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EVIDENCE FOR BACTERIAL PRESENCE IN THE ADULT FEMALE BLADDER

Hypothesis / aims of study

The etiology of many lower urinary tract disorders is unknown. Unlike many areas of the human body, the normal urinary tract is assumed to be sterile using culture-dependent methods. While urine cultures are currently the gold standard for clinical assessment of bacteria in the urinary tract, culture-dependent techniques are of limited value because the vast majority of bacteria are not cultured.

The aims of this study were 1) to determine if a urinary microbiome exists in adult women (i.e. do bacteria typically reside in the urinary tracts of women?), 2) to compare transurethral catheterization (TUC) and suprapubic aspirate (SPA) methods of urine collection for bacteriologic research in women and 3) to generate pilot data that compare the urinary microbiome of women without urinary symptoms to the urinary microbiome of those with prolapse (POP) and/or urinary incontinence (UI) symptoms.

Study design, materials & methods:

Subjects & Sample Collection: Participants were women undergoing surgery for treatment of POP and/or UI (POP/UI subjects) and a comparison group of women undergoing benign gynecologic surgery (Controls). All subjects completed the long-version of the Pelvic Floor Distress Inventory (PFDI) and did not have urinary tract infection (urine culture negative & absence of clinical UTI diagnosis). POP/UI participants had at least one bothersome POP/UI symptom, while control participants endorsed no POP/UI symptom on the PFDI. After preparation of the suprapubic skin, perineum and vagina with iodine and prior to administration of peri-operative antibiotics, we collected four samples: 1: suprapubic skin swab; 2: needle passed through suprapubic skin and subcutaneous tissues but not into the bladder; 3: urine by SPA; 4: urine by TUC. The urine samples were divided: 5 mL were sent to the Clinical Microbiology Laboratory for culturing and the rest placed at 4°C for microbial DNA isolation.

Microbial DNA Isolation: Within 24 h, the urine samples were centrifuged and the pellet re-suspended in DNA stabilization buffer. The swabs were washed with sterile phosphate buffered saline supplemented with DNA stabilization buffer. Both types of samples were maintained frozen at -80°C until microbial DNA isolation and sequence analysis. Isolation of DNA from urine specimens and swabs were performed in a laminar flow hood, according to strict SOPs developed through the Human Microbiome Project consortium. Genomic DNA (gDNA) was isolated from urines using the Qiagen DNeasy tissue extraction kit. gDNA was stored at 4°C until 16S PCR amplification and was quantified by Quant-It fluorescent dsDNA assay.

Bar-coded Multiplex 16S rDNA Pyrosequencing: A bar-coded multiplex V1-V3 pyrosequence approach was used with specific refinement for use with urines. From pyrosequencing reads, we trimmed primers & discarded poor quality sequences. We removed human sequence contamination identified by BLAST against the NCBI human genome database. We grouped 16S rDNA sequences into operational taxonomic units (OTUs), using the MOTHUR package (www.mothur.org) to identify OTUs with different identity cut-offs. We assigned bacterial taxonomy using RDP Classifier. We computed diversity & richness indices (e.g. Shannon and Chao I) using the EstimateS program.

Interpretation of results

From 12 culture-negative POP/UI patients and 12 culture-negative controls, we obtained urines by TUC and SPA. Because SPA gains access to the bladder via the abdomen, we controlled for skin contamination with skin swabs and needle sticks. All of the controls and 10 of the 12 POP/UI patients had molecular evidence of urinary bacteria. Using a multiplex V1-V3 pyrosequencing protocol, we obtained a total of 425,849 high-quality sequences for subsequent analysis. For each of these sequences, we assigned taxonomic classifications using RDP Classifier v.2.2 at 90% confidence. In total, from all samples, we identified 395 genera from 22 phyla.

Both TUC and SPA samples contained bacterial gDNA evidence for the presence of bacteria and there appeared to be no obvious difference between the control and POP/UI cohorts. For controls, we obtained 166 genera from TUC (0.34% genera/reads) and 227 from SPA (0.42%). For the POP/UI patients, we obtained 153 from TUC (0.42%) & 192 from SPA (0.47).

The TUC and SPA microbiomes resembled each other. Only 22 genera out of 307 total (<7.2%) were present in different relative abundance in TUC and SPA ($p<0.05$). Only 2 represented a substantial proportion of the microbiome [>1% sequence reads in either TUC or SPA (*Burkholderia* 2.9% in SPA, and *Lactobacillus* 6.2% & 24.5% in SPA & TUC, respectively)]. The rest represented a tiny fraction of the urinary microbiome or their significant difference reflects unreliable measurement due to their low abundance. Thus, they do not represent a substantial impediment to using TUC sampling, which does not require anesthesia, in lieu of SPA, which does.

Concluding message

The confirmatory evidence of a urinary microbiome from high-quality samples, in the absence of known UTI, may provide insight into the etiology of certain lower urinary tract conditions. The clinical implications of these findings require further study.

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Was this study approved by an ethics committee?	**Yes**
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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**