The Male Bladder Microbiome is Associated with Lower Urinary Tract Symptoms

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Introduction

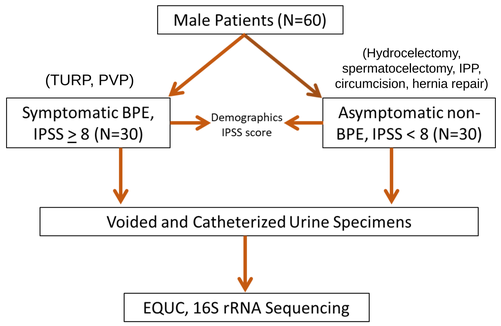
Our current understanding of the pathophysiology of benign prostatic enlargement (BPE) and lower urinary tract symptoms(LUTS) relies on the entrenched dogma that the bladder is sterile. This has recently been invalidated by numerous reports describing the existence of bacterial communities (microbiota) 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 UUI, 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 resembled those 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 mainly bladder microbiota while voided urine samples the entire lower urinary tract.***

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

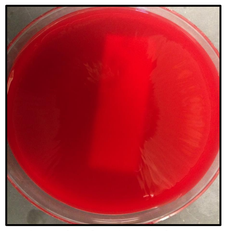
- We performed a prospective, cross-sectional study of consecutive surgical patients at an academic tertiary care center presenting to Urology and General Surgery for BPE and non-BPE related surgery.
- Urine was obtained the day of surgery (voided urine in pre-op, catheterized under anesthesia, before abx)



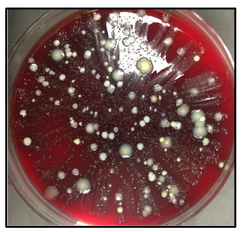
Exclusion Criteria:
- Symptomatic UTI
- Urolithiasis
- Antibiotics within 30d
- GU malignancy
- Immunocompromised
- Urethral stricture
- Urinary retention
- Prior catheterization
- Any prior GU instrumentation
- Recent abdominal/pelvic surgery
- Dx of BPE (non-BPE group)
- H/o LUTS medications (non-BPE group)

- 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
(1µL, Blood agar, Aerobic 35°C, 24 hr)



Enhanced Quantitative Urine Culture (EQUC)
(100µL, 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 distinct morphologies were identified using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry.

Methods

16 S ribosomal RNA Sequencing

- Genomic DNA (gDNA) was extracted from urine with the Qiagen 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 dsDNA assay.
- The 16S rRNA genes were PCR amplified from gDNA using degenerate primers with index sequences and sequenced in pools on an Ilumina MiSeq on site.
- To assess potential DNA contamination, an extraction (no 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 mothur pipeline was used to assign OTUs based on the 97% similarity cutoff for putative species-level OTUs. Classification was performed using mothur's RDP classifier.
- The threshold for sequencing positivity was set at a conservative ≥ 2000 reads.

Results

	Overall n=49	*BPE n=28*	*Non-BPE n=21*	*p-value[b]*
Age, mean (SD)	61.7 (10.3)	64.3 (10.2)	56.0 (8.5)	**0.004**
Non-Caucasian, n (%)	4 (8.2)	2 (7.1)	2 (9.5)	0.99
Hispanic, n (%)	8 (16.3)	6 (21.4)	2 (9.5)	0.44
Body mass index, median (IQR)	28.8 (26.5-34.0)	30.0 (26.6-34.2)	27.7 (26.5-30.3)	0.21
Current tobacco use, n (%)	6 (12.5)	1 (3.6)	5 (25.0)	0.069
Hypertension, n (%)	23 (46.9)	18 (64.3)	5 (23.8)	**0.005**
Diabetes, n (%)	9 (18.4)	5 (17.9)	4 (19.0)	0.99
Circumcised, n (%)	18 (54.5)	12 (54.5)	6 (54.5)	0.99
IPSS, median (IQR)	17 (5-25)	25 (20-27)	5 (3-7)	**<0.001**

[a]Missing values: n=1 current tobacco use, n=16 circumcision

[b]p-values from t-tests (age), Wilcoxon rank sum tests (medians), and chi-square or Fisher's exact test as appropriate (all others)

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

By a combination of EQUC and 16S sequencing, bacteria were detected in 98% (48/49) of voided specimens and 39% (19/49) of catheterized specimens. Voided and catheterized urine from the same patients appeared markedly different by both modalities.

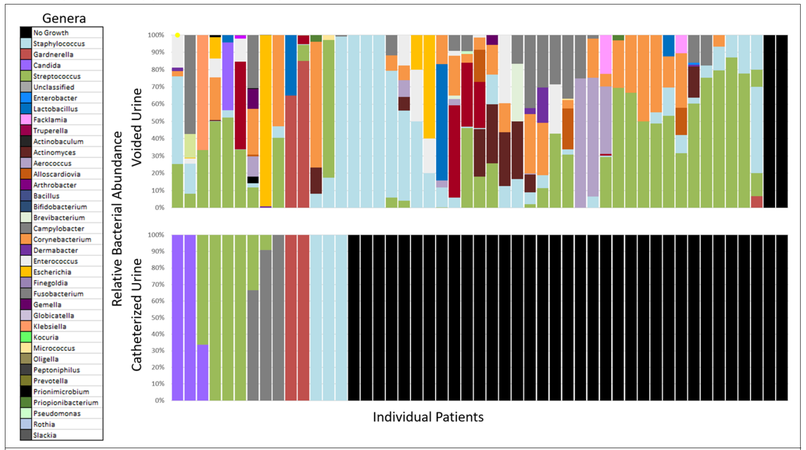


Figure 1. Relative bacterial abundance in voided (top) and catheterized (bottom) urine, as determined by EQUC. Vertical lines represent individual patients. Colors represent various genera. **Voided urine shows significant contamination compared to catheterized urine.** 16S sequencing showed similar results.

There were no statistically significant differences between patients with and without detectable bladder microbiota with respect to age, race, ethnicity, BMI, tobacco, HTN, DM2 or circumcision status. There were no significant differences between specific genera identified from patients based on degree of LUTS.

Disclosures

The investigators have no relevant financial disclosures

Results

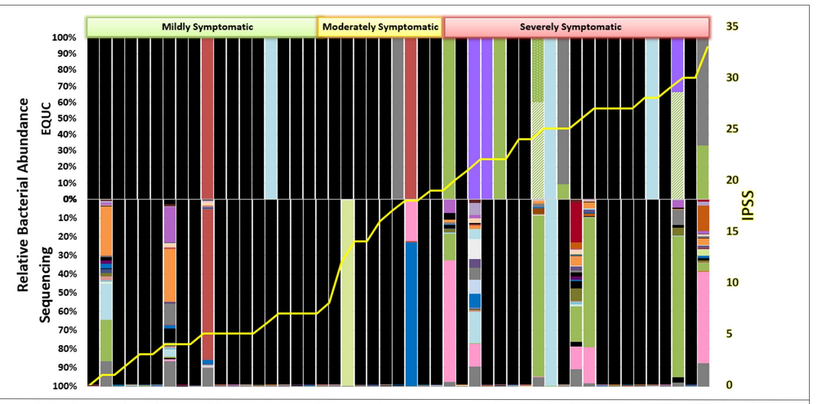


Figure 2. Relative abundance of bacteria in catheterized urine compared to IPPS. Columns represent individual patients. Colors and patterns represent bacterial genera (EQUC and 16S) and species (EQUC only). **Patients with increasing IPSS category are more often found to have detectable bacteria.**

	Overall n=49	*Bacteria present n=19*	*No bacteria present n=30*	*p-value[a]*	*Odds ratio (95% Confidence interval)[b]*	*p-value*
IPSS category						
< 8	18 (36.7)	4 (21.1)	14 (46.7)			
8-19	10 (20.4)	3 (15.7)	7 (23.3)	**0.024**	2.21 (1.09-4.49)	**0.029**
> 19	21 (42.9)	12 (63.2)	9 (30.0)			

[a]p-value from Cochran-Armitage test for trend [b]IPSS category as a linear term

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

Bacteria were detected from **catheterized** specimens in 22% (4/18) men with mild LUTS (IPSS 1-7), 30% (3/10) men with moderate LUTS (IPSS 8-19) and 57% (12/21) men with severe LUTS (IPSS 20-35, p=0.024,) (Figure 2). An increase in IPSS category was associated with significantly higher odds of detectable bacteria in catheterized urine (OR: 2.21, 95% CI: 1.09-4.49, Table 2). IPSS voiding and storage sub-scores showed similar results.

No association was found between the presence of bacteria in **voided urine** and increasing IPSS. There was no statistically significant association between presence of bacteria in voided urine and increasing IPSS voiding or storage subscores.

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.

Identification and characterization of a lower urinary tract "dysbiosis" could pave the way for novel individualized therapies for symptomatic BPE targeted at specific microbiota and their products. Improved symptom management would not only benefit patients but could also reduce healthcare costs associated with long term medical therapy, surgical intervention, emergency department presentations and inpatient hospitalizations.

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References Available Upon Request