**Introduction**

Our current understanding of the pathophysiology of benign prostatic enlargement (BPE) and lower urinary tract symptoms (LUTS) relies on the entrenched dogma that the bladder is sterile. This has recently been invalidated by numerous reports describing the existence of bacterial communities (microbiota) in urine obtained in the absence of clinical urinary tract infection (UTI). Additional studies provide evidence that female bladder microbiota may have clinical implications in UUT response to OAB treatment, and risk of post-instrumentation UTI. No published study to date has explored relationships between male lower urinary tract microbiota (LUTM) and BPE-associated LUTS.

Prior work from our group noted that the bacterial DNA detected in catheterized urine closely resembles that obtained by SPA and diverged substantially from mid-stream voided urine. Another study found that the microbiome of male voided urine closely resembled that of distal urethral swabs. Thus, catheterized urine samples may reflect the bladder microbiome. In most patients without clinical UTI, standard urine culture does not detect the presence of many bacterial species, including most uropathogens. Instead, two more sensitive assays have been employed for the detection of these microbes: Expanded Quantitative Urine Culture (EQUC) and 16S ribosomal RNA (rRNA) gene sequencing (16S sequencing). Using a combination of these modalities, we aimed to characterize the relationship between male LUTM as sampled by voided and catheterized urine and BPE/LUTS.

**Methods**

- We performed a prospective, cross-sectional study of consecutive surgical patients at an academic tertiary care center presenting to and General Surgery for BPE and non-BPE related surgery.
- Urine was obtained the day of surgery (voided urine in pre-op, catheterized under anesthesia, after abx).
- Complete data were collected on 49 surgical patients (28 BPE, 21 non-BPE). Baseline characteristics were compared using descriptive statistics.
- Odds ratios for IPSS category (mild, moderate, severe) and presence of detectable bladder bacteria in catheterized urine were reported from a univariable logistic regression model.
- Relative proportions of specific bacterial species genera identified were compared to IPSS category using chi square tests. P-values less than 0.05 were considered statistically significant. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

Enhanced Quantitative Urine Culture (EQUC)

- **Standard Urine Culture** (3ul, Blood agar, Aerobic 35°C, 24 hr)
- **Enhanced Quantitative Urine Culture (EQUC)** (100ul, Blood agar, Aerobic 35°C, 48 hr)

- EQUC uses more urine and various growth conditions to isolate diverse Gram-negative and Gram-positive bacteria, including slow growing anaerobes and fastidious bacteria.
- Colonies with unique distinctive morphologies were identified using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.
- Genomic DNA (gDNA) was extracted from urine with the Qiaegen DNeasy Blood and Tissue kit.
- Controls were included in all steps to monitor for potential reagent contamination. DNA quality and quantity were monitored by gel electrophoresis and fluorescent gDNA assay.
- The 16S rRNA genes were PCR amplified from gDNA using degenerate primers with index sequences and sequenced in pools on an Illumina MiSeq on site.

**Results**

- To assess potential DNA contamination, an (non urine) control was processed with the samples and sequenced in every run. To ensure reproducibility, samples were independently extracted and sequenced at least twice.
- De-multiplexing was completed with MiSeq Control software and MiSeq Reporter. The mother pipeline was used to assign OTUs based on the 97% similarity cutoff for putative species-level OTUs. Classification was performed using mother's RDP classifier.
- The threshold for sequencing positivity was set at a conservative ≥ 2000 reads.

**Table 1. Patient demographics and clinical characteristics by type of surgery – BPE or non-BPE**

<table>
<thead>
<tr>
<th>Type of Surgery</th>
<th>Overall</th>
<th>BPE</th>
<th>Non-BPE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPE</td>
<td>22 (44.9)</td>
<td>12 (54.5)</td>
<td>9 (30.0)</td>
<td>0.024</td>
</tr>
<tr>
<td>Non-BPE</td>
<td>27</td>
<td>10 (37.0)</td>
<td>17 (63.0)</td>
<td>0.562</td>
</tr>
</tbody>
</table>

**Table 2. Univariate logistic regression comparing IPSS category to presence of detectable bladder microbiota**

<table>
<thead>
<tr>
<th>IPSS category</th>
<th>Overall</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 8</td>
<td>19</td>
<td>0.029</td>
</tr>
<tr>
<td>8-19</td>
<td>21 (42.9)</td>
<td>0.024</td>
</tr>
<tr>
<td>≥ 20</td>
<td>12 (63.2)</td>
<td>0.024</td>
</tr>
</tbody>
</table>

**Conclusion**

Mid-stream clean catch voided urine was found to be a poor sampling method for studying the male bladder microbiome, likely due to contamination from higher biomass in the distal urethra. This is an important finding for design of future urinary microbiome studies. Presence of bladder microbiota identified in catheterized urine was associated with increasing IPSS category. It is unclear if these microbiota are a cause of LUTS or a result of BPE. Larger scale studies are needed to define the exact organisms and pathophysiology at play.

**Acknowledgements**

The investigators would like to thank Evann Hilt, Travis Price, Kristal Thomas-White, Roberto Limaera, and Thomas Halverson for their support, as well as Gina Kuffel and Michael Zilliox for sequencing our samples.

**Funding**

Internal Funding - Loyola University Stritch School of Medicine Research Funding Committee. LU-20790

**References Available Upon Request**

**Disclosures**

The investigators have no relevant financial disclosures.