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Characterization of urinary microbiome dysbiosis in prostate cancer and benign prostatic hyperplasia: A metagenomics study from North-Eastern Algeria

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Article

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Abstract

Purpose

Prostate cancer (PCa) is a leading cause of cancer-related mortality in men worldwide. Chronic prostate inflammation, often associated with benign prostatic hyperplasia (BPH), is a recognized contributor to prostate carcinogenesis. Recent evidence suggests that urinary microbiota dysbiosis may play a role in this process and serve as a source of microbial biomarkers.

Methods

Here, we characterized the urinary microbiome in PCa and BPH patients compared to controls using targeted 16S rRNA gene sequencing.

Results

Urine samples from 68 male participants (22 PCa, 32 BPH, 14 controls) were analyzed. Significant differences in microbial diversity and composition were observed across the three group. BPH samples exhibited the highest alpha diversity, followed by PCa samples, while controls showed the lowest diversity (Shannon index, p = 0.001). PCa urines were dominated by *Streptococcus, Gardnerella*, and *Klebsiella*, whereas BPH urines showed higher abundances of pro-inflammatory anaerobic or microaerophilic bacteria such as *Fusobacterium*, *Prevotella*, and *Campylobacter*. Beta diversity analysis revealed distinct clustering of PCa samples (p = 0.001), significantly associated with *Gardnerella vaginalis* and *Klebsiella pneumonia*. Chemotherapy significantly altered the urinary microbiome, increasing the abundance of uropathogenic Proteobacteria (56.2%) (*Escherichia coli*, *Enterococcus faecalis)*, opportunistic bacteria (*Finegoldia magna* and *Prevotella bivia)*, and depleting beneficial bacteria *Lactobacillus crispatus* and *Corynebacterium amycolatum*.

Conclusions

This study identifies the urinary microbiome as a promising non-invasive biomarker for prostate diseases. Microbial dysbiosis promotes pro-inflammatory and uropathogenic bacteria and contribute to prostate inflammation and cancer progression, while chemotherapy further destabilizes this fragile ecosystem. Microbiota-based probiotic supplementation, could help to restore microbial balance and support urogenital health in prostate diseases.

Introduction

Prostate cancer (PCa) remains one of the most frequently diagnosed malignancies in men and is a leading cause of cancer-related mortality worldwide, with over 1.4 million new cases annually, and

approximately 375,000 deaths reported in 2020 [1]. Its incidence increases with age and is influenced by multiple factors, including genetic predisposition, ethnicity, diet, hormonal imbalances, and chronic inflammation. Meanwhile, benign prostatic hyperplasia (BPH), although non-malignant, affects approximately 50% of men over the age of 50 and up to 90% of those over 80, significantly contributing to lower urinary tract symptoms and impaired quality of life [2,3]. Despite its benign nature, BPH shares pathophysiological features with PCa, particularly regarding androgen signaling and the inflammatory microenvironment. Chronic inflammation, commonly observed in BPH, is considered a key factor in neoplastic transformation, suggesting a potential continuum from BPH to PCa [4,5]. Emerging evidence underscores the role of the gut microbiome in systemic inflammation and hormonal regulation, with *Helicobacter pylori* being implicated in modulating testosterone levels, immune responses, and intestinal permeability factors that may contribute to prostate carcinogenesis [6]. Additionally, gut dysbiosis has been associated with activation of the NF-κB-IL6-STAT3 axis, promoting tumor proliferation and resistance to chemotherapy [7].

Recent studies highlighted the key role played by microbiomes in the development of prostate disease. The urinary tract, previously considered sterile, revealed diverse microbial communities that differ between healthy individuals and patients with prostate disease [8]. Dysbiosis characterized by reduced microbial diversity and increased abundance of pro-inflammatory taxa such as *Streptococcus anginosus*, *Cutibacterium acnes*, and *Sphingomonas* sp. has been linked to chronic inflammation, immune activation, and altered androgen metabolism, potentially compromising hormone-based therapies and facilitating tumor progression [9].

To date, no studies have investigated the urinary microbiome profiles in prostate diseases within the Algerian population. To address this gap, we conducted a retrospective study to explore the urine microbiome in patients with PCa and BPH, compared to healthy individuals. This study provides the first regional overview of urinary microbiome dynamics in prostate disease and identifies potential microbial biomarkers relevant to diagnosis and disease stratification in North African populations.

Methods

Patients and Ethics

We conducted a pilot retrospective study on urine samples collected at the Urology Departments (one university hospital and three clinics) in Annaba (Algeria) between April 2022 and December 2024. Samples were obtained from three groups: patients diagnosed with PCa, patients with BPH, and individuals undergoing urological consultation without any identified pathology, who served as controls (Fig. 1). Urine samples from all PCa and BPH patients were routinely collected as part of their clinical follow-up. All specimens were systematically sent to the Department of Microbiology at Annaba University Hospital and stored at -80°C for up to one year. For urinary microbiome analysis, a minimum volume of 10 mL was required; only samples meeting this criterion were included in the study. Selected samples were then shipped to the Department of Microbiology at Nîmes University Hospital (France) for

sequencing and analysis. All participants were older than 40 years and provided written informed consent prior to inclusion in the study. The study protocol was approved by the Ethics Committee of Annaba University Hospital under reference number 001/CE/CHUA/2025. All methods were performed in accordance with the relevant guidelines and regulations.

DNA Extraction

Total DNA was extracted using a customized protocol adapted for the EZ1 Advanced XL instrument (Qiagen, Courtaboeuf, France). Briefly, 10 mL of urine were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was discarded. The pellet was resuspended in 200 µL of G2 buffer (Qiagen) and incubated at 56°C for 30 minutes with 20 µL of Proteinase K. DNA extraction was then performed using the EZ1 DNA Tissue Kit (Qiagen), and eluted in a final volume of 50 µL. DNA concentration was measured using a Qubit™ Fluorometer with the Qubit™ dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, Villebon-sur-Yvette, France).

16s rRNA Library Preparation, Sequencing, and Analysis

Targeted metagenomic analysis was performed using 16S rRNA gene sequencing, following the Illumina protocol for the V3–V4 hypervariable regions. Final libraries were diluted to 9 nM and pooled with 25% PhiX control to improve sequencing diversity. Sequencing was conducted in a single 2×300 bp paired-end run on the Illumina MiSeq platform (Illumina, San Diego, CA, USA). Raw sequences underwent quality filtering, chimera removal, error correction, and taxonomic classification using the DADA2 pipeline with the R package (version 1.34.0). Taxonomic assignments were based on the SILVA reference database (version 2022), generating amplicon sequence variant (ASV) tables.

Statistical analysis

Microbiome data were processed using the *phyloseq* package (version 1.46.0). Initial quality control included contaminant detection and removal using the *decontam* package (version 1.26.0), based on their prevalence in negative controls and their frequency with an inverse correlation with DNA concentration. Microbiome data were rarefied to a minimum depth of 5,000 sequences to ensure comparability (this threshold was reasonable considering the aspect of rarefaction curves). They were also transformed into relative abundance (%) for graphical representation. Alpha diversity was assessed using ASV counts and the Shannon index, reflecting taxonomic richness and evenness. Differences in alpha diversity among groups were tested using multiple linear regression, adjusting for potential confounders such as age. Beta diversity was evaluated by Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity, with significance assessed via PERMANOVA (including age as a covariate). Differential abundance analysis was performed using Kruskal-Wallis tests on rarefied data. All analyses were conducted in R (version 4.4.2), with significance set at alpha = 0.05. Multiple testing correction was applied using the Benjamini-Hochberg method.

Results

Demographical data

Across all participating centers, 75 urine samples (≥ 10 mL) were collected. After applying the inclusion criteria, 68 samples from 68 patients with complete clinical and microbiological data were retained for microbiota analysis. The seven excluded samples were obtained from patients with bladder cancer and were removed from further analysis **(**Fig. 1). The final cohort included 22 patients with PCa (mean age: 75 years), 32 with BPH (mean age: 70 years), and 14 controls (mean age: 54 years). Among PCa patients, 11 were receiving chemotherapy, including 7 with metastatic disease. Comorbidity profiles revealed that 26 participants were diabetic (14 BPH, 8 PCa, 4 controls), and 38 had hypertension (22 BPH, 9 PCa, 7 controls). Reported urological symptoms included urinary burning (n = 22 patients), urgency (n = 21, including 3 with hematuria), frequency (n = 40), pelvic pain (n = 18), lower back pain (n = 17), fever (n = 14), and general weakness (n = 19). Cyto-bacteriological urine analysis revealed three positive cultures, all among BPH patients, yielding *Enterobacter cloacae* (n = 2) and *Klebsiella pneumoniae* (n = 1) (Table 1).

Table 1
Demographic and clinical characteristics of the 68 male participants included in the study.

Characteristic	Overall	Prostate cancer	controls	Benign prostate hyperplasia	p-value[2]
	N = 68[1]	N = 22[1]	N = 14[1]	N = 32[1]	
Age	70 (60, 75)	75 (67, 77)	54 (46, 64)	70 (61, 74)	< 0.001
Cancer	22 (32%)	22 (100%)	0 (0%)	0 (0%)	< 0.001
Metastasis	7 (32%)	7 (32%)	NA	NA	ND
Chemotherapy	11 (52%)	11 (52%)	NA	NA	ND
Comorbidities					
Smoking	11 (16%)	3 (14%)	2 (14%)	6 (19%)	> 0.9
Diabetes	26 (38%)	8 (36%)	4 (29%)	14 (44%)	0.6
Arterial hypertension	38 (56%)	9 (41%)	7 (50%)	22 (69%)	0.11
Urological symptoms					
Urinary burning	22 (32%)	7 (32%)	0 (0%)	15 (47%)	0.004
Urinary urgency	21 (31%)	7 (32%)	1 (7.1%)	13 (41%)	0.068
Hematuria	3 (4.4%)	0 (0%)	1 (7.1%)	2 (6.3%)	0.4
Urinary frequency	40 (59%)	14 (64%)	2 (14%)	24 (75%)	< 0.001
Pelvic pain	18 (26%)	7 (32%)	3 (21%)	8 (25%)	0.8
Lower back pain	17 (25%)	6 (27%)	2 (14%)	9 (28%)	0.7
Fever	14 (21%)	4 (18%)	0 (0%)	10 (31%)	0.041
Tiredness	19 (28%)	10 (45%)	1 (7.1%)	8 (25%)	0.043
Microbiological findings					
Macro exam trouble	3 (4.4%)	0 (0%)	0 (0%)	3 (9.4%)	0.3
Positive Urinalysis	3 (4.4%)	0 (0%)	0 (0%)	3 (9.4%)	0.3
Species identified					> 0.9
Enterobacter cloacae	2 (67%)	0 (0%)	NA	2 (67%)	

[1] Median (Q1, Q3); n (%), [2] Kruskal-Wallis rank sum test; Fisher's exact test; Pearson's Chi-squared test. NA, not applicable; ND, not determined

Characteristic	Overall	Prostate cancer	controls	Benign prostate hyperplasia	p-value[2]
	N = 68[1]	N = 22[1]	N = 14[1]	N = 32[1]	
Klebsiella pneumoniae	1 (33%)	0 (0%)	NA	1 (33%)	

[1] Median (Q1, Q3); n (%), [2] Kruskal-Wallis rank sum test; Fisher's exact test; Pearson's Chi-squared test. NA, not applicable; ND, not determined

Urinary Microbiome in Prostate Cancer

Across all samples, a total of 2,916 ASVs were identified, corresponding to 27 phyla, 579 genera, and 795 species. The urinary microbiome profiles of PCa patients exhibited a distinct composition, dominated by six phyla: Firmicutes (29.99%), Proteobacteria (23.74%), Actinobacteriota (18.25%), Bacteroidota (13.63%), Fusobacteriota (9.32%), and Campylobacterota (3.23%).

At the genus level, *Streptococcus* (13.45%), *Gardnerella* (10.80%), and *Klebsiella* (10.48%) were most abundant, all previously associated with urogenital dysbiosis and chronic inflammatory. Other notable genera included *Prevotella* (6.8%), *Fusobacterium* (5.9%), and *Veillonella* (5.6%), implicated in inflammatory cascades and tumour-promoting microenvironments.

Dominant species included *Gardnerella vaginalis* (10.7%), *Streptococcus mitis* (3.8%), *Veillonella atypica* (2.7%) and *Fusobacterium periodonticum* (2.6%) (Fig. 2, **Supplementary data 1**).

Urinary Microbiome in Benign Prostate Hyperplasia

The urinary microbiome of BPH patients comprised 22 phyla, 431 genera, and 588 species. The most prevalent phyla were Firmicutes (38.5%), followed by Proteobacteria (22.6%), Actinobacteriota (15.1%), Bacteroidota (11.4%) and Fusobacteriota (6.7%).

At genus level, *Escherichia-Shigella* (12.0%) and *Streptococcus* (11.5%) were dominant, alongside *Staphylococcus* (6.8%), *Veillonella* (5.0%), *Corynebacterium* (4.9%), *Fusobacterium* (4.8%) and *Prevotella* (4.4%). Other clinically relevant genera included *Gardnerella* (4.0%), *Gemella* (2.9%), *Lactobacillus* (2.4%) and *Campylobacter* (2.2%).

At the species level, *Gardnerella vaginalis* (3.7%) was the most prevalent, followed by *Fusobacterium periodonticum* (2.1%), *Lactobacillus iners* (1.8%), *Campylobacter concisus* (1.4%), *Leuconostoc carnosum* (1.3%), and *Staphylococcus haemolyticus* (1.0%) (Fig. 2, **Supplementary data 1**).These findings suggest a complex microbial landscape potentially involved in prostate and urinary tract health.

Urinary Microbiome in Controls

The urinary microbiomes of controls were less diverse than that of PCa and BPH patients, comprising 11 phyla, 177 genera, and 257 species. Dominant phyla included Firmicutes (27.4%) and Bacteroidota (26.3%), followed by Fusobacteriota (19.9%), Proteobacteria (10.1%), Campylobacterota (7.7%) and Actinobacteriota (6.2%).

At the genus level, *Fusobacterium* (16.4%) was most abundant, followed by *Prevotella* (10.7%), an unclassified genus group (10.1%), *Campylobacter* (7.5%), *Streptococcus* (6.8%), *Veillonella* (6.3%) and *Escherichia-Shigella* (5.5%).

Key species included *Fusobacterium periodonticum* (7.1%), *Campylobacter concisus* (4.9%), *Haemophilus* spp. (3.9%), *Veillonella atypica* (1.2%), *Alloscardovia omnicolens* (0.9%), *Prevotella timonensis* (0.6%), and *Campylobacter ureolyticus* (0.6%). The presence of anaerobic and commensal taxa in the urinary microbiome of asymptomatic individuals may contribute to urinary homeostasis and urogenital health (Fig. 2, **Supplementary data 1**).

Comparative Microbiome Analysis between PCa, BPH, and Controls

Alpha diversity differed significantly between PCa and controls (Shannon index, $p = 0.02$) and was near significant between BPH and controls (Shannon index, $p = 0.07$; Fig. 3A). Both comparisons were significant using the Inverse Simpson index ($p = 0.02$ and $p = 0.002$, respectively). Beta diversity analysis using PCoA based on Bray-Curtis dissimilarity revealed distinct clustering of PCa samples, indicating reduced intra-group variability and a more conserved microbial community (Fig. 3B). BPH and controls samples showed greater dispersion and partial overlap, suggesting higher microbial heterogeneity. PERMANOVA test confirmed these differences ($p = 0.002$). Age was included as a covariate representing a potential confounding factor. No significant association was observed ($p = 0.74$), whereas diagnosis remained the only factor significantly influencing microbiome composition ($p = 0.002$).

At the phylum level, BPH patients exhibited significantly higher relative abundances of Bacteroidota (26.3%), Fusobacteriota (19.9%), and Campylobacterota (7.6%) compared to PCa (13.6%, 9.4%, and 3.2%, respectively) and controls (11.7%, 7.0%, and 2.3%, respectively) ($p < 0.001$; Fig. 2A). Cyanobacteria were enriched in controls (0.48%) but nearly absent in disease groups.

Genus-level analysis revealed significant enrichment of *Fusobacterium* (16.4%), *Prevotella* (10.7%), *Campylobacter* (7.43%), *Leptotrichia* (3.18%), and *Porphyromonas* (2.93%) in BPH compared to PCa and controls ($p < 0.001$; Fig. 2B). Other genera such as *Capnocytophaga*, TM7x, *Selenomonas*, and *Alloprevotella* were also more abundant in BPH, suggesting predominance of anaerobic and pro-inflammatory taxa in BPH samples. *Escherichia-Shigella* genus was significantly more prevalent in controls than in disease groups ($p < 0.0001$), while *Gardnerella* and *Klebsiella* were markedly elevated in PCa patients ($p = 0.04$ and 0.005, respectively), suggesting potential associations with carcinogenesis. *Veillonella*, *Haemophilus*, *Actinomyces* and *Granulicatella* were significantly more abundant in BPH group

($p < 0.05$; Fig. 2C). Pairwise comparisons between BPH and PCa revealed significant associations of *Klebsiella pneumonia* with BPH and *Veillonella montpellierensis* and *Escherichia-Shigella_Other* with PCa ($p = 0.007$, 0.011, and 0.016, respectively).

Treatment Impact on Urinary Microbiome in Prostate Cancer

Within the PCa subgroup, no significant differences in alpha or beta diversity were observed between metastatic and non-metastatic patients. However, subtle taxonomic variations were noted: *Lactobacillus* was relatively more abundant in non-metastatic samples, whereas *Streptococcus* and *Gardnerella* were enriched in metastatic urine. Chemotherapy exerted a pronounced effect on urinary microbiome composition (Fig. 4). Patients not receiving chemotherapy exhibited significantly higher alpha diversify ($p = 0.004$), and beta diversity analysis revealed distinct microbial clustering between treated and untreated individuals ($p < 0.001$; **Fig. 4**). Chemotherapy induced notable shifts at multiple taxonomic levels. Proteobacteria showed a marked increase in samples of post-chemotherapy patients compared to samples of pre-treatment patients or controls (mean relative abundance: 56.2% vs. 29.8%, $p = 0.0012$), indicating a dysbiotic shift toward potentially pathogenic taxa. Conversely, Firmicutes and Actinobacteria, typically associated with a balanced and protective urinary microbiome, were significantly reduced following chemotherapy (Firmicutes: 18.7% vs. 34.1%, $p = 0.003$; Actinobacteria: 4.5% vs. 11.2%, $p = 0.019$).

At genus level, beneficial taxa such as *Lactobacillus* ($p = 0.012$) and *Corynebacterium* ($p = 0.021$) declined significantly, while pathogenic genera including *Escherichia* ($p = 0.008$) and *Enterococcus* ($p = 0.015$) were enriched in samples of patients post-treatment.

Species-level analysis revealed significant increases in *Escherichia coli* (mean abundance: 12.6% vs. 3.9%, $p = 0.005$) and *Enterococcus faecalis* (6.8% vs. 1.5%, $p = 0.009$), both commonly associated with urinary tract infections, especially in immunocompromised patients. Beneficial taxa such as *Lactobacillus crispatus* (0.4% vs. 5.6%, $p = 0.007$) and *Corynebacterium amycolatum* (1.1% vs. 4.8%, $p = 0.018$), *Streptococcus mitis* (0.3% vs. 2.9%, $p = 0.011$) and *Actinomyces neuii* ($p = 0.025$) were significantly depleted in samples of post-treated patients compared to those of pre-treated patients, potentially reflecting the cytotoxic impact of chemotherapy. Interestingly, *Finegoldia magna* and *Prevotella bivia*, occasionally implicated in uropathogenic processes, were significantly enriched in post-chemotherapy samples ($p = 0.031$ and 0.044, respectively).

Study Limitations

This study presents several limitations. First, the sample size was relatively small in each group, particularly in the PCa group ($n = 22$), and only 14 controls were included. Second, the lack of longitudinal data prevented assessment of microbiome dynamics before and after chemotherapy. Third, although age differences between groups were accounted for in all analyses., residual confounding cannot be fully excluded. Partial 16S rDNA sequencing limited species-level resolution, restricting comparison at

the species level and the ability to highlight species microbial associations. Finally, no follow-up was available for BPH patients who may have progressed to PCa, limiting insights into transitional microbial signatures.

Discussion

Urine has long been considered a sterile environment, and its microbial composition has therefore remained underexplored. Although recent work has highlighted the gut microbiota as a contributor to inflammation and prostate carcinogenesis [7], far fewer studies have examined the urinary microbiota, with urine traditionally used only for detecting urinary tract infections in PCa and BPH patients [10,11]. Most microbiome studies in PCa rely on prostate biopsy specimens [12–14], yet accumulating evidence suggests that urine may serve as a non-invasive conduit of identifying microbial biomarkers. A previous investigation has reported genera such as *Streptococcus, Prevotella, Peptoniphilus, Negativicoccus, Actinomyces, Propionimicrobium, and Facklamia* as dominant in PCa urine [15]. Our findings confirm and extend these observations, revealing distinct urinary microbiome signatures across PCa, BPH, and control groups. Notably, BPH patients exhibited higher alpha and beta diversity (Fig. 3), and a microbiome enriched in anaerobic and pro-inflammatory genera, including *Fusobacterium*, *Prevotella*, *Campylobacter*, *Leptotrichia*, and *Porphyromonas*. These taxa are well known for promoting mucosal inflammation and immune modulation and may contribute to the transition from BPH to PCa, as previously proposed [16]. In contrast, *Escherichia–Shigella*, classically associated with urinary tract infections, was significantly more prevalent in controls ($p < 0.0001$), whereas pathogenic genera such as *Gardnerella, Klebsiella*, and *Veillonella montpelliierensis* were strongly associated with PCa ($p = 0.04$, 0.005, and 0.011, respectively). Their biofilm-forming and immunomodulatory properties support the hypothesis that a pathogenic urinary dysbiosis may contribute to prostate tumorigenesis. Similar microbial signatures have also been documented in bladder cancer, suggesting a possible shared dysbiotic profile across urogenital malignancies [17].

A key contribution of this study is the characterization of chemotherapy-associated dysbiosis in the urinary microbiome of PCa patients. Chemotherapy significantly decreased microbial diversity and shifted the urinary microbiome toward a Proteobacteria-dominant state (56.2%), alongside reductions in Firmicutes and Actinobacteria, phyla typically associated with a balanced and protective urinary ecosystem. Pathogenic species such as *Escherichia coli* and *Enterococcus faecalis*, commonly implicated in infections among immunocompromised individuals, were significantly enriched post-chemotherapy [18,19]. Conversely, beneficial taxa including *Lactobacillus crispatus* and *Corynebacterium amycolatum*, knownfor their anti-inflammatory and uroprotective roles [20], were nearly absent in the post-chemotherapy urine microbiomes. Opportunistic micro-organisms such as *Streptococcus mitis*, *Actinomyces neuii*, *Finegoldia magna*, and *Prevotella bivia* also increased following chemotherapy, further highlighting the vulnerability of the urinary microbiome to immunosuppression and the associated risk of infection [21]. Although age differed between groups, diagnosis rather than age remained the primary determinant of microbiome structure, consistent with previous reports [22].

These findings have several clinical implications. Chemotherapy-induced dysbiosis may compromise urinary mucosal integrity and heighten susceptibility to infection. Microbiota-based interventions—including targeted probiotic supplementation—may offer a promising strategy to restore microbial balance, reduce inflammation, and potentially improve clinical outcomes in PCa and BPH. Restoring protective genera such as *Lactobacillus* could help mitigate treatment-related adverse effects and support overall urogenital health.

Conclusion

This study provides novel insights into the association between urinary microbiome and prostate diseases. We showed that microbial dysbiosis is a key factor in prostate inflammation and carcinogenesis, with distinct microbial signatures observed in PCa and BPH patients. These findings open new avenues for microbiome-based diagnostics and prognostics in prostate disease, potentially enabling earlier detection and improved patient stratification. Importantly, our data suggest that chemotherapy was associated with profound urinary microbiome disruption, characterized by loss of protective species and enrichment of pathogenic taxa, which may increase infection risk and compromise mucosal integrity. These observations underscore the need for strategies to preserve or restore microbial balance during treatment. Probiotic interventions aimed at restoring microbial balance —particularly through *Lactobacillus* supplementation—represent a promising adjunct to conventional therapy, with potential to reduce inflammation and enhance treatment tolerance.. Future research should focus on larger, longitudinal cohorts to validate these findings and explore the therapeutic potential of urinary microbiota modulation in prostate disease management.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Annaba University (approval number: 001/CE/CHUA/2025). Informed consent was obtained from all participants prior to sample collection and data analysis.

Competing interests

The authors declare no competing interests.

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Author Contribution

AA: Sampling, methodology, data collection, microbiological analysis, metagenomics, writing the original draft of the manuscript. FS: Statistical analysis, data interpretation, validation, and writing of the original draft of the manuscript. ND and SN: routine laboratory analysis, data collection. MR, MA, KA, EMB, GKD, CK: Patient recruitment, sampling, and clinical data collection and follow-up. CDR: conceptualization, formal analysis, methodology and validation. JPL: study direction, conceptualization, validation, writing of the original manuscript. MM: study direction, conceptualization, metagenomics analysis, methodology, bioinformatics, data cleaning and validation, writing the original manuscript: MM& JPL: critical review of the manuscript, approval, and validation and submission. All the authors have read and approved the final version of the manuscript.

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Data Availability

Sequencing data are available on NCBI GenBank database under the accession number: PRJNA1308502.

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Figures

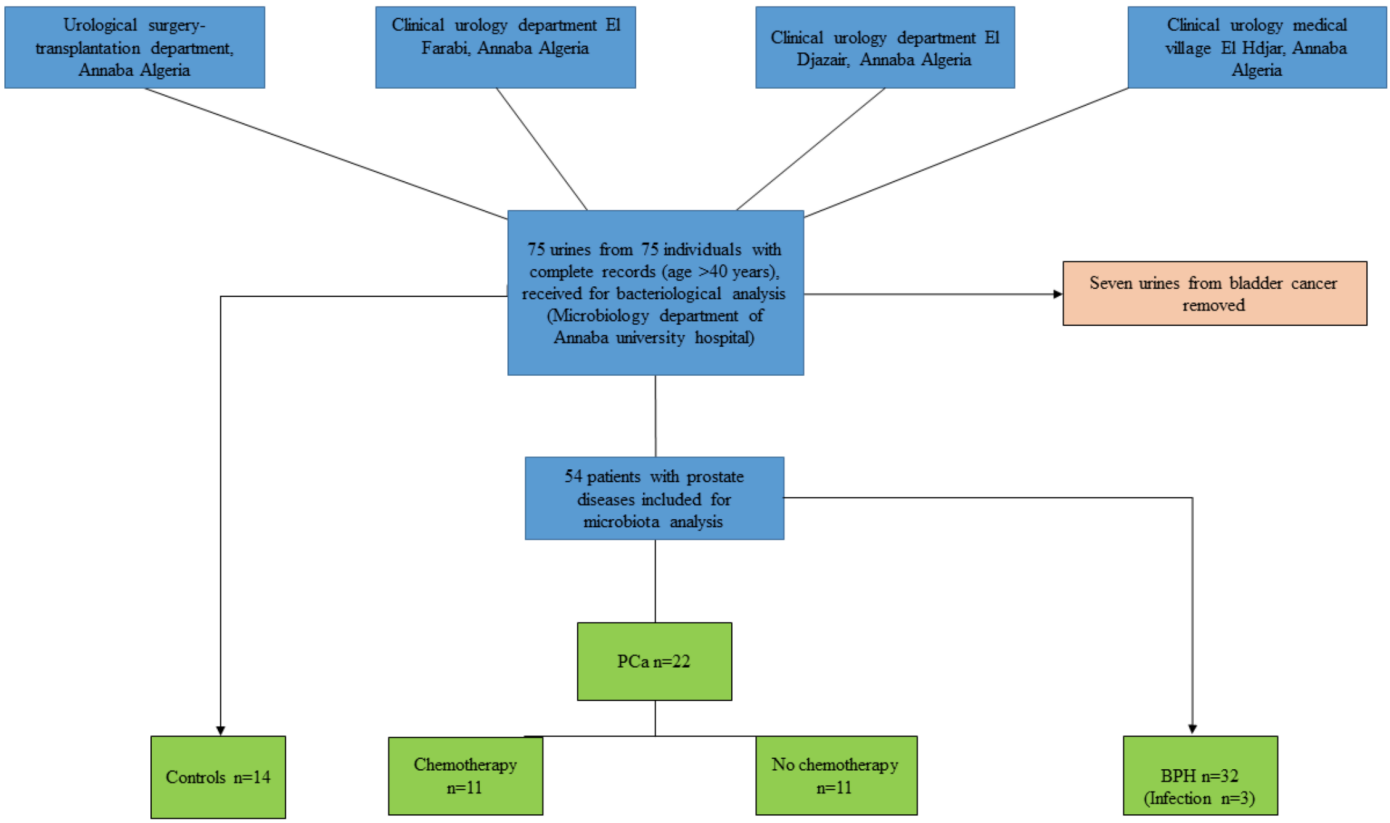


Figure 1

Flowchart of patient selection and inclusion for microbiome analysis. From all the collected urine samples, 68 were selected for microbiota analysis, including 22 patients with prostate cancer (PCa), 32 benign prostatic hyperplasia (BPH), and 14 controls. Patients with incomplete clinical records or other cancer types were excluded.

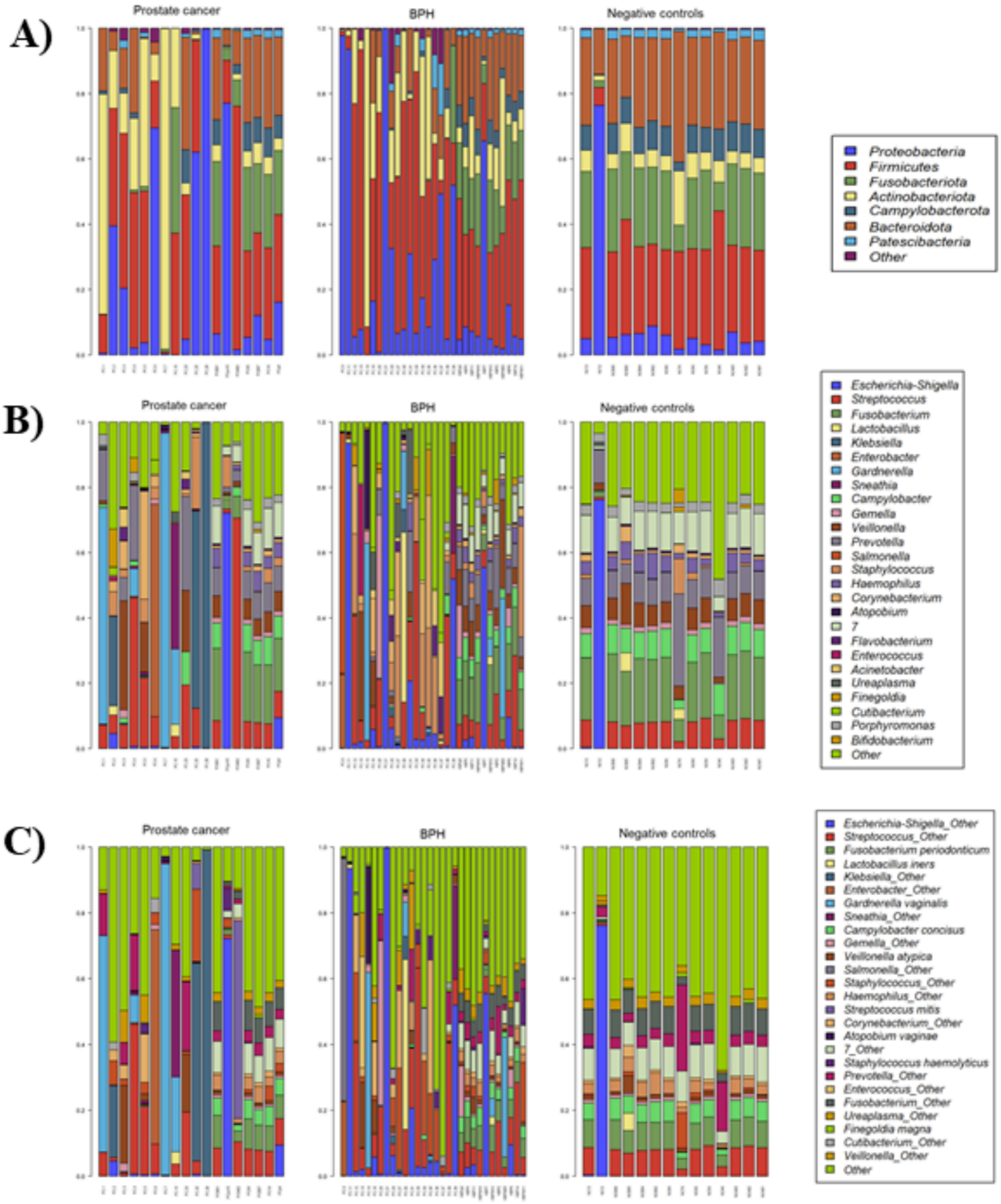


Figure 2

Taxonomic composition of urinary microbiome in patients with prostate cancer (PCa), benign prostatic hyperplasia (BPH), and controls. **A)** At the phylum level, Proteobacteria, Firmicutes, and Actinobacteriota were predominant in PCa and BPH samples, while Fusobacteriota and Bacteriodota were more abundant in controls. **B)** At genus level, *Fusobacterium*, *Prevotella*, *Campylobacter*, *Leptotrichia*, and *Porphyromonas* were enriched in BPH. *Gardnerella* and *Klebsiella* were significantly associated with PCa. **C)** At species

level, *Klebsiella pneumonia* was more abundant in BPH. *Veillonella montpelliierensis*and *Escherichia-Shigella_Other* were enriched in PCa samples.

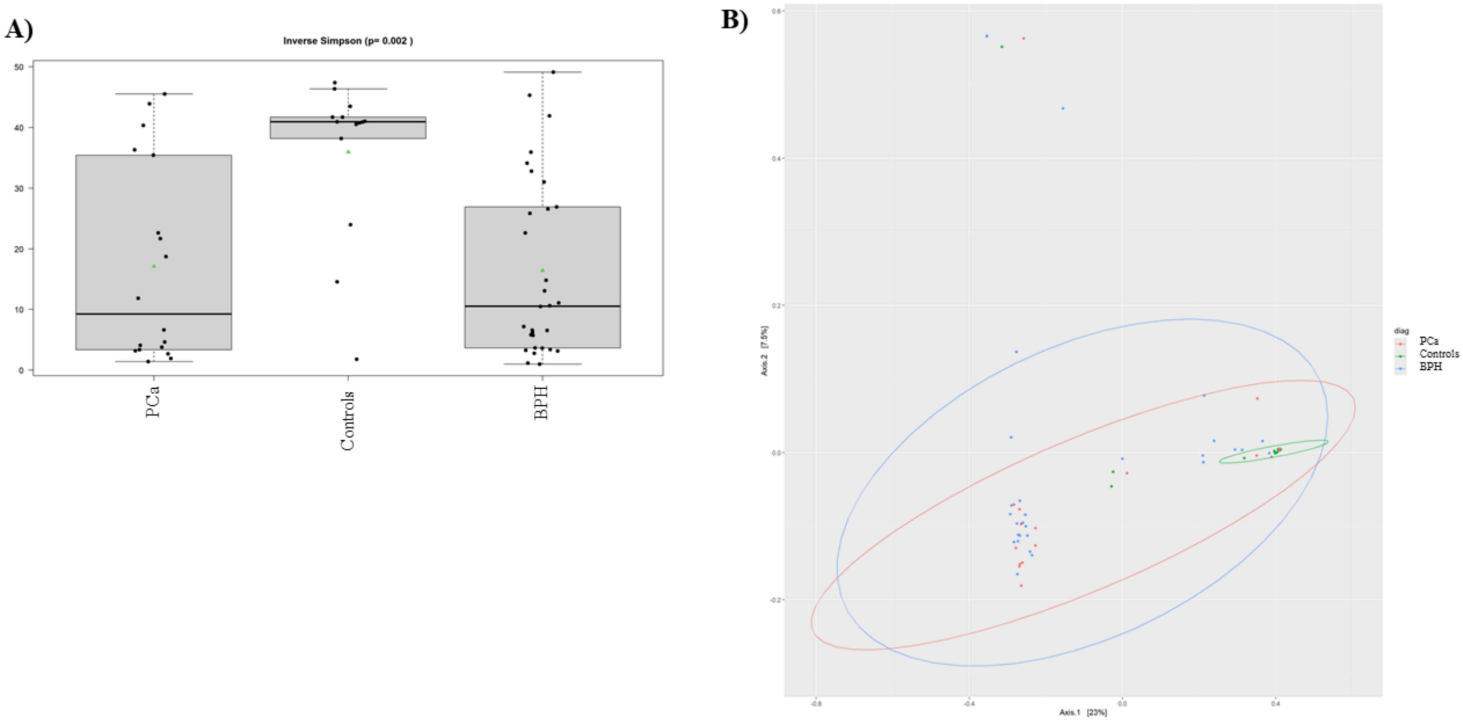


Figure 3

Comparative analysis of microbial urinary diversity across study groups (patients with prostate cancer (PCa), benign prostatic hyperplasia (BPH), and controls). A) Alpha diversity, assessed using the invers Simpson, was significantly higher in BPH and PCa groups compared to controls (p= 0.002). B) Beta diversity analysis using PCoA based on Bray-Curtis dissimilarity revealed distinct clustering of PCa samples, with partial overlap between BPH and controls groups, indicating differences in microbial community structure (*P*=0.002).

Supplementary Files

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