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DEFINING THE RELATIONSHIP BETWEEN VAGINAL AND URINARY MICROBIOMES

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Abstract

Background: Although the vaginal and urinary microbiomes have been increasingly well-characterized in health and disease, few have described the relationship between these neighboring environments. Elucidating this relationship has implications for understanding how manipulation of the vaginal microbiome may affect the urinary microbiome and treatment of common urinary conditions.

Objective: To describe the relationship between urinary and vaginal microbiomes using 16S rRNA gene sequencing. We hypothesized that the composition of the urinary and vaginal microbiomes would be significantly associated, with similarities in predominant taxa.

Study Design: This multicenter study collected vaginal swabs and catheterized urine samples from 186 women with mixed urinary incontinence (MUI) enrolled in a parent study and 84 similarly aged controls. Investigators decided a priori that if vaginal and/or urinary microbiomes differed between continent and incontinent women, the groups would be analyzed separately; if similar, samples from continent and incontinent women would be pooled and analyzed together. A central laboratory sequenced variable regions 1–3 (v1–3) and characterized bacteria to the genus level. Operational taxonomic unit (OTU) abundance was described for paired vaginal and urine samples. Pearson's correlation characterized the relationship between individual OTUs of paired samples. Canonical Correlation Analysis (CCA) evaluated the association between clinical variables (including MUI and control status) and vaginal and urinary OTUs, using the CCA function in the Vegan package (R version 3.5). Linear discriminant analysis effect size (LEfSe) was used to find taxa that discriminated between vaginal and urinary samples.

Results: Urinary and vaginal samples were collected from 212 women [mean age 53 (±11 years)] and results from 197-paired samples were available for analysis. As OTUs in MUI and control samples were related in CCA and since taxa did not discriminate between MUI or controls in either vagina or urine, MUI and control samples were pooled for further analysis. CCA of vaginal and urinary samples indicated that that 60 of the 100 most abundant OTUs in the samples largely overlapped. *Lactobacillus* was the most abundant genus in both urine and vagina (contributing on average 53% to an individual's urine sample and 64% to an individual's vaginal sample) (Pearson correlation r=0.53). Though less abundant than *Lactobacillus,* other bacteria with high Pearson correlation coefficients also commonly found in vagina and urine included: *Gardnerella* (r=0.70), *Prevotella* (r=0.64), and *Ureaplasma* (r=0.50). LEfSe analysis identified *Tepidomonas* and *Flavobacterium* as bacteria that distinguished the urinary environment for both MUI and controls as these bacteria were absent in the vagina (*Tepidimonas* effect size 2.38, $p<0.001$, *Flavobacterium* effect size 2.15, $p<0.001$). Though Lactobacillus was the most abundant bacteria in both urine and vagina, it was more abundant in the vagina (LEfSe effect size 2.72, $p<0.001$).

Conclusions: Significant associations between vaginal and urinary microbiomes were demonstrated with *Lactobacillus* being predominant in both urine and vagina. Abundance of other bacteria also correlated highly between the vagina and urine. This inter-relatedness has implications for studying manipulation of the urogenital microbiome in treating conditions such as urgency urinary incontinence and urinary tract infections.

Condensation:

Significant associations between vaginal and urinary bacterial microbiomes exist, largely due to presence of the genus *Lactobacillus*.

Keywords

Urologic conditions; lactobacillus; mixed urinary incontinence; urinary microbiome; vaginal microbiome

Introduction

The microbiome is important in genitourinary health and disease. Associations between the vaginal microbiome and diseases such as bacterial vaginosis, human papilloma and human immunodeficiency virus infections are well-known.[1,2,3] Likewise, the urinary microbiome is associated with lower urinary tract conditions such as urgency urinary incontinence,[4] recurrent urinary tract infections,[5] and responsiveness to therapy for lower urinary tract symptoms.[6,7] Although observations support associations between the microbiome and urogenital conditions, evidence exploring interactions between vaginal and urinary microbiomes is sparse.

Urinary pathogens are thought to stem from the gastrointestinal tract, with an intermediary step of vaginal colonization.[5,7] Manipulation of the vaginal microbiome may decrease occurrence of lower urinary tract disease suggesting that the vaginal microbiome also directly interacts with the bladder. For example, predominance of *Lactobacillus crispatus* in the vagina has been associated with lower risk of urinary tract infections.[5,6] Modifying the vaginal microbiome to treat lower urinary tract disease may provide a novel approach. However, the link between the vaginal and urinary microbiome must first be characterized.

The primary objective of this study was to compare the composition of the vaginal and urinary microbiome. The populations studied included women with and without urinary incontinence. The study not only compared potential differences and similarities in vagina and urine, it also explored whether the relationship between vaginal and urinary microbiomes differed in women with and without urinary incontinence. This work compared the overall relationship between vaginal and urinary microbiomes as well as their predominant taxa. We hypothesized that vaginal and urinary microbiomes, including predominant taxa, would be associated.

Materials & Methods

Study Population & Study Design

The methodology and inclusion and exclusion criteria for this study, an exploratory analysis comparing vaginal and urinary microbiomes, have been described.[8] Briefly, participants were non-pregnant, had not used antibiotics within the last month, were not using vaginal probiotics/spermicides, and were without urinary or vaginal infection symptoms (absent discharge, dysuria, pyuria). This IRB-approved (UNMHSC IRB#14–259, 10/14/14), multi-site, observational study enrolled women with MUI participating in a parent randomized trial of mid-urethral sling alone versus sling plus behavioral and pelvic floor therapy[9] and

similarly aged controls without MUI. All participants provided written consent. Paired vaginal swabs and catheterized urine samples were collected.[8]

Laboratory Methods

This study's laboratory methods for amplicon-based 16S rRNA gene sequencing have been described.[8] Other vaginal microbiome studies have amplified variable regions 1–3 (v1–3).[1,2,10,11] To avoid potential measurement bias arising from amplification of discrepant variable regions, this study amplified v1–3 regions for both urine and vaginal samples. Variable regions 4–6 were also amplified and only minor differences from v1–3 were noted; therefore, the v1–3 results are reported. Vaginal and catheterized urine samples were placed in nucleic acid protectant or Assay Assure® (Thermo Fisher Scientific, Waltham, MA) treated vials, respectively. Samples were shipped on dry ice within 24 hours and stored at −80° Centigrade prior to processing. DNA was extracted and PCR amplification was performed. Primers A17F and 515R amplified an ~500bp region of the 16S rRNA gene, with addition of the Nextera® (Illumina, San Diego, CA) linker sequence, allowing generation of Illumina sequencing libraries.[8,12] Libraries were pooled in equimolar ratios sequenced on Illumina Miseq® with 300bp paired end reads and v3 sequencing chemistry. Amplicon sequences were classified through alignment and comparison to the GreenGenes database using the Ribosomal Database Project Classifier.[13,14]

Statistical & Bioinformatics Analysis

Bacterial taxa were evaluated to the genus level. Because distribution of Lactobacillus species in urine and vagina may be clinically relevant,[1,2,4,5] the study also more fully described *Lactobacillus* to the species level.[8] Genus relative abundance (referred to as OTU abundance) was quantified based on contributions of a specific genus to each of the individual paired urine and vaginal samples. Alpha diversity calculations for urine and vagina were reported using Shannon indices. Alpha diversity results were compared using paired t-tests.

Separately for vaginal and urine samples, OTUs that contributed greater than 2–3% of the total OTUs of at least one sample were considered for further analysis. This choice was made pragmatically as these bacteria typically represented the 20–25 most abundant OTUs overall. Abundant OTUs were described by their mean, minimum and maximum contributions to the vaginal and urine samples.

Pearson correlation assessed the strength of association in the relative abundance of individual OTUs between paired vaginal and urine samples from the same participant. This was performed for the most abundant genera and for the *Lactobacillus* species. Pearson correlation was also used to calculate specific relationships between age and OTUs, further refining the multivariate analysis findings. Results were reported as r-values..

Canonical Correlation Analysis (CCA), a dimensionality reduction method,[13] was used to evaluate multivariate correlations between clinical characteristics and pooled vaginal and urinary OTUs. CCA was also used to assess whether MUI or control group membership correlated with OTUs.[14] The investigative team agreed a priori that if CCA found that the vaginal or urinary OTUs differed between MUI and controls, MUI and control OTUs would

be described separately; whereas if CCA found that OTUs did not differentiate MUI and controls, they would be pooled and described in aggregate. The latter alternative, by increasing the sample size, would offer greater power for the multivariate analysis. Clinical characteristics assessed for correlation with OTUs using CCA were participant age and body mass index (BMI), as these have been associated with differing OTUs.[15] Age, rather than menopausal status, was used in the multivariate analysis because approximately 20% of participants were of uncertain menopausal status[15] and because age, as a continuous variable, offered greater power to discriminate OTU differences in the multivariate analysis.

OTUs were filtered to include the 100 most abundant in vagina and urine. CCA was performed using the CCorA function in the Vegan package (software R version 3.5).[16] The resulting canonical axes represented linear combinations of each group of input variables that were maximally correlated with each other. To explore which characteristics and OTUs were responsible for correlations, the envfit function was used to obtain the correlation between each characteristic or OTU and the CCA axes, and these correlations were plotted. Associations between clinical characteristics and OTUs were further assessed by calculating the Pearson correlation between them. LEfSe[17] was used to find OTUs that discriminated between vagina and urine. A LEfSe, a score > 2.0 was considered significant. Counts for OTUs with significant LEfSe scores were normalized to the approximate mean sum of all OTU counts per sample to minimize false positives, or inflated results. The threshold for statistical significance for all other evaluations was $P<0.05$.

Results

Participants, OTU Abundance, Correlations between Paired Vaginal & Urine Samples

Urine and vaginal samples were obtained from 212 women (128 MUI, 84 controls) between January 2015-April 2016. A total of 197 paired urine and vaginal samples were available for analyses. Participant clinical characteristics have been described[15]: the majority of women were white, non-smoking, aged 53 (±11) years with a BMI ranging from overweight to obese.[15]

Urinary bacterial 16S rRNA v1–3 gene sequencing resulted in a median sequencing read depth of 90,805 reads per sample (range 10,572–734,491) and classification of 28 phyla, 58 classes, 114 orders, 245 families, and 705 genera. Vaginal bacterial 16S rRNA gene sequencing resulted in a median sequencing read depth per sample of 121,894 reads (range 25,950–660,243) and classification of 24 phyla, 45 classes, 91 orders, 195 families, and 476 genera.

There were no differences in OTUs between MUI and controls. In CCA, the centroid ellipses of MUI and control OTUs were virtually indistinguishable (Supplement Figure 1); thus MUI and control samples were combined for the remaining analyses.

Alpha diversity was greater in urine samples (Shannon index mean 1.27±0.88) relative to vaginal samples (Shannon index mean 0.80±0.75), $P<0.001$. Table 1 lists the most abundant OTUs in either vaginal or urine samples, with nine of these (*Lactobacillus, Streptococcus, Prevotella, Escherichia, Ureaplasma, Shuttleworthia, Gardnerella, Veillonella, Sneathia)*

present in both vagina and urine. *Lactobacillus* contributed, on average, 53% to the make-up of an individual's urinary and 64% to an individual's vaginal samples.

Figure 1 illustrates the most abundant genera in paired samples for each participant and gives the Pearson correlation for each OTU between the two sites. Women are sorted from left to right according to the percentage of Lactobacillus in their urine specimen, and the figure shows the paired samples from each person (urine in Figure 1a and vagina in Figure 1b). Many women had high levels of *Lactobacillus* in both sites, and there was moderate Pearson correlation (r=0.53) between the levels of *Lactobacillus* in paired urine and vaginal samples. Other OTUs with moderate to high Pearson correlation coefficients between vagina and urine included *Gardnerella* (r=0.70), with the highest correlation, followed by *Prevotella* (r=0.64), *Ureaplasma* (r=0.50), and *Escherichia* (r=0.47).

Lactobacillus Species

Evaluation of *Lactobacillus* species identified presence of six predominant species in the urine and vagina including: *L. iners* (urine=39%, vagina=35%), *L. crispatus* (urine=18%, vagina=16%), *L. gasseri* (urine=13%, vagina=14%), *L. jensenii* (urine=7.1%, vagina=5.5%), *L. taiwanensis* (urine=11%, vagina=14%), *L. ultanensis* (urine=8.3%, vagina=9.7%). Because *L. ultanensis* and *L. taiwanensis species* have not been routinely reported in previous vaginal or urinary microbiome studies, we further investigated the possibility of misclassification of these *Lactobacillus* species. We completed identical sequencing and bioinformatics processing of a mock community consisting of known quantities of only *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii.* Indeed, a small percentage of sequencing reads from the mock community (<3%) were classified as *L. ultanensis, L. taiwanensis,* indicating minor bioinformatics misclassifications.

Lactobacillus sp.—Pearson correlation in paired vaginal and urine samples demonstrated correlation coefficients >0.50 for *L. iners* (r=0.72), *L. crispatus* (r=0.67), *L. jensenii* (r=0.77), and *L. gasseri* (r=0.73). Correlations were lower for *L. ultanensis* (r=0.41) and *L. taiwanensis* (r=0.37).

Multivariate Analysis

Figure 2 illustrates correlations with the CCA axes for clinical characteristics and OTUs. In this figure, associations can be interpreted based on vector length (longer vectors represent stronger correlations with one or both of the CCA axes) and direction (positive associations between vectors in the same direction, and negative associations for vectors in opposite directions). The first axis is strongly associated with the site of the sample (vagina or urine). *Flavobacterium* and *Tepidimonas* were also correlated with the first axis; both are more abundant in urine and almost absent from the vaginal samples. This was reinforced by the LEfSe analysis in which these two OTUs differentiated the urinary environment (*Tepidomonas* effect size 2.38, p<0.001 and *Flavobacterium* effect size 2.15, p<0.001). *Lactobacillus'* was negatively correlated with the first CCA axis, consistent with a greater presence in the vagina compared to urine. *Lactobacillus* also differentiated the vaginal environment on LEfSe: despite the fact that this genus is the most abundant in both urine

and vagina, it distinguished the vaginal environment due to its greater abundance in vagina compared to urine (effect size 2.72, p<0.001).

The second CCA axis showed a negative association between age and *Lactobacillus* (Figure 2); the Pearson correlation between the two was r=−0.30 in urine and r=−0.28 in the vagina. Other vaginal OTUs also associated with age (i.e. comparable r-values) but of far lesser abundance included *Peptoniphilus* (r=0.33), *Corynebacterium* (r=0.31), *Luteococcus* (r=0.30), *Finegoldia* (r=0.30), and *Marinobacter* (r=0.29), all of which increased with age. In urine, *Jannaschia* increased with age (r=0.29). In contrast to *Lactobacillus,* these OTUs only contributed a mean of >0%−1% each to the samples. BMI was not associated with OTUs in CCA (Figure 2).

Comment

Main Findings

The results of this study demonstrate a definitive association between vaginal and urinary genera based on 16S rRNA gene sequencing. This was true irrespective of MUI or control status, as bacteria in the vaginal and urinary microbiomes largely overlapped based on both CCA and descriptive analysis. On average, *Lactobacillus* contributed > 50% to the composition of individual samples, and by virtue of its abundance, was largely responsible for overlap between urine and vagina. Overlap was also due, though to a lesser extent, to bacteria that contributed a mean of <7% to individual samples, including *Streptococcus, Prevotella, Ureaplasma, Shuttleworthia, Escherichia, Veillonella, Sneathia and Gardnerella*. OTUs with the highest correlation coefficients between paired vaginal and urine samples included some of these same bacteria: *Gardnerella, Prevotella, Ureaplasma* and *Lactobacillus*. These findings indicate that the vagina and bladder share a common urogenital microbiome that may serve as potential reservoir for manipulation in treating lower urinary tract conditions.

Clinical Implications

This study's results are similar to another report in peri-menopausal women, with respect to vaginal *Lactobacillus* species.[10] The current results are enriched by the finding that the presence of these similar *Lactobacillus* species were highly correlated between the vagina and urine. The current analysis also differentiated bladder from vagina by the lack of *Tepidomonas* and *Flavobacterium* in the vagina. *Lactobacillus* also differentiated vagina and bladder; although the most abundant OTU in both vagina and urine, it was more abundant in vagina.

The only clinical characteristic that correlated with a specific OTU was age. *Lactobacillus* had the strongest relationship to age. Increased age was associated with decreased *Lactobacillus* in both vaginal and urinary microbiomes, as reported in other studies.[5,18] The identification of the high predominance of *Lactobacillus* in both urine and vaginal niches and its decrease with aging may serve as theoretical basis for future studies examining the role of *Lactobacillus* in treatment and prevention of common urinary conditions. Notably,

other vaginal and urinary OTUs that increased with age were of low abundance in both environments and are of uncertain significance.

The current work specifically addressed relationships between vaginal and urinary microbiomes using 16S rRNA sequencing in 197 samples, the largest collection of paired vaginal and urinary samples available for analysis. A prior study evaluated the microbiota of urine from 38 asymptomatic and 39 symptomatic women compared to publicly available vaginal and gastrointestinal microbiome data, and reported that 23 common species were found in both bladder and vaginal microbiota.[23] That study also showed that shared protein functions existed between the bladder and vagina. Although of high interest, the vast majority of samples studied in that report were from unrelated individuals of diverse and uncharacterized populations. Findings from the previously reported study are difficult to compare to our current study, given differences in study design. Nonetheless, both studies found overlap between vaginal and urinary microbiomes, supporting our conclusion that the two are inter-related.

Our findings of similarity between bladder and vaginal OTUs, coupled with the work by others, also suggest these niches and their OTUs interact and affect urogenital health. For example, Fok reported that presence of *Atopobium vaginae* in urine correlated with its presence in the vagina and perineum.[22] Higher overactive bladder symptoms were associated with higher urinary abundance of *Atopobium vaginae,* suggesting OTUs in these anatomic sites may influence organ function/dysfunction. As another example, a systematic review concluded that vaginal estrogen improved genitourinary syndrome of menopause symptoms in both the vagina and bladder, decreasing vaginal dryness, dyspareunia, UUI, stress urinary incontinence and urinary tract infections (UTIs).[24] Researchers hypothesize that vaginal estrogen and increased *Lactobacillus*[25] (and specific *Lactobacillus* species [4,5,22,23]) improve vaginal and bladder health,[5,6,24,26,27] and that *Lactobacillus* decreases vaginal dysbiosis by maintaining an acidic pH via hydrogen peroxide production or other mechanisms.[26,27] The importance of vaginal and urinary *Lactobacillus* and *Atopobium* are but two examples of the clinical relevance of OTU similarity between these neighboring environments. It is possible that other vaginal and bladder associated OTUs may be future targets for clinical interventions. OTU similarity between these neighboring niches and their observed clinical associations, suggests an intriguing possibility that the microbiome of the vagina and bladder not only interact, but that they represent a shared urogenital microbiome.[8,23]

The current study characterized *Lactobacillus* to the species level using 16S rRNA sequencing and found four species that correlated highly between vagina and urine: *L. iners*, *L. crispatus*, *L. jensenii*, and *L. gasseri*. These four species are characteristic of the vaginal microbiome and have been well documented.[1,2,10] Previously, their presence in urine has been less commonly reported using 16S sequencing techniques, but has been documented in urine using an expanded quantitative urine culture (EQUC) protocol.[23,28] The *L. species* identified in the current microbiome analysis are consistent with previous vaginal 16S and urinary EQUC findings and are noteworthy for their demonstration of a significant correlation of these bacterial species in vagina and urine. The current classification methodology identified a small proportion of *L. ultanensis, L. taiwanensis*; however, this should be interpreted with caution as further evaluation revealed that a minority of

sequencing reads from *L. iners*, *L. crispatus*, *L. jensenii*, and *L. gasseri* could be misidentified as *L. ultanensis* or *taiwanensis.*

Classification of bacteria to a more granular level, such as to the species level rather than the genus level, is increasingly important in examining the impact of bacteria on health and disease. For example, the genus *Prevotella* has been known to be associated with bacterial vaginosis, but recently, the specific species, *Prevotella bivia* has been more specifically implicated in bacterial vaginosis.[19,29] Furthermore, classification of *Escherichia coli* to the sub-species, or strain level, has allowed identification of the virulent strains (ie. UPEC or uropathogenic *E. coli*) associated with urinary tract infections.[21]

Regarding urogenital health, it may be insufficient to merely classify the relative abundance of *Lactobacillus* to the genus level and assume that dominance is associated with health. Evidence suggests that specific species of *Lactobacillus* may be associated with differences in health. Understanding protective or deleterious associations between *L. species* and urogenital abnormalities is in its formative stages with unclear and conflicting findings. For example, *L. iners* has been reported to have both positive and negative health-related associations. In the vagina, evidence suggests *L.iners* may be associated with dysbiosis and increased susceptibility to certain sexually transmitted infections such as HIV-1 and Chlamydia.[30,32,31] In contrast, *L. iners* in the bladder has been interpreted to be protective against post-operative urinary infections in women undergoing pelvic reconstruction.[7] As associations cannot be assumed to be causal, some have suggested that the associations between L. *iners* and disease could reflect its environmental adaptation to specific niches, rather than reflect a causal role in disease.[33] Clearly, further work to the species level is required to refine our understanding of bacterial contributions to health.

Similar to our previous publication, the current study did not find an overall difference in OTUs between MUI and controls.[15] A post-hoc analysis of the prior work, however, did find differences in a sub-group of samples comparing younger MUI and controls.[15] When samples were categorized into “community types” using Dirichlet multinomial mixture methods, a high-*Lactobacillus* (89.2% *Lactobacillus*) community type had a greater proportion of controls relative to MUI in women younger than 51 years. Clustering samples into smaller, specific communities (as done in the prior work) may have a role in distinguishing samples by certain clinical characteristics, but these sub-groups represent only a portion of an entire population. The current study considered overall OTU abundance in all samples of the entire population; MUI and control samples were similar, and vaginal and urinary samples were strikingly similar.

Study Strengths & Weaknesses

Limitations of this study include that its population largely consisted of women in their 50’s with and without MUI and identified most bacteria to the genus level. Despite the fact that the current study found OTUs were not associated with MUI or control status, future study of matched vaginal and urinary samples to the species level in large groups of women across a larger spectrum of ages and bladder health is warranted. Additionally, the limitations and bias of 16S rRNA gene sequencing are well documented.[34,35] Lack of consensus regarding DNA extraction methods, variable regions used, sequencing read length and depth, and

bioinformatics methodologies limits comparative study of the urogenital and other microbiomes. In order to compare results across different studies, it is critical to understand how samples collected from different laboratories, different niches, using different methodologies, influence study findings. To this end, the current study used matched vaginal and urine samples from the same individuals, assessed the microbiome using the same laboratory methodology, and found a high concordance between the two niches. Study strengths include its large number of well-characterized participants who simultaneously provided matched catheterized urine and vaginal specimens, using a standardized protocol. Controls and cases were of similar age, which is particularly important, as *Lactobacillus* abundance has been demonstrated to be age-related.

Conclusion

In summary, this study demonstrates definitive associations between vaginal and urinary genera. *Lactobacillus* was abundant in both urine and vagina. Presence of other bacteria also correlated highly between the vagina and urine. This inter-relatedness has implications for future treatment of urogenital conditions, although gaps remain in advancing knowledge regarding the urogenital microbiome into clinically effective treatment. Future studies should address microbiome-based biomarkers developed to predict disease and treatment response.[36]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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AJOG at a Glance:

A. The relationship between vaginal and urinary microbiomes has not been fully established.

B. In both women with mixed urinary incontinence and asymptomatic controls, there are strong similarities between vaginal and urinary microbiomes.

C. The association between urinary and vaginal microbiomes is largely due to *Lactobacillus,* although other bacteria also contributed. These associations may have implications for future treatment strategies and microbiome research in women.

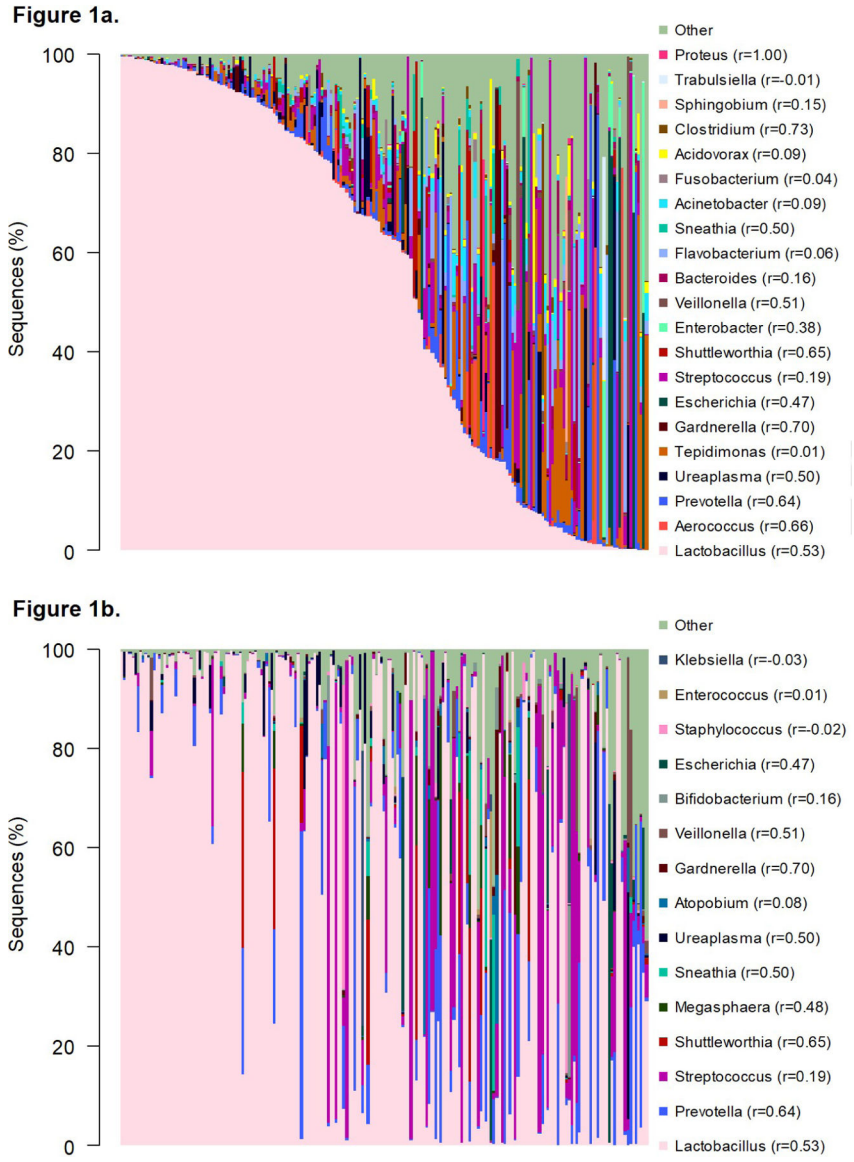

Figure 1. Direct Comparison of OTU Distribution for paired urine and vaginal samples 1a. Urine samples. 1b. Vaginal samples.

Urinary samples for each participant represented in 1a with the paired vaginal samples directly below in 1b, and most abundant OTUs are represented. R-values for the OTUs correlating presence in paired vaginal and urinary samples are represented to the right of the specific OTUs (in parentheses). Note: 1a. *Proteus* r=1.0 represents measured values in a single subject.

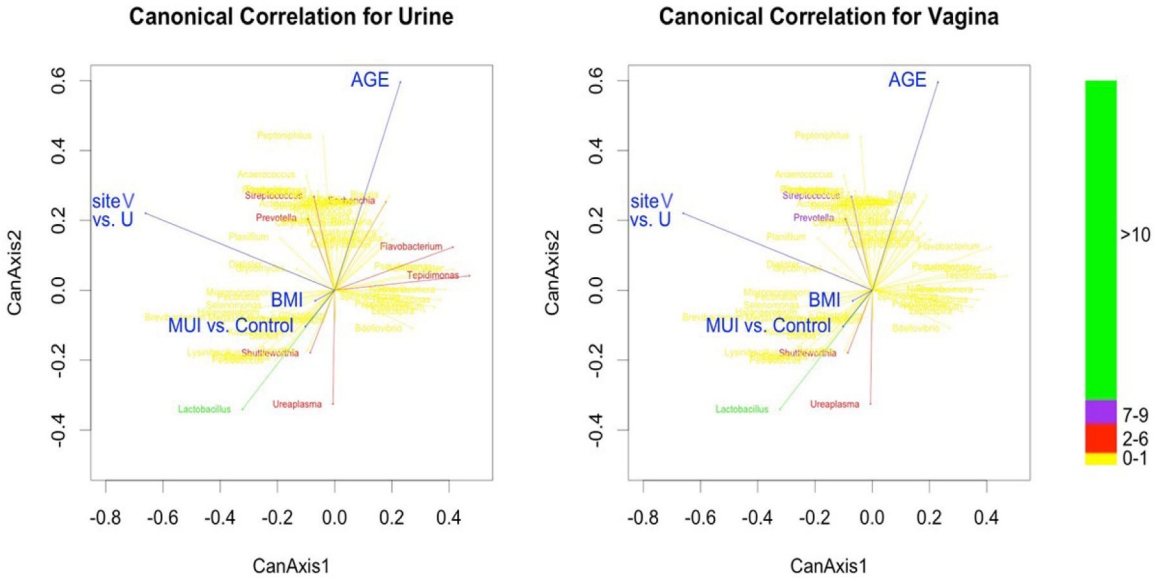

Figure 2. Canonical correlation of OTUs pooled across urine and vaginal samples

(color-coded by percent contribution to urine [left panel] or vaginal [right panel] samples of 0 to >10% [from Table 1]) vs. variables (in blue: MUI status, site [represented by Vagina (V) versus Urine (U)], age, BMI). Correlations with the first two canonical axes (representing linear combination of OTUs and clinical variables that were maximally correlated with each other) are plotted. A cluster of OTUs (including *Flavobacterium* and *Tepidimonas*) are negatively correlated with vaginal site (almost absent in vagina, but more abundant in urine). There was a strong negative correlation between age in both vagina and urine (e.g. as age increased, abundance of the *Lactobacillus* decreased in both sample types).

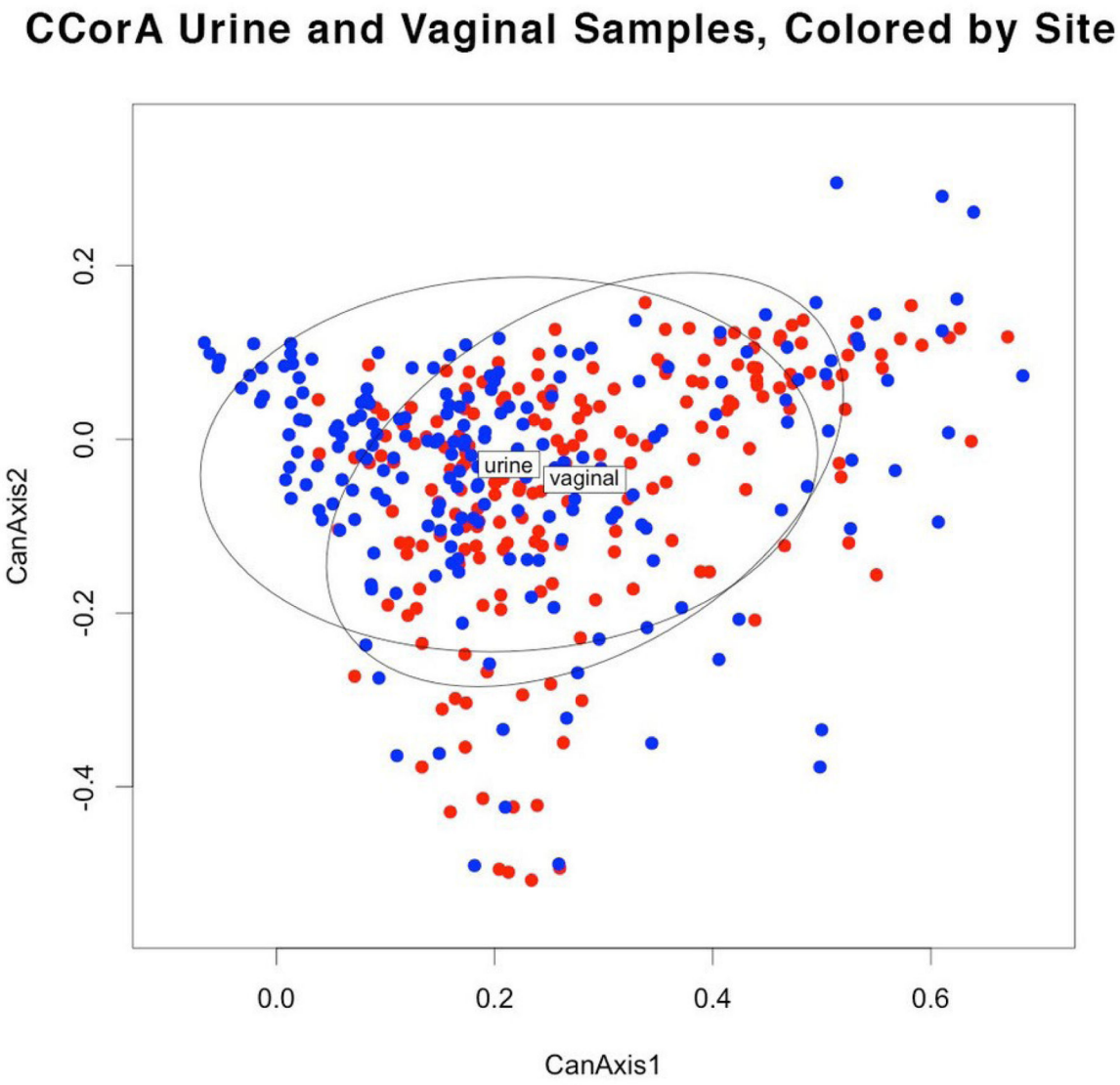

Figure 3. Canonical correlation of urine (blue) vs. vaginal (red) samples.
Centroid ellipses are standard deviation, 75% confidence intervals. Vaginal and urine samples are closely related; urine samples show a cluster in the upper left that does not overlap with vagina.

Table 1.

Contribution of Most Abundant Bacterial Genera/OTUs to Individuals Urine and Vaginal Samples

Genera	Percent Contribution in Urine Samples			Percent Contribution in Vaginal Sample		
	Mean	Min	Max	Mean	Min	Max
Lactobacillus	53.20	0.05	99.52	64.25	0.11	99.60
Streptococcus	5.17	0.00	95.86	9.58	0.01	98.02
Tepidimonas	5.06	0.00	65.98			
Prevotella	4.32	0.00	87.62	6.96	0.00	81.75
Flavobacterium	3.02	0.00	58.11			
Escherichia	2.50	0.00	79.96	1.25	0.00	68.01
Ureaplasma	2.48	0.00	46.85	1.70	0.00	45.70
Shuttleworthia	1.59	0.00	55.35	1.59	0.00	39.19
Aerococcus	1.41	0.00	76.54			
Gardnerella	1.34	0.00	74.25	0.60	0.00	29.17
Veillonella	0.97	0.00	73.63	0.86	0.00	37.21
Bacteroides	0.89	0.00	53.84			
Enterobacter	0.81	0.00	46.32			
Acidovorax	0.76	0.00	38.30			
Sneathia	0.73	0.00	32.00	0.78	0.00	35.51
Clostridium	0.45	0.00	54.66			
Fusobacterium	0.40	0.00	41.11			
Sphingobium	0.29	0.00	53.59			
Proteus	0.29	0.00	54.71			
Trabulsiella	0.24	0.00	45.00			
Staphylococcus				0.78	0.00	74.40
Atopobium				0.76	0.00	67.62
Klebsiella				0.52	0.00	55.44
Bifidobacterium				0.36	0.00	34.56
Enterococcus				0.18	0.00	25.61
Megasphaera				1.11	0.00	43.99
Other	14.08	0.32	65.38	8.72	0.25	58.46