Introduction

Lower urinary tract disorders [e.g., urinary tract infection (UTI), urinary incontinence (UI), overactive bladder (OAB) & bladder pain syndromes] are highly prevalent in American women. Unfortunately, reliance on culture-dependent techniques to assess urinary bacteria has severely limited understanding of the urinary tract in health and disease, as the vast majority of bacteria are not cultured by routine clinical laboratory techniques.

We previously reported our results, obtained using culture-independent high-throughput DNA sequencing, to document that "normal" urine obtained directly from the female bladder often is not "sterile" (1). Other groups have used similar approaches to provide culture-independent evidence of bacterial communities (microbiota) in the urine of apparently healthy women, affected female patients and adolescent/young men (1-4). The exciting discovery of the female urinary microbiota (FUM) opens a novel line of potentially high-impact investigations that should enhance our understanding of etiology, prevention and treatment of all lower urinary tract disorders. To date, DNA sequence-based (microbiome) approaches have not been meaningfully incorporated into urinary research.

We also recently reported the use of quantitative PCR (qPCR) to detect the presence of urinary microbiota in adult women with urgency urinary incontinence (UUI) who were participants in the NICHD-sponsored Anti-cholinergic versus Botulinum Comparison (ABC) trial, a registered, randomized trial of intravesical botulinum A versus oral UUI medication (5) for treatment of UUI. We found that, at baseline, daily UUI episodes were significantly greater in qPCR positive participants [5.70 (±2.60) vs. 4.73 (±2.86), P=0.041].

Objectives

The aim of this study was to determine whether urinary microbiota are associated with specific UI subtypes.

Methods

With IRB approval, we obtained catheterized urine samples from women segregated into 4 different UI subtypes by symptoms [pure stress urinary incontinence (SUI), predominately SUI (SUI>SUI), predominately UUI (UUI>SUI), and pure UUI] using the Pelvic Floor Distress Inventory (a validated symptom questionnaire). Each sample was split: one portion was sent for conventional urine culture and Gram stain analysis; the remaining portion was sent for DNA sequence analysis using the 454 platform to sequence the V1-V3 regions of the 16S rRNA gene of each bacterium. The detected 16S rRNA sequences were first classified by taxonomy, permitting us to identify the bacterial community (microbiota) in each sample. These microbiota were compared using a statistical method called Advanced Principal Coordinate Analysis (PCoA), which determined the degree of similarity amongst the microbiota in the patient samples. To validate our results, we re-analyzed the same 16 samples by sequencing the V4 region of the 16S rRNA gene using MiSeq, a newer sequencing platform that provides increased sensitivity over the 454 method because it yields many more sequences per sample.

Results

Principal Coordinate Analysis (Figure 1) of V1-V3 sequences obtained by the 454 method shows that the microbiomes of UUI and UUI>SUI patients (Aqua & Blue) differ from those of SUI and SUI>SUI patients (Red & Lime). Patients with UUI symptoms fell into at least two distinct groups.

Another way to assess the difference of the bladder microbiota in distinct UI subtypes is to measure the taxonomic difference in terms of abundance. Given the closer clustering of SUI and UUI>SUI microbiota in Figure 1, it is not surprisingly that this second analysis using the MiSeq 2X250bp data set not indicated that the microbiota of these 2 cohorts were less diverse than those of the UUI and UUI>SUI cohorts. As an example, Figure 2 shows 4 taxonomic units at three different taxonomic levels (order, class and family). With 3 of these 4 taxa, the UUI cohorts tended to be more diverse than the SUI cohorts.

Conclusion

These preliminary data are consistent with the hypothesis that bladder microbiota differ among different UI subtypes and that differing components or entire bacterial communities could affect UUI symptoms.

References