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Vaginal Estrogen Therapy Is Associated with Increased *Lactobacillus* in the Urine of Post-Menopausal Women with Overactive Bladder Symptoms

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Abstract

Introduction—Previous work has shown that the vaginal microbiome decreases in *Lactobacillus* predominance and becomes more diverse following menopause. It has also been shown that estrogen therapy restores *Lactobacillus-dominance* in the vagina, and that topical estrogen is associated with OAB symptom improvement. We now know that the bladder contains a unique microbiome, and increased bladder microbiome diversity is associated with OAB. However, there is no understanding of how quickly each pelvic floor microbiome responds to estrogen or if those changes are associated with symptom improvement.

Study Design—Analysis of data from post-menopausal participants in two trials (NCT02524769 and NCT02835846) who chose vaginal estrogen as their primary OAB treatment and used 0.5 grams of conjugated estrogen (Premarin Cream, (Pfizer, New York City, NY)) twice weekly for 12 weeks. Baseline and 12-week follow-up data included the OAB-q questionnaire and participants provided catheterized urine, vaginal swabs, perineal swabs, and voided urine. Microbes were detected by an enhanced culture protocol. Linear mixed models were used to estimate microbiome changes over time. Urinary AMP activity was assessed by a bacterial growth inhibition assay and correlated with relative abundance of members of the urobiome.

Results—Twelve weeks of estrogen treatment resulted in decreased microbial diversity within the vagina (Shannon, p=0.047; Richness, p=0.043), but not in the other niches. A significant increase in *Lactobacillus* was detected in the bladder (p=0.037), but not the vagina (p=0.33), perineum (p=0.56), or voided urine (p=0.28). The change in *Lactobacillus* levels in the bladder was associated with modest changes in urgency incontinence symptoms (p=0.02). The relative abundance of the genus *Corynebacterium* correlated positively with urinary AMP activity after estrogen treatment.

Conclusion—Estrogen therapy may change the microbiome of different pelvic floor niches. The vagina begins to decrease in diversity and the bladder experiences a significant increase in *Lactobacillus* levels; the latter is correlated with a modest improvement in the symptom severity sub-scale of the OAB-q.

Condensation

Estrogen therapy for overactive bladder resulted in decreased bladder bacterial diversity and increased bladder *Lactobacillus,* which were associated with modest changes in urgency incontinence symptoms.

Keywords

enhanced urine culture; estrogen; overactive bladder; urgency urinary incontinence; urinary microbiome; urinary urgency; vaginal microbiome; 16S rRNA gene sequencing; antimicrobial peptides

INTRODUCTION

Loss of estrogen in post-menopausal women has long been associated with increased pelvic floor symptoms and disorders, such as urinary tract infections and overactive bladder (OAB). The emergence of these symptoms is associated with a change of the vaginal

microbiota. Premenopausal women tend to have low microbial diversity and are more likely to be predominated by *Lactobacillus* than postmenopausal women[1,2], and hormone replacement therapy reverses this change[3,4].

In post-menopausal women, use of vaginal estrogen improves symptoms of OAB[5], a syndrome characterized by urinary urgency, often associated with frequency and nocturia, with or without urgency urinary incontinence in the absence of infection or other pathology[6]. Although the precise mechanism of symptom relief is not understood, vaginal estrogen increases vaginal blood flow and reduces density of vaginal autonomic and sensory nerves[5,7].

The normal loss of estrogen during menopause promotes structural and chemical changes throughout the urogenital tract by reducing urothelial thickness and the abundance of tight junction proteins[8,9], which can presumably facilitate pathogen colonization *via* impaired urothelial barrier function. Low estrogen also reduces the production of several endogenous anti-microbial peptides (AMPs). Elements of both the vaginal and urinary innate immune systems that exhibit direct microbicidal activity[9], AMPs help minimize bacterial dysbiosis and facilitate normal epithelial barrier function in the female urogenital tract. Although transvaginal medications likely alter nearby bacterial niches (e.g. the bladder), no study has reported the response of the urinary microbiota to vaginal estrogen.

In the last decade, the female urinary microbiota (urobiome) has been confirmed and associations with clinical conditions of interest have been reported. Relevant to this study, we previously reported increased urobiome diversity in women with OAB[10]. We further reported that, in women planning oral anti-cholinergic therapy for OAB treatment, pre-treatment urobiome status stratifies patients into treatment response groups; women with less diverse urobiomes are more likely to respond to anti-cholinergic OAB therapy[11]. These reports provide evidence that the urobiome could factor in lower urinary tract symptoms and that urobiome diversity contributes to lower urinary tract symptoms and lower urinary tract symptom treatment response.

We also have reported that AMP levels and/or activity correlate with urinary tract infection risk in women undergoing urogynecological surgery[12]. Although urinary AMP activity has been assessed in several urinary pathologies[9,12–14], no studies have assessed the role of estrogen in modulating urinary AMP activity as a therapeutic for women with OAB. Given that estrogen promotes epithelial differentiation, which is associated with greater AMP expression and activity, estrogen likely regulates urinary AMPs to optimize urobiome equilibrium[9,14,15] and impact the severity of OAB.

Given the proximity of the bladder and vaginal microbiota[16], we hypothesize that the urobiome may become less diverse following vaginal estrogen treatment, which may improve OAB symptoms. We further hypothesize that vaginal estrogen treatment in hypoestrogenic women with OAB will be associated with 1) alteration of other urobiome characteristics 2) a correlation between OAB symptoms and reduced urobiome diversity, and 3) altered urinary AMP activity.

MATERIALS AND METHODS

This study features a quasi-experimental design and pools data from participants in two IRB-approved single-armed interventional registered trials with identical study aims and study protocol and statistical analysis plans. The first (NCT02524769, local IRB LU#207152) enrolled 27 participants as a fellowship thesis project, which continued as an investigator-initiated project (NCT02835846, local IRB LU#207777 funded by Kimberly-Clark Corporation) that enrolled 37 additional participants.

Study design and patient recruitment

Women seeking OAB treatment were recruited in the ambulatory urogynecology clinic at Loyola University Medical Center (12/2015–11/2017) after being clinically counseled on evidence-based first-line options for OAB treatment, including behavioral modifications, physical therapy, and vaginal estrogen for treatment of atrophy, as appropriate. Women who chose vaginal estrogen as their primary OAB treatment were invited to participate. Women recruited to the study were also allowed, but not required, to participate in pelvic floor physical therapy as part of their treatment. Eligibility criteria were broad to facilitate subsequent generalization. We included women with OAB symptoms who (1) had higher weighted scores on urge incontinence questions as compared to stress incontinence questions on the Medical, Epidemiological and Social Aspects of Aging (MESA) urinary incontinence questionnaire[17]; (2) had clinical indication for vaginal estrogen use; (3) were postmenopausal by history, defined as twelve months or greater since last menstrual period or over the age of 55 if they have had a previous hysterectomy; (4) had no current vaginal estrogen therapy and (5) had English skills sufficient to complete questionnaires. Women were excluded for the following: (1) use of systemic hormone replacement therapy (HRT) currently or within the past three months; (2) personal history of estrogen-dependent malignancies (breast, endometrial); (3) contraindication or allergy to local estrogen therapy; (4) insufficient language skills to complete study tasks; (5) urinary tract infection at baseline assessment; (6) use of antibiotics within the past two weeks; (7) pelvic organ prolapse stage III (>1 cm beyond hymen); (8) unwillingness to use vaginal estrogen preparation; or (9) currently on anti-cholinergic medication, desire for anti-cholinergic medication at this visit, or who have received anti-cholinergic medication within the past three months.

Symptom assessment and sample collection

Consented participants were asked to complete the OAB-q, a 33 item self-administered validated questionnaire for OAB-related symptoms and quality of life. It has an 8-item symptom bother scale and a 25-item health related quality of life scale with four domains; coping, concern, sleep, and social interaction. The subscale and total scores are transformed into a 0 – 100 scale. For the symptoms severity subscale, higher values indicate more severe symptoms whereas for the quality of life outcome higher scores indicate improved quality of life[18]. Demographics and clinical characteristics were abstracted from medical records. Physical examination included pelvic organ prolapse quantification (POP-q)[19]. Each participant contributed baseline samples in the following order: voided urine, perineal swab, vaginal swab, and catheterized urine. Free of charge, participants were provided 0.625 mg conjugated estrogen/gram of cream (Premarin, Pfizer, New York City, NY) and instructed to

use 0.5 grams with an applicator twice weekly for 12 weeks based on the Society for Gynecological Surgeons' clinical practice guidelines for vaginal estrogen use in postmenopausal women with urogynecological complaints[20]. Participants kept a simple medication diary for estrogen use during the course of the study. Follow-up occurred 12 weeks after the initial visit. Participants contributed follow-up samples identical to baseline and again completed the OAB-q.

Sample collection and preparation

Voided and catheterized urine specimens were collected in separate sterile blue cap-collection cups (BD #364956) and distributed as follows: a gray-top culture tube was filled for processing within 4 h of collection by the Expanded Quantitative Urine Culture protocol.[28] Vaginal and perineal swabs were collected using the BD ESwab (BD#220245); each swab was swirled in 1 ml of bacterial preservative and an aliquot was provided for Expanded Quantitative Urine Culture as described for urine samples.

Expanded Quantitative Urine Culture

Catheterized urine samples were tested by two culture techniques (details in Supplementary Table 1): a standard urine culture method with detection level of 1000 colony forming units (CFU)/mL and the Expanded Quantitative Urine Culture method with detection level of 10 CFU/mL. Due to large biomass, voided urine and swabs were tested by the Expanded Quantitative Urine Culture method with detection levels of 100 or 1000 CFU/mL. The taxonomic identity of all distinct colony morphologies were tested by MALDI-TOF, as described[21].

Cluster analysis and diversity measurements

The relative abundance of each taxon was determined for both the baseline and 12-week time points and clustered using the Bray Curtis Dissimilarity index, a measure of beta (between sample) diversity. Bacterial compositions of urine and swab specimens were compared using four measures of alpha diversity. Richness was determined by counting the number of unique species. The distribution of microbial species within samples (evenness) was calculated with Pielou's Index. Combined interactions were calculated with the Shannon Index (richness and evenness) and Simpson Index (richness and species abundance). All calculations were performed in RStudio (R version 3.5.1)[22].

HPLC fractionation of urine and antimicrobial radial diffusion assay

AMP activity is highly dependent upon the degree of peptide hydrophobicity[23]. High pressure liquid chromatography can be used to isolate and purify AMPs using increasing concentration of acetonitrile to elute peptides based upon their degree of hydrophobicity (Supplementary Figure 1A). Thus, voided urine was subjected to high pressure liquid chromatography fractionation using a C18 column and tested for antimicrobial activity by radial diffusion assay, as described [12,13]. The 20 fractions were classified into 4 levels (groups) based on increasing levels of eluted peptide hydrophobicity: Level 1: Fractions 1–2: 10–15% Acetonitrile; Level 2: F3–6: 16–40% Acetonitrile; Level 3: F7–F11: 40–55% Acetonitrile; Level 4: F12–20: 56–90% Acetonitrile. Each fraction was subjected to a radial

diffusion assay to assess their capacity to inhibit the growth of a Gram-positive AMP-susceptible mutant *(Staphylococcus aureus* ΔmprF). The resultant zone of bacterial growth inhibition in mm^2 was measured using ImageJ Software and normalized to the total peptide bond concentration measured at UV 214nm (Supplementary Figure 1B).

Statistical methods

Descriptive statistics for baseline demographics and clinical characteristics were calculated; comparisons were tested using Wilcoxon rank sum tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. Medians and interquartile ranges were presented for OAB-q subscales at each time point, and Wilcoxon signed rank tests assessed statistical significance of change in OAB-q. Each diversity measure was modeled as a separate dependent variable in a linear mixed effects regression, with independent variables including time period, OAB-q symptom severity, physical therapy completed, weeks of estrogen compliance, and body mass index and included random intercepts for subjects. Spearman's rho was calculated for change in *Lactobacillus* with change in the OAB-q symptom severity (12-week minus baseline values) for each sample type. Culture data analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). For AMP data analyses, Spearman's Rank Correlation was used to test associations between change in relative bacterial abundance at the genus level and change in area of bacterial growth inhibition per fraction. A Bonferroni correction was used to compensate for multiple testing (type 1 error). All AMP data analyses were performed in RStudio (R version 3.5.1)[22].

RESULTS

Baseline samples were collected from 62 participants; 41 participants contributed data at the 12-week follow-up visit. Ten of the 21 women who did not return for follow-up stopped using vaginal estrogen due to concerns about risks or side effects of the medication; two additional participants found the study medication "cumbersome" and discontinued use. Eight participants were not responsive to follow-up calls and one participant was unexpectedly out of the country for family care. Study completers were similar to non-completers in demographics, pelvic floor history, physical exam findings and baseline OAB-q subscales, although prolapse stage differed slightly (Table 1). Among those who completed the study, all were compliant to estrogen treatment for at least 6 weeks, and 24 (58.5%) were compliant for 12 weeks. Physical therapy was also offered to patients; 13 (31.7%) did not do any physical therapy, 8 (19.5%) completed some sessions, and 20 (48.8%) completed all sessions.

At the 12-week visit, all OAB-q sub-scale scores improved (Table 2). Most participants (n=30, 73%) reported at least a 10-point improvement in symptom severity. For those who completed the study, the median symptom severity score decreased from 48 (IQR:43–70) to 25 (IQR:8–50). Older women were less likely to report symptom improvement. When we compared women with a symptom severity improvement of > 10 points to those with less than 10 points (minimal clinically difference (MCID)) [24], there were no differences in physical therapy attendance rates (53% with symptom improvement attended all sessions versus 36% without symptom improvement; p=0.34) or compliance with estrogen use (57%

with symptom improvement were compliant all weeks versus 64% without symptom improvement; p=0.74).

Linear mixed regression model showed minimal differences in diversity between baseline and the 12-week follow-up. Specifically, catheterized and voided samples showed no change in diversity while some decreases in diversity were seen in the vaginal swabs (Shannon index p=0.047, species richness p=0.043) and perineal swabs (Peilou p=0.034) (Table 3).

Measuring overall diversity did not provide insight into any increase or decrease in the abundance of specific taxa. For that reason, we also used a separate model to identify if there were significant differences in the abundance of *Lactobacillus* from baseline to 12 weeks. Catheterized urines had significantly more *Lactobacillus* by 12-weeks (p=0.037), but no corresponding increase was seen in vaginal (p=0.33), perineal (p=0.56), or voided (p=0.28) samples (Table 3). The observation of more *Lactobacillus-predominance* was apparent on a participant-by-participant basis for catheterized urine samples (Figure 1A). While the figures do seem to indicate a greater predominance of *Lactobacillus* by 12-weeks in the vagina (Figure 1B), perineum (Figure 1C), and voided urines (Figure 1D), our model did not show this to be statistically significant (Table 3). Additionally, the change in *Lactobacillus* levels in catheterized urine from baseline to 12-weeks was associated with an improvement in the symptom severity sub-scale of the OAB-q (p=0.02), but no correlations was seen for *Lactobacillus* levels in the vagina (p=0.22), perineum (p=0.72), or voided urine (p=0.14) (Table 4).

We then assessed the correlation between the bacteria cultured from voided urine and urinary AMP activity. In terms of relative abundance, the genera *Corynebacterium* and *Streptococcus* (rho=0.56–0.79) correlated with increased AMP activity, whereas the genus *Actinomyces* correlated negatively (rho=−0.66) after estrogen treatment. Significance for *Corynebacterium* and *Actinomyces* was mostly observed for urine fractions eluted at moderately high or high levels of hydrophobicity; significance for *Streptococcus* was observed at moderately low hydrophobicity levels (Table 5). Following Bonferroni correction, only *Corynebacterium* remained highly significant (p=0.005). The results were similar when calculated in terms of CFU/mL (Supplementary Table 2).

DISCUSSION/COMMENT

Principal Findings

Our findings indicate that post-menopausal women seeking treatment for OAB who are treated with vaginal estrogen for twelve weeks do have measurable changes in their pelvic floor microbiota. The vagina and perineum decrease in diversity, and catheterized urine (but not vaginal swabs) increases in *Lactobacillus.* The increased *Lactobacillus* in catheterized urine correlates with clinically significant differences in the symptom severity sub-scale of the OAB-q. Finally, AMP activity correlates positively with detection of *Corynebacterium.*

Results

This study provides longitudinal data to supplement previous cohort comparison studies that provided evidence for depletion of vaginal *Lactobacillus* species in untreated post-

menopausal women[1]. It also provides evidence that similar changes occur in the bladder and that these changes can be reversed with topical estrogen treatment.

Clinical Implications

It is already known that low dose vaginal estrogen can improve symptoms of OAB in post-menopausal women[25–29]. This study suggests changes in the urinary microbiome as one possible mechanism for symptom improvement. It is a relatively low risk treatment option that may be added in combination with other evidence-based treatments for OAB.

Research Implications

Previous work has shown that postmenopausal women using topical estrogen cream have a greater amount of vaginal *Lactobacillus* compared to age-matched controls not using the cream[3,30]. However, because these studies used populations of women who had been on hormones for years, we do not know how long it takes for *Lactobacillus* predominance to occur. Shen and colleagues observed an increase in vaginal *Lactobacillus* predominance following 4 weeks of low dose oral estrogen in a population of postmenopausal women with atrophic vaginitis[4]. However, no one has looked at the changes in the microbiota over 12 weeks of topical treatment in a population of women with OAB. In this study, we took hypoestrogenic women, sampled them prior to and following 12-weeks of topical estrogen treatment and we observed changes in the microbiota in different pelvic floor niches. Although we did see a decrease in diversity in the vagina, we did not see the expected increase in *Lactobacillus.* We hypothesize that this is due to the short duration of our study.

Bacterial communities, like other ecosystems, can withstand different amounts of disturbance without changing state. This is called ecological resilience[31]. It is possible that 12 weeks of hormone are enough of a disturbance to change the state of the bladder ecosystem, but not enough for the vaginal ecosystem. Longer-term studies are needed to determine if longer time points (e.g. 6 months or 1 year) would be sufficient to observe shifts in the vaginal microbiota. The mechanisms of change in the urobiome will require further study to determine whether they are modulated directly via an effect of vaginal estrogen[15] or as a secondary phenomenon related to changes in the vulva/vagina that favor *Lactobacillus*[32].

As hypothesized, estrogen treatment increased the urinary AMP activity in several patients, which correlated with higher *Corynebacterium* abundance. The urinary microenvironment following estrogen treatment may enable the proliferation of *Corynebacterium* by providing key metabolites that are normally limited in the urine of hypoestrogenic women. Multiple recent reports of *Corynebacterium* isolation from urine samples, particularly from females, indicate that members of this genus may play an important role in female urinary health[33]. It is also possible that enhanced AMP activity against other bacteria following estrogen treatment serves as a selective advantage to promote *Corynebacterium* colonization and/or proliferation in the urinary tract of OAB patients. Estrogen may promote a shift in urinary AMPs to encourage bacterial tolerance in the urinary tract and protect the bladder from other microbes associated with OAB symptoms, as we previously determined that hBD1 was critical in protecting women with pelvic organ prolapse from UTI [12]. Further studies are

necessary to better define the role that *Corynebacterium* and AMP activity may play in OAB development and persistence.

Strengths and Limitations

Strengths of this study include its longitudinal aspect, samples from four relevant pelvic floor niches, and the use of each woman as her own control. Another strength was that we supplied estrogen therapy at no cost to the participant. This eliminated issues of insurance coverage and monetary constraints and helped to avoid recruitment bias. The use of the expanded quantitative urine culture protocol allowed for speciation and quantification of the bacteria in each niche, and limited the errors inherent with 16S sequencing (such as PCR and sequencing errors or errors in classification). However, our expanded quantitative urine culture protocol does miss detection of strict anaerobes and *non-Candida* fungi.

A major weakness of the study is the lack of a control group, which does not allow a causal connection to be made between estrogen treatment and OAB symptom improvement. Patients were also allowed to participate in pelvic floor physical therapy and counseled on behavioral changes, both of which may have been confounders and played a part in symptom improvement, although women with no symptom improvement had the same physical therapy compliance as those who had symptom improvement. Another weakness was the high dropout rate. Approximately one-third of the patients who enrolled in the study discontinued use of the estrogen cream prior to completion of the study. Other studies have also found discontinuation rates as high as 89% in the first year of use of locally applied estrogen creams[34]. Those who discontinued use may have been less likely to note symptom improvement and the burden of use was greater than perceived benefit. This does have the potential to introduce bias causing overestimation of symptom improvement and amplify the change in microbiomes and AMPs. Finally, we modeled the microbiome data in terms of relative abundance. It should be noted that a relative increase of one taxon does not necessarily represent an absolute increase in that taxon, but could represent decreases of other taxa.

As observed clinically[35], some participants were not compliant with vaginal estrogen therapy despite appropriate counseling about the benefits and risks. The patient information sheet that accompanies prescribed vaginal estrogen cream is identical to the sheet provided for all doses and routes of estrogen, essentially equating higher dose oral ingestion with low dose vaginal use and causing patients to avoid low-dose therapy. Changes to this federally mandated information might be warranted to more appropriately advise patients concerning dose, systemic absorption, and route-related risks.

Conclusion

This study builds on the growing knowledge of the urobiome in various health states and provides direct evidence of urobiome changes, comparable with changes in other nearby relevant pelvic microbial niches. These findings can be used to inform future interventional studies, and refine hypotheses for further testing in both clinical and laboratory settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a Glance

Why was this study conducted?

- Determine if estrogen treatment of post-menopausal women with overactive bladder decreases urobiome diversity.

What are the key findings?

- Estrogen therapy increased *Lactobacillus* levels in bladder, but not vagina.
- This increase associated with modest changes in urgency incontinence symptoms.

What does this study add to what is already known?

- Others analyzed estrogen's effect on vaginal microbiota of atrophic vaginitis patients, or compared cohorts not on estrogen to those on estrogen for years.
- We tracked women with overactive bladder over 12 weeks of estrogen treatment.
- We compared microbiota of vaginal swabs, perineal swabs, urine obtained by void (which samples the genitourinary tract) and urine obtained by transurethral catheter (which samples the bladder).
- We observed the estrogen effect on microbiota of multiple pelvic floor niches.

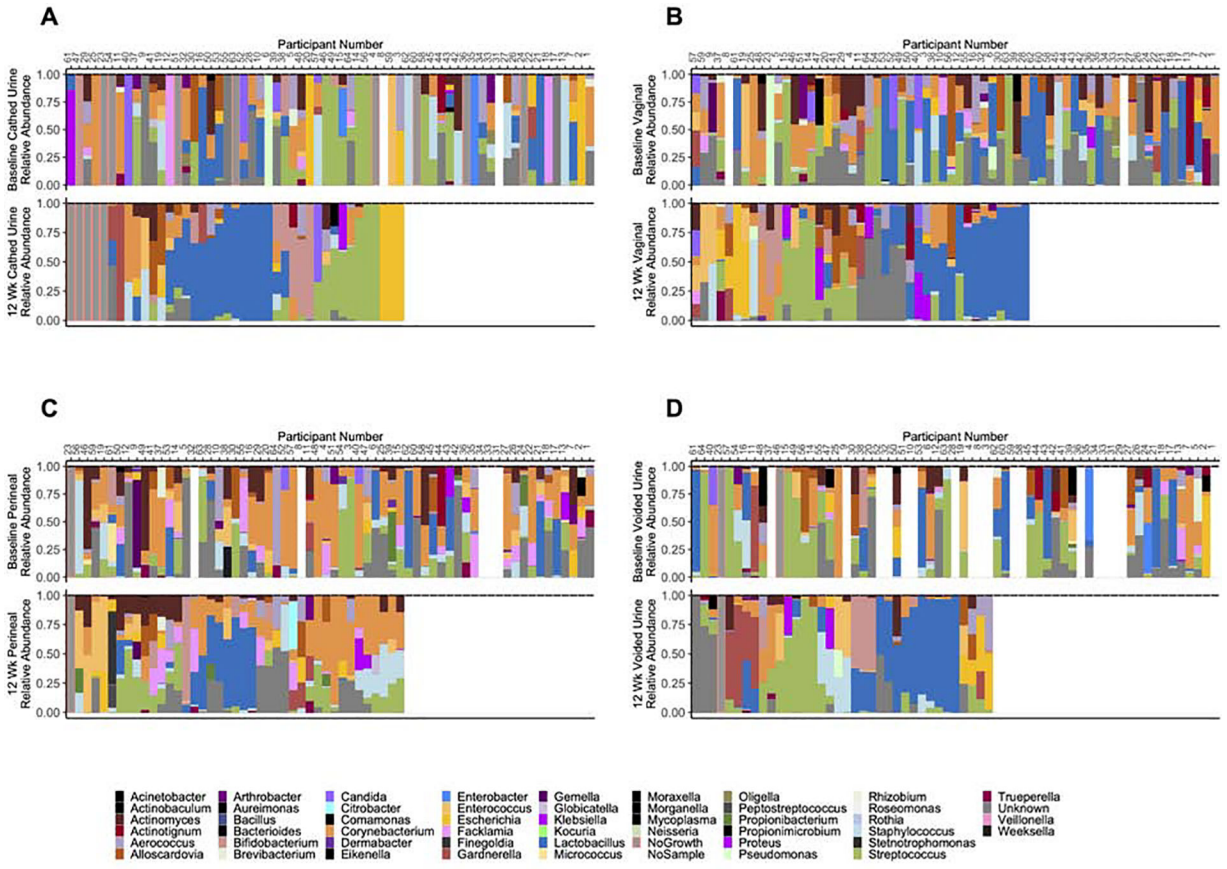

Figure 1. Microbiota of catheterized urine (A), vaginal swabs (B), perineal swabs (C) and voided urine (D) at baseline and after 12 weeks of estrogen therapy.

Top: baseline; bottom: 12-week. Baseline data arranged according to 12-week microbiota. Figure constructed in R (version 3.5.1) with Vegan (version 2.5–5) [36], Cowplots (version 1.0.0) [37], ggplotify (version 0. 0.5), and ggplot2 (version 3.2.1) [38,39] packages.

Table 1:

Baseline characteristics by study completion

	Completed study n=41 (66.1%)	Did Not Complete Study n=21 (33.9%)	p-value
Age, median (IQR *)	68 (62–73)	69 (64–74)	0.84
Race/ethnicity, n (%)			
Caucasian	28 (68.3)	14 (66.7)	0.56
African American	8 (19.5)	5 (23.8)	
Asian	1 (2.4)	2 (9.5)	
Hispanic	2 (4.9)	0 (0.0)	
Other	2 (4.9)	0 (0.0)	
BMI, median (IQR)	29 (26–35)	30 (26–36)	0.46
Vaginal deliveries, median (IQR)	3 (2–3)	2 (2–4)	0.99
Prior hysterectomy, n (%)	18 (43.9)	11 (52.4)	0.53
Stage of prolapse, n (%)			
0	13 (31.7)	8 (38.1)	0.008
1	9 (22.0)	11 (52.4)	
2	19 (46.3)	2 (9.5)	
Ovaries removed, n (%)	11 (28.2)	5 (25.0)	0.79
Previous incontinence surgery, n (%)	4 (9.8)	2 (9.5)	0.99
Post Void Residual (mL), median (IQR)	30 (15–60)	40 (25–60)	0.30
Baseline OAB-q ** subscales, median (IQR)			
Symptom severity	48 (43–70)	55 (40–65)	0.69
Coping	65 (43–83)	55 (20–78)	0.61
Concern	57 (37–71)	46 (17–83)	0.55
Sleeping	64 (32–76)	52 (36–84)	0.92
Social	92 (80–100)	80 (68–100)	0.29
HRQOL ***	67 (46–78)	60 (33–77)	0.59

* IQR: interquartile range

** OAB-q: Overactive Bladder quantified

*** HRQOL: Health Related Quality of Life

Table 2:

OAB-q* subscale scores between baseline and follow-up

	Baseline	Follow-up	Change Post - Pre	p-value
OAB-q* subscales, median (IQR)**				
Symptom severity	48 (43–70)	25 (8–50)	−23 (−38– −5)	**<0.001**
Coping	65 (43–83)	88 (75–100)	18 (3–38)	**<0.001**
Concern	57 (37–71)	86 (57–97)	23 (6–34)	**<0.001**
Sleeping	64 (32–76)	84 (60–92)	16 (0–24)	**<0.001**
Social	92 (80–100)	100 (95–100)	4 (0–16)	**0.006**
HRQOL*	67 (46–78)	86 (71–97)	17 (6–27)	**<0.001**

* OAB-q: Overactive Bladder quantified

** IQR: interquartile range

*** HRQOL: Health Related Quality of Life

bold: statistically significant, p<0.05

Table 3:

Microbiome diversity measurements between baseline and follow-up based on expanded quantitative urine culture results

	Baseline, Adjusted mean (SE)	Follow-up, Adjusted mean (SE)	Change, Adjusted mean (SE)	p-value
	Catheterized urine samples			
Shannon index	0.83 (0.12)	0.65 (0.12)	−0.17 (0.16)	0.30
Simpson index	0.49 (0.06)	0.49 (0.06)	0.00 (0.09)	0.97
Species richness	3.94 (0.49)	3.41 (0.48)	−0.53 (0.66)	0.43
Pielou's evenness	0.65 (0.07)	0.57 (0.07)	−0.09 (0.10)	0.39
% Lactobacillus	10.8 (5.4)	25.1 (5.3)	14.4 (6.6)	**0.037**
	Vaginal swab samples			
Shannon index	1.29 (0.08)	1.04 (0.08)	−0.25 (0.12)	**0.047**
Simpson index	0.61 (0.04)	0.54 (0.04)	−0.07 (0.05)	0.19
Species richness	8.83 (0.52)	7.36 (0.52)	−1.46 (0.70)	**0.043**
Pielou's evenness	0.62 (0.03)	0.56 (0.03)	−0.06 (0.05)	0.21
% Lactobacillus	17.7 (5.2)	24.2 (5.1)	6.6 (6.6)	0.33
	Perineal swab samples			
Shannon index	1.37 (0.09)	1.46 (0.09)	0.08 (0.11)	0.47
Simpson index	0.65 (0.03)	0.71 (0.03)	0.06 (0.04)	0.15
Species richness	9.66 (0.58)	8.30 (0.56)	−1.36 (0.72)	0.07
Pielou's evenness	0.60 (0.04)	0.71 (0.04)	0.10 (0.05)	**0.034**
% Lactobacillus	6.7 (3.1)	9.2 (3.0)	2.5 (4.2)	0.56
	Voided urine samples			
Shannon index	1.10 (0.11)	0.99 (0.10)	−0.12 (0.15)	0.45
Simpson index	0.59 (0.05)	0.54 (0.05)	−0.05 (0.07)	0.43
Species richness	7.56 (0.73)	7.08 (0.69)	−0.48 (0.78)	0.55
Pielou's evenness	0.54 (0.05)	0.55 (0.05)	0.01 (0.07)	0.87
% Lactobacillus	13.4 (6.3)	22.5 (5.5)	9.2 (8.3)	0.28

Bold: statistically significant, $p<0.05$

Table 4:

Comparison of change in *Lactobacillus* levels to change in symptom over 12 weeks

	Spearman's rho	95% CI	p-value
Catheterized Urine	−0.42	−0.67, −0.06	**0.02**
Vaginal swab	−0.21	−0.49, 0.12	0.22
Perineal swab	−0.06	−0.38, 0.27	0.72
Voided urine	−0.32	−0.64, 0.12	0.14

Bold: statistically significant, $p<0.05$

Table 5:

Correlation between changes in urinary AMP activity and genus relative abundance

Genus	AMP fraction/level	p value	S	rho	adjusted p value
Actinomyces	11/3	**0.015**	603.3	−0.66	0.058
Corynebacterium	16/4	**0.001**	76.2	0.79	**0.005**
Corynebacterium	20/4	**0.048**	161.0	0.56	0.191
Streptococcus	5/2	**0.031**	146.9	0.60	0.126

Fractions were classified into 4 Levels (L1-L4) of increasing levels of hydrophobicity: Level 1: F1−2, 10−15% Acetonitrile; Level 2: F3−6, 16−40% Acetonitrile; Level 3: F7-F11, 40−55% Acetonitrile; Level 4: F12−20, 56−90% Acetonitrile; Bold: statistically significant, $p<0.05$