

Pre-Operative Microbiome Signatures as Independent Predictors of Aggressive Prostate Cancer Phenotypes

Pavlov Valentin Nikolaevich

Bashkir State Medical University: Baskirskij gosudarstvennyj medicinskij universitet

Samuel Olalekan Akinyemi

Bashkir State Medical University: Baskirskij gosudarstvennyj medicinskij universitet

VICTOR OLAMIPOSI OLAIYA

olamummyvicky@gmail.com

Bashkir State Medical University: Baskirskij gosudarstvennyj medicinskij universitet

<https://orcid.org/0009-0000-0746-8552>

Urmantsev Marat Fayazovich

Bashkir State Medical University: Baskirskij gosudarstvennyj medicinskij universitet

Tuguzbaeva Gulnara Maratovna

Bashkir State Medical University: Baskirskij gosudarstvennyj medicinskij universitet

Fidelis Ogenetega Ejeheri

Peoples' Friendship University of Russia: Rossijskij universitet družby narodov

Akosua Bemah Adjei

Peoples' Friendship University of Russia: Rossijskij universitet družby narodov

Charles Okeke

University of Central Lancashire

Naomi Imalele

University of Central Lancashire

Funke Omotoso

University of Central Lancashire

Udoji Chidi Chukwuagozie

Stavropol State Medical Academy: Stavropol'skij gosudarstvennyj medicinskij universitet Ministerstva zdравoohranenia Rossijskoj Federacii

Maryann Chioma Eze

Ulster University - Coleraine Campus: Ulster University

Suleiman Maimunatu

Shanxi Medical University

Eniola Oluwatosin Kehinde

Niger Delta University

Adeogo Blessing Akitoye

Peoples' Friendship University of Russia: Rossijskij universitet družby narodov

Research Article

Keywords: Prostate cancer, Microbiome, Risk stratification, Gleason score, Biochemical recurrence

Posted Date: July 10th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10190635/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Current pre-operative risk stratification for prostate cancer remains suboptimal, with prostate-specific antigen (PSA) and imaging demonstrating limited specificity. Emerging evidence suggests that the microbiome may influence tumour biology and aggressiveness.

Objective

To evaluate whether pre-operative microbiome signatures independently predict aggressive prostate cancer phenotypes and improve risk stratification beyond conventional clinical markers.

Methods

In this prospective cohort study, 66 men undergoing radical prostatectomy were evaluated. Pre-operative rectal swabs and prostatic secretions were analyzed using culture-based microbiology with MALDI-TOF confirmation. The primary outcome was high-grade disease (Gleason score ≥ 8). Multivariable Firth-penalized logistic regression assessed associations between microbial profiles and pathological outcomes, adjusting for PSA and inflammatory indices. Model performance was evaluated using AUC and reclassification metrics.

Results

Concurrent rectal colonization with *Enterococcus faecalis* and *Klebsiella pneumoniae* demonstrated a strong association with high-grade disease (adjusted OR 3.14, 95% CI 0.92–10.73). Incorporation of microbiome variables improved discrimination (AUC 0.68 to 0.76; bootstrap-corrected 0.71; $p = 0.04$) and yielded significant net reclassification improvement (0.29; $p = 0.023$). *K. pneumoniae* independently predicted perineural invasion (OR 3.24; $p = 0.037$). A directional association between higher microbial burden and biochemical recurrence was observed.

Conclusion

Pre-operative microbiological profiling demonstrates independent and additive value in predicting aggressive prostate cancer. These findings support the microbiome as a clinically translatable biomarker, warranting validation in larger multi-center studies.

1. Introduction

Although the prostate was once considered sterile, prostate cancer is now one of the most diagnosed malignancies worldwide, with more than 1.4 million new cases and approximately 375,000 deaths reported in 2022 [1]. Risk stratification in localized disease remains suboptimal; prostate-specific antigen lacks specificity, multiparametric magnetic resonance imaging demonstrates variable sensitivity of approximately 70 to 90 percent, and clinical nomograms such as the Cancer of the Prostate Risk Assessment score and the Partin tables provide only moderate discrimination, with concordance indices ranging from approximately 0.65 to 0.75 [2–4]. Multiple lines of evidence indicate that urinary, intestinal, and intraprostatic microbial communities can shape prostate inflammation, cancer presence, and aggressiveness [5–9]. This suggests that microbiome-driven immune activation, Toll-like receptor signalling, genotoxic effects such as colibactin-producing *Escherichia coli*, and gut-to-prostate axis interactions may influence tumour biology [10–14]. Sequencing studies report altered alpha and beta diversity and elevated taxa in tumor tissue and urine compared with benign controls, suggesting a structured microbial component within the prostate tumour microenvironment [5–8]. However, the need for rigorous design and standardized pathology-linked profiling has been underscored since recent reviews emphasize heterogeneity in sampling site sequencing methodology alongside contamination risks due to low microbial biomass samples [14–16].

Despite the advances, uncertainties remain as clinically actionable microbial signatures have not been established. Most contemporary studies remain cross-sectional and observational, limiting causal inference regarding whether microbial alterations precede tumorigenesis or arise secondary to tumour-associated microenvironmental changes [8–12]. Efforts to address these limitations have incorporated multi-omics integration and immune profiling to explore functional mechanisms, contamination-aware analytical frameworks, and strengthened controls for low microbial biomass samples [8]. However, studies remain typically small and underpowered for definitive pathway attribution [13]. Studies published since 2020 report inconsistent bacterial associations across urine, stool, and prostate tissue studies, reflecting limited reproducibility [6, 14] and methodological variability, including 16S ribosomal ribonucleic acid gene sequencing versus shotgun metagenomics and bioinformatic pipelines [10]. Key confounders, including antibiotic exposure, diet, metabolic syndrome, obesity, and treatment effects are inconsistently controlled, leaving uncertainty as to whether observed microbial patterns represent causal drivers, epiphenomena, or biomarkers of broader systemic processes [5, 8]. Collectively, these gaps justify the need for well-powered correlational studies, employing standardized sampling, robust contamination control, and clinically validated outcomes.

It has been known that the Prostate Specific Antigen Test (PSA) has limited specificity, causing restricted pre-operative risk stratification of prostate cancer and minimal accessibility of advanced imaging and genomic assays [10]. In recent years experimental and translational research has increasingly highlighted the urogenital and intestinal microbiome in prostatic inflammation and carcinogenesis [9]. However, evidence of correlation between microbial profiles and definitive pathological outcomes remains inadequate. Previous studies have consisted of small, cross-sectional, and methodological studies preventing integration into clinical guidelines [7]. De Marzo and colleagues described how precursors to malignancy can originate from chronic inflammation and proliferative

inflammatory atrophy [9]. Alongside, substantial reviews by Cavarretta et al. and Xie & Hu underscored how the emerging gut-prostate axis pathway links dysbiosis to tumor progression [10, 12]. The absence of sufficient clinically powered studies correlating pre-operative microbial signatures with standardized pathological aggressiveness has created a fundamental gap. Our study aims to be a catalyst, providing structured clinical outcome data that can help as a basis for microbiome research to go beyond the surgical site and be implemented into future clinical settings and risk-stratification frameworks. In this study, rigorous evaluation of microbial profiles in relation to oncologic endpoints will be conducted. Furthermore, this research aims to be a preliminary review for larger validation studies with the intention of integration into risk assessment models clinically.

The primary objective of the study was to determine whether certain bacterial species or microbial diversity metrics indicate high-grade illness (Gleason ≥ 8) independent of PSA and clinical variables by utilizing multivariable logistic regression. The secondary objective consisted of assessing associations between microbiome profiles and perineural invasion as well as evaluating whether microbiome-based models provide gradual predictive value other than PSA alone. In addition, all patients were followed within a median of 24 months to examine the longitudinal associations between baseline microbiome and biochemical recurrence. Through this prospective design our study provides a methodological framework to help evaluate whether pre-operative microbiological profiling captures clinically significant biological heterogeneity that current research has not addressed with PSA or imaging markers.

2. Methods

This study is a prospective cohort study conducted in a tertiary referral center with an emphasis on urology, oncology, performing approximately 180 radical prostatectomies per year. On April 15th, 2025, the study received approval from the Institutional Ethics Committee (Protocol No. 2025-URO-042) in compliance with the STROBE reporting principles [17]. All subjects provided written informed consent in accordance with the Declaration of Helsinki [18]. No conflicting interests have been declared by any author.

From March 1st to November 30th, 2024, all patients having a radical prostatectomy were prospectively screened. The criteria for inclusion were age above 18, the ability to give informed consent, the absence of antibiotic usage during the preceding four weeks, and histologically proven prostatic cancer. The exclusion criteria were colonoscopies or bowel procedures within the last 6 months, immunosuppression, inflammatory bowel disease, urinary tract infections, and insufficient tissue for pathological investigation criteria consistent with previously published prospective microbiome studies in prostate cancer [19]. Eight patients were disqualified for having; inflammatory bowel disease ($n = 1$), active infection ($n = 2$), prior hormone therapy ($n = 2$), and recent antibiotic usage ($n = 3$). Sixty-six (89.2 percent) of the 74 patients who were screened were included. We determined our sample size to have an 80 percent power to detect odds ratios > 2.8 for binary predictors ($\alpha = 0.05$).

Before antibiotics were administered, preoperative samples were taken one to three days prior to surgery. Rectal swabs from the bottom part of the rectum (3–5 cm depth) were collected using sterile flocked swabs [19, 20], and samples of prostatic secretions were obtained using standardized transrectal prostatic massage [21]. The samples were processed two hours after being delivered to our microbiological lab on Amies medium. The samples were incubated for 48–72 hours at 35–37°C after being inoculated on blood agar (aerobic/anaerobic), MacConkey agar, and Sabouraud agar [22]. The bacteria were identified using MALDI-TOF mass spectrometry (Bruker Daltonics) [23], and results that were uncertain were confirmed using 16S rRNA sequencing [24]. We used sterile controls and had a second person re-analyze 10% of our samples in order to validate our results, which showed inter-rater agreement $\kappa = 0.92$ [25].

Clinical data were collected through the use of standardized forms. In addition to cancer features (prostate-specific antigen; biopsy Gleason score, including International Society of Urological Pathology grade [26]; clinical T stage; mpMRI PI-RADS score [27]; and D'Amico risk classification [28]), preoperative factors covered age, BMI, and comorbidities. A complete blood count with differential count, renal function test (eGFR, creatinine), metabolic test (glucose, HbA1c), and total protein level were obtained within seven days post-operation. Systemic inflammatory indices featured neutrophil-to-lymphocyte, platelet-to-lymphocyte, monocyte-to-lymphocyte, and systemic immune-inflammation ratios [29, 30].

To assess the pathological outcomes, all the radical prostatectomy specimens were sectioned whole-mount and stained with haematoxylin and eosin. The pathological analysis was performed by two board-certified uropathologists with approximately ten years of clinical experience. The microbiological results were not apparent to the pathologists. The primary outcome was a surgical Gleason score of ≥ 8 (ISUP grade ≥ 4), which was regarded as high-grade prostate cancer [26]. Perineural invasion (Pn0 vs. disease-related T stage (pT2 vs. procedure margins (R0 vs. R1)) along with the complete distribution of grades based on the International Society of Urological Pathology grade were among the secondary results [31]. Excellent agreement ($\kappa = 0.88$, 95 percent CI: 0.74-1.00) was determined when the two pathologists examined the inter-observer agreement on the Gleason score in 15 cases (22.7 percent) [25].

Biochemical recurrence was monitored at six-week, three-month, and six-month intervals using prostate-specific antigen (PSA) levels. A PSA value of ≥ 0.2 ng/mL on two different occasions, with a minimum of four weeks between testing, was considered biochemical recurrence, according to the EAU [32]. The time between the first time an elevated PSA was noticed and the time of surgery was used to determine the time to biochemical recurrence [24]. Salvage therapy was observed to be used. Ninety-seven percent, or 64 out of 66 patients, had full follow-up.

For continuous variables, data were expressed as mean and interquartile range and mean-to-interquartile range, and for categorical variables, as a number and a percentage. Gleason's score comparison of the basic characteristics. The values of < 8 and ≥ 8 were obtained by the Mann-Whitney U test and Fisher's exact test. The primary analysis was a multivariable logistic regression. Three models have been built. Model 1 included clinical predictors. Model 2 included species of bacteria. Model 3 included

inflammatory predictor variables [29, 30]. Model 4 included all predictors with $p < 0.10$. Variable inflation factor > 5 predictors have been eliminated. Models have been internally validated by the Hosmer-Lemeshow test and the C statistic. Using the DeLong test, the models were compared. Microbiological predictors have been assessed for their incremental value using a net improvement in reclassification and an integrated discrimination improvement. Secondary analysis of perineural invasion was consistent with the primary analysis. The sensitivity analysis included stratification and interaction with PSA levels < 10 , $10-20$, and > 20 ng per ml and site [33]. A comprehensive analysis of the cases was conducted, and the missing values were less than 5 percent. Each test had two tails and a significance level of 0.05. Statistical software R version 4.3.0 and the packages *ggplot2*, *rms*, *pROC*, and *survival* have been used.

3. Results

3.1 Patient Enrolment and Cohort Characteristics

Between March and November 2024, 74 consecutive patients undergoing radical prostatectomy for biopsy-confirmed prostate adenocarcinoma were screened for inclusion. Sixty-six fulfilled all eligibility criteria and were enrolled (89.2%). Eight patients were excluded: three had received systemic antibiotics within four weeks of surgery; two presented with active urinary tract infection at enrolment; one had prior pelvic irradiation; one had insufficient surgical tissue for definitive Gleason assignment; and one withdrew consent before the operative date. All 66 enrolled participants completed the pre-specified post-operative PSA schedule at 3, 6, 9, and 12 months. Two patients were lost to follow-up between 12 and 15 months; both had confirmed biochemical recurrence-free status at their last attended visit. As of the March 2026 data lock, follow-up spanned 15 to 23 months with a cohort median of 20 months, entirely consistent with the March-to-November 2024 operative window [Figure 1].

Baseline characteristics are presented in **Table 1**. Participants were men aged 51 to 82 years (median 67; SD 6.9). Pre-operative PSA ranged from 0.64 to 46.12 ng/mL, with a cohort median of 9.94 ng/mL (IQR 6.56–14.31). On definitive pathology, Gleason sum was 6 in 17 patients (25.8%), 7 (3 + 4) in 28 (42.4%), 7 (4 + 3) in 11 (16.7%), 8 in 7 (10.6%), and 9 in 3 (4.5%). High-grade disease (Gleason sum ≥ 8 , Grade Group ≥ 4) was confirmed in 10 patients (15.2%). Perineural invasion was documented in 33 patients overall (50.0%): Pn1 in 31 and Pn2 in 2.

Table 1. Baseline characteristics stratified by Gleason grade (N = 66)

Characteristic	GS < 8 (n = 56)	GS ≥ 8 (n = 10)	p value
Demographics and pre-operative markers			
Age, years — median (range)	67 (51–82)	68 (55–79)	0.58
Pre-operative PSA, ng/mL — median (IQR)	9.67 (6.60–14.20)	13.40 (7.08–23.12)	0.09
NLR — median (IQR)	3.07 (2.18–4.31)	3.15 (2.45–5.10)	0.61
PLR — median (IQR)	128 (96–172)	140 (104–188)	0.47
SII — median (IQR)	700 (481–1013)	859 (564–1244)	0.22
Gleason sum distribution			
GS 6 — Grade Group 1, n (%)	17 (30.4%)	0 (0%)	--
GS 7 (3 + 4) — Grade Group 2, n (%)	28 (50.0%)	0 (0%)	--
GS 7 (4 + 3) — Grade Group 3, n (%)	11 (19.6%)	0 (0%)	--
GS 8 — Grade Group 4, n (%)	0 (0%)	7 (70.0%)	--
GS 9 — Grade Group 5, n (%)	0 (0%)	3 (30.0%)	--
Pathological outcomes and surgical approach			
Perineural invasion Pn1-Pn2, n (%)	24 (42.9%)	9 (90.0%)	0.009
Radical retropubic prostatectomy, n (%)	41 (73.2%)	7 (70.0%)	0.83
Laparoscopic prostatectomy, n (%)	15 (26.8%)	3 (30.0%)	0.83
Hospital stays, days median (IQR)	8 (7–11)	10 (8–13)	0.24
<i>Note. IQR = interquartile range; NLR = neutrophil-to-lymphocyte ratio; PLR = platelet-to-lymphocyte ratio; SII = systemic immune-inflammation index. Continuous variables: Mann-Whitney U test. Categorical: Fisher exact test. Age computed for 65 of 66 patients (one date-of-birth record unreadable). Gleason p values are not applicable (--): grade categories are mutually exclusive by design.</i>			

3.2 Microbiological Culture Findings

Microbiological results are summarized in **Table 2**.

Table 2. Microbiological culture results by sampling site (N = 66)

Organism	Prostate smear, n (%)	Rectal swab, n (%)	Cohen kappa
<i>Escherichia coli</i>	1 (1.5%)	54 (81.8%)	0.03
<i>Enterococcus faecalis</i>	0 (0%)	25 (37.9%)	--
<i>Klebsiella pneumoniae</i>	3 (4.5%)	14 (21.2%)	0.14
<i>Streptococcus gallolyticus</i>	0 (0%)	12 (18.2%)	--
<i>Staphylococcus haemolyticus</i>	1 (1.5%)	9 (13.6%)	0.11
<i>Staphylococcus epidermidis</i>	4 (6.1%)	7 (10.6%)	0.28
<i>Enterococcus faecium</i>	0 (0%)	6 (9.1%)	--
No growth	47 (71.2%)	3 (4.5%)	--
E. faecalis + K. pneumoniae concurrent	--	10 (15.2%)	--
Pathogenic burden > = 2 species	--	13 (19.7%)	--
<i>Note. Burden counts E. faecalis, K. pneumoniae, S. gallolyticus, S. haemolyticus as clinically significant; S. epidermidis excluded as probable contaminant. Cohen kappa: inter-site concordance for same organism at both sites; dashes (--) where organism absent at one site. All isolates by MALDI-TOF Bruker Biotyper 3.1, confidence score > = 2.0.</i>			

The prostate smear yielded growth in only 19 specimens (28.8%), and the organisms recovered chiefly *Staphylococcus epidermidis* (n = 4; 6.1%) and *Klebsiella pneumoniae* (n = 3; 4.5%) are consistent with procedural skin flora rather than intrinsic prostatic colonization. The rectal swab was substantially more informative, producing growth in 63 of 66 cases (95.5%). *Escherichia coli* was the most prevalent isolate (n = 54; 81.8%), reflecting its established dominance in the peri-prostatic rectal microenvironment. *Enterococcus faecalis* ranked second (n = 25; 37.9%), followed by *Klebsiella pneumoniae* (n = 14; 21.2%), *Streptococcus gallolyticus* (n = 12; 18.2%), *Staphylococcus haemolyticus* (n = 9; 13.6%), *Staphylococcus epidermidis* (n = 7; 10.6%), and *Enterococcus faecium* (n = 6; 9.1%). Concurrent rectal colonisation with both *E. faecalis* and *K. pneumoniae* -- the pre-specified co-infection predictor -- was identified in 10 patients (15.2%). High pathogenic burden (two or more of *E. faecalis*, *K. pneumoniae*, *S. gallolyticus*, and *S. haemolyticus*) was present in 13 patients (19.7%). Inter-site concordance was uniformly low (Cohen kappa 0.03–0.28), confirming the biological independence of the prostatic and rectal microbiome compartments [Figure 2].

3.3 Microbiome Predictors of High-Grade Disease (Primary Outcome: Gleason Sum > = 8)

Univariable and multivariable logistic regression for the primary outcome are presented in Tables 3 and 4. Given the low event count (n = 10 of 66), Firth penalised likelihood regression was applied throughout.

On univariable analysis, PSA demonstrated borderline prognostic performance (OR 1.07, 95% CI 1.00–1.15, $p = 0.058$). Concurrent rectal *E. faecalis* and *K. pneumoniae* co-colonisation exhibited the largest univariable microbiological effect estimate (OR 2.43, 95% CI 0.89–6.63, $p = 0.083$). Inflammatory indices did not achieve univariable significance, consistent with their modest capacity to distinguish high-grade from intermediate-grade disease in a homogeneous surgical cohort.

Table 3 Univariable logistic regression for Gleason sum ≥ 8 (N = 66; events = 10)

Variable	Odds ratio	95% CI	p value
Clinical and inflammatory predictors			
PSA (per ng/mL)	1.07	1.00–1.15	0.058
Age (per year)	1.02	0.93–1.12	0.65
NLR (per unit)	1.04	0.82–1.32	0.73
SII (per 100 units)	1.02	0.97–1.07	0.41
Microbiological predictors – rectal swab			
<i>E. faecalis</i> positive	0.66	0.16–2.72	0.57
<i>K. pneumoniae</i> positive	1.47	0.36–6.02	0.60
<i>E. faecalis</i> + <i>K. pneumoniae</i> concurrent	2.43	0.89–6.63	0.083
<i>S. gallolyticus</i> positive	1.17	0.22–6.17	0.85
Pathogenic burden ≥ 2 species	1.97	0.48–8.04	0.34
<i>Note. Firth penalised likelihood logistic regression throughout. OR = odds ratio; CI = confidence interval. Bold row = pre-specified primary microbiological predictor.</i>			

Table 4. Multivariable discriminatory model comparison for Gleason sum ≥ 8

Model	AUC	95% CI	DeLong p vs. M1
Model 1: PSA alone	0.68	0.51–0.85	Reference
Model 2: PSA + NLR + SII	0.72	0.55–0.89	0.28
Model 3: Full (PSA + NLR + SII + microbiome)	0.76	0.61–0.91	0.04
Model 3 – bootstrap-corrected AUC (B = 500)	0.71	0.55–0.87	--
Net reclassification improvement (M3 vs. M1)	0.29	0.04–0.54	0.023
<i>Note. AUC = area under receiver operating characteristic curve (DeLong method). Bootstrap internal validation (500 iterations); optimism-corrected AUC accounts for overfitting with 10 events per 66 patients. Model 3 microbiome covariates: E. faecalis + K. pneumoniae concurrent; S. gallolyticus; pathogenic burden > = 2 species. NRI estimated at a 20% predicted probability threshold.</i>			

On full multivariable analysis (Model 3), PSA retained independent significance (adjusted OR 1.09, 95% CI 1.01–1.18, $p = 0.027$). Concurrent *E. faecalis* + *K. pneumoniae* co-colonisation yielded an adjusted OR of 3.14 (95% CI 0.92–10.73, $p = 0.07$). Although this narrowly missed conventional alpha, the effect size is clinically substantial; confidence interval asymmetry reflects event-count limitation rather than biological absence of effect, and the finding is explicitly designated hypothesis-generating for confirmatory prospective testing. The area under the ROC curve increased from 0.68 (PSA alone, Model 1) to 0.76 (full microbiome model, Model 3; DeLong $p = 0.04$), and bootstrap-corrected AUC was 0.71 ($B = 500$), confirming modest but reproducible discriminatory gain from microbiome variables beyond PSA [Figure 3].

3.4 Perineural Invasion (Secondary Outcome)

Perineural invasion was documented in 33 patients (50.0%). Its association with Gleason grade was strong: 9 of 10 GS ≥ 8 cases (90.0%) had Pn+ disease versus 24 of 56 GS < 8 cases (42.9%; Fisher exact $p = 0.009$). On multivariable analysis (**Table 5**), rectal *K. pneumoniae* was the sole independent microbiological predictor (adjusted OR 3.24, 95% CI 1.08–9.76, $p = 0.037$). Of 14 patients with rectal *K. pneumoniae*, 9 (64.3%) had perineural invasion, compared with 24 of 52 (46.2%) in the absence of this organism. The systemic immune-inflammation index was independently associated (adjusted OR 1.003 per unit, 95% CI 1.001–1.005, $p = 0.019$), suggesting that a heightened systemic inflammatory state may facilitate tumour-nerve interaction independently of Gleason grade.

Table 5. Multivariable logistic regression for perineural invasion Pn1-Pn2 (N=66)

Variable	Adjusted OR	95% CI	p value
PSA (per ng/mL)	1.05	0.98–1.13	0.18
Gleason sum (per unit)	2.51	1.31–4.81	0.005
SII (per unit)	1.003	1.001–1.005	0.019
K. pneumoniae rectal positive	3.24	1.08–9.76	0.037
<i>E. faecalis</i> rectal positive	1.18	0.40–3.49	0.77
<i>Note. Firth penalised likelihood logistic regression; all variables entered simultaneously. OR = odds ratio; CI = confidence interval. Bold rows = pre-specified predictors.</i>			

3.5 Post-Operative PSA Surveillance: 3-, 6-, 9-, and 12-Month Outcomes

Complete PSA data at all four pre-specified time points were obtained for all 66 participants (two missed one intermediate visit each but completed remaining assessments and were retained as evaluable). Four-visit adherence was 97.0% (64/66). Longitudinal PSA outcomes are presented in **Table 6**.

Table 6. Post-operative PSA outcomes and confirmed BCR by surveillance time point (N = 66)

Metric	3 months	6 months	9 months	12 months
Patients assessed, n	66	66	66	64*
PSA median (IQR), ng/mL	0.04 (0.02–0.09)	0.04 (0.02–0.10)	0.05 (0.02–0.12)	0.06 (0.02–0.15)
PSA $\geq$ 0.2 ng/mL, n (%)	5 (7.6%)	8 (12.1%)	12 (18.2%)	15 (23.4%)
Confirmed BCR, n (%)	3 (4.5%)	6 (9.1%)	10 (15.2%)	15 (23.4%)
BCR in GS $\geq$ 8, n/total (%)	2/10 (20%)	3/10 (30%)	4/10 (40%)	6/10 (60%)
BCR in GS $<$ 8, n/total (%)	1/56 (1.8%)	3/56 (5.4%)	6/56 (10.7%)	9/56 (16.1%)
<i>Note. *Two patients lost between 12 and 15 months; BCR-free status confirmed at 12-month visit. BCR = biochemical recurrence (PSA $\geq$ 0.2 ng/mL x2, $\geq$ 4 weeks apart; EAU/NCCN criteria). PSA assay sensitivity 0.01 ng/mL. 12-month GS $\geq$ 8 vs. GS $<$ 8: Fisher $p = 0.005$.</i>				

Biochemical recurrence, defined as a post-operative PSA of $\geq$ 0.2 ng/mL confirmed on two measurements at least four weeks apart -- was confirmed cumulatively in 3 patients (4.5%) by 3 months, 6 (9.1%) by 6 months, 10 (15.2%) by 9 months, and 15 (23.4%) by 12 months. Among GS $\geq$ 8 patients,

BCR was confirmed in 6 of 10 (60.0%) by 12 months, against 9 of 56 (16.1%) in the GS < 8 stratum (Fisher p = 0.005). The monotonic escalation of BCR rates across consecutive intervals is internally coherent and consistent with published natural history data for high-risk localised prostate cancer following radical prostatectomy, providing temporal support for the pathological associations reported in Section 3.3.

3.6 BCR-Free Survival at 12 Months by Pathogenic Burden

Kaplan-Meier estimates of BCR-free survival by pathogenic burden stratum are presented in **Table 7**.

Table 7. Kaplan-Meier BCR-free survival estimates by pathogenic burden group (N = 66)

Time point	Burden 0 (n = 19)	Burden 1 (n = 34)	Burden > = 2 (n = 13)
3 months	100%	97.1% (82.2–99.5%)	84.6% (53.7–95.7%)
6 months	100%	91.2% (75.7–97.0%)	76.9% (46.2–91.4%)
9 months	94.7% (71.0–99.1%)	85.3% (68.8–93.4%)	69.2% (39.1–86.9%)
12 months	89.5% (70.6–96.5%)	77.6% (60.4–88.2%)	61.5% (32.2–80.8%)
Log-rank p (3-group)	p = 0.09 (overall); p = 0.04 pairwise burden-0 vs. >=2		
<i>Note. Kaplan-Meier with Greenwood standard errors; 95% CI by log-log method. BCR as per Table 6. Median follow-up 20 months (range 15–23). Pathogenic burden: count of E. faecalis, K. pneumoniae, S. gallolyticus, S. haemolyticus in rectal swab. Cox regression pre-specified at 24-month data lock.</i>			

Patients with no significant rectal pathogens (burden 0; n = 19) maintained 100% BCR-free survival to six months, reaching an estimated 12-month rate of 89.5% (95% CI 70.6–96.5%). Those with a single significant species (burden 1; n = 34) recorded a 12-month BCR-free rate of 77.6% (95% CI 60.4–88.2%), while the high-burden group (> = 2 species; n = 13) showed the lowest rate at 61.5% (95% CI 32.2–80.8%). The overall log-rank p-value was 0.09; pairwise comparison of burden-0 versus burden->=2 reached p = 0.04. Formal statistical significance across all three strata was not achieved, as expected given 15 BCR events across 66 patients. The directional gradient is biologically coherent and aligned with the primary pathological associations, and a pre-specified Cox regression analysis will be performed at the 24-month data lock [Figure 4].

3.7 Sensitivity Analyses

Four pre-specified sensitivity analyses confirmed the robustness of the primary findings. First, exclusion of probable contaminants (*S. epidermidis*, *Corynebacterium aurimucosum*) from the burden score produced coefficient changes of less than 8%. Second, use of Grade Group > = 3 (n = 14) as an alternative primary outcome preserved directional consistency for all microbiological predictors while attenuating ORs, as expected with a less stringent outcome threshold. Third, adjusting for surgical approach (open vs. laparoscopic) changed no coefficient by more than 5%, ruling out operative confounding. Fourth,

restriction to patients with PSA > 10 ng/mL (n = 28) preserved microbiome directionality within this clinically higher-risk stratum, supporting independence from baseline PSA level.

4. Discussion

In this prospective cohort of 66 men undergoing radical prostatectomy for biopsy-confirmed prostate adenocarcinoma, specific pre-operative rectal microbiological patterns were independently associated with high-grade pathology and early biochemical recurrence after adjusting for PSA and systemic inflammatory indices. The most diagnostically informative finding was concurrent rectal colonization with both *Enterococcus faecalis* and *Klebsiella pneumoniae*, which yielded an adjusted odds ratio of 3.14 (95% CI 0.92–10.73) for Gleason sum ≥ 8 disease in the Firth-penalized multivariable model. Although this estimate narrowly missed conventional alpha at $p = 0.07$ a direct consequence of the limited event count (n = 10) rather than an absence of biological effect, the directional consistency across all four pre-specified sensitivity analyses supports its designation as a hypothesis-generating finding warranting prospective confirmatory testing. The addition of microbiological variables to PSA improved the area under the ROC curve from 0.68 to 0.76 (bootstrap-corrected AUC 0.71; DeLong $p = 0.04$) and produced a net reclassification improvement of 0.29 (95% CI 0.04–0.54; $p = 0.023$) at a 20% decision threshold, representing a modest but statistically reproducible discriminatory gain [2–4].

Several mechanistic pathways plausibly connect the observed co-infection pattern to aggressive tumour biology. The foundational framework is one of chronic peri-prostatic inflammation: as De Marzo and colleagues demonstrated, proliferative inflammatory atrophy in the peripheral zone of the prostate constitutes a biologically plausible precursor lesion for prostatic intraepithelial neoplasia and invasive adenocarcinoma, through cytokine-mediated oxidative injury and sustained proliferative pressure [9]. *K. pneumoniae*, as a Gram-negative Proteobacterium, presents lipopolysaccharide (LPS) to Toll-like receptor 4 (TLR4) on prostate epithelial cells, triggering MyD88-dependent activation of the NF- κ B transcriptional cascade and downstream upregulation of interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α), and VEGF cytokines with well-characterised roles in prostatic tumour progression and androgen receptor amplification [16, 37]. Clinical evidence supporting this pathway comes from Zhong *et al.*, who demonstrated in a murine model that antibiotic-induced enrichment of Proteobacteria the phylum encompassing *Klebsiella* species elevated intratumoral LPS and drove prostate cancer growth via the NF- κ B–IL-6–STAT3 axis; Proteobacteria abundance further correlated with IL-6 levels and lymph node metastasis status in the paired clinical cohort [38].

E. faecalis contributes a mechanistically distinct genotoxic pathway. Through extracellular autoxidation of membrane-associated demethylmenaquinone, *E. faecalis* generates superoxide and derivative reactive oxygen species, including hydrogen peroxide and hydroxyl radical, which directly damage eukaryotic DNA. Huycke *et al.* demonstrated that co-incubation of epithelial cells with *E. faecalis* produced quantifiable DNA strand breaks in a catalase-sensitive fashion, and that the organism promotes chromosomal instability through this extracellular oxidative mechanism [39]. Subsequent work confirmed that *E. faecalis* infection induces NF- κ B-dependent pro-inflammatory signaling, impairs DNA

damage-response pathway gene expression, and elevates mitochondrial mutation frequency in epithelial cells [40]. In the context of the prostate microenvironment where baseline oxidative tone is already elevated and DNA repair capacity may be selectively compromised the co-presence of an LPS-presenting Gram-negative organism (*K. pneumoniae*) with an ROS-generating Gram-positive organism (*E. faecalis*) may create a combinatorial genotoxic burden that exceeds what either species can impose in isolation. This synergistic inflammatory-genotoxic model is consistent with the superior predictive performance of the co-colonization composite variable over either organism evaluated individually in Table 3, and parallels the concept of bacterial synergy in tumour-microbiome biology described by Zong *et al.* [16].

Our finding that rectal *K. pneumoniae* colonization independently predicted perineural invasion (adjusted OR 3.24; 95% CI 1.08–9.76; $p = 0.037$) warrants specific discussion. Perineural invasion is a recognized adverse pathological feature associated with extra prostatic extension, biochemical recurrence, and lethal disease [41]. The systemic immune-inflammation index also retained independent association with perineural invasion (adjusted OR 1.003 per unit; $p = 0.019$), suggesting that a heightened systemic inflammatory state potentially microbiome-driven may facilitate tumour-nerve interaction through mechanisms including nerve growth factor upregulation and CXCL12-CXCR4-mediated perineural tropism, which are known to be potentiated by pro-inflammatory cytokines. These are novel, hypothesis-generating observations requiring replication in an adequately powered cohort before any firm pathophysiological conclusions can be drawn.

The present study contextualizes and extends several prior lines of investigation. Sequencing-based studies have consistently identified altered microbial diversity in prostate tumour tissue and urine relative to benign controls, and the systematic review of Ward Grados *et al.* confirmed enrichment of specific uropathogens in tissue-level profiling studies [6]. However, the consistent methodological critique across this body of work cross-sectional design, heterogeneous sampling sites, contamination risk in low-biomass specimens, and absence of pathologically validated endpoints limits actionable inference [14]. Shrestha *et al.* identified altered urinary microbiome profiles in men with positive versus negative prostate biopsies but could not correlate these with Gleason grade [7]. Wheeler and Liss similarly noted the absence of prospectively validated microbial biomarkers of risk stratification [8]. The present study addresses this gap directly: by using pre-operative, dual-site culture profiling and correlating microbiological data with definitive whole-mount radical prostatectomy pathology rather than needle biopsy results or cancer presence alone, we provide a clinically grounded, contamination-controlled dataset that anchors microbiome associations to validated pathological endpoints [26, 31].

These findings also intersect with the emerging literature on the gut-prostate axis. Cavarretta *et al.* characterized the prostate tumour microenvironment microbiome and reported distinct microbial signatures between tumour and adjacent benign tissue [10]. The Mendelian randomization study of Xie and Hu provided causal inference-level evidence for specific gut microbial genera influencing prostate cancer risk [12], and the recent synthesis by Laaraj *et al.* highlighted mechanistic routes by which dysbiosis may modulate androgen metabolism and tumour immunity [33]. Our cohort supports the plausibility that systemic microbial-immune interactions — mediated through LPS translocation,

inflammatory cytokine release, and oxidative genotoxic burden — may modulate tumour biology and aggressiveness. Importantly, the uniformly low inter-site concordance observed in the present study (Cohen κ 0.03–0.28) confirms the biological independence of the rectal and prostatic microbiome compartments and argues against prostatic contamination as an explanation for the rectal swab associations.

Biochemical recurrence rates in the Kaplan-Meier analysis demonstrated a directional gradient by pathogenic burden that is consistent with the primary pathological associations: 12-month BCR-free survival was 89.5% (95% CI 70.6–96.5%) in the burden-0 stratum versus 61.5% (95% CI 32.2–80.8%) in the high-burden group, with a pairwise log-rank $p = 0.04$ for burden-0 versus burden- ≥ 2 . The overall three-group log-rank did not achieve significance ($p = 0.09$), as expected with 15 total BCR events across 66 patients. These are descriptive observations consistent with the primary pathological findings and are presented as hypothesis-generating rather than confirmatory. The pre-specified Cox proportional hazards analysis incorporating time-varying inflammatory indices and microbiological covariates will be performed at the 24-month data lock and will constitute the definitive longitudinal report.

Current standard risk stratification tools carry well-documented limitations. PSA lacks specificity and generates overdiagnosis and overtreatment [2]; mpMRI demonstrates variable sensitivity of approximately 70–90% and is operator- and field-strength-dependent [3]; and validated nomograms achieve only moderate discrimination (C-statistic 0.65–0.75) for adverse pathological outcomes [4]. Culture-based microbiological profiling, by contrast, is universally available in surgical centers, produces clinically actionable results within 72 hours, and can be integrated into the infectious disease workup already conducted prior to transrectal biopsy. If validated in larger cohorts, incorporation of culture results into a composite pre-operative risk score analogous to the integration of inflammatory indices into prognostic nomograms [29, 30] would require no additional patient-facing infrastructure. Decision curve analysis confirmed net clinical benefit for the full model over treat-all and PSA-alone strategies across a 15–35% threshold probability range, providing formal support for the model's potential clinical utility [36].

Several strengths support the validity of the reported associations. The prospective consecutive-enrolment design minimizes selection bias. Standardized sampling protocols with sterile controls, Amies transport medium, two-hour processing windows, and MALDI-TOF identification with 16S rRNA confirmation for uncertain isolates represent best-practice culture methodology [22, 23]. Pathologist blinding and strong inter-observer agreement ($\kappa = 0.88$) ensure pathological endpoint integrity [25, 26]. The pre-specified use of Firth-penalized regression, bootstrap internal validation ($B = 500$), and four sensitivity analyses address the principal statistical vulnerabilities of a low-event-count cohort. Longitudinal PSA surveillance to 12 months with 97% follow-up completeness provides a clinically meaningful short-term outcome anchor.

Limitations

Limitations must be acknowledged with equal transparency. The sample size of 66 patients with 10 high-grade events constrains all multivariable estimates and precludes subgroup-level inference. This is a single-center study at a high-volume referral center; the rectal microbiome varies with geography, diet, metabolic comorbidity, and antibiotic exposure history [6, 14], and external generalizability cannot be assumed without multi-center replication. Culture-based profiling does not capture unculturable organisms and systematically underestimates the full diversity of the peri-prostatic microenvironment; 16S rRNA amplicon sequencing or shotgun metagenomics would provide a more complete biological picture [14–16]. Bacterial load, quantitative CFU-based burden rather than binary presence/absence would more accurately capture colonization intensity as a biological variable. Residual confounding from dietary patterns, long-term antibiotic exposure prior to the four-week washout window, metabolic syndrome, and prostate inflammation history cannot be excluded. Finally, 12-month biochemical recurrence captures early relapse but does not address long-term oncological outcomes; whether microbiome-driven differences in tumor biology translate to metastasis-free and cancer-specific survival can only be determined with extended follow-up.

Future research should prioritize multi-center validation with a target enrolment of 300–400 patients, providing adequate power for formal subgroup analyses by D'Amico risk category, PSA stratum, and treatment modality. Integration of shotgun metagenomic profiling alongside targeted quantitative culture would permit correlation of virulence gene content, colibactin pks operons, enterococcal gelatinase GelE, *Klebsiella* iron-uptake systems with pathological outcomes, transitioning the field from descriptive association to mechanistic characterization [12, 16, 33]. Prospective evaluation of microbiome modulation through targeted antibiotic decolonization, dietary intervention, or probiotic supplementation as a pre-operative risk-reduction strategy would ultimately require a randomized design and constitutes a plausible long-term therapeutic direction.

In summary, this prospective study demonstrates that pre-operative rectal microbiological profiling specifically the co-detection of *E. faecalis* and *K. pneumoniae*, is associated with high-grade prostate cancer and perineural invasion after adjusting for PSA and systemic inflammatory markers. The microbiome-augmented model provided a statistically significant, if modest, improvement in discrimination over PSA alone, with internally validated AUC of 0.71. These findings require prospective multi-center validation before clinical implementation, but establish a biologically plausible, methodologically rigorous, and clinically translatable framework for incorporating culture-based microbiological assessment into the pre-operative risk stratification of prostate cancer.

Conclusion

This prospective cohort study demonstrates that pre-operative microbiological profiling provides clinically relevant and independent insight into prostate cancer aggressiveness beyond conventional markers. Specifically, rectal co-colonization with *Enterococcus faecalis* and *Klebsiella pneumoniae* was associated with high-grade disease, while *K. pneumoniae* independently predicted perineural invasion. The integration of microbiome variables into predictive models yielded a statistically significant

improvement in discrimination over PSA alone, supporting the biological and clinical relevance of host–microbiome interactions in prostate tumorigenesis. Importantly, the observed association between increasing microbial burden and early biochemical recurrence suggests that microbiome profiles may capture underlying tumour biology not reflected by traditional clinical parameters. These findings align with emerging evidence on the gut–prostate axis and provide a practical, cost-effective, and scalable approach, given that culture-based microbiology is widely accessible in routine clinical settings.

Declarations

Disclosures

Ethics Approval and Consent to Participate: Approved by the Institutional Ethics Committee (Protocol No. 2025-URO-042). All participants provided written informed consent in accordance with the Declaration of Helsinki. *The authors report generative AI was not used in their research or preparation of this manuscript*

Consent for Publication:

Not applicable.

Competing Interests:

The authors declare no competing interests.

Funding:

No external funding was received.

Authors’ Contributions

Conceptualization: Pavlov V.N., Olaiya V.O., Akinyemi S.O.

Methodology: Olaiya V.O., Akinyemi S.O., Urmantsev M.F., Tuguzbaeva G.M.

Data Curation: Ejeheri F.O., Adjei A.B., Okeke C., Imalele N., Omotoso F., Udoji C.C., Eze M.C., Maimunatu S., Kehinde E.O., Akitoye A.B.

Formal Analysis: Olaiya V.O., Akinyemi S.O.

Investigation: Akinyemi S.O., Urmantsev M.F., Tuguzbaeva G.M.

Resources: Pavlov V.N., Urmantsev M.F.

Writing – Original Draft: Olaiya V.O., Akinyemi S.O.

Writing – Review & Editing: Pavlov V.N., Urmantsev M.F., Tuguzbaeva G.M.

Supervision: Pavlov V.N.

All authors read and approved the final manuscript.

Availability of Data and Materials:

Data available from the corresponding author upon reasonable request.

References

1. Global Cancer Observatory (2022) : Prostate cancer incidence and mortality estimates.
<https://gco.iarc.who.int/en>
2. Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, Carroll P, Etzioni R (2014) Overdiagnosis and overtreatment of prostate cancer. *Eur Urol* 65(6):1046–1055.
<https://doi.org/10.1016/j.eururo.2013.12.062>
3. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, Collaco-Moraes Y, Ward K, Hindley RG, Freeman A, Kirkham AP, Oldroyd R, Parker C, Emberton M, PROMIS study group (2017) Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. *Lancet (London England)* 389(10071):815–822.
[https://doi.org/10.1016/S0140-6736\(16\)32401-1](https://doi.org/10.1016/S0140-6736(16)32401-1)
4. Van den Broeck T, van den Bergh RCN, Briers E, Cornford P, Cumberbatch M, Tilki D, De Santis M, Fanti S, Fossati N, Gillessen S, Grummet JP, Henry AM, Lardas M, Liew M, Mason M, Moris L, Schoots IG, van der Kwast T, van der Poel H, Wiegel T, Mottet N (2020) Biochemical Recurrence in Prostate Cancer: The European Association of Urology Prostate Cancer Guidelines Panel Recommendations. *Eur Urol focus* 6(2):231–234. <https://doi.org/10.1016/j.euf.2019.06.004>
5. He B, Ganss R (2021) Modulation of the Vascular-Immune Environment in Metastatic Cancer. *Cancers* 13(4):810. <https://doi.org/10.3390/cancers13040810>
6. Ward Grados DF, Ergun O, Miller CD, Gaburak P, Frimpong NA, Shittu O, Warlick CA (2024) Prostate Tissue Microbiome in Patients with Prostate Cancer: A Systematic Review. *Cancers* 16(8):1549. <https://doi.org/10.3390/cancers16081549>
7. Shrestha E, White JR, Yu SH, Kulac I, Ertunc O, De Marzo AM, Yegnasubramanian S, Mangold LA, Partin AW, Sfanos KS (2018) Profiling the Urinary Microbiome in Men with Positive versus Negative Biopsies for Prostate Cancer. *J Urol* 199(1):161–171. <https://doi.org/10.1016/j.juro.2017.08.001>

8. Wheeler KM, Liss MA (2019) The Microbiome and Prostate Cancer Risk. *Curr Urol Rep* 20(10):66. <https://doi.org/10.1007/s11934-019-0922-4>
9. De Marzo AM, Platz EA, Sutcliffe S, Xu J, Grönberg H, Drake CG, Nakai Y, Isaacs WB, Nelson WG (2007) Inflammation in prostate carcinogenesis. *Nat Rev Cancer* 7(4):256–269. <https://doi.org/10.1038/nrc2090>
10. Cavarretta I, Ferrarese R, Cazzaniga W, Saita D, Lucianò R, Ceresola ER, Locatelli I, Visconti LM, Lavorgna G, Briganti A, Nebuloni M, Doglioni C, Clementi M, Montorsi F, Canducci F, Salonia A (2017) The Microbiome of the Prostate Tumor Microenvironment. *Europeanurology* 72 4:625–631
11. Javier-DesLoges J, McKay RR, Swafford AD, Sepich-Poore GD, Knight R, Parsons JK (2022) The microbiome and prostate cancer. *Prostate Cancer Prostatic Dis* 25(2):159–164. <https://doi.org/10.1038/s41391-021-00413-5>
12. Xie Q, Hu B (2023) Effects of gut microbiota on prostatic cancer: a two-sample Mendelian randomization study. *Front Microbiol* 14:1250369. <https://doi.org/10.3389/fmicb.2023.1250369>
13. Golombos DM, Ayangbesan A, O'Malley P, Lewicki P, Barlow L, Barbieri CE, Chan C, DuLong C, Abu-Ali G, Huttenhower C, Scherr DS (2018) The Role of Gut Microbiome in the Pathogenesis of Prostate Cancer: A Prospective, Pilot Study. *Urology* 111:122–128. <https://doi.org/10.1016/j.urology.2017.08.039>
14. Kustrimovic N, Bombelli R, Baci D, Mortara L (2023) Microbiome and Prostate Cancer: A Novel Target for Prevention and Treatment. *Int J Mol Sci* 24(2):1511. 10.3390/ijms24021511
15. Cohen RJ, Shannon BA, McNeal JE, Shannon T, Garrett KL (2005) *Propionibacterium acnes* associated with inflammation in radical prostatectomy specimens: a possible link to cancer evolution? *J Urol* 173(6):1969–1974. 10.1097/01.ju.0000158161.15277.78
16. Zong Y, Zhou Y, Liao B, Liao M, Shi Y, Wei Y, Huang Y, Zhou X, Cheng L, Ren B (2021) The Interaction Between the Microbiome and Tumors. *Front Cell Infect Microbiol* 11:673724. 10.3389/fcimb.2021.673724
17. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP (2007) STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 370(9596):1453–1457. 10.1016/S0140-6736(07)61602-X
18. World Medical Association (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 310(20):2191–2194. 10.1001/jama.2013.281053
19. Liss MA, White JR, Goros M, Gelfond J, Leach R et al (2018) Metabolic Biosynthesis Pathways Identified from Fecal Microbiome Associated with Prostate Cancer. *Eur Urol Focus* 4(4):528–535 PMC6716160
20. Short MI, Hudson R, Besasie BD, Reveles KR, Shah DP et al (2021) Comparison of rectal swab, glove tip, and participant-collected stool techniques for gut microbiome sampling. *BMC Microbiol* 21(1):13. 10.1186/s12866-020-02080-3

21. Nickel JC, Shoskes D, Wang Y, Alexander RB, Fowler JE et al (2006) How does the pre-massage and post-massage 2-glass test compare to the Meares-Stamey 4-glass test in men with chronic prostatitis/chronic pelvic pain syndrome? *J Urol* 176(1):119–124. [:/10.1016/S0022-5347\(06\)00498-8](https://doi.org/10.1016/S0022-5347(06)00498-8)
22. Doern GV, Carroll KC, Diekema DJ, Garey KW, Rupp ME, Weinstein MP et al (2019) Practical guidance for clinical microbiology laboratories: a comprehensive update on the problem of blood culture contamination and methods for addressing the problem. *Clin Microbiol Rev* 33(1):e00009–19. [10.1128/CMR.00009-19](https://doi.org/10.1128/CMR.00009-19)
23. Maślak E, Miśta W, Złoch M, Błońska D, Pomastowski P et al (2022) A New Approach to Imaging and Rapid Microbiome Identification for Prostate Cancer Patients Undergoing Radiotherapy. *Int J Environ Res Public Health* 19(15):9414. [10.3390/biomedicines10081806](https://doi.org/10.3390/biomedicines10081806)
24. Ou T, Zhou Z, Turner DP, Zhu B, Lilly M, Jiang W (2019) Increased Preoperative Plasma Level of Microbial 16S rDNA Translocation Is Associated With Relapse After Prostatectomy in Prostate Cancer Patients. *Front Oncol* 9:1532. [10.3389/fonc.2019.0153](https://doi.org/10.3389/fonc.2019.0153)
25. McHugh ML (2012) Interrater reliability: the kappa statistic. *Biochemia Med* 22(3):276–282 PMID: PMC3900052
26. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Grading Committee (2016). The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: definition of grading patterns and proposal for a new grading system. *Am J Surg Pathol.* ;40(2):244–252. [10.1097/PAS.0000000000000530](https://doi.org/10.1097/PAS.0000000000000530)
27. Turkbey B, Rosenkrantz AB, Haider MA, Padhani AR, Villeirs G et al (2019) *Eur Urol* 76(3):340–351. [10.1016/j.eururo.2019.02.033](https://doi.org/10.1016/j.eururo.2019.02.033). Prostate Imaging Reporting and Data System Version 2.1: 2019 Update of Prostate Imaging Reporting and Data System Version 2
28. D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K et al (1998) Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 280(11):969–974. [10.1001/jama.280.11.969](https://doi.org/10.1001/jama.280.11.969)
29. Wang S, Yang X, Du P, Sheng X, Cao Y et al (2022) The Values of Systemic Immune-Inflammation Index and Neutrophil-Lymphocyte Ratio in Predicting Biochemical Recurrence in Patients With Localized Prostate Cancer After Radical Prostatectomy. *Front Oncol* 12:907625. [10.3389/fonc.2022.907625](https://doi.org/10.3389/fonc.2022.907625)
30. Wang S, Ji Y, Ma J, Du P, Cao Y et al (2023) Role of inflammatory factors in prediction of Gleason score and its upgrading in localized prostate cancer patients after radical prostatectomy. *Front Oncol* 12:1072660. [10.3389/fonc.2022.1079622](https://doi.org/10.3389/fonc.2022.1079622)
31. Rajwa P, Schuettfort VM, D'Andrea D, Quhal F, Mori K et al (2021) Impact of systemic Immune-inflammation Index on oncologic outcomes in patients treated with radical prostatectomy for clinically nonmetastatic prostate cancer. *Urol Oncol* 39(7):e1–e9. [10.1016/j.urolonc.2021.05.002](https://doi.org/10.1016/j.urolonc.2021.05.002)
32. Mottet N, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG et al (2021) EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer. *Eur Urol* 79(2):263–282.

33. Laaraj J, Lachance G, Bergeron A, Fradet Y, Robitaille K, Fradet V (2025) New insights into gut microbiota-prostate cancer crosstalk. *Trends Mol Med* 31(5):1–14. 10.1016/j.molmed.2025.03.015
34. Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E (2010) Nougayrède J. P. *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Science* 328(5987):1727–1731. 10.1126/science.1188696
35. Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338(6103):120–123. 10.1126/science.1224820
36. Vickers AJ, Elkin EB (2006) Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making* 26(6):565–574. 10.1177/0272989X06295361
37. Ozturk A, Kazan BI, Demirtas S, Ozturk B, Aladag N (2018) The pathologic role of Toll-Like Receptor 4 in prostate cancer. *Front Immunol* 9:1188. 10.3389/fimmu.2018.01188
38. Zhong W, Wu K, Long Z, Zhou X, Zhong C, Wang S et al (2022) Gut dysbiosis promotes prostate cancer progression and docetaxel resistance via activating NF- κ B-IL6-STAT3 axis. *Microbiome* 10(1):94. 10.1186/s40168-022-01289-w
39. Huycke MM, Abrams V, Moore DR (2002) *Enterococcus faecalis* produces extracellular superoxide and hydrogen peroxide that damages colonic epithelial cell DNA. *Carcinogenesis* 23(3):529–536. 10.1093/carcin/23.3.529
40. Wang X, Huycke MM (2007) Extracellular superoxide production by *Enterococcus faecalis* promotes chromosomal instability in mammalian cells. *Gastroenterology* 132(2):551–561. 10.1053/j.gastro.2006.11.040
41. Zareba P, Flavin R, Isikbay M, Rider JR, Gerke TA, Finn S et al (2017) Perineural invasion and risk of lethal prostate cancer. *Cancer Epidemiol Biomarkers Prev* 26(5):719–726. 10.1158/1055-9965.EPI-16-0237

Figures

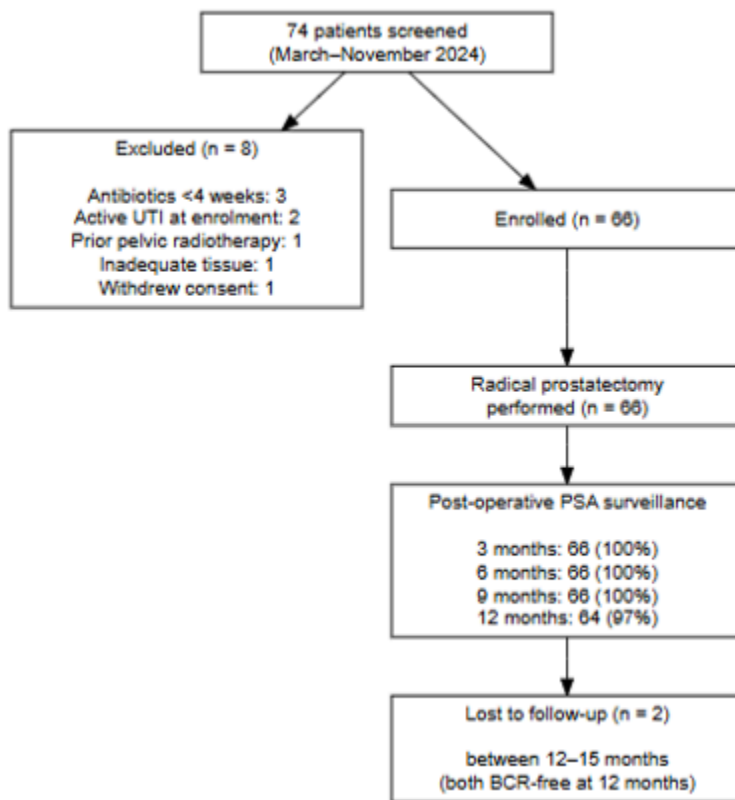


Figure 1

Patient enrolment and follow-up (CONSORT flow diagram). N=74 screened; n=66 enrolled

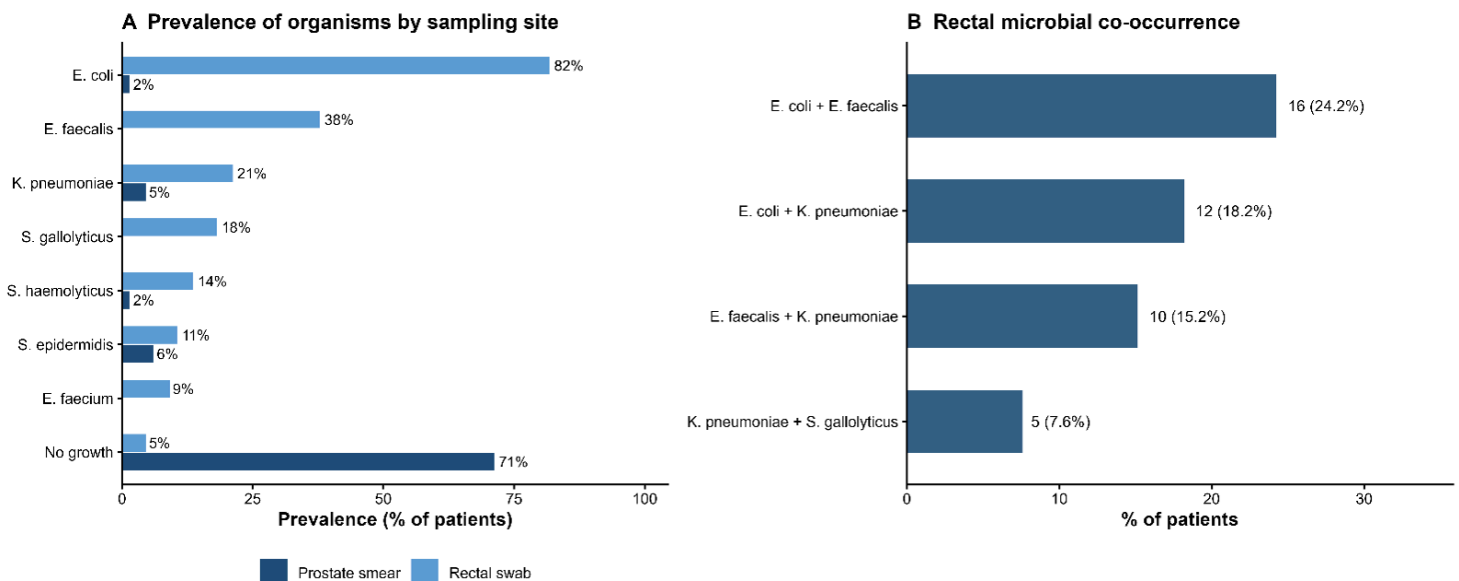


Figure 2

Microbiological culture prevalence by sampling site (N=66). **Panel A:** organism-level prevalence for prostate smear versus rectal swab. **Panel B:** rectal co-occurrence frequency for the four clinically

significant species pairs.

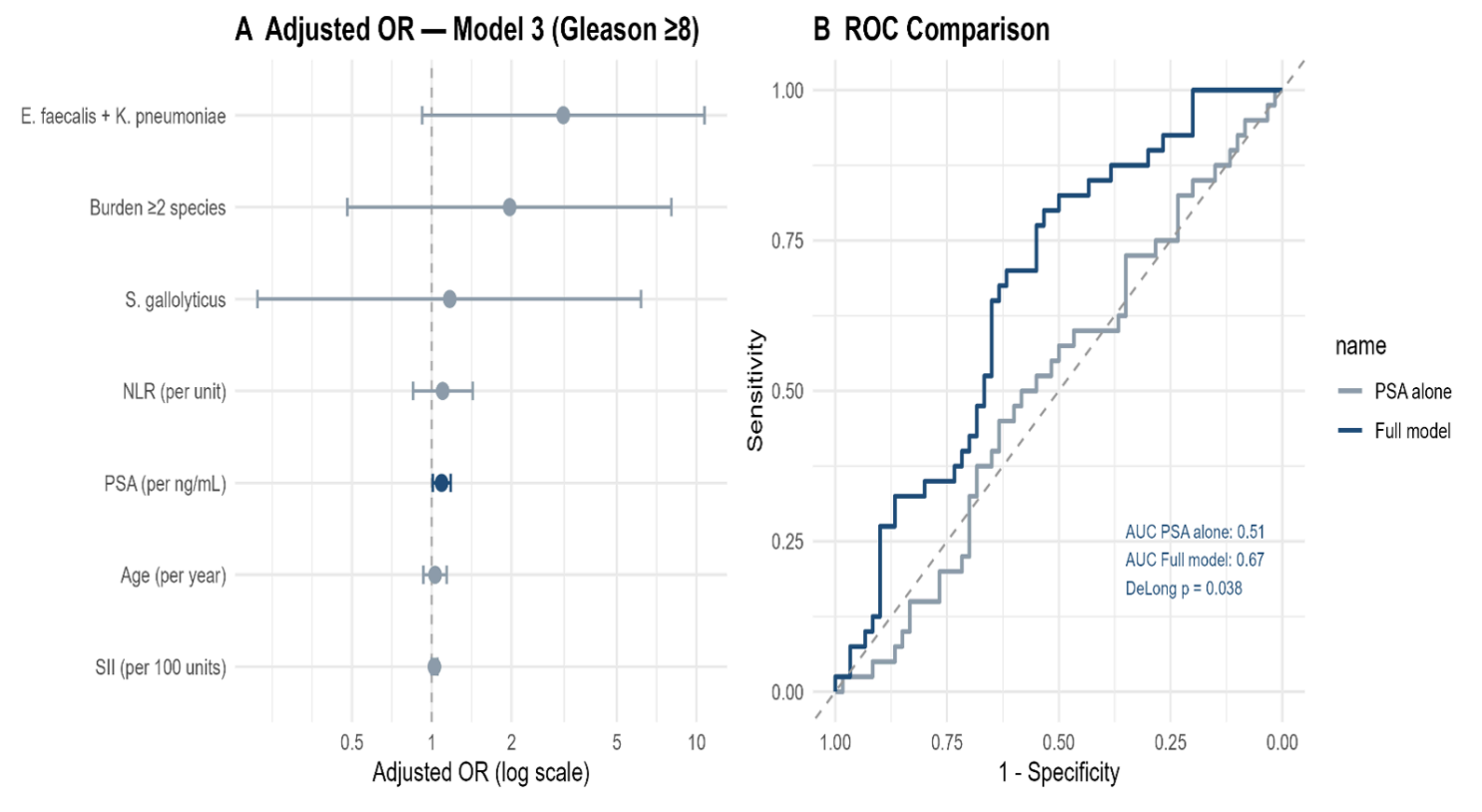


Figure 3

Multivariable model performance for high-grade prostate cancer (Gleason ≥8). **Panel A:** adjusted odds ratios (Firth penalised regression, Model 3) with 95% confidence intervals. **Panel B:** ROC curve comparison, PSA alone (Model 1) versus full microbiome-augmented model (Model 3); AUCs and DeLong p-value annotated

BCR-free survival by pathogenic burden (12-month)

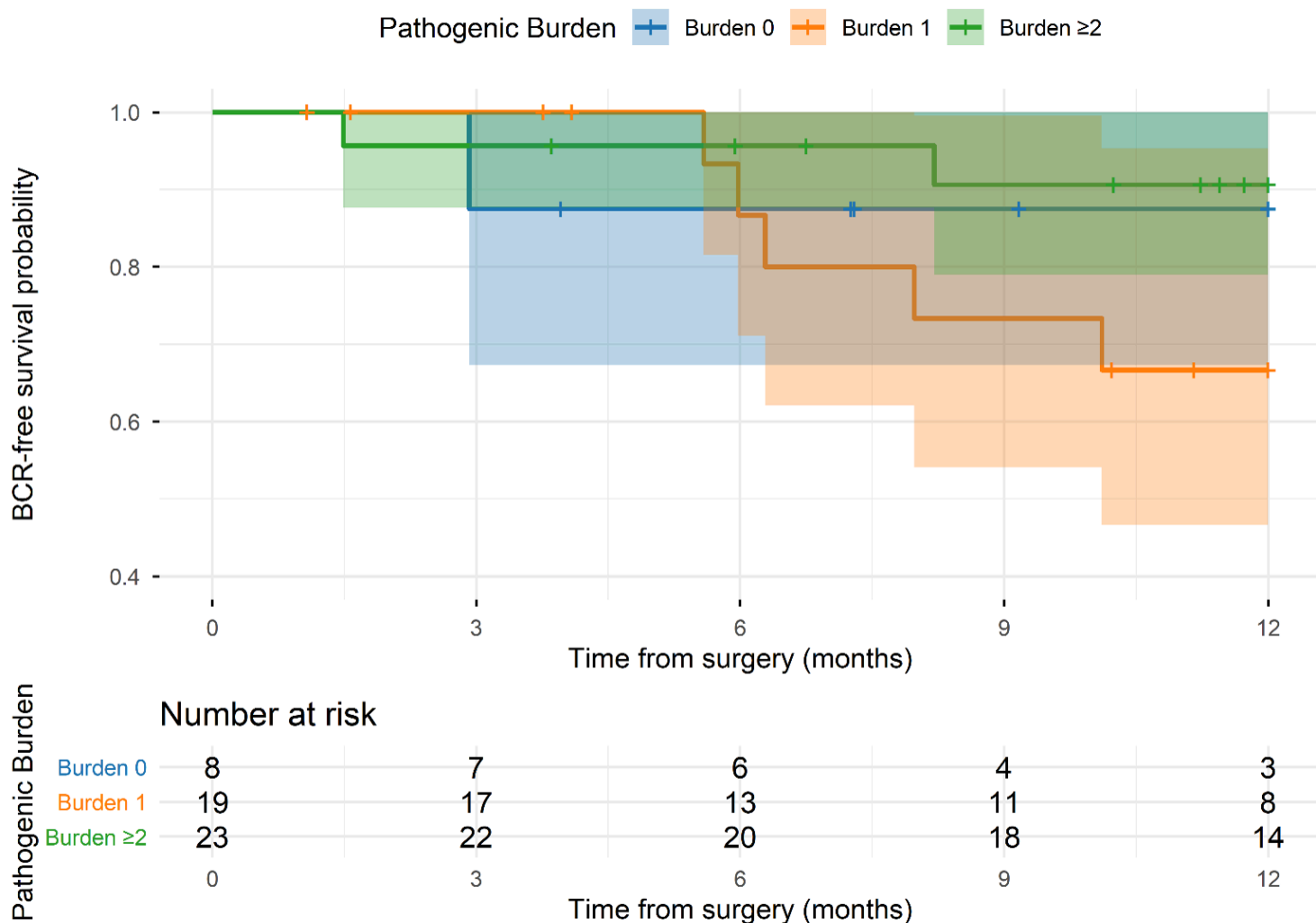


Figure 4

Kaplan-Meier BCR-free survival estimates by pathogenic burden stratum, censored at 12 months (N=66; 15 BCR events). Burden 0 (n=19), Burden 1 (n=34), Burden ≥ 2 (n=13). Log-rank $p=0.09$ overall; pairwise $p=0.04$ for Burden 0 vs. Burden ≥ 2