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EVALUATION OF THE URINARY MICROBIOTA OF WOMEN WITH UNCOMPLICATED STRESS URINARY INCONTINENCE

Hypothesis / aims of study

The influence of the human microbiota on health and disease is increasingly appreciated. The urinary microbiota of adult women with stress urinary incontinence (SUI) has not been investigated. This analysis used a biorepository of urine samples collected from a large, multi-center, National Institutes of Health sponsored clinical trial of women with uncomplicated SUI planning surgery to characterize the cross-sectional relationships between urinary microbiota parameters and demographic and clinical characteristics of adult women with SUI.

Study design, materials and methods

Participants provided written research consent to contribute a single baseline urine specimen to the biorepository. Eligibility included adult women who reported symptoms of SUI ≥3 months, had a post-void residual <150 mL, a negative urinalysis/standard urine culture, clinical assessment of urethral mobility, desire for SUI surgery, a positive provocative stress urinary test and a qualifying Medical, Epidemiologic, and Social Aspects of Aging (MESA) questionnaire [1] subscale score (stress > urge). Demographic and clinical characteristics were obtained by self-report including hormonal status, which was categorized by the study team into the hormone group that most appropriately described the patient's hormone use: pre-menopausal, post-menopausal (with or without self-reported, current exogenous hormone use) or uncertain about status.

Urine specimens were collected prior to surgery by a standard protocol and tested by dipstick to exclude UTI at study entry. Specimens were centrifuged and the supernatant frozen at -80°C until shipped on dry ice to the National Institute of Diabetes and Digestive and Kidney biorepository. Available baseline urine specimens (174 clean catch samples, 23 catheterized samples) were stored at -80°C until processed for sequence analysis.

Microbial composition was determined by sequencing the variable 4 (V4) region of the bacterial 16S rRNA gene, as described [2]. DNA isolation was performed in a laminar flow hood to avoid contamination. Genomic DNA was extracted from 1 ml of urine, using validated protocols [2]. The V4 region was amplified by a two-step polymerase chain reaction (PCR), using modified universal primers 515F and 806R. Each specimen was sequenced in duplicate and classified by phylogenetic diversity as measured by Bray-Curtis dissimilarity. A phylogenetic tree was generated and compared to percent total classified reads (relative abundance) at each taxonomic level (phyla, class, order, family, genus). For a genus level example, see Figure 1.

Each major branch or clade (termed urotype) in the phylogenetic tree was named for the predominant classified taxon (e.g., *Lactobacillus*). When there was no predominant taxon, we used the term "non-predominant" to describe the urotype. Due to read depths less than 2000, two samples from replica 1 and one sample from replica 2 were also classified as "below the detection threshold", for a total of 28 in replica 1 and 27 in replica 2.

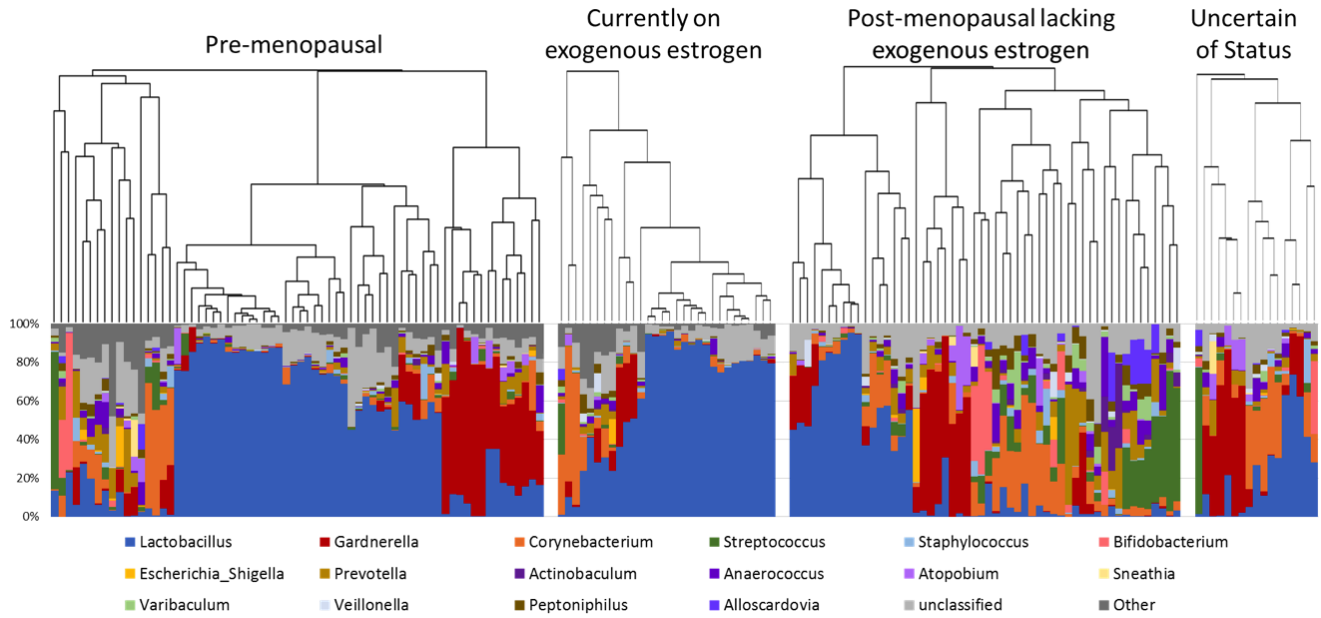
Generalized Estimating Equations (GEE), extensions of Generalized Linear Models that account for correlation between replicas, were used to describe associations between demographic and clinical factors with diversity measurements after adjusting for genus urotype. There was insufficient sample size and power to compare urotypes between catheterized and voided samples. All statistical analyses were conducted using SAS v9.4 (SAS Software Cary, NC) and statistical significance was assessed at the α=0.05 level.

Results

The majority of 197 participants were non-Hispanic Caucasian (79%) and currently married (74%) with a mean age of 51 (SD:9.7) years. Bacterial diversity was significantly associated with higher BMI ($p=0.02$), increased MESA urge index score ($p=0.04$), and hormonal status ($p<0.001$). Post-menopausal women not on exogenous hormones had more diversity than did pre-menopausal women as measured by the Peilou evenness index ($p<0.001$), a measure of the total number of unique taxa within a given individual without accounting for distribution of those taxa. Because community evenness associates strongly with hormonal status, we constructed a visual comparison of microbial diversity subdivided by hormonal use (Figure 1). Compared to other groups, the hormone-positive women (pre-menopausal and post-menopausal on exogenous hormones) had a higher frequency of *Lactobacillus* or *Gardnerella* urotypes (66%) and a lower frequency of 'non-predominant' urotypes, while the hormone-negative women (post-menopausal not on exogenous hormones) had a lower frequency of *Lactobacillus* or *Gardnerella* urotypes (38%) and greater frequency of 'non-predominant' urotypes ($p<0.001$).

Figure 1: Phylogenetic diversity and urotype distribution between estrogen status

Relative abundance of the microbial community at the genus level for the 4 estrogen groups. Each bar is a separate individual with the percent of total classified reads to the genus level represented on the y-axis. Phylogenetic relatedness as measured by Bray-Curtis dissimilarity is depicted in the dendrograms above each group.



Interpretation of results

Hormonal status appears to be related to FUM diversity/evenness in this study of women undergoing surgery for uncomplicated SUI. Menopausal women not on exogenous hormones had increased microbial evenness and their FUM was less likely to be predominated by a single microbe. These results suggest that predominance (most often by *Lactobacillus* species) is typical of hormone-positive women.

We observed an association between microbial diversity/evenness and UUI symptoms despite the possibility of vulvo-vaginal contamination in these voided urine samples, similar to catheterized urine samples from women with UUI but not SUI [2, 3]. The cause versus effect relationship between UUI and microbial diversity/evenness will require further study. It is plausible that *de novo*/persistent UUI could be predicted prior to surgery using the baseline FUM. This study also provides evidence that voided urine samples can be usefully analyzed, despite potential vulvo-vaginal contamination, with one important caveat. The FUM detected in pre-surgical voided urine samples of this cohort was similar to those assessed in catheterized samples obtained from other cohorts of women [2, 3].

Concluding message

Women undergoing SUI surgery have detectable urinary microbiota. The diversity/evenness of the microbiota was associated cross-sectionally with hormonal status, UUI symptoms and BMI. Voided urine samples appear feasible for certain future FUM studies.

References

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Disclosures

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