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RESEARCH PAPER

Assessing the role of the microbiome in the pathogenesis of prostate cancer and its relationship with patient clinical characteristics

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Abstract

Background The occurrence of prostate cancer showed a 3% yearly rise between 2014 and 2019, marking a reversal from two decades of declining rates. This resulted in an additional 99,000 new cases. Age, diet, genetics/hereditary, lifestyle, ignorance, environment, etc. are known to contribute to cancer progression. Research has determined that microbial dysbiosis contributes to cancer prognosis. The high-throughput sequencing technologies help in understanding the role of uncultured bacteria in a profound manner with great clarity.

Methods The research involves 10 histopathological confirmed patients, 3 diagnosed with benign prostatic hyperplasia (BPH) and 7 with PCa. Tissue samples were procured from patients undergoing TRUS and further tissue bacterial genomic DNA was isolated. The microbial composition was assessed using the advanced 16S metagenomic sequencing method. This comprehensive approach aims to characterise the microbial landscape associated with BPH and PCa pathology in the studied patients. The analysis data was compared with the patient's clinical characteristics.

Results A Diverse and noteworthy rise in the abundance of bacterial species in BPH tissue samples compared to PCa samples. Our results indicated a potential correlation between earlier tumour stages heightened bacterial presence and more advanced stages where bacterial abundance was comparatively lower.

Conclusion Our report suggests that the variations in abundances, whether higher or lower, of specific species in malignant prostate tissue suggest potential diagnostic and prognostic applications in the future. It would help in the urgent need to develop or enhance existing tools to prevent unnecessary over-treatment or inadequate treatment for patients.

Keywords Prostate cancer · Benign prostatic hyperplasia · Microbiome · Metagenomics sequencing · PSA

Abbreviations

PCa	Prostate cancer
BPH	Begning prostatic hyperplasia
NGS	Next generation sequencing
PSA	Prostate specific antigen
OUT	Operational taxonomical units
PCoA	Principal Coordinate Analysis
TRUS	Tran rectal ultrasound guided prostate biopsy

Introduction

Prostate cancer is the most prevalent cancer among older men and has been extensively studied by scientists since the 1950s. Despite significant research efforts, the evidence supporting the causes of the disease remains inconclusive (Stangelberger et al. 2008). In India, the epidemiological prevalence of prostate cancer ranges from 5.0 to 9.0 cases per 10,000 individuals per year, surpassing rates in Asia and Africa. Notably, the 5-year survival rate for prostate cancer patients exceeds 99% (Gupta et al. 2017). In the United States, approximately 60% of prostate adenocarcinoma has been detected in men aged 60 years and above.

Prostate cancer prognosis can be influenced by various factors, which play a crucial role in the progression, development, and outcomes of the disease. Certain factors that are known to impact prostate cancer prognosis include age, obesity, diet, ignorance, ethnicity, genetic factors, and access to health care (Bashir et al. 2015). Despite

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significant research efforts and evidence, the causes of the disease, remains inconclusive. Trillions of microbial cell assemblies residing within the human body engage in a symbiotic relationship with their host, significantly influencing the host's overall health (Xavier et al. 2020; Wheeler et al. 2019). The difficulty in effectively identifying and isolating unculturable bacteria, along with the absence of reliable tests, hinders scientists from differentiating between aggressive and indolent prostate cancer at any stage, thereby restricting advancements in microbiome research. There is an urgent need to develop or enhance existing tools to prevent over-treatment or inadequate treatment (Kustrimovic et al. 2023).

Recent advancements in high-throughput sequencing techniques, including Sanger sequencing, Next-Generation Sequencing (NGS), and pan-pathogen microarray metagenomics analysis (PathoChip), have enabled the scientists to identify both anaerobic and aerobic bacteria that are difficult to culture, facilitating the study of microbiome dysbiosis and its impact on various disorders or diseases (Sarkar et al. 2022; Hurst et al. 2022; Sfanos et al. 2008). Multicentric microbiome studies have determined that dysbiosis may contribute to a pro-inflammatory response, exerting direct or indirect effects on the development and progression of cancer (Feng et al. 2019). However, the precise mechanisms remains unclear.

Specific opportunistic bacteria, such as *Enterobacteriaceae, Proteobacteria,* and *Fusobacteria sp.,* are recognized for inducing inflammatory responses. Inflammation caused by the microbiome could contribute to prostate carcinogenesis. The most commonly found bacteria, *Propionibacterium acnes* isolated from prostate cancer patients, is positively associated with prostate inflammation (Yow et al. 2017). Similarly*, Actinomyces, Acinetobacter,* and *Streptococcus spp.* are other common bacteria found in benign and prostate cancer positive patients (Kustrimovic et al. 2023; Cohen et al. 2005). Recent research has determined that 90% of samples are positive for the presence o*f Helicobacter pylori. H. pylori* is known to have a cytotoxic gene A (cagA) having a cytotoxic effect on prostate DNA. It is one of the major organisms of concern, causing gastric cancer. Furthermore, research suggests that organisms infecting patients may differ genotypically and phenotypically from the common strains (Khatoon et al. 2016).

Short-chain fatty acids derived from the microbes are known to facilitate prostate cancer carcinogenesis and affect immunotherapy response (Yang et al. 2023). Studies have also determined the positive effect of microbiome in cancer treatment. Clinical trials have suggested that fecal microbiota transplantation (FMT) along with immunotherapy has enabled the researchers to explore new cancer treatments having tumor growth suppressor properties (Pernigoni et al. 2023).

Various studies have also established links between sexually transmitted bacteria *(Ureaplasma, M. genitalium, M. hyorhinis, Neisseria gonorrhoeae, and Chlamydia trachomatis)* and cancer progression (Chattopadhyay et al. 2021). Similarly, viruses are known to have a major contribution to carcinogenesis for example Human papillomavirus 16/ 18, human cytomegalovirus etc. Approximately 80% of the microbiome studies have detected the presence of bacterial DNA in the prostate gland (Cavarretta et al. 2017). Microbiome investigation could potentially serve as biomarker for screening high-risk versus lower-risk population.

The primary goal of this research was to genetically characterize the microbiome present in prostate tissue. Moreover, the research aimed to explore the potential connection between the identified microbiome and levels of prostate-specific antigen (PSA) in histopathological confirmed cases of benign prostatic hyperplasia (BPH) and prostate cancer (PCa) samples. By exploring the genotypic aspects of the prostate tissue microbiome and its potential correlation with patient's clinical characteristics s, this study seeks to provide new insights into the role of the microbiome in prostate health.

Materials and methods

Study design and patient population

All the male patients aged > 50 years, complaining of Lower Urinary Tract Symptoms (LUTS) ($n = 10$), including benign prostatic hyperplasia (BPH) patients ($n = 3$), and prostate cancer (PCa) patients ($n = 7$), visiting the Department of Urology KLE's Dr. Prabhakar Kore Medical and Research Centre advised for TRUS- Tran rectal ultrasound-guided prostate biopsy, were considered for the study. Patients with antibiotic prophylaxis were excluded from the study. Patients diagnosed with BPH were considered as "controls" with prostate-specific antigen levels, while histopathologically confirmed prostate cancer patients were considered as positive samples. The Ethics Committee on Human Subjects of KAHER **[Ref. No. approved the study KAHER/ EC/22–23/113 (iv)].**

Specimen collection

The prostate tissue biopsy chips (2–3 mm in size) were collected from each patient undergoing a biopsy and immediately immersed in a saline-containing container. The samples were transported to the laboratory for further analysis within one hour. After analysis, the tissue samples were stored in 70% ethanol at – 20 ºC.

DNA extraction

All prostate samples ($n = 10$) were brought up to room temperature before beginning the DNA extraction procedure. Each specimen was macerated into small pieces using a mortar and pestle and then transferred into 1.5 ml graduated plastic tubes containing lysis buffer and proteinase K. Individual sample metagenomic DNA extraction was carried out using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instruction manual. The purity of the isolated DNA was evaluated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the DNA quality and quantity were assessed through agarose gel electrophoresis (1% Agarose). The isolated DNA was stored at − 20 until further assessment.

PCR conditions

All the procedures from sample collection to PCR amplification were carried out under strict sterile conditions. 16S rRNA gene universal primers 101F/533R and 519F/806R were used to amplify targeted hypervariable regions V2-V3 and V4. All the primers were taken from Yow et al. 2017, for species identification and characterization of bacterial taxonomy **(Table** 1**)** Cycling conditions considered for PCR amplification were 95% for 5 min, 35cycles for 45 s at 95, 56 ºC for 60 s and 72 ºC for 90 s with final extension at 72 ºC 5 min (Yow et al. 2017). he PCR amplicon results were assessed using 1.8% agarose gel electrophoresis.

MiSeq Library preparation using Illumina

The Illumine amplicons qualified for quality control were amplified following the Illumina standard protocol. The purification of amplicons utilized AMPure XP beads (Beckman Coulter, Brea, CA, USA), and the quantification of these amplicons were conducted with a Qubit Fluorometer. Following the manufacturer's library protocol, amplicon analysis was performed using D1000 Screen tape on the 4200 Tape Station system (Agilent Technologies, USA). MiSeq (20 pM) was used to load the determined libraries for cluster generation and sequencing, for forward as well as reverse direction Paired-end sequencing was used. Reagents from the kit were utilized for binding complementary oligo adapters to the samples on a paired-end flow cell**.** The adapter design enabled specific cleavage of the selected forward and reverse strands during sequencing.

Metagenomics pipeline

An overview of 16S metagenomics sequencing involved the following steps a) quality control, b) align sequences c) clean alignments d) pre-cluster sequences e) chimera detection f) classify sequences g) Remove non-bacterial sequences h) generate a distance matrix i) OUT clustering j) classify OUT k) Taxonomy table. The Roadmap (ampliseq v1.1.2) framework through DADA2 and QIIME2 was used to analyze the paired-end reads of Illumina for amplicon sequencing following the FAIR principle. Several scientific tools like FASTQC and MultiQC were used to assess the quality of raw data. Primer fragments were eliminated from the reads by using the cut adapt plugin. Similarly, to discard the noise, bind and chimeric sequences, the DADA2 plugin was used. Chimeric regions were flagged using the UCHIME algorithm. The GREENGENESv.13.8–99 database was used taxonomically classify the filtered contigs. (Operational Taxonomic Units) OTUs were clustered and their abundance was estimated. According to the Human Microbiome Project database, the determined sequence were divided based on the degree of taxonomy, i.e., phylum, genus, and species. The taxonomical classification was used to state the relative abundance of OTUs. The diversity of the OTUs was assessed using a rarefaction curve. PCoA plot was used to determine the similarity among the samples based on their taxonomy. Ampvis2 R package tool helped to determine heatmaps per sample for the top 20 taxonomical classes. Beta diversity measured the richness and variations among the taxonomy, where alpha diversity (Chao1 and ACE) measures the abundance-based Coverage Estimator indices and the richness and distribution of taxa. These were assessed using Shannon, Fisher, Simpson, and InvSimpson indices. Beta diversity was assessed using Whites-non-parametric test in STAMP, which determines the statistical significance differences in OTUs.

Statistical analysis

To study the association between microbiome and host, the patients were categorized into clinical groups, including, age, PSA, risk level, TNM stage, and Gleason score

Table 1 16S hypervariable region V2F-V3R and V4F-V4R

Sr. no	Oligo Name	Seq 5' to 3'	Molecular weight
	V2- 101F	AGGGCG(INO)ACGGGTGAGTAA	6200.62
2	V3-533R	ATTACCGCGGCTGCTGG	5202.38
3	V4-519F	TGCCAGCAGCCGCGGTAA	5509.60
4	V4-806R	GGACACAGGTATCTAAT	5843.85

(Table 2). The software ggplot2 in R studio was used to build the graphs, similarly, phyloseq package in R studio was used to determine Constrained Analysis of Principal Coordinates (CAP).

Table 2 Clinicopathological Characteristics

	No. samples
Patients, (N)	14
Tissue samples	10
Age (median)	70.4
Tumour	
Benign	3 (30%)
Malignant	7(70%)
Gleason Score	
< 7	4 (40%)
> 7	6(60%)
PSA (ng/ml)	
< 6.5	3 (30%)
> 6.5	7 (70%)
TNM stage	
pT1	4 (40%)
pT2	4 (40%)
pT3	2 (20%)
Risk	
Low	3 (30%)
Medium	4 (40%)
High	3 (30%)

Results

Characteristics of case series

In this study, a total of 10 patients undergoing TRUS-guided biopsy were considered, among which 7 (70%) patients were prostate cancer-positive (malignant), and the remaining 3 (30%) were benign prostatitis hyperplasia patients. All the patients considered for the study had a mean age of 70.4 years. Patients were evaluated based on histopathological reports, Gleason Score, PSA, and TNM staging. (Table 2). About 40% of samples had a Gleason score of less than 7, while the remaining 60% had a score of 7 or higher. An equal distribution of samples was observed, with 30% PSA < 6.5 ng/ml and another 70% having a PSA value > 6.5 ng/ml. In terms of pathological TNM stage, approximately 40% of the samples were categorized as T1, 40% as T2, and 20% were classified as T3.We systematically compared the microbiome between aggressive and less aggressive samples using patient clinical data.

PCR amplification

The samples underwent amplification using 16S universal bacterial primers. All amplified samples tested positive for 16S universal bacterial primers, specifically within the V2-V3 and V4 hypervariable regions, displaying a distinct band at 460-478 bp. (Fig. 1a and b).

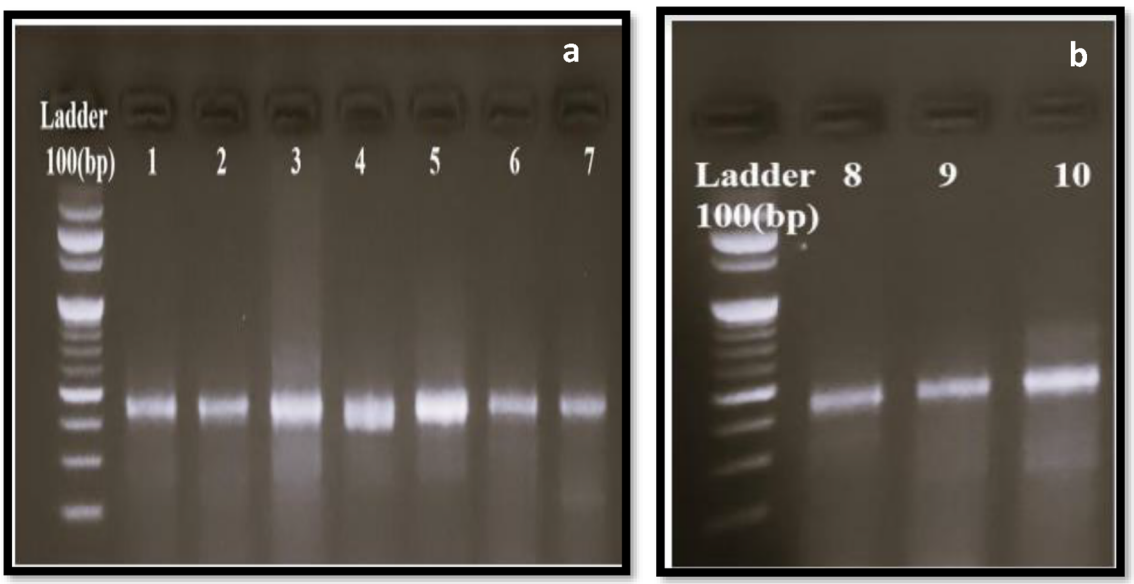


Fig. 1. 1% Gel Electrophoresis imaging of 16S hypervariable region. Bands around 460-478 bp

16 s Metagenomics sequencing data analysis

A total of 10 samples were sequenced using a MiSeq platform, yielding 2, 83,482 reads on average after eliminating low-quality reads from the raw data having a read length of 251 bp and GC content higher than 50%. All samples exhibited a smooth and steady progression in rarefaction curves (Fig. 2), suggesting that the sequencing depth was adequate for conducting diversity analysis. Principal Coordinate Analysis (PCoA) plots were used to visually depict the compositional differences of Operational Taxonomic Units (OTUs) among individual samples (Fig. 3). A total of seven hundred and eighty-seven unique OTUs were identified in all 10 samples, from which 26 phylum, 343 genera, and 330 species were determined. Bar plots of 16S metagenomics sequencing represent the top 10 and top 20 families, phyla, genera, and species (Fig. 4) White's non-parametric test was used to identify the common and unique OTUs between two groups: Group 1 with PSA $\leq$ 6.5 and Group 2 with PSA $\geq$ 6.5. A total of 398 OTUs were common between both groups, whereas Group 1 had 158 unique OTUs, and Group 2 had 230 unique OTUs. The heat map illustrates the species-specific abundance, providing a visual representation of the varying abundance of different species based on PSA values (Fig. 5).

Alpha diversity

Six different alpha diversity measures were employed to investigate overall differences in species richness. This included assessing the total number of species (Observed, Chao1, and ACE) as well as their even distribution, indicated as relative abundances (Shannon, Simpson, and Inverse Simpson) in both benign and malignant prostate tissue samples. Variations in alpha diversity observed between benign and malignant prostate tissue samples suggest the presence of microbial dysbiosis, which may be associated with PCa. This potential microbial imbalance could contribute to our understanding of the role of the microbiome in the development or progression of PCa. Further analysis and exploration of these differences may provide valuable insights into the complex relationship between microbial communities and prostate health or disease. Upon visual examination of our data, there appears to be a consistent decrease in species diversity in PCa malignant tissue when compared to benign tissue, as indicated by all the measures of alpha diversity. This reduction in diversity across multiple metrics suggests a potential shift in microbial composition associated with malignancy in the prostate tissue. Among the six alpha diversity measures tested, Chao1, ACE, and Shannon indices showed

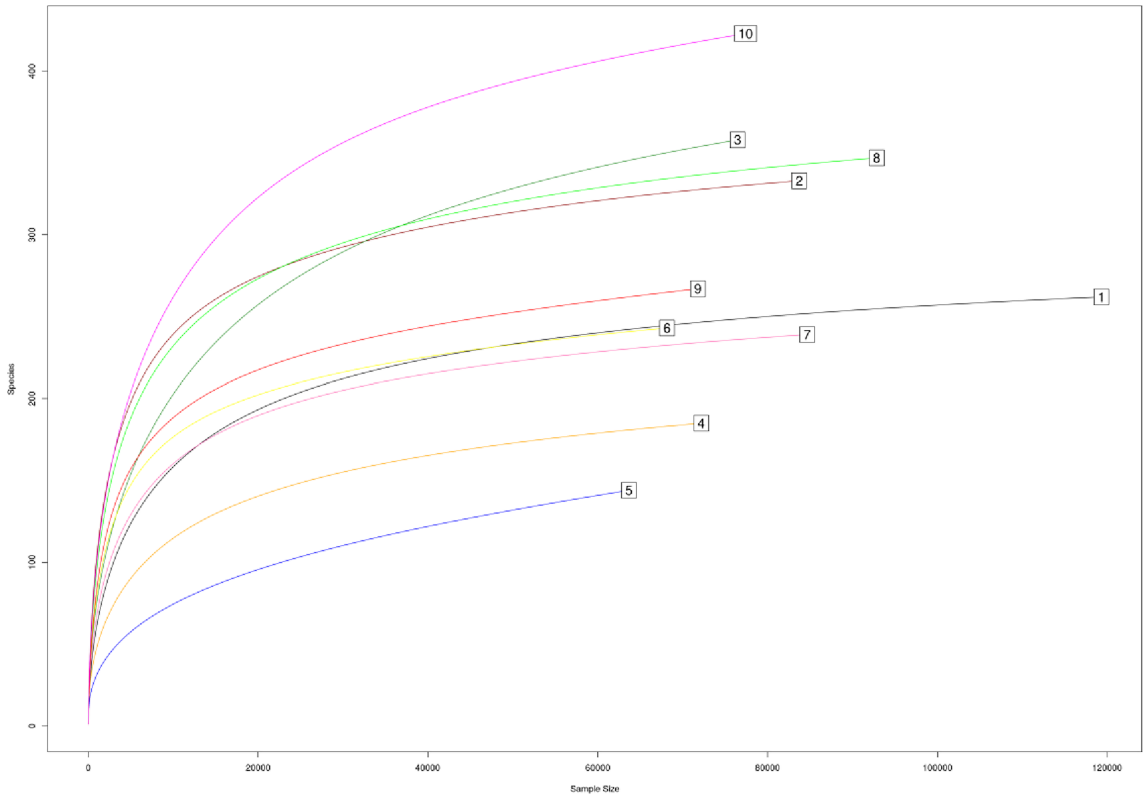


Fig. 2 - Rarefaction plot determines the Number of OUT reads per samples

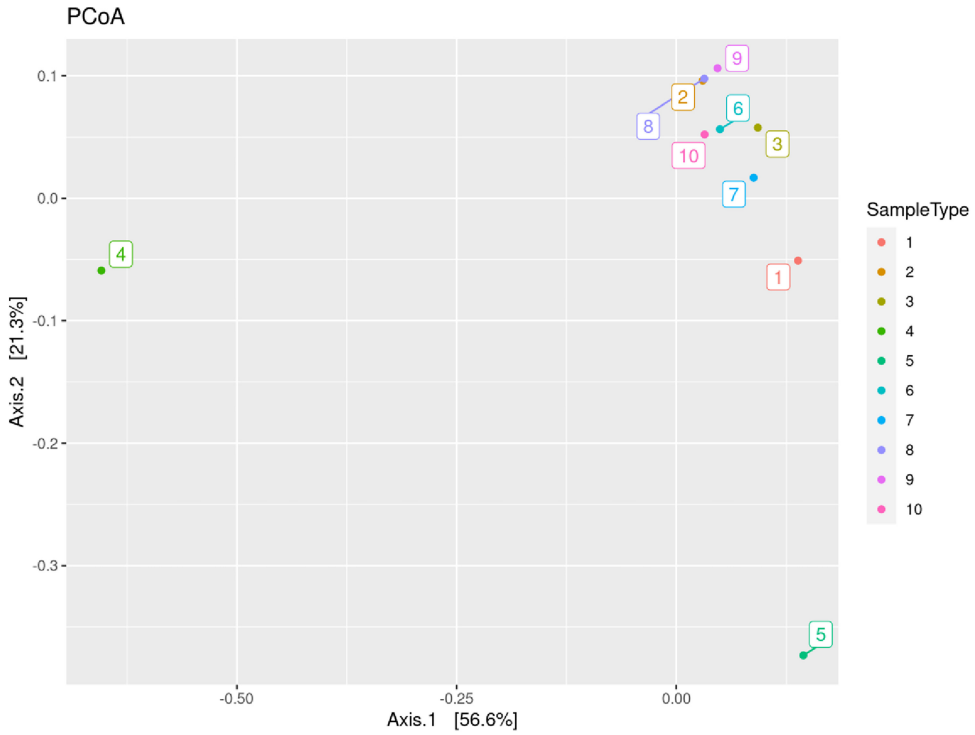


Fig. 3 - PCoA Plot determines the similarity of OTUs between each sample

significant differences with p-values of < 0.039, < 0.036, **and** < 0.026, respectively (Fig. 6 a, b).

Microbial abundance

The abundance of bacteria was greater in samples from individuals with benign prostatic hyperplasia (BPH) when compared to those with prostate cancer (PCa). *Kocuria, Rhizophila, Nocardioides, Pseudonocardiaceae, Flavobacteriales, Streptophyta, Brunensis, Cibaria, Phyllobacteriaceae, Paracoccus, Rhodobacter,* and *Rheinheimera perlucida* were the common species found in both BPH and PCa samples.

Microbial species differentiation among BPH and PCa tissue samples

The most abundant taxa present in both prostate cancer-positive as well as benign samples were *Proteobacteria* accounting for about *54%* followed by *Firmicute (40%), Actinobacteria (5%), Deinococci (2%), Bacteroides (2%), cyanobacteria. (0.5%) and Fusobacteria (0.2%).*

Beta diversity was used to measure the sequential microbial difference between BPH and PCa tissue samples in relation to Gleason score, tumour staging and PSA (Shoskes., et al., 2016). Distinct microbial species were identified, highlighting differences between tissue samples from individuals with BPH and PCa. In BPH samples, *Varibaculum, Solirubrobacterales, Lactobacillaceae, Pediococcus, Skermanella,* and *Erythrobacteraceae* were identified. In contrast, PCa tissue samples exhibited the presence of *Defectiva, Cruenta*, and *Aerolata*, along with the bacterial families *Clostridiaceae* and *Tissierellaceae*. This microbial species differentiation relates to the unique microbial compositions associated with BPH and PCa.

Clinical characteristics and microbial differentiation

There was no statistically significant difference observed between the age of patients and the microbial variations. This suggests that the microbial differences identified in the study are not strongly influenced by the age of the individuals. Indeed, when considering the PSA values, Gleason score, and tumor types of all the patients, it was observed that BPH exhibited a higher abundance of pathogenic bacteria compared to PCa. The bacterial abundance was observed to be higher **in** tumor stages T1 and T2 when compared to T3. This finding indicates a potential correlation between earlier tumor stages (T1 and T2) and increased bacterial presence, as opposed to the more advanced T3 stage, where bacterial abundance appears to be comparatively lower. *Staphylococcus saprophytic* was significantly more abundant in the T3 stage in comparison to T1 and T2. Similarly, the abundance of *Lactobacillus helveticus* was decreased in PCa samples in comparison to BPH.

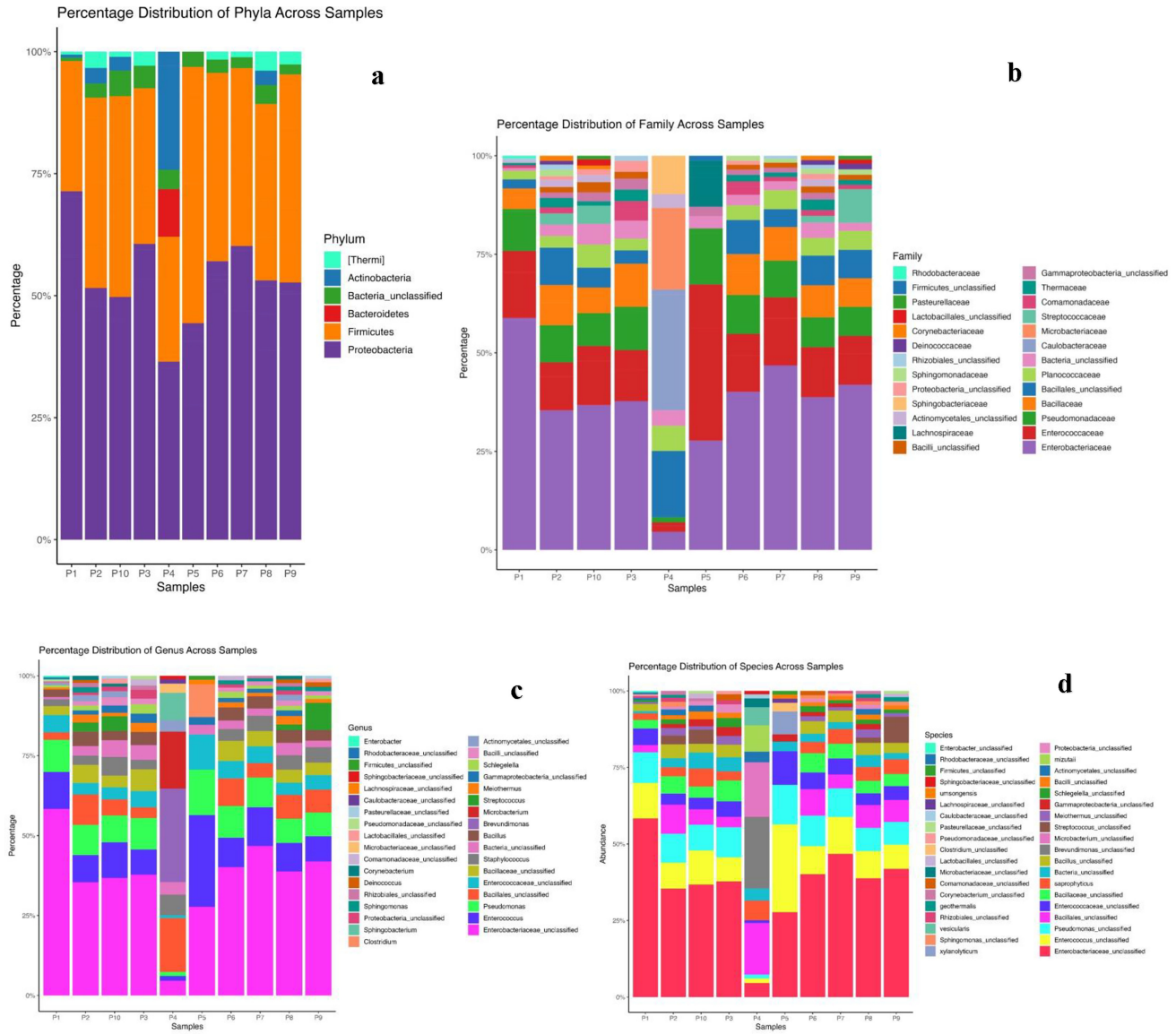


Fig. 4 - Bar plot visualising the percentage distribution of (**a**). phylum in each samples **b**. Family **c.** Genus **d**. Species

Discussion

Since decades ago, there has been a notable urge in research investigating the link between the host microbiome and cancer, primarily driven by significant advancements in the sensitivity and specificity of molecular technology (Yang et al.2023). Numerous studies have elucidated the impact of microbiome dysbiosis on the prognosis of various cancers, including colorectal cancer, gastric cancer, oral cancer, and prostate cancer (Sfanos et al. 2008; Pernigoni et al. 2023; Chattopadhyay et al. 2021). The continuous development in molecular technology is crucial for the ongoing improvement and advancement of research methodologies in the exploration of complex scientific questions. Therefore, microbial mapping is indeed necessary to improve prognostic and diagnostic perspectives in cancer. Prostate cancer is a complex disease influenced by a combination of genetic, environmental, microbiota, and lifestyle factors. The interplay of these elements makes it challenging to identify the specific causative factors. Ongoing research aims to study the complexities of prostate cancer etiology and identify the intricate relationships between the microbiome and its host. As our study continues to evolve, it is anticipated that new insights will contribute to more effective prevention strategies and targeted interventions for prostate cancer. Studies have described the presence of unique prostate regional microbiota *Helicobacter pylori* as one of the species of concern. The World Health Organisation has categorized this organism as a carcinogen and is known to be the major reason for gastric cancer (Sarkar et al. 2022). Trillions of

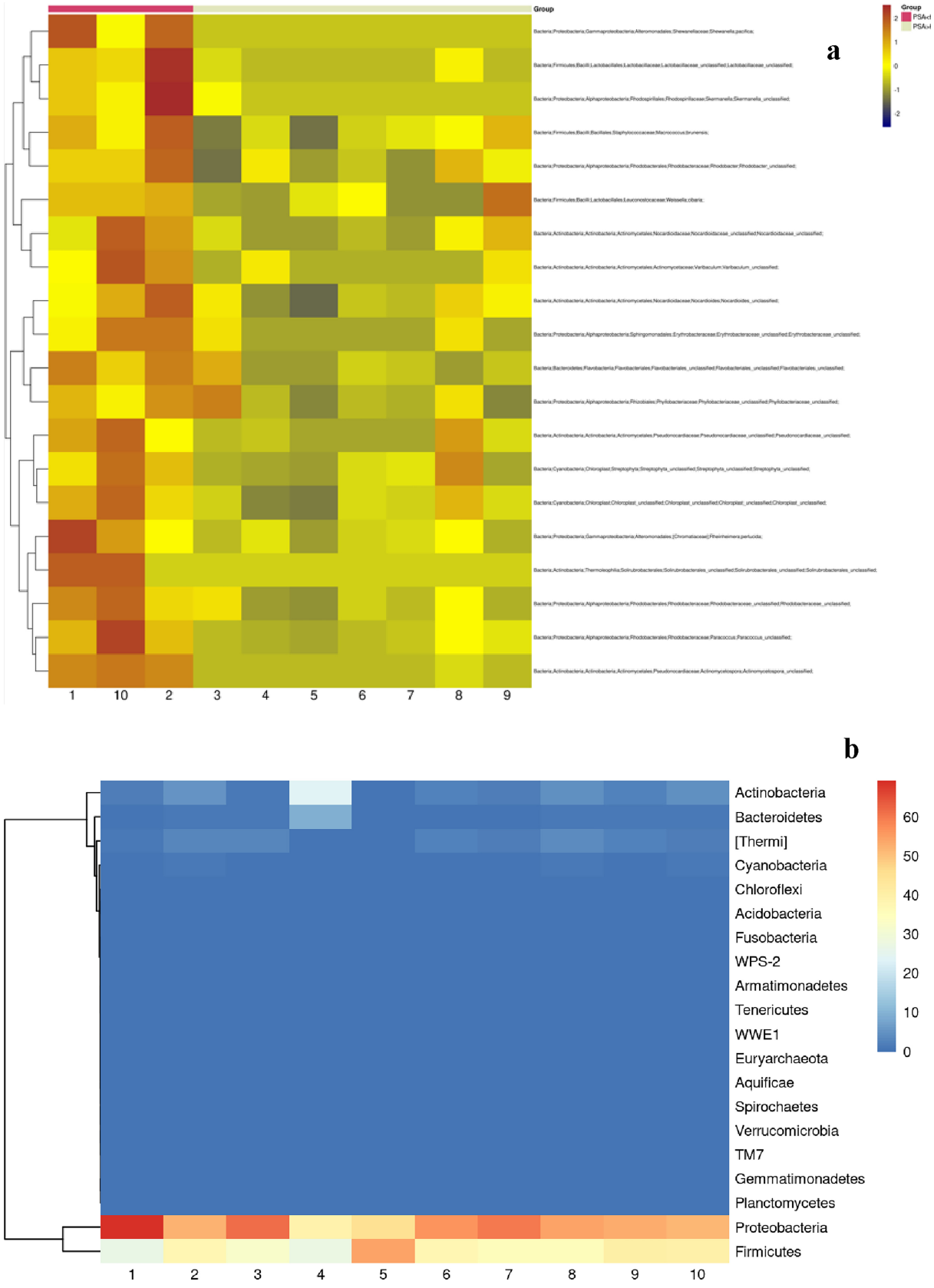


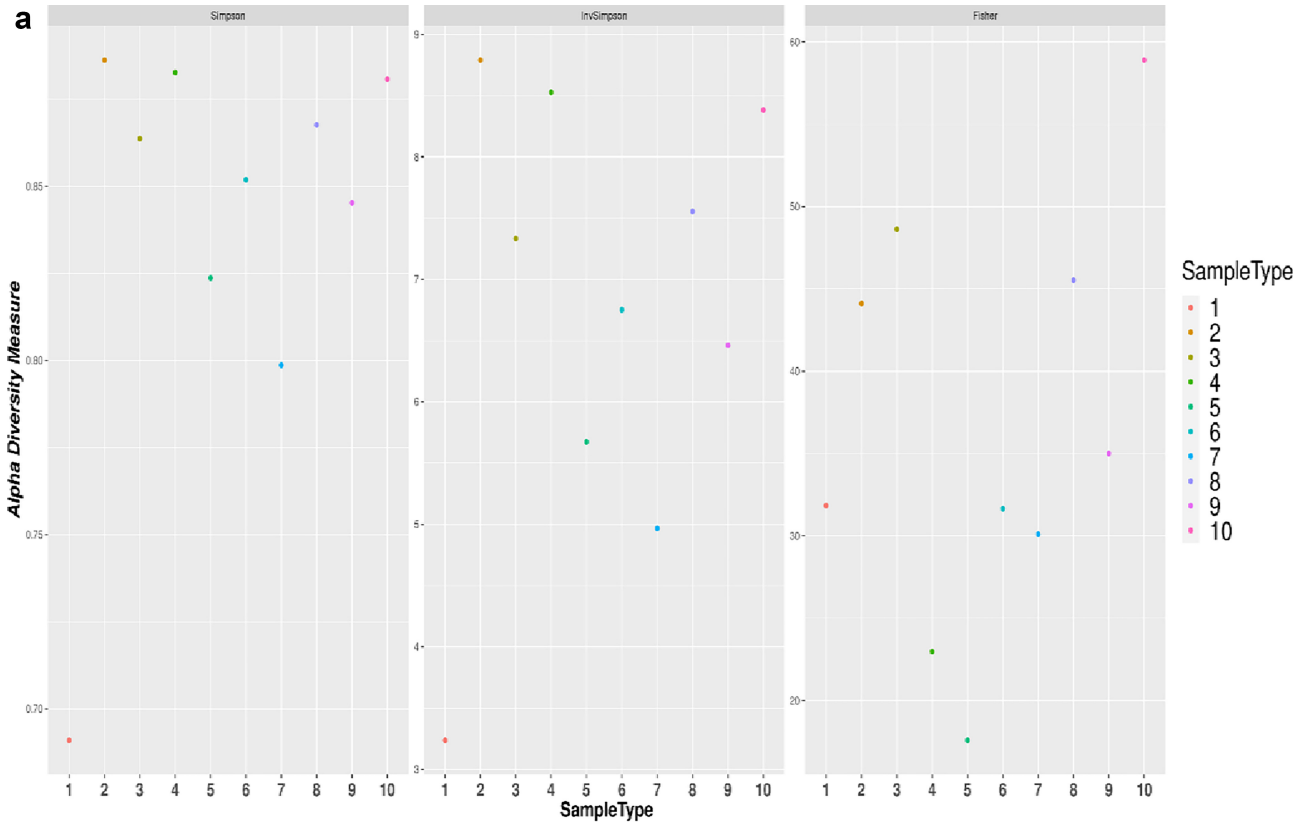
Fig.5 **a.** Heat map represents the percentage abundance of specific species in each sample**. b.** Heat map representing the abundance of specific bacteria in correlating it with PSA < 6.5 and > 6.5

bacteria reside within and around the gland, being one of the major mainstays at the site of inflammation yet in all the research carried out to date the relation between dysbiosis of the microbial community and its influence on prognosis of carcinogenesis remains unclear (Cavarretta et al. 2017). In our study utilizing 16S metagenomics sequencing, we found a noteworthy increase in the abundance of bacterial species in benign prostatic hyperplasia (BPH) tissue samples compared to prostate cancer (PCa) samples. Additionally, our results indicated a potential correlation between earlier tumour stages (T1 and T2) and heightened bacterial presence, contrasting with the more advanced T3 stage where bacterial abundance seemed to be comparatively lower. These findings provide insights into the microbial dynamics associated with BPH and different tumour stages in prostate cancer. *Porphyromonas sp. nov, Varibaculum sp. nov., Peptoniphilus sp. nov., and Fenollaria sp. nov* are four novel bacteria isolated and identified by researchers in the urine of prostate cancer-positive patients (Gupta et al. 2017). Similar to our study, previously, Ye Feng along with other researchers studied the metagenomic and metatranscriptomic analysis of prostate microbiota in prostate cancer patients. Their results determined that *Escherichia, Propionibacterium, Acinetobacter, and Pseudomonas* were the dominant bacteria present inside the core of prostate cancer patients (Feng et al. 2019). The presence of *E.coli* in benign prostate samples is known to induce DNA damage and further lead to inflammation (Jain et al. 2020). Our study, determined that all the samples were positive for *Pseudomonas* spp. These species are known to have an outstanding capability to adapt. They are known to affect patients suffering from AIDs, wounds, and cancer, extensively produce extracellular virulence factors and cell-associated virulence factors causing tissue damage, host cell damage, exotoxins, lipopolysaccharides, chronic infections, T3SS secretion leading to damage to the host immune system, etc. (Paprocka et al. 2022). All the samples were positive for *Enterobacteriaceae sp*. The percentage of these in all the samples was above 25% being the most abundant bacteria. No significance was shown between PCa versus BPH patients. *Enterobacteriaceae* has been widely studied in colorectal cancer, intestinal bowel disease, etc. for its role in causing inflammation and its carcinogenic response (Arthur et al. 2014). *Mycobacterium sp*. a common nosocomial bacteria found to be abundant in T3 stage samples then T2 and T1. Our results coincided with a similar research conducted in 2022 that found the presence of *Mycobacterium sp*. and determined the spp. to be abundant in patients with higher Gleason score (Zhang et al. 2018). Ilaria Cavarretta et al. 2017 conducted a microbiome study and determined that *Streptococcus* was highly abundant in non-tumour regions, playing a role in maintaining the balance of the host extracellular environment (Kustrimovic et al. 2023). A study also revealed a significantly higher proportion of *Streptococcus* in the cancer group compared to the Non-cancerous group, possibly attributed to differences in sample types (Cavarretta et al. 2017). In this study, we also observed a pronounced rise in *Streptococcus* in high-grade samples as opposed to low-grade ones. *Staphylococcus* often takes on a pathogenic role by evading the host immune system through surface adhesion molecules such as PVL (Panton-Valentine leukocidin), SpA (staphylococcal protein A), α-hemolysin, TSST-1 (Toxic shock syndrome toxin-1), and SEs (staphylococcal enterotoxins) (Wei et al. 2022). *Enterobacteriaceae Staphylococcus sp, Streptococcus sp., Moraxella spp.,* and *Propionibacterium acnes* were identified as members of the 'core' microbial community in prostate cancer patients (Xavier et al. 2020; Wheeler et al. 2019; Salachan et al. 2022). Aligning with our results, previous research indicated that specific quantities of *Lactobacillus crispatus* lead to a decrease in tumour size and a reduction in Cox2 expression within tumour tissues in a mouse model of experimental breast cancer (Ma el al. 2019). They discovered that specific microbes, including *Lactobacillus crispatus, Listeria monocytogenes,* and *Thermus thermophilus*, contribute to the inhibition of tumour growth. Conversely, other microbes such as *Nevskia ramosa* and *Staphylococcus* promote cancer by inducing inflammation, fostering immunosuppression, and regulating the expression of prostate cancer stem cells (PCSCs) that drive metastases (Motevaseli et al. 2018). Elevation in *Escherichia coli* coincided with a reduction in probiotics like *Lactobacillus helveticus* and *Lactobacillus iners*. (Yin et al. 2021). *Actinobacteria* species are high producers of a wide range of bioactive natural products which has anticancer and ant proliferative effects on colorectal cancer (Yodolla Bahrami et al. 2022). There was a significant difference in the abundance of actinobacteria between BPH and PCa patients. Species abundance was decreased in PCa having a higher Gleason score. Proteobacteria phylum is the most prevalent bacteria isolated from bladder, colitis-associated colorectal cancer (Bonnet, et al. 2014) and gut (Human Microbiome Project Cosortium, 2012) cancer patients. These changes could potentially serve to predict risk and initiating early treatment for prostate diseases.

Our study has certain limitations, one major limitation is the smaller sample size, Secondly, and the microbiome assessment of normal prostate tissue samples has not been carried out due to ethical issues and the impracticality of the medical code of conduct.

Conclusion

In conclusion, our study demonstrates an association between prostate cancer and dysbiosis within the prostate microbial ecosystem, revealing a decreased overall species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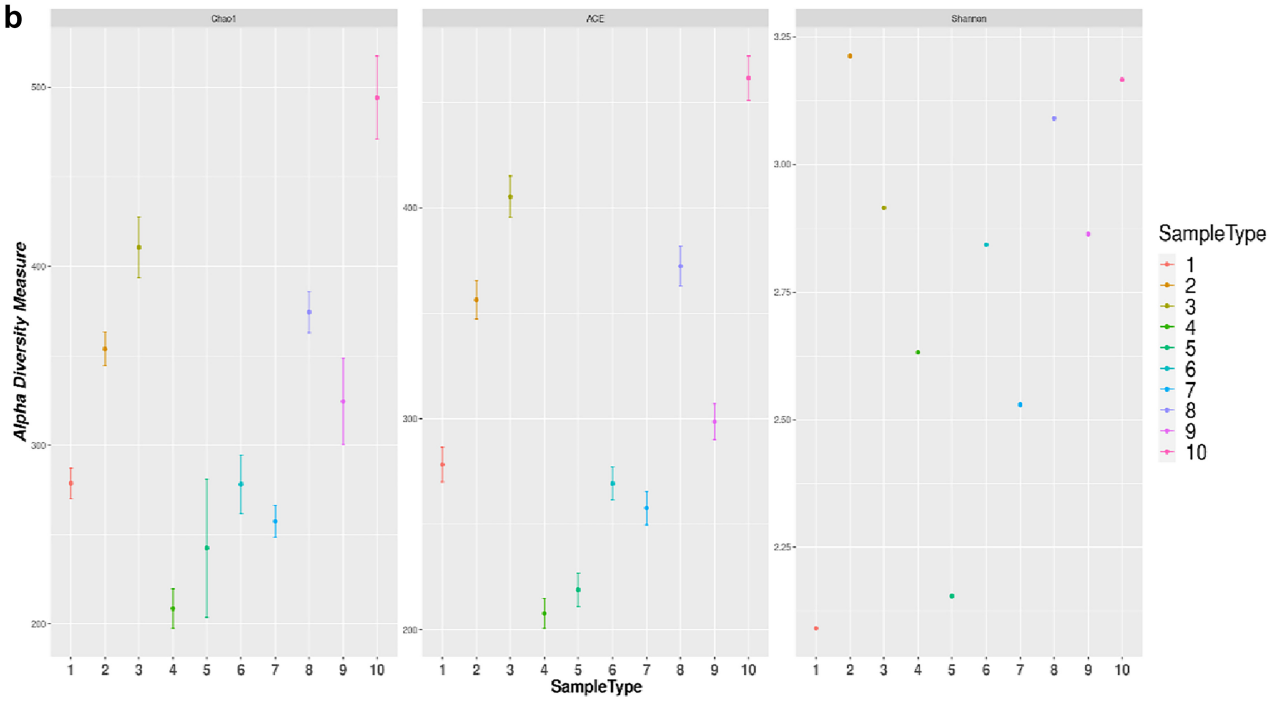
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◂**Fig.6 a, b.** Chao1 and ACE focus on aspects of species richness, while Shannon, Simpson, and Inverse Simpson encompass both species richness and evenness. Samples 2, 3, and 10 were benign while samples no. 1, 4, 5, 6, 7, and 8 were BPH samples. A Wilcoxon rank sum test was employed to assess diversity differences between the two groups. Notably, malignant tissue samples exhibited a significant overall reduction in species diversity compared to benign tissue in the discovery cohort, reaching statistical significance at a p-value threshold of 0.05. This suggests a substantial alteration in microbial composition associated with malignancy in the analysed tissue samples

diversity in malignant tissue compared to benign samples. The variations in abundances, whether higher or lower, of specific species in malignant prostate tissue suggest potential diagnostic and prognostic applications in the future. In our research, *Staphylococcus* merged as the most prevalent bacteria across all samples. Notably, there was a substantial elevation in the abundance of this bacterium observed in patients with T3-stage samples. Similarly, we determined *Lactobacillus helveticus* was decreased in PCa patients. *Pseudomonas sp* were highly abundance in PCa samples. Therefore, our findings substantiate the presence of a significant biological connection between the microbiota in the prostate and the development/progression of prostate cancer (PCa). Future research should focus on exploring the mechanistic relationships between specific microbial species and prostate cancer progression. Large-scale studies involving diverse patient cohorts could validate and expand upon our results. Furthermore, integrating multi-omics approaches, such as metabolomics and transcriptomics, may uncover new biomarkers for early detection and prognosis

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Declarations

Conflict of interest The authors declare no conflict of interest.

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