Urinary microbiota is associated to clinicopathological features in benign prostatic hyperplasia

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Abstract

Background

The urinary microbiota of patients with benign prostatic hyperplasia (BPH) has been associated with lower urinary tract symptoms (LUTS), however, little is known about urinary microbiota correlations with clinical clinicopathological parameters associated with BPH. Here, we investigate associations between the urinary microbiota and clinical parameters of patients with BPH undergoing surgery.

Methods

Forty-one patients with BPH undergoing surgery were recruited from two medical centers. Catheterized urine specimens were collected and the microbiota was characterized by 16S rRNA gene sequencing. Patients were segregated into two groups according to each clinical parameter and differences in urinary microbiota diversity and composition were evaluated.

Results

Higher prostate weight and PSA levels were associated with higher alpha-diversity in the urinary microbiota of BPH patients. At the specific-microbe level, we found that the greater the prostatic weight, the lower the relative abundance of *Streptococcus*, while the greater the PSA levels, the higher the abundance of *Lactobacillus*. Treatment with 5-α-reductase inhibitor was associated with overall urinary microbiota composition, in part due to a higher abundance of *Corynebacterium* and *Anaerococcus* in this group.

Conclusions

We demonstrated that the urinary microbiota of BPH patients is associated with clinicopathological features, highlighting a possible role of urinary microbes in the BPH clinical course.

1. INTRODUCTION

Sterile urine is a paradigm that is being widely refuted. The urinary system has a set of microorganisms that cannot be identified by traditional culture methods, but novel strategies involving genetic sequencing of urinary lavage have been changing this scenario. These findings have expanded the frontiers of research on the contribution of the urinary microbiota to the maintenance of a healthy urinary environment, as well as on how its dysbiosis can contribute to the development of various pathologies, ranging from urinary incontinence and bladder cancer to lower urinary tract symptoms (LUTS) in men and women.
Benign prostatic hyperplasia (BPH) is a common urological disease that affects elderly men, causing LUTS. These symptoms can be divided into storage, micturition, and post-micturition symptoms. LUTS relate to bladder outlet obstruction caused by BPH due to histological changes in the prostate gland. α-1 adrenergic receptor antagonists (also called alpha-blockers) and 5-α-reductase inhibitors are the drugs of choice for BPH, but many patients require surgical treatment. The absolute indications for BPH prostate surgery are persistent macroscopic haematuria, LUTS refractory to drug treatment, recurrent urinary infections, renal failure, hydronephrosis, and acute urinary retention.

The pathophysiologic mechanisms of BPH are poorly understood, however, inflammation has been associated with disease progression and bacterial infections are possible inducers of prostatic inflammation. In this scenario and given the anatomical proximity between the prostate gland and the urinary system, a possible link between the urinary microbiota and BPH has been suggested, but more studies are needed to assess the impact of urinary microbes in the different pathophysiological aspects of this condition.

In this study, to evaluate the involvement of the urinary microbiota in the context of BPH, we collected catheterized urine samples from BPH patients undergoing surgery and analyzed their urinary microbiota by sequencing the 16S rRNA gene. We searched for associations between the urinary microbiota and clinical parameters relevant to BPH.

2. MATERIALS AND METHODS

2.1 Sample collection

We included in this prospective study patients with BPH undergoing transurethral resection of the prostate (TURP) who had at least one surgical indication for BPH. All included patients signed an informed consent form for the study, which was approved by the institutional ethics committees.

From each patient, a urine sample was collected during a sterile surgical procedure for TURP performed at Hospital Sírio-Libanês (São Paulo, Brazil) or Hospital Nossa Senhora das Graças (Presidente Prudente, Brazil) for the treatment of BPH. The samples were collected using a urinary catheter immediately after the start of TURP for the treatment of BPH and always before antibiotic prophylaxis. All urine samples were placed in sterile 80 ml collection tubes and stored at -80°C until DNA extraction.

2.2 DNA extraction and 16S rRNA amplicon sequencing

We extracted bacterial DNA from urinary samples using the QIAamp DNA Microbiome kit (Qiagen, Germany) and prepared sequencing libraries using the QIAseq 16S/ITS Region Panel kit (Qiagen). We chose to amplify and sequence the 16S gene hypervariable regions V1V2, as recommended for male urinary samples. Libraries with a final concentration < 0.4 nM were deemed to have undetectable microbiota and were not sequenced. Libraries with detectable microbes were sequenced at a final
concentration of 10 pM in the Illumina MiSeq System using the MiSeq Reagent Kit v3 (Illumina, San Diego, USA).

2.3 Bioinformatic analysis

Read processing was carried out in QIIME2\(^\text{20}\). DADA2 was used to generate amplicon sequence variants (ASVs) and chimeric ASVs were removed using VSEARCH\(^\text{21,22}\). After taxonomic classification of ASVs using a naive-Bayes-based classifier and the SILVA database, non-bacterial ASVs were removed\(^\text{23}\).

2.4 Microbiome and statistical analysis

The dataset was normalized by scaling with ranked subsampling\(^\text{24}\), where we established 905 as the minimum number of reads needed to characterize the microbiota. Samples with < 905 reads were also considered to have undetectable microbiota. Next, we calculated microbiota alpha- (ASV richness, Gini-Simpson, Shannon, and Faith phylogenetic diversity) and beta-diversity metrics (weighted UniFrac).

Patients were segregated into two groups, according to each clinical parameter. Mann-Whitney was used to compare the alpha-diversity between groups. The difference in composition between groups and the influence of covariates on the bacterial compositions were evaluated by PERMANOVA. Differences in genera abundances between groups were assessed using the MaAsLin2 method\(^\text{25}\).

3. RESULTS

3.1 Characteristics of the study patients

Forty-one patients were recruited between March 2019 and May 2022. All patients had at least one clinical indication for TURP, whether due to LUTS, paradoxical incontinence, acute urinary retention, recurrent urinary tract infection, haematuria, urinary flow abnormality, voiding residue, or structural changes in the bladder. None of the patients had hydronephrosis, uremia, or lithiasis. The clinical and demographic data are shown in Table 1. We observed a high rate of changes in the bladder structure, such as trabeculations, diverticula, and/or detrusor hypertrophy, affecting 63.4% of the patients. The mean prostate-specific antigen (PSA) was 3.63 [0.37–22.96]. The prostate, assessed by digital rectal examination, had a mean weight of 56.25 g [25–110 g]. Five patients needed to have an indwelling urinary catheter due to acute urinary retention. More than a third of the patients (36.7%) did not use drug therapy before TURP.
Table 1  
Clinical and demographic data of study patients.

<table>
<thead>
<tr>
<th></th>
<th>BPH</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>66.48 [48–79]</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnic group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasians</td>
<td>38</td>
<td>92.7</td>
</tr>
<tr>
<td>African-American</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td>Mulatto</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Western ethnicities</td>
<td>2</td>
<td>4.9</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>8</td>
<td>19.5</td>
</tr>
<tr>
<td><strong>ECOG performance status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>36</td>
<td>87.8</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>9.8</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2.4</td>
</tr>
<tr>
<td><strong>Urinary tract infection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9.8</td>
</tr>
<tr>
<td><strong>Antibiotic use</strong></td>
<td>4</td>
<td>9.8</td>
</tr>
<tr>
<td><strong>Bladder catheter use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td><strong>Prostate weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50</td>
<td>16</td>
<td>39.0</td>
</tr>
<tr>
<td>≥ 50</td>
<td>25</td>
<td>61.0</td>
</tr>
<tr>
<td><strong>Post-voiding residual volume (ml)</strong></td>
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<td></td>
</tr>
<tr>
<td>&lt; 80</td>
<td>30</td>
<td>73.2</td>
</tr>
<tr>
<td>≥ 80</td>
<td>11</td>
<td>26.8</td>
</tr>
<tr>
<td><strong>Structural changes of the bladder</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>63.4</td>
</tr>
</tbody>
</table>

*: detrusor hypertrophy, diverticulum and/or trabeculation.

### 3.2 Urinary microbiota detectability association with clinical/demographic data

Each patient provided a single catheterized urine sample during TURP. Among the 41 samples analyzed, 31 (76%) were considered to have detectable microbiota (see Materials and Methods) and had their
urinary microbiota characterized.

We did not find a significant association between urinary microbiota detectability and clinical/demographic data, such as age, smoking status, PSA, and residual urinary volume (Fisher’s exact test, p > 0.05; Supplementary Table 1). However, a trend towards higher detectability among patients with greater prostate weight (≥ 50 g) was noticed (Fisher’s exact test, p = 0.13).

### 3.3 Urinary microbiota alpha-diversity association with clinicopathological parameters

Considering different metrics (see Materials and Methods), we searched for associations between the alpha-diversity of the urinary microbiota and clinicopathological parameters associated with the context of BPH. We found that the urinary microbiota of BPH patients with higher PSA showed greater Faith's phylogenetic diversity and higher ASV richness (Fig. 1A). In addition, patients with greater prostate weight showed higher Faith's phylogenetic diversity (Fig. 1B). Other parameters, such as age and presence of structural changes of the bladder, were not associated with urinary microbiota alpha-diversity (Supplementary Fig. 1).

### 3.4 Urinary microbiota beta-diversity association with clinicopathological parameters

Next, we evaluated associations between the composition of the urinary microbiota and clinicopathological parameters. In multivariate PERMANOVA including all parameters of interest, we found that the prior use of 5-α-reductase inhibitors was the only parameter significantly associated with the composition of the urinary microbiota, but it explained only ~7% of the variance observed across the whole cohort (Fig. 2A). Principal coordinate analysis with groups stratified by previous use of 5-α-reductase inhibitors and univariate PERMANOVA test also indicated a significant difference in composition between groups (Fig. 2B).

### 3.5 Urinary microbiota taxonomic composition association with clinicopathological parameters

The most relevant bacterial genera in the urinary microbiota of the population evaluated in terms of median relative abundance were *Corynebacterium* (14.4%), *Lactobacillus* (8.0%), *Variovorax* (7.9%), *Staphylococcus* (7.5%), and *Cutibacterium* (6.5%). However, the overall taxonomic composition of the urinary microbiota varied considerably between BPH patients (Fig. 3A). Nevertheless, some genera showed high prevalence, such as *Anaerococcus*, *Corynebacterium*, *Cutibacterium*, *Finegoldia*, *Lactobacillus*, *Peptoniphilus*, SN8, *Staphylococcus*, and *Streptococcus*, which were present (relative abundance ≥ 1%) in at least a fourth of patients with detectable microbiota.

Focusing on the most prevalent genera mentioned above, we searched for associations between the urinary microbiota taxonomic composition and clinicopathological parameters of the BPH context using the MaAsLin2 tool (Fig. 3B). After correcting for the number of genera tested, we found, for example, that
the relative abundance of *Streptococcus* was significantly lower in patients with greater prostate weight and that the relative abundance of *Lactobacillus* was significantly higher in patients with higher PSA levels. We also found that BPH patients who had previously used 5α-reductase inhibitors showed higher relative abundances of *Corynebacterium* and *Anaerococcus*, which partly explains the significantly different urinary microbiota composition observed previously for this group.

4. DISCUSSION

Since the discovery that urine is not sterile, the impact of urinary microbiota on BPH has been investigated. In this study, we analyzed catheterized urine microbiota from patients with BPH undergoing surgery and investigated the association between urinary microbiota detectability, diversity, and composition with clinical parameters.

We showed that greater prostate weight and higher PSA levels are both associated with higher urinary microbiota alpha-diversity in BPH patients. We also found associations between clinical parameters and the abundance of specific genera, such as a higher abundance of *Streptococcus* in patients with greater prostatic weight and a higher abundance of *Lactobacillus* in patients with higher PSA levels. The latter is in line with a recent study showing a higher abundance of *Lactobacillus* in the urinary microbiota of BPH patients in comparison with controls. The aforementioned study further described an association between *Haemophilus* abundance and PSA levels that we did not find, but this is possibly due to their adoption of midstream urine collection as sampling strategy.

Many studies have shown that catheterized urine is more appropriate than voided urine samples for urinary microbiota analyses, since catheterization avoids urine sample contamination with distal urinary tract and skin bacteria. One such study also investigated BPH patients. They showed an association between the detectability of microbiota in catheterized urine samples, but not in the midstream voided ones, with the severity of LUTS. This is in contrast with our findings showing that microbiota detectability was not associated with clinicopathological features. Of note, detectability was considerably higher in our cohort (76% vs. 27%), likely reflecting the optimized protocol we adopted.

Chronic prostatic inflammation has been associated with BPH pathogenesis and clinical progression. Although the mechanisms inducing inflammation are not yet completely understood, recent studies suggest a role of the urinary microbiota in this process. While bacterial infections may induce the secretion of proinflammatory cytokines, chemokines, and growth factors, resulting in the inflammation of the prostate gland, participation of the commensal urinary microbiota has not been shown.

The use of alpha-blockers and/or 5α-reductase inhibitors is the gold standard first-line therapy for patients with BPH, reducing inflammation and preventing disease progression. Interestingly, we showed that treatment with a 5α-reductase inhibitor was associated with a higher abundance of urinary *Corynebacterium* and *Anaerococcus*, both of which have been reported to be part of the healthy human urinary microbiota. Although causality cannot be evaluated, we can hypothesize that this drug might
modulate the urinary microbiota of patients with BPH toward a non-inflammatory composition. Of note, due to the small sample size, we considered the use of 5-α-reductase inhibitors as an independent variable for statistical analysis, so that we could not evaluate interactions between drugs (e.g., 5-α-reductase inhibitor + alpha-blocker). Besides, all patients analyzed in our study underwent surgery. Thus, it is possible that the microbiota differences between groups could be even sharper if patients responsive to the drugs — and, consequently, that had not had to undergo TURP — were considered in our analysis.

5. CONCLUSION

In this study, we analyzed the catheterized urine microbiota from patients with BPH undergoing surgery and investigated the association between the urinary microbiota detectability, diversity, and composition with clinicopathological parameters. Urinary microbiota diversity and composition were associated with clinical parameters in BPH, such as the link between the abundance of commensal urinary bacterial genera and the use of 5-α-reductase inhibitors. Future studies investigating those associations in more depth may pave the way for new therapeutic strategies for BPH.

DECLARATIONS

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None.

Competing interests

None.

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Contributions

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Drafting of the manuscript: Antonio CH Mariotti, Vitor Heidrich, Lilian T Inoue, Hugo DB dos Santos.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Vitor Heidrich.

Obtaining funding: Vitor Heidrich and Anamaria A Camargo.

Administrative, technical, or material support: None.

Supervision: Anamaria A Camargo and Marco A Arap.

REFERENCES


**Figures**

![Figures](image)

**Figure 1**
Association between urinary microbiota alpha-diversity and PSA (A) or prostate weight (B). Different alpha-diversity metrics were evaluated in each subplot. Mann-Whitney test was used.

Figure 2

(A) Association between urinary microbiota composition and clinicopathological parameters. Multivariate PERMANOVA test was used. (B) Principal coordinate analysis (PCoA) representing taxonomic compositions among patients that used or did not use 5-α-reductase inhibitors. Compositional distances were calculated using weighted UniFrac. Ellipsoids represent 95% confidence intervals. PERMANOVA test was used.
Figure 3

(A) Taxonomic compositions of the urinary microbiota of each patient with detectable microbiota included in the study. Only genera present at relative abundance $\geq 5\%$ in at least 2 patients are depicted.

(B) Multivariate association between highly prevalent genera (relative abundance $\geq 5\%$ in $\geq 25\%$ of patients) and clinicopathological parameters. The MaAsLin2 tool was used and the linear model...
coefficients are depicted as a surrogate of effect size. +: positive significant association; -: negative significant association.

**Supplementary Files**

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- [Supplementarymaterial.docx](#)