysis that are normal flora of the intestinal tract. Previously thought to be a member of the genus *Streptococcus*, it is now recognized as a separate genus.
Tolumonas	.06	.10	—
Varibaculum	.04	.10	—
Sphingomonas	.06	.10	A genus of gram-negative, aerobic, rod-shaped bacteria characterized by an outer membrane that contains glycosphingolipids but lacks lipopolysaccharide. They have the ability to degrade a broad range of substituted aromatic compounds.
Litoricola	.10	.11	—
Peptostreptococcus	.10	.11	A genus of gram-positive, anaerobic, coccoid bacteria that is part of the normal flora of humans. Its organisms are opportunistic pathogens causing bacteremias and soft tissue infections.
Nesterenkonia	.10	.14	—
Veillonella	.24	.17	A genus of gram-negative, anaerobic cocci parasitic in the mouth and intestinal and respiratory tracts of humans and other animals.
Saccharopolyspora	.12	.17	A genus of gram-positive bacteria whose spores are round to oval and covered by a sheath.
Brevibacterium	.06	.20	A gram-positive organism found in dairy products, fresh and salt waters, marine organisms, insects, and decaying organic matter.
Luteococcus	.06	.21	—
Propionispora	.14	.24	—

FDR-corrected *P* values were calculated using unadjusted and age-adjusted generalized linear models implemented with MaAsLin, testing for the association of normalized and transformed vaginal OTU data and Δ UDI. Results shown include any OTU with a corrected *P* value of <.25 in the age-adjusted model. MeSH scope notes in blanks indicate no known information for these genera.

FDR, false discovery rate; *MeSH*, Medical Subject Headings; *OTU*, operational taxonomic unit; *UDI*, Urogenital Distress Inventory.

[a] OTUs with an FDR-corrected *P*<.10. All significant OTUs had increased abundances with a higher Δ UDI (lesser reduction in UDI indicating worse response).

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.

SUPPLEMENTAL TABLE 3
Association with vaginal OTUs and UI subtypes

Vaginal OTU	*P* value of the unadjusted model	*P* value of the age-adjusted model	UI subtypes	Tukey HSD *P* value
Lactobacillus	.18	.15		
			MUI vs none	.17
			UUI vs none	1.00
			MUI vs UUI	.23
Prevotella	.09	.09		
			MUI vs none	.20
			UUI vs none	.19
			MUI vs UUI	.90
Campylobacter	1.54E-05[a]	1.30E-05[a]		
			MUI vs none	6.67E-05[a]
			UUI vs none	.005[a]
			MUI vs UUI	.12
Facklamia	.0004[a]	.0003[a]		
			MUI vs none	.001[a]
			UUI vs none	.03[a]
			MUI vs UUI	0.22

Results shown are from unadjusted and age-adjusted analyses of variance comparing UI subtypes with normalized and transformed vaginal OTU data. The Tukey HSD results are for pairwise comparisons of subtype means. MUI, UUI, and none categories were defined as reporting both stress and urgency UI episodes, only urgency UI episodes, or no UI episodes, respectively, on 3-day diaries at 12 months.

HSD, honest significant difference; *MUI*, mixed urinary incontinence; *OTU*, operational taxonomic unit; *UI*, urinary incontinence; *UUI*, urgency urinary incontinence.

[a] Results that met the significance threshold of $P<.05$.

Richter et al. Urogenital microbiome and surgical treatment response. Am J Obstet Gynecol 2021.