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Association Between the Urogenital Microbiome and Surgical Treatment Response in Women Undergoing Mid-urethral Sling Surgery for Mixed Urinary Incontinence

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Trial Registry: http://www.clinical **trials.gov** (NCT01959347)

Abstract

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

Objective: To describe whether the preoperative urinary and vaginal microbiomes were associated with treatment response 12-months after mid-urethral sling surgery 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 mid-urethral sling surgery in the ESTEEM (Effects of Surgical Treatment Enhanced with Exercise for Mixed Urinary Incontinence) trial (NCT01959347) and compared the microbiota in women who were surgical responders versus surgical non-responders. Twelve-month objective response was defined as a ≥70% reduction from baseline urinary incontinence episodes on three-day diary. Subjective response was defined as change from baseline in Urogenital Distress Inventory scores. Bacterial abundance and beta diversity were assessed using 16S rRNA (ribosomal ribonucleic acid) sequencing. The primary differential abundance analysis described predominant bacterial operational taxonomic units associated with responders vs. non-responders using unadjusted and age-adjusted linear models.

Results: Objective non-responders (N=28) compared to responders (N=72) were older (58.5±10.7 vs 51.6±10.2 years) and more likely post-menopausal without hormone use (Odds Ratio [95% Confidence Interval] 6.4 [1.8–22.6]). Vaginal and urinary microbiota beta diversity was associated with age ($p<0.05$) for both responders and non-responders. Overall predominant operational taxonomic units (genera) were: *Lactobacillus*, *Gardnerella*, *Tepidomonas*, *Escherichia*, *Streptococcus*, and *Prevotella*. Operational taxonomic units from baseline urine samples were not significantly associated with treatment response. A greater abundance of baseline vaginal *Lactobacillus* was associated with objective response($p=0.04$) *and Prevotella* with objective non-response ($p=0.01$). Adjusting for age, only a greater abundance of baseline vaginal *Prevotella* was associated with objective non-response ($p=0.01$). Less abundant vaginal operational taxonomic units were also associated with objective and subjective responses and persistent urinary incontinence symptoms ($p<0.05$).

Conclusions: Women meeting a 70% reduction of urinary incontinence treatment episodes (objective responders) had greater vaginal *Lactobacillus* at the time of surgery, however controlling for age diminished this association. Women not meeting a 70% reduction of urinary incontinence episodes one year after mid-urethral sling surgery had greater vaginal *Prevotella* at the time of mid-urethral sling surgery. Further research is needed to determine whether therapy altering the vaginal microbiome may impact surgical treatment response in women with mixed urinary incontinence.

Condensation:

At the time of surgery, the vaginal microbiome was associated with the success of mid-urethral sling in women with combined stress and urgency urinary incontinence.

Keywords

urinary microbiome; vaginal microbiome; mid-urethral sling procedure; mixed urinary incontinence; surgical outcomes

Introduction

The urogenital microbiome is associated with lower urinary tract health, although the relationship is complicated. While some investigators have found that urgency urinary incontinence (UUI) symptoms are associated with the urinary microbiome,[1,2,3] 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* (≤61% of genera) was associated with MUI while a higher proportion of controls had *High-Lactobacillus* communities (89% *Lactobacillus*).[4]

We recently demonstrated that the composition of the urinary microbiome is correlated with the vaginal microbiome in women with MUI.[5] This complements prior genome-level work showing that vaginal and bladder microbiota are functionally distinct from gastrointestinal microbiota, but similar to each other.[6] The inter-relatedness of the vaginal and urinary microbiomes may offer unique opportunities to optimize lower urinary tract health and treatment outcomes.[5,6,7] For example, vaginal therapies could have beneficial downstream effects on the urinary microenvironment and treatment outcomes. Alternatively, just as differences in the urinary microenvironment are associated with differential treatment response to medication for UUI,[2] the vaginal microenvironment at the time of mid-urethral sling surgery in women with MUI could be related to differential treatment outcomes. This is plausible given that surgical outcomes following pancreatic and colon surgery may be associated with specific gut microbiome compositions and targeting of the gut microbiome has been suggested as a means of improving surgical outcome. [8,9,10]

This is a planned secondary analysis in a subset of women participating in the Effect of Behavioral and Pelvic Floor Muscle Therapy Combined with Surgery versus Surgery Alone on Incontinence Symptoms Among Women With Mixed Urinary Incontinence (ESTEEM) Trial (NCT01959347).[11] The objective of this study was to describe pre-operative urinary and vaginal bacterial taxa associated with 12-month responses after mid-urethral sling surgery in women with MUI. It was hypothesized that relative abundance of *Lactobacillus* among preoperative urinary or vaginal microbiota would be associated with surgical treatment response.

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 (126/480) with MUI enrolled in the ESTEEM trial at one time-point, prior to surgery. As previously described, ESTEEM participants were randomized in a 1:1 ratio to either mid-urethral sling surgery or mid-urethral sling plus perioperative behavioral and pelvic floor muscle therapy.[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 three-day bladder diary characterizing incontinence episodes. Two subjective measures and one objective measure of response were evaluated. The binary subjective response was defined as a reduction in UDI score from baseline to 12 months meeting the minimal clinically important difference (MCID) threshold (−26.1 points).[12] The second subjective measure was change from baseline in UDI score (ΔUDI) at 12 months. For participants who underwent additional urinary incontinence treatment prior to 12 months, ΔUDI was determined using the participant's UDI score from the last questionnaire completed before initiation of additional treatment. Binary objective response to treatment was defined as ≥70% reduction from baseline in urinary incontinence episodes on the three-day diary at 12 months, as well as no additional treatment, as previously described.[14]

Sample processing

Sample processing has previously been described.[4,15] Briefly, after DNA (Deoxyribonucleic Acid) extraction, the 16S rRNA variable region 4-6 was amplified using PCR (Polymerase Chain Reaction) primers 515F and 1114R and sequenced with 300 bp paired-reads on the Illumina MiSeq®. 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 (v1.01) for sequencing data from urine and vaginal samples. The pipeline classified OTUs to the genus level using the Ribosomal Database Project (RDP) Classifier,[16] and a curated version of the Greengenes database.

Statistical analyses

Clinical and demographic differences between surgical responders and non-responders were assessed using unadjusted general linear and logistic models and Wilcoxon rank-sum tests implemented in SAS statistical software version 9.4 (SAS Institute, Inc., Cary, North Carolina). 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 treatment response, Bray-Curtis dissimilarity metrics were calculated for urinary and vaginal sequencing data and analyzed using permutational multivariate analysis of variance.[17,18] These analyses were implemented in R (v3.6.0) using the vegdist and adonis functions in the vegan package (v2.5-6).[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 three or more subjects were considered. Differential abundance of predominant genera (N=6) were compared between treatment responders and non-responders, using the normalized and arcsine square-root transformed OTU data with unadjusted and adjusted generalized linear models (GLM) implemented with MaAslin[20] with a significance threshold of $p\text{-value} < 0.05$. GLM implemented in MaAslin was also used to evaluate the relationship between differential OTUs and participant characteristics selected as model covariates.

The differential abundance analysis was then expanded to include an analysis of less abundant OTUs, defined as 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 0.10 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 treatment response were more closely associated with the stress or urgency components of UI. The relative abundance of four OTUs of interest were compared between woman who had persistent stress urinary incontinence (SUI), UUI, MUI, or none (defined as reporting only SUI, only UUI, both, or neither on the 12-month diary) using unadjusted and adjusted analysis of variance (ANOVA) and Tukey’s HSD (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, N=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, due to the small number of non-responders, this binary subjective outcome was not analyzed further and change in UDI (ΔUDI) score was assessed as the subjective outcome.

Objective non-responders (N=28), compared to responders (N=72), were older and more likely to be post-menopausal not on hormones. Other clinical and demographic variables are noted in Table 1. Mean UI episodes at 12 months were higher in non-responders by 2.9 incontinence episodes per day (95% CI 2.0 to 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 to 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 with respect to objective response, along with age and menopausal status (all p<0.05). Significant differences in microbiome composition were found in urine specimens with respect to age (p=0.001; Table 3). When age, menopausal status and hormonal status were analyzed together, only age remained significant. Therefore, only age was included in adjusted models.

Six predominant OTUs, defined as the most abundant OTU in 3 or more samples, were identified, four of which were predominant in both urine and vagina *(Lactobacillus, Prevotella, Gardnerella* and *Streptococcus)* and two were predominant in only urine

(Escherichia and *Tepidimonas).* There were no significant associations of urinary OTUs with objective and subjective (ΔUDI) treatment responses (data not shown).

In unadjusted models, both vaginal *Lactobacillus* and *Prevotella* were associated with objective treatment response. After controlling for age, *Prevotella* remained the only predominant vaginal OTU associated with objective treatment response, and no genera were associated with subjective response (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 treatment. None of the 274 less abundant OTUs present in urine were associated with treatment responses (data not shown). However, 16 of the 133 less abundant vaginal OTUs were significantly associated with objective response. Four were significantly associated with subjective treatment response, and two (*Campylobacter* and *Facklamia*) were associated with both outcomes (Supplemental Table S1–S2). These less abundant OTUs were all inversely correlated with *Lactobacillus* (Figure 3) and were all increased in women with worse response to treatment (e.g., objective non-responders).

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. Due to 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 significant associations between incontinence subtype persistence and vaginal *Lactobacillus* or *Prevotella* was seen (Supplemental Figure S1; Supplemental Table S3). Less abundant vaginal OTUs associated with both objective and subjective response, *Campylobacter* and *Facklamia*, were more abundant in the persistent MUI and UUI-only groups compared to the group without SUI or UUI (Supplemental Table S3). However, these OTUs did not distinguish persistent MUI from UUI alone.

Structured Discussion/Comment

Principal Findings:

This planned secondary analysis of the ESTEEM trial characterizes the urogenital microbiome of women undergoing mid-urethral sling surgery for mixed urinary incontinence. Greater abundance of vaginal *Prevotella*, more commonly found in non-responders, 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 response. The urinary microbiome was not associated with surgical treatment response.

Results:

This study found that two predominant OTUs in the vagina, *Lactobacillus* and *Prevotella*, were associated with objective treatment response. *Lactobacillus* was associated with women attaining a ≥70% improvement in UI episodes although this association was not statistically significant after adjusting for age. Greater abundance of vaginal *Prevotella* was associated with women failing to meet this threshold of UI episode improvement and this relationship remained significant after adjusting for age. Interestingly, although a relationship existed between predominant vaginal OTUs and treatment response, a relationship between the urinary microbiome and responder status was not noted. Exploratory analyses also revealed a relationship between less abundant vaginal OTUs with regard to objective and subjective outcomes and persistent/recurrent UI symptoms at 12 months.

Clinical Implications:

Taken together, the study findings suggest a framework in which the vaginal microbiome may modulate treatment response following mid-urethral sling surgery.

Significant evidence exists surrounding the protective nature of *Lactobacillus* in stabilization of the vaginal environment. There is greater abundance of *Lactobacillus* in well estrogenized pre-menopausal women compared with atrophic post-menopausal women. *Lactobacillus* plays a role in many lower urinary tract disorders including urinary tract infections and also in protection from certain vaginal infections.[22,23] *Prevotella*, a gram-negative anaerobe, is a common vaginal commensal bacteria, but its association with bacterial vaginosis, (especially *Prevotella bivia*), also 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 mixed urinary incontinence 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 urinary tract infections. 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. It is important, however, 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 treatment outcomes.[25]

Since 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.[4,5,6] Differences were identified in the vaginal microbiome that were associated with surgical treatment response, 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 due to the relatively small numbers of non-responders or the fact that the bacterial community in the urine is somewhat isolated from the connective tissue environment of the mid-urethral sling.

It is also possible that technical factors in urinary microbiome assessment in this study limited the sensitivity of our analysis to find associations between urinary microbiota and treatment response. Compared to 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 mid-urethral sling while urinary microbiota are not, but these urinary results are a surrogate for a different interaction. It is possible that higher *Prevotella* exists in 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 with respect to surgical responder status.

In exploratory, hypothesis-generating analyses, it was noted that several of the less abundant OTUs were significantly associated with objective treatment response (e.g., *Sphingomonas*, *Campylobacter*, *Varibaculum*, *Arcobacter*, and others). Interestingly, two, *Campylobacter* and *Facklamia*, were increased in both objective and subjective non-responders. *Campylobacter* is a gram negative spiral zoonotic bacterium and 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 vagina, urine and cerebrospinal fluid. Though 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.

Treatment response decreased with age, as did *Lactobacillus* abundance. Whether *Lactobacillus* is a determinant or marker of improved treatment response cannot be ascertained in this study due to its close association with age, and due to 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 non-response, only that they are noted to be associated with treatment response.

Research Implications:

There are abundant opportunities for continued evaluation of treatment outcomes associated with the genitourinary microbiome. Future work will be required to explore whether increasing *Lactobacillus* abundance with vaginal supplementation or manipulation will improve treatment outcomes and whether this would affect the abundance of OTUs associated with poor treatment outcomes. Additionally, with advancement 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:

Strengths of this study include 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 one core lab reducing technical artifacts. Weaknesses of this study include 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 response to treatment as a continuous trait for both the urine and vaginal samples. The study is limited by 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 non-responders. Additionally, analyses of rare OTUs can be biased due to 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 non-responder 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:

In conclusion, the genera *Lactobacillus* and *Prevotella* were associated with objective treatment response and after controlling for age, one predominant (*Prevotella*) and other less abundant OTUs were associated with surgical treatment non-response in women undergoing mid-urethral sling surgery for MUI. Further research is needed in order to corroborate the current findings and to further explore potential clinical applications in the setting of surgery for pelvic floor disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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. [PubMed: 25006228]
2. Thomas-White KJ, Hilt EE, Fok C, et al. Incontinence medication response relates to the female urinary microbiota. Int Urogynecol J 2016;27:723–733. [PubMed: 26423260]
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. [PubMed: 24515544]
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; 29:1785–1795. [PubMed: 29909556]
5. Komesu YM, Dinwiddie D, Richter HE, et al. Defining the relationship between the vagina and urinary microbiomes. Am J Obstet Gynecol 2020;222:154.e1–10. [PubMed: 31421123]
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. [PubMed: 29674608]
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. Published 2020 Mar 26. doi:10.3205/id000046 [PubMed: 32373427]

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. [PubMed: 30777006]
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–e141. [PubMed: 29341151]
10. Macalister Kinross J, Markar S, Karthikesalingam A, et al. A meta-analysis of probiotic and symbiotic use in elective surgery: does nutrition modulation of the gut microbiome improve clinical outcome? J Parenteral and Enteral Nutrition 2013; 37:243–253.
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;322:1066–1076. [PubMed: 31529007]
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;27:1479–1490. [PubMed: 27287818]
13. Shumaker SA, Wyman JF, Uebersax JS, et al. Health-related quality of life measures for women with urinary incontinence: the Incontinence Impact Questionnaire and the Urogenital Distress Inventory. Qual of Life Res 1994; 3:291–306. [PubMed: 7841963]
14. Sung VW, Richter HE, Moalli P, et al. Characteristics associated with treatment failure one year after midurethral sling surgery in women with mixed urinary incontinence. Obstet Gynecol, 2020;136:482–491. [PubMed: 32769647]
15. Komesu YM, Richter HE, Dinwiddie D, et al. Methodology for a vaginal and urinary microbiome study in women with mixed urinary incontinence. Int Urogynecol J 2016;28:711–720. [PubMed: 27738739]
16. Wang Q, Garrity GM, Tiedje JM, et al. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 2007; 73:5261–5267. [PubMed: 17586664]
17. Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 1957; 27(4):325–349, 10.2307/1942268
18. Warton DI, Wright TW, Wang Y. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution 2011;3:89–101.
19. Oksanen J, Guillaume Blanchet F, Friendly M, et al. (2019). vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan
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. [PubMed: 23013615]
21. Benjamini Y, Hochberg Y. Controlling the false delivery rate: a practical and powerful approach to multiple testing. J.R. Statist Soc Series 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. [PubMed: 30837959]
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. [PubMed: 31119104]
24. Randis TM, Ratner AJ. Gardnerella And Prevotella: Co-conspirators in the pathogenesis of bacterial vaginosis. J Infect Dis 2019;220:1085–1088. [PubMed: 30715397]
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 Aug 11:S0002-9378(20)30834-6. doi: 10.1016/j.ajog.2020.08.006. Epub ahead of print.
26. Karstens L, et al. Community profiling of the urinary microbiota: considertions for low-biomass samples. Nat Rev Urology 2018; 15:735–749. [PubMed: 30315209]
27. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of Campylobacter infection. Clin Microbiol Rev 2015;28:687–720. [PubMed: 26062576]

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. [PubMed: 26380331]

29. LaClaire L, Facklam R. Antimicrobial susceptibilities and clinical sources of Facklamia species. Antimicrob Agents Chemother 2000;44:2130–2. [PubMed: 10898686]

30. Popovic VB, Situm M, Chow CET, et al. The urinary microbiome associated with bladder cancer. Nature Scientific Reports 2018;8:12157.

Take Home Message

In women with mixed urinary incontinence, urinary microbiota were not associated with outcomes after mid-urethral sling surgery. However, vaginal microbiota at the time of surgery were associated with subjective and objective treatment responses one 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 impacts surgical outcomes.

AJOG at a Glance:

Why was the study conducted?

- To evaluate whether preoperative urinary or vaginal microbiomes are associated with surgical response at 12 months in women with mixed urinary incontinence undergoing mid-urethral sling placement.

What are the key findings?

- Older and post-menopausal women without hormone replacement more commonly were surgical non-responders
- Greater abundance of pre-operative vaginal *Lactobacillus* was associated with improved objective surgical response (decreased urinary incontinence episodes); this effect diminished after adjusting for age
- Greater abundance of pre-operative vaginal *Prevotella* was associated with worsened objective surgical response even after adjusting for age
- Urinary microbiota were not associated with surgical treatment response

What does this study add to what is already known?

- Baseline vaginal microbiome findings were associated with treatment response to mid-urethral slings in women with mixed urinary incontinence

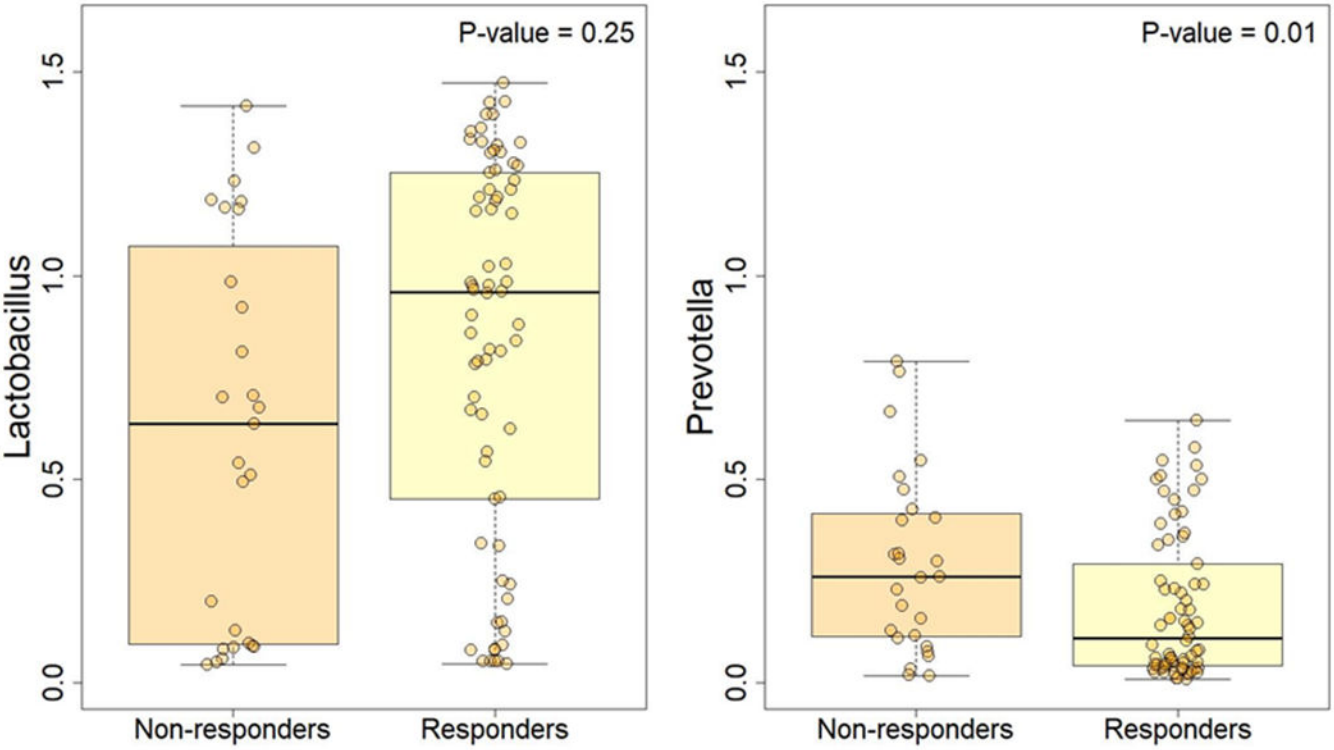

Figure 1:

Associations of Vaginal *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).

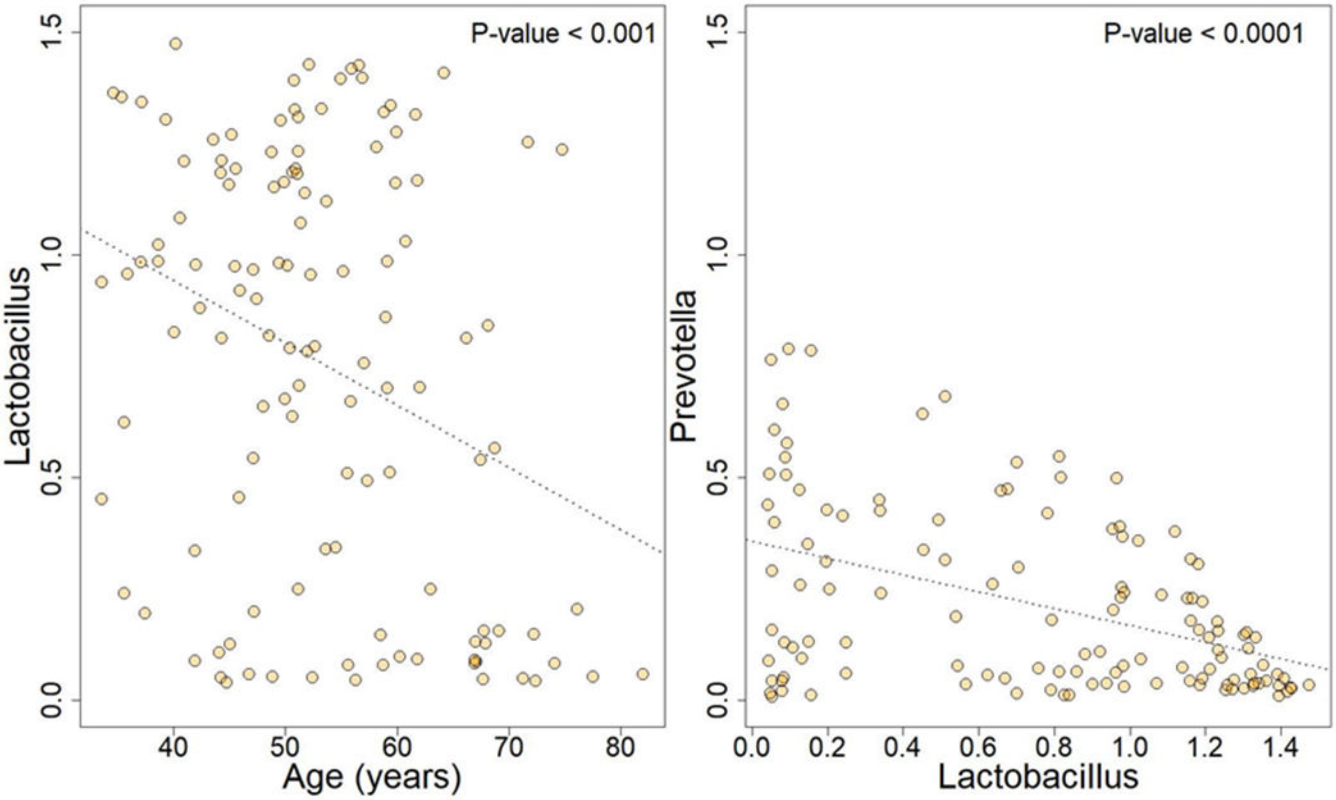

Figure 2: Association of Vaginal *Lactobacillus* with Age and Vaginal *Prevotella*

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

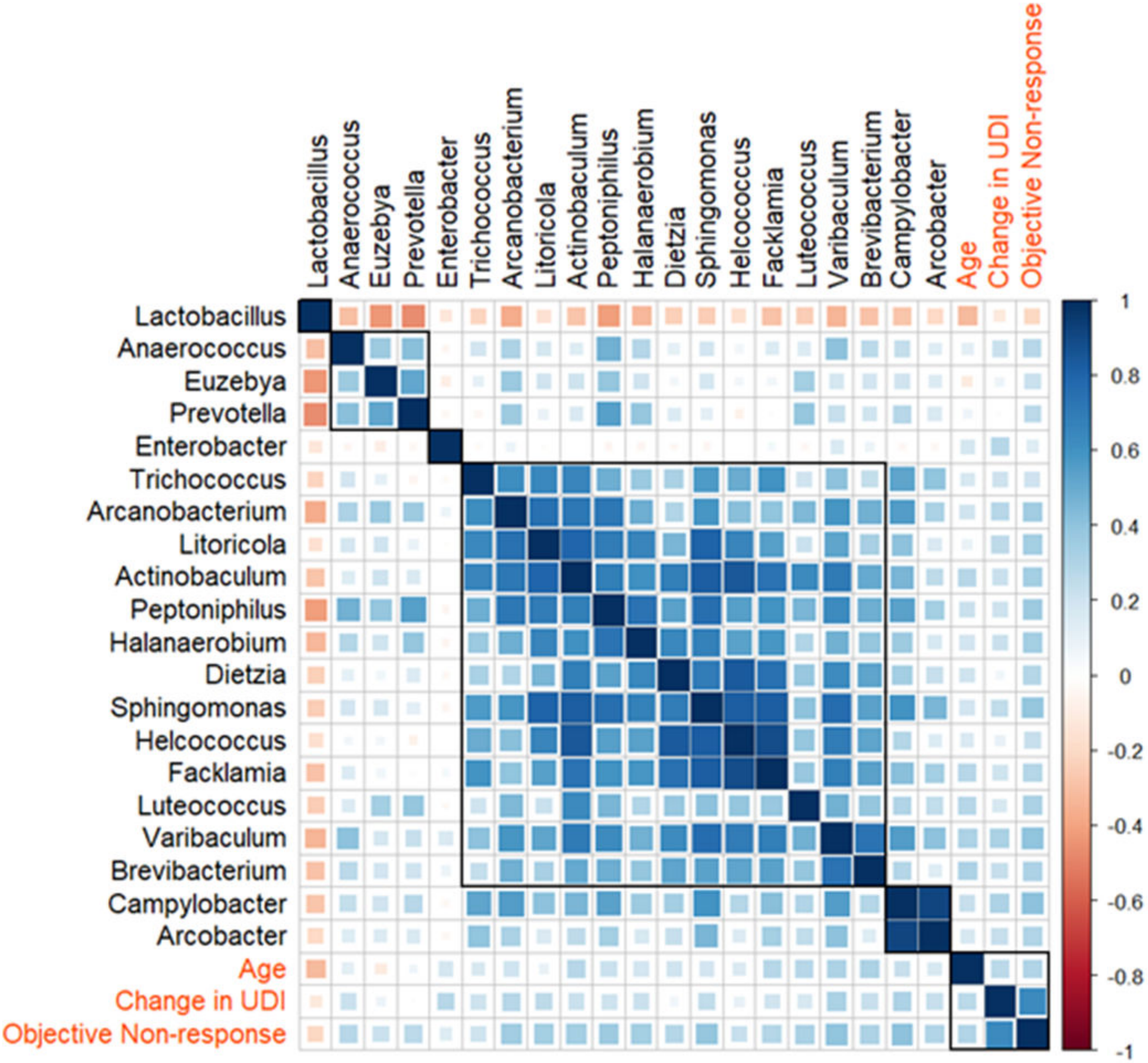

Figure 3: Correlation matrix of genera associated with response to treatment and age

Correlation matrix showing the relationship of the most significant genera associated with objective non-response and change in UDI in the vagina, including predominant (*Lactobacillus* and *Prevotella*; Table 4) and less abundant (Supplemental Tables S1–2) OTUs. 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.

Table 1.

Baseline Characteristics of HMS-ESTEEM Participants with 12 Month Objective Response Data

Variable	Category	Total (N=100)	Objective Non-responders (N=28)	Objective Responders (N=72)	Unadjusted Mean or Median Difference or Odds Ratio [95% CI]
Age, mean (SD), y		53.5 (10.8)	58.5 (10.7)	51.6 (10.2)	6.9 [2.2, 11.6]
Body Mass Index, mean (SD)		32.3 (7.2)	34.2 (7.2)	31.6 (7.1)	2.6 [−0.6, 5.8]
Race, No. (%)	White	71 (71.0)	16 (57.1)	55 (76.4)	ref.
	More than one race	1 (1.0)	1 (3.6)	0 (0.0)	--
	Asian	2 (2.0)	1 (3.6)	1 (1.4)	3.4 [0.2, 60.3]
	Black/African American	12 (12.0)	5 (17.9)	7 (9.7)	2.5 [0.7, 8.9]
	American Indian/ Alaskan Native	1 (1.0)	0 (0.0)	1 (1.4)	--
	Other	13 (13.0)	5 (17.9)	8 (11.1)	2.1 [0.6, 7.6]
Ethnicity, No. (%)	Not Hispanic/Not Latina	72 (72.0)	18 (64.3)	54 (75.0)	ref.
	Hispanic/Latina	26 (26.0)	10 (35.7)	16 (22.2)	1.9 [0.7, 4.9]
	Unknown/Not Reported	2 (2.0)	0 (0.0)	2 (2.8)	--
Smoking status, No. (%)	Never smoked	58 (58.0)	16 (57.1)	42 (58.3)	ref.
	Quit smoking six or more months ago	29 (29.0)	9 (32.1)	20 (27.8)	1.2 [0.4, 3.2]
	Quit smoking less than six months ago	2 (2.0)	0 (0.0)	2 (2.8)	--
	Currently smoking	11 (11.0)	3 (10.7)	8 (11.1)	1.0 [0.2, 4.3]
Current UTI symptoms, No. (%)		2 (2.0)	0 (0.0)	2 (2.8)	--
Vaginal deliveries, median [min, max], No.		2 [0, 8]	2 [0, 6]	2 [0, 8]	0 [−1, 1]
Menopause and hormone status, No. (%)	pre-menopausal	31 (31.0)	4 (14.3)	27 (37.5)	ref.
	post-menopausal w/ ahormones	19 (19.0)	4 (14.3)	15 (20.8)	1.8 [0.4, 8.4]
	post-menopausal w/o 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]

-- Could not be calculated due to small sample size; UTI = Urinary Tract Infection

Table 2.

Outcomes of HMS-ESTEEM Participants with 12 Month Objective Response Data

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

Table 3:

Microbiome Beta Diversity Analysis Results (global association)

	Urine		Vagina	
Variable	**R^2**	**P-value**	**R^2**	**P-value**
Age, mean (SD), y	0.04	**0.001**	0.06	**0.001**
Body Mass Index, mean (SD)	0.01	0.15	0.01	0.30
Race	0.01	0.83	0.01	0.67
Ethnicity	0.01	0.80	0.01	0.64
Current smoking	0.01	0.10	0.01	0.23
Current UTI symptoms	0.004	0.96	0.01	0.73
Ever pregnant	0.01	0.46	0.01	0.60
Menopause and hormone status	0.03	0.08	0.05	**0.01**
Oral or patch hormone use	0.01	0.72	0.01	0.14
Vaginal cream/tablets hormone use	0.01	0.76	0.01	0.46
Any hormone use	0.01	0.47	0.01	0.19
ΔUDI at 12 M	0.02	0.09	0.02	0.07
Objective Response	0.01	0.39	0.02	**0.03**

Beta diversity analyses were conducted using permutational multivariate analysis of variance implemented in Adonis with a significance threshold of p-value<0.05. Results shown are from unadjusted analyses of one demographic or clinical variable at a time.

Table 4:

Associations of Predominant OTUs in Vagina with Objective and Subjective Response at 12-Months

Vagina		Objective Response (27 Non-responders / 70 Responders)		Subjective Response (N=101)	
	Predominant in N (%) of Samples	**P-value Unadjusted model**	**P-value Age-adjusted model**	**P-value Unadjusted model**	**P-value Age-adjusted model**
Lactobacillus	78 (63%)	**0.04**	0.25	0.22	0.69
Prevotella	11 (10%)	**0.01**	**0.01**	0.91	0.99
Gardnerella	12 (11%)	0.97	0.65	0.22	0.41
Streptococcus	6 (5%)	0.97	0.26	0.32	0.92

For each sample, the predominant OTU was defined as the OTU with the highest relative abundance. This analysis included OTUs that were predominant in 3 or more 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). Results in bold met the significance threshold of 0.05.