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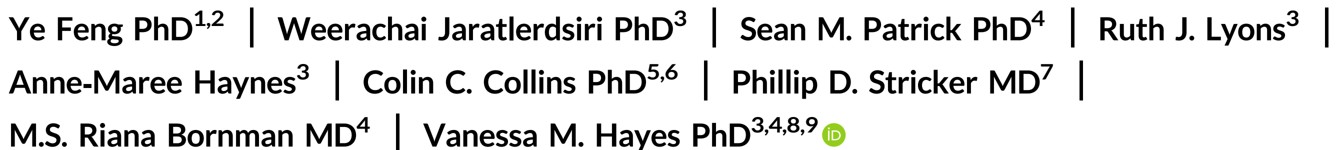
Metagenomic analysis reveals a rich bacterial content in high-risk prostate tumors from African men



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Abstract

Background: Inflammation is a hallmark of prostate cancer (PCa), yet no pathogenic agent has been identified. Men from Africa are at increased risk for both aggressive prostate disease and infection. We hypothesize that pathogenic microbes may be contributing, at least in part, to high-risk PCa presentation within Africa and in turn the observed ethnic disparity.

Methods: Here we reveal through metagenomic analysis of host-derived whole-genome sequencing data, the microbial content within prostate tumor tissue from 22 men. What is unique about this study is that patients were separated by ethnicity, African vs European, and environments, Africa vs Australia.

Results: We identified 23 common bacterial genera between the African, Australian, and Chinese prostate tumor samples, while nonbacterial microbes were notably absent. While the most abundant genera across all samples included: *Escherichia*, *Propionibacterium*, and *Pseudomonas*, the core prostate tumor microbiota was enriched for *Proteobacteria*. We observed a significant increase in the richness of the bacterial communities within the African vs Australian samples ($t = 4.6\text{-}5.5$; $P = .0004\text{-}.001$), largely driven by eight predominant genera. Considering core human gut microbiota, African prostate tissue samples appear enriched for *Escherichia* and *Acidovorax*, with an abundance of *Eubacterium* associated with host tumor hypermutation.

Conclusions: Our study provides suggestive evidence for the presence of a core, bacteria-rich, prostate microbiome. While unable to exclude for fecal contamination, the observed increased bacterial content and richness within the African vs non-African samples, together with elevated tumor mutational burden, suggests the possibility that bacterially-driven oncogenic transformation within the prostate microenvironment may be contributing to aggressive disease presentation in Africa.

Ye Feng and Weerachai Jaratlerdsiri contributed equally to this study.

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KEYWORDS
Africa, bacterial burden, ethnic disparity, microbiome, prostate cancer, prostate microenvironment, tumor mutational burden

1 | INTRODUCTION

Prostate cancer (PCa) is the most common male malignancy and the third cause of cancer-related death in men in the United States, with disproportionate incidence and mortality in African Americans.[1] In the Southern African Prostate Cancer Study (SAPCS), we showed black South African men to present with the significantly increased high-risk disease compared with age-matched African Americans.[2] Besides genetic and socioeconomic contributions, a protective association with increased sexual activity and an inverse association with erectile dysfunction, have suggested a possible role for pathogen shedding in reducing PCa risk.[3] Additional epidemiological evidence for a pathogenic link to PCa risk, as reviewed, includes: prostatitis (inflammation of the prostate), sexually transmitted infections, and history of acne.[4] With the highest rate of infectious diseases and infection-related cancers,[5] our hypothesis is that the elevated high-risk PCa presentation observed within men from Africa, at least in part, be contributed by a microbial pathogenic agent(s).

Next-generation sequencing (NGS) has allowed for an unbiased assessment of the human microbiome, particularly shifts in microbial content (dysbiosis) associated with the disease. While the microbial content of the human gut, skin, oral cavity, vagina, and, more recently, the urinary tract have received much attention, the prostate has largely been overlooked.[6] Before the application of NGS approaches, the most promising candidate to emerge from culture-based and amplification studies included *Propionibacterium*, which was further shown to enhance cell proliferation both in vivo and in vitro.[7] Two studies using ultradeep massively parallel 16S ribosomal RNA sequencing provided evidence for an abundance of bacteria within the human prostate.[8,9] Focusing on Australian men of European ancestry, core tissue was formalin-fixed and paraffin-embedded or snap-frozen after surgery, with a range of pathologies and tissue types (tumor, peritumor, or nontumor; 16 patients), and some were of aggressive disease (Gleason score ≥8; 10 patients). Key observations included significant enrichment for *Actinobacteria* and the possible correlation between microbial dysbiosis and pathophysiology,[8] and an abundance of *Enterobacteriaceae* with additional low levels of endogenous retroviruses detected using additional total RNA massively parallel sequencing.[9] More recently, we used unbiased shotgun sequencing of fresh prostate tumor and matched adjacent benign tissue from 65 Chinese men having undergone prostate surgery, identifying 47 bacterial genera, and confirming previous reports of enrichment for *Propionibacterium* and *Actinobacteria*.[10] No microbial differentiation between patient-matched tumor-benign tissue was observed—most likely a consequence of field effect. Together with a recent review that includes genitourinary microbiota detected in urine, seminal fluid and expressed prostatic secretions, and associated prostatic disease,[11] these studies provide tantalizing evidence for the role of bacterial infection in PCa pathogenesis.

2 | PATIENTS AND METHODS

2.1 | Patients and ethics

African patients were recruited at the time of diagnosis from the University of Pretoria's Steve Biko Academic Hospital in South Africa (n = 6), while Australian patients (all European descent) were recruited at the time of surgery from St Vincent's Hospital in New South Wales (n = 16). Histopathological Gleason score was used to classify tumors as high risk (Gleason score ≥8; all African and nine Australian patients) or low risk (Gleason score = 6; seven Australian patients) (see Table 1 for clinical characteristics). African patients consented as part of the previously described SAPCS.[2]

South African patients consented understudy approval granted by the University of Pretoria Human Research Ethics Committee (HREC #43/2010) including US Federal-wide assurance (FWA00002567 and IRB00002235 IORG0001762). Biospecimens were shipped under the Republic of South Africa Department of Health Export Permit, in accordance with the National Health Act 2003 (J1/2/4/2 #1/12) and interinstitutional Material Transfer Agreement to the Garvan Institute of Medical Research in Australia. Australian patients consented under the St Vincent's Hospital HREC approval (#SVH/12/231). Data generation and analysis were performed in accordance with site-specific approval granted by St Vincent's Hospital HREC (#SVH 15/227), with Garvan Governance approval (#GHRP 1522).

2.2 | Sample processing

Fresh prostate tissue was obtained either at routine diagnostic sampling, via a condom sheathed needle transrectal core biopsy (African) or immediately post radical prostatectomy, via core biopsy needle sampling under sterile conditions (Australian). It should be noted that all patients would have received antibiotics, specifically Ciproxin before biopsy (African) or Ceftriaxone during surgery (Australian). All samples were immediately placed in a droplet of optimal cutting temperature cryopreservation

TABLE 1 Sequencing statistics and bacterial content for 22 African and Australian patients

	UP2003	UP2039	UP2099	UP2113	UP2116	UP2133	5545	13104	13179	10651	15917	5684	16599	12543	5287	5958	10738	6359	5656	11452	5060	5902
Ethnicity/Country	African	African	African	African	African	African	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus	Eur/Aus
HR/LRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	HRPCa	LRPCa	LRPCa	LRPCa	LRPCa	LRPCa	LRPCa	LRPCa
Clinical characteristics and sequencing statistics																						
Gleason score	9	8	8	8	10	9	8	9	9	9	9	9	9	9	9	6	6	6	6	6	6	6
Tumor purity	42%	68%	78%	41%	44%	43%	79%	41%	52%	75%	42%	75%	68%	52%	55%	56%	84%	52%	33%	56%	53%	70%
hg38 coverage	86×	75×	82×	86×	87×	81×	67×	76×	85×	91×	86×	71×	89×	77×	80×	68×	80×	79×	86×	68×	74×	74×
TMB (Mut/Mb)	4.6	3.3	3.4	55	3	4.7[a]	2.2	1.9	2.2	2.6	2.1	1.9	2.8	1.7	1.8	1.7	1.6	1.8	3	1.1	1.7	1.3
Unmapped reads[b]	1.16%	0.73%	1.07%	1.04%	0.74%	1.08%	0.92%	1.26%	1.17%	0.78%	1.31%	0.97%	0.82%	0.80%	0.90%	0.90%	0.71%	0.96%	0.64%	1.57%	1%	0.65%
Bacterial content and load (no reads[c])																						
Total no of genera	23	39	18	55	24	47	10	17	7	5	21	13	7	16	10	9	18	17	10	22	18	7
Bacteroides	32.9	5267.7	91	4339.7	221.2	8085.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Escherichia	481.9	310.6	314.6	982.8	458	2236.9	281.6	433.8	391.1	167.3	310.1	244.5	199.8	148.3	200.7	298.8	259	297.7	120.5	530.8	338	107.6
Eubacterium	8.8	16.9	10.4	3862.6	5.9	892.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Acidovorax	329.3	279.3	110.7	1569	193.7	183.8	NA	5.5	NA	NA	NA	9.1	NA	NA	NA	NA	18.3	13.2	NA	8.9	NA	1.3
Propionibacterium	35.7	57.7	88.1	84.4	25.4	65.5	38	158.5	120.8	145.1	441.9	87.4	57.8	80.2	31.4	62.9	51.9	376.3	90.4	194	63.8	50
Acinetobacter	172.9	94.1	33.6	60.7	17.8	7	NA	6.1	5	NA	7.1	NA	NA	NA	5.8	6.9	10	8.4	NA	1099.9	33.5	NA
Pseudomonas	66.4	45.8	40	53.5	49.1	58	44.4	79.9	41.4	25.3	31.2	26.1	16.8	29.8	26.2	71.2	187	171.7	134.2	225.4	24.6	24
Total burden	1419.2	6841	889.4	13 605.7	1294.6	13 404.8	487.9	948	653.7	390.4	1232.2	539.3	347.5	590	418.2	595.5	827.8	1465	494.1	2366.4	717.1	281

Abbreviations: Aus, Australian; Eur, European; HRPCa, high-risk prostate cancer; LRPCa, low-risk prostate cancer; NA, not applicable; TMB, tumor mutational burden.

[a]Hyperduplicated tumor genome.

[b]Percentage of reads not mapping to the human reference genome (hg38).

[c]Normalized number of reads.

medium on sterilized foil, the foil folded and placed in a histology cassette before being snap-frozen. All samples were stored at −80°C. African samples were shipped on dry ice to Australia. Total DNA was extracted for all samples within a single laboratory using Qiagen columns (Qiagen Pty Ltd, Doncaster, VIC). DNA was stored at 4°C in elution buffer (AE: 10 mM Tris-Cl, 0.5 mM EDTA, at pH 9.0) before sequencing.

2.3 | Whole-genome sequencing

All samples underwent whole-genome 2 × 150 cycle paired-end sequencing on an Illumina HiSeq X Ten instrument (Kinghorn Center for Clinical Genomics [KCCG], Garvan Institute) and were sequenced at the same time to eliminate potential for batch effects. On average, over 80X coverage per prostate tissue specimen was generated, as previously described.[12] Reads were adapter-trimmed and filtered to remove low-quality bases (<Q15), short reads (<70 bp), and missing read pairs, before aligned to the hg38 reference using bwa-mem v0.7.12.[13] While mapped reads were used to establish the host genomic landscape of the African vs Australian-derived tumors,[12] unmapped data was used for further microbiome analyses.

2.4 | Host tumor mutational burden

Tumor mutational burden (TMB), that is, the total number per megabase (Mb) of single-nucleotide variants and small insertions and deletions (indels) acquired during tumorigenesis, was calculated against matched blood, as previously described by Jaratlerdsiri[12] (see genomic data summarized in Table 1). We report a 1.2-fold increase in TMB between the low- and high-risk Australian-derived tumors and a 1.8-fold increase in TMB between the African- and Australian-derived high-risk tumors, excluding for the single hypermutated African tumor UP2113 (55 somatic mutations/Mb). A second African-derived tumor (UP2133) was uniquely hyperduplicated with 234 tandem duplications.

2.5 | Microbial and statistical analysis

Reads unmapped to the human reference, were mapped by BWA to National Center for Biotechnology Information (NCBI) full set of microbial reference genomes, using a minimum cut-off of five mapped reads per sample. RepeatMasker was used to identify repeat and low complexity reads. The coverage uniformity of the bacterial genomes was assessed as described previously.[14] The relative abundance of the identified microorganisms was measured based on their normalized read counts.

The indices of alpha- and beta-diversity were calculated using the QIIME package. Comparison of these indices between groups was conducted using the Student *t* test. The nonmetric multidimensional scaling (NMDS) analysis was performed using the vegan package in R software.[15]

3 | RESULTS

3.1 | Prostate tissue and tumor microbiome

Unmapped human sequences derived from cancerous prostate tissue were retrieved and mapped against microbial reference genomes. While read counts for viruses were negligible and unlikely significant, we observed 281 to 49 809 bacterial reads per 10^9 human genome-mapped reads (Table 1). No other nonbacterial/nonviral reads were identified. Using a minimum cut-off of five mapped reads per sample, we identified a total of 75 bacterial genera within the African (Table S1) and 48 in the Australian prostate tumor tissue (Table S2), with an overlap of 28 genera, the vast majority (20 of 28) Proteobacteria (Table 2). The most abundant genera across the study included: *Escherichia*, *Propionibacterium*, and *Pseudomonas* (Figure 1A), in agreement with the complimentary study out of China.[10] While *Escherichia* has been shown to stimulate PCa progression in vitro,[17] *Propionibacterium acne* has reported being significantly more common in prostate tissue from men with, compared with men without PCa.[18] Comparing our African and Australian data with the 47 bacterial genera from the Chinese study,[10] we identified 23 commonly represented genera (Table 2).

3.2 | Microbial biodiversity and abundance associated with clinical presentation and ethnicity

Neither clustering nor NMDS based on beta-diversity measures (differences in species composition) could distinguish the high-risk and low-risk Australian-European tumors (Figure 1A and 1B). Complimentary to the Chinese study,[10] we showed a lack of association between bacterial abundance and clinical presentation. In contrast, we observed a significant increase in the community richness (alpha-diversity) in the African over the European-derived tumors (ACE and chao1, $t = 5.5$ and 4.6, $P = .0004$ and .001, two-tailed t test) (Figure 1C). Four genera, *Streptococcus*, *Alicycliphilus*, *Acidovorax*, and *Escherichia*, were elevated and four, *Bacteroides*, *Eubacterium*, *Parabacteroides*, and *Odoribacter*, were exclusive to our African-derived prostate tumor tissue (Figure 1D). These anaerobic genera accounted for 62% to 89% of the total African-derived bacterial burden. *Streptococcus* was reported as one of 11 predominant bacterial genera (>1% threshold) in European Italian prostate and cancer tissue.[8] Irrespective of country of origin, we observed a predominance of Gram-negative over Gram-positive bacteria (all $P < 7.1e{-}5$), while anaerobic to aerobic ratio was significantly associated with African-derived tumors ($P = .0002$).

4 | DISCUSSION

Taking advantage of discarded unmapped human genomic data generated in whole-genome sequencing efforts, we were ideally positioned in this study to perform metagenomic analysis for experimentally controlled data generated from cancerous prostate tissue derived from patients from geographically distinct continents,

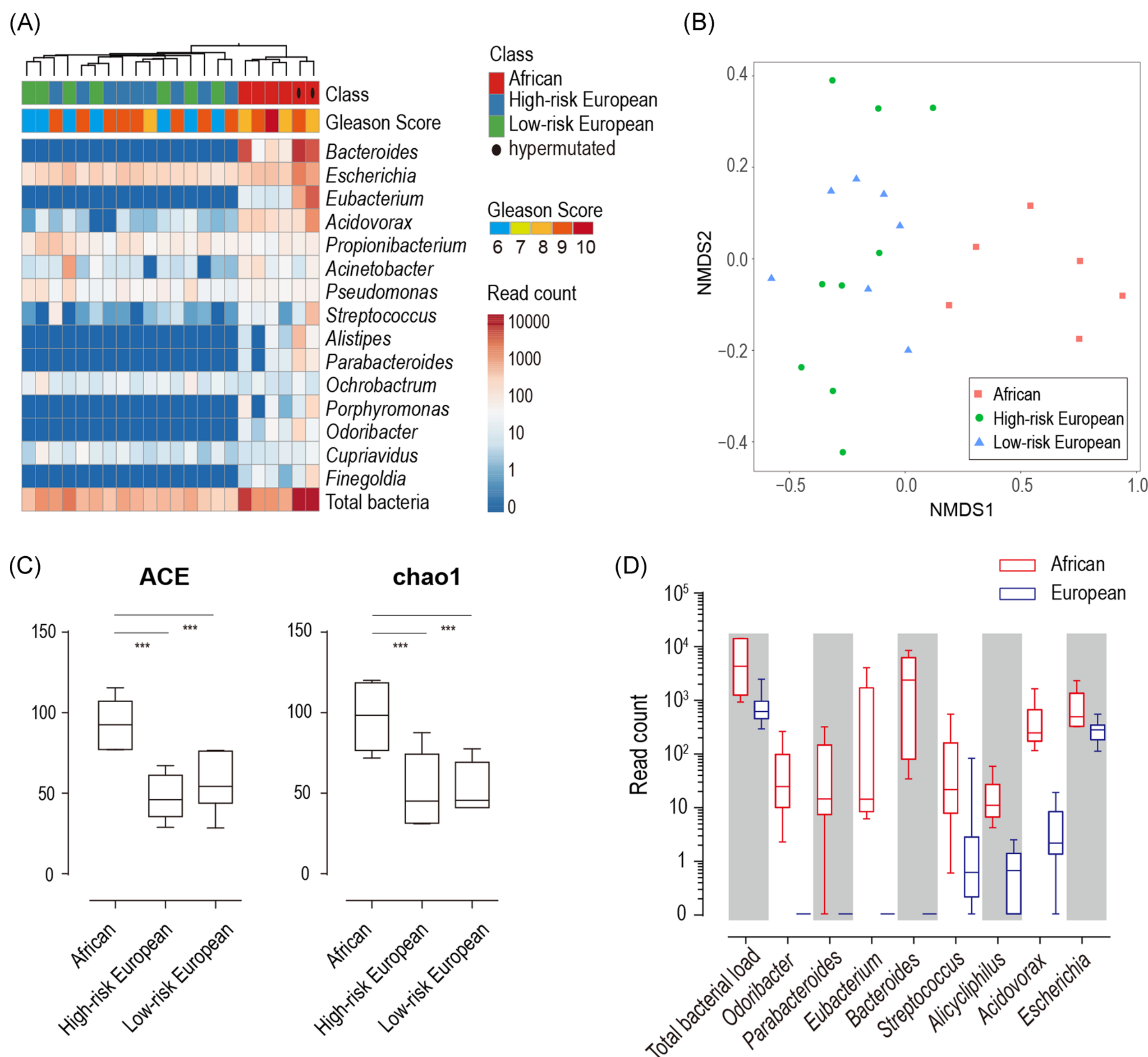

FIGURE 1 Prostate microbiomes shown in this study. A, Heatmap showing the read count of the top 15 abundant bacterial genera identified in the patients in this study, with the UPGMA tree constructed based on the weighted_UniFrac distance between specimens. B, The NMDS plots in which the African and European samples are distinguishable but the high- and low-risk European samples are not distinguishable. C, Comparison of the alpha-diversity indices, ACE and chao1, between the prostate microbiomes of the African and European patients. D, Bacterial genera that are differentially abundant between the African and the European patients. NMDS, nonmetric multidimensional scaling

Africa and Australia,[12] while allowing for further correlation with recently published data from China.[10] Avoiding for sequencing-derived batch effects, it should be noted that as a host genome-profiling study, negative sequencing controls were not included. Synergies observed across the cohorts include: (a) a lack of nonbacterial microbes and (b) a 1.6-fold increase in the total number of bacterial genera represented within the African vs Australian and African vs Chinese tissues. The most abundant bacterial genera across the cohorts include *Escherichia*, *Propionibacterium*, and *Pseudomonas*. Overall, we observed an over-representation of Proteobacteria across the study samples (71%, 20 of 28 genera) and between the continents (74%, 17 of 23 genera), including well-known human pathogens, such as *Escherichia*, *Salmonella*, and *Acinetobacter*.[19] Although we found no correlation between the presence of these bacteria and clinical presentation (high- vs low-risk PCa), larger studies are required to make further clinical correlations. Conversely, the possibility exists that the 23 bacterial genera common across the three ethnic groups from three continents, may constitute the core prostate human microbiome, irrespectively of disease status.

A major limitation of metagenomic studies is controlling for contaminants. While we were unable to control for differences in sampling procedures, transrectal biopsy sampling in Africans vs postsurgical sampling in Australians, ensuring samples were fresh reduced contaminants commonly introduced during tissue fixation, and centralizing the sequencing to a single laboratory, workflow and time

TABLE 2 Bacterial genera shared between African and Australian prostate tumor samples (n=28)

Genus	Phylum	Chinese prostate tissue study[10]	Fecal-derived core gut microbiome (34 genera)[16]
Achromobacter	Proteobacteria	Absent	Absent
Propionibacterium	Actinobacteria	Present	Absent
Sphingomonas	Proteobacteria	Present	Absent
Ralstonia	Proteobacteria	Present	Absent
Acidovorax	Proteobacteria	Present	Absent
Micrococcus	Actinobacteria	Absent	Absent
Kluyvera	Proteobacteria	Absent	Absent
Moraxella	Proteobacteria	Present	Absent
Rhodococcus	Actinobacteria	Present	Absent
Dechlorosoma	Proteobacteria	Present	Absent
Delftia	Proteobacteria	Present	Absent
Klebsiella	Proteobacteria	Present	Present (rank 28)
Escherichia-Shigella	Proteobacteria	Present	Present (rank 19)
Pantoea	Proteobacteria	Present	Absent
Cupriavidus	Proteobacteria	Present	Absent
Enterobacter	Proteobacteria	Present	Absent
Corynebacterium	Actinobacteria	Present	Absent
Citrobacter	Proteobacteria	Present	Absent
Thermus	Deinococcus-Thermus	Absent	Absent
Acinetobacter	Proteobacteria	Present	Absent
Streptococcus	Firmicutes	Present	Present (rank 14)
Stenotrophomonas	Proteobacteria	Present	Absent
Salmonella	Proteobacteria	Present	Absent
Pseudomonas	Proteobacteria	Present	Absent
Ochrobactrum	Proteobacteria	Present	Absent
Comamonas	Proteobacteria	Absent	Absent
Staphylococcus	Firmicutes	Present	Absent
Lactobacillus	Firmicutes	Present	Absent

point, further minimized for potential downstream confounders. It is therefore essential that we consider the potential for fecal contamination of the African samples. The healthy human fecal-derived gut microbiome is characterized by an abundance of *Bacteroidetes* and *Firmicutes*,[6] specifically *Bacteroides* and *Eubacterium*.[16] Notably, Bacteroidetes: *Bacteroides*, *Parabacteroides*, and *Odoribacter*, and the Firmicutes: *Eubacterium*, were found to be uniquely represented within our African tissues. Although absent from our Australian tissues, both *Bacteroides* and *Eubacterium* were present within the postsurgically derived Chinese tissue. It is also well-established that the gut microbiome will differ considerably between ethnic groups and continents, with most studies focused on non-Africans. A study comparing the healthy fecal bacterial content in children from Africa (rural village in Burkina Faso) and Europe (urban area in Florence, Italy) for example, confirmed an abundance of *Firmicutes* (51%) and *Bacteroides* (23%) in the Europeans, while *Prevotella* (53%) and *Xylanibacter* (20%) were predominant in the African-derived stools.[20] While *Xylanibacter* was absent in our African prostate samples, *Prevotella* was present, as reported also for the Chinese samples.

In the absence of patient-specific rectal swabs, to further assess the source of abundance for the African-derived prostate microbiota, we performed an extensive comparative analysis with 34 published core gut bacterial genera identified from the stools of 364 healthy volunteers from Denmark, Spain, China, or the United States.[16] Of the 15 most abundant African-derived prostate bacterial genera identified in our study (Figure 1A), 8 were notably absent within the core gut microbiome and include (in order of abundance): *Acidovorax*, *Propionibacterium*, *Acinetobacter*, *Pseudomonas*, *Ochrobactrum*, *Porphyromonas*, *Cupriavidus*, and *Finegoldia*, of which only *Porphyromonas* and *Finegoldia* were absent in our Australian samples, although *Finegoldia* was present in the Chinese samples. Conversely, 27 of the 34 core gut genera were not rated abundant in our African prostate samples, with 17 notably absent and including (in order of fecal core abundance): *Faecalibacterium*, *Coprococcus*, *Dorea*, *Blautia*, *Colinsella*, *Capnocytophaga*, *Bilophila*, *Sutterella*, *Dialister*, *Paraprevotella*, *Haemophilus*, *Methanobrevibacter*, *Desulfovibrio*, *Megasphaera*, *Butyrivibrio*, *Mitsuokella*, and *Phascolarctobacterium*. Of the prostate-derived genera common across the African, Australian, and Chinese tissues, only three were ranked as core fecal microbiota (Table 2), with a notable absence of Bacteroidetes and underrepresentation of Firmicutes (3 of 23 genera).

A recent study using 16S sequencing from rectal swabs from men undergoing transrectal biopsy reported enrichment for core gut genera *Bacteroides* and *Streptococcus* in cancerous over noncancerous patients.[21] In our African-derived tumor tissue, these genera were ranked first and eighth, respectively. While *Bacteroides* was absent within our Australian tumors and present in the Chinese, *Streptococcus* was present across all studies. Two additional studies have reported *Streptococcus* to be enriched in rectal swabs and/or voided urine from men with PCa.[22,23] Taken together, we cannot exclude for a possible contribution of these core gut microbiota in promoting advanced PCa presentation within Africa. Associations to consider include, *Bacteroides* abundance within vaginal communities of women with bacterial vaginosis (BV), a common condition impacting southern African women,[24] and *Bacteroides* abundance within the gut mucosa of patients with colorectal cancer.[25]

Irrespective of *Bacteroides* abundance, eight genera were common across all the African samples of which *Escherichia* and *Acidovorax* appear to be the most significant to all tumors (Figure 2). While *Escherichia* is a well-known human infectious pathogen,[19] *Acidovorax*, although a common phytopathogen found in the environment, has been associated with rare cases of human infection.[26,27] Besides total bacterial burden, *Eubacterium* abundance was significantly associated with host tumor genomic instability, including the most hypermutated prostate tumor identified to date, 55 mutations/Mb ($t = -1650$, $P = 4.9e{-10}$) and a uniquely hyperduplicated tumor with 33.4-fold

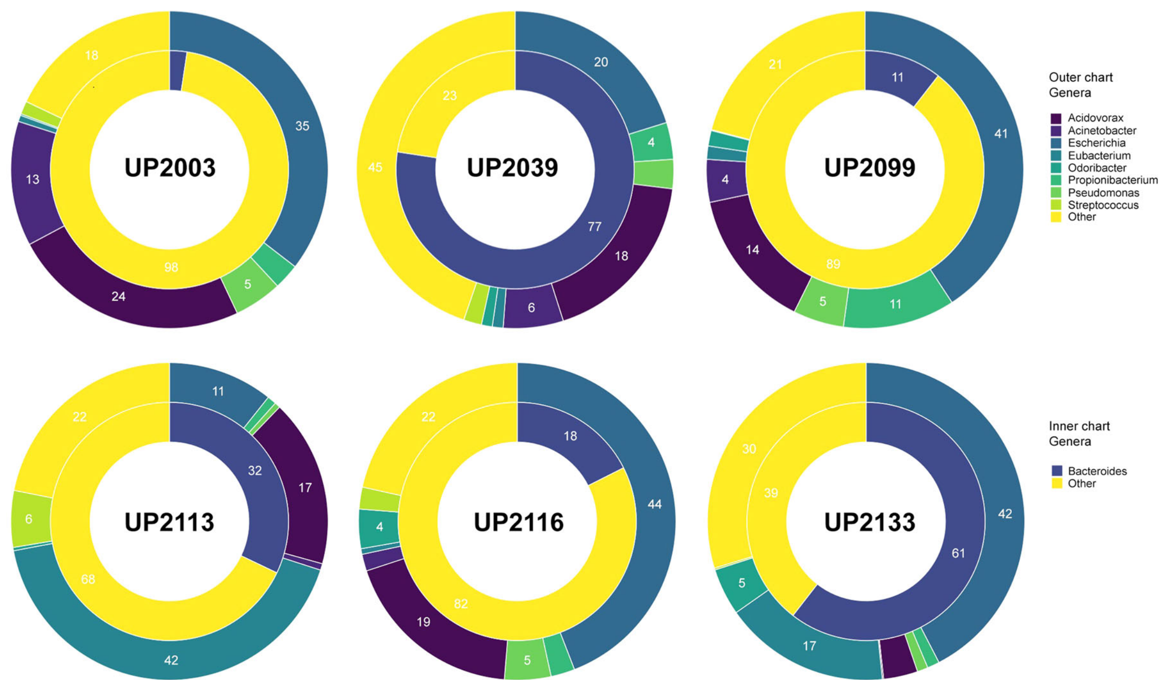

FIGURE 2 Distribution of common bacterial genera within six African high-risk prostate tumors, with *Bacteroides*, included (inner panel) and excluded (outer panel). Number in a chart indicates the percentage of bacterial abundance

increase in acquired tandem duplications ($t = -378$, $P = 4.1e{-}08$). Increased TMB is a hallmark of carcinogenic induced cancers, namely ultraviolet exposure in melanoma (TMB 13.5 mutations/Mb) and tobacco smoke in lung cancer (TMB 7.2 mutations/Mb), leading to associated genomic instability and/or DNA damage.[28] PCa with no known mutagenic agent, has a TMB of <1 mutations/Mb across pathologies.[29] Having observed a 1.8-fold increase in TMB between our African- vs European-derived high-risk tumors ($t = 4.4544$, $P = .007$; two-tailed t test), this is roughly fourfold greater than reported overall for PCa.[12] As is the case of *Helicobacter pylori* and *Salmonella enterica*, bacteria can induce DNA damage influencing oncogenic transformation.[30] As with *Bacteroides*, *Eubacterium* abundance has been observed in South African women with BV.[31]

5 | SUMMARY

In conclusion, we have observed 23 common bacterial genera within prostate tissue derived from patients from three continents, with a predominance for *Proteobacteria*. Including known pathogens, such as *Escherichia*, *Salmonella*, and *Acinetobacter*, we found no link with clinical presentation. Compared with Australian and Chinese data, our African-derived high-risk PCa tissue showed a 1.6-fold increased bacterial burden, with an abundance of anaerobic bacteria. While unable to exclude for fecal contamination within our African samples, notable observations included: half of the core human gut bacterial genera were absent, *Escherichia* and *Acidovorax* were significantly abundant, while total bacterial burden and *Eubacterium* abundance correlated with host tumor hypermutation. Further studies controlling for possible contaminants are required to elucidate if microbes are contributing to adverse disease presentation within Africa.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

VMH designed and initiated the study with support from MSRB. SMP, RJL, A-MH, PDS, and MSRB were responsible for patient

recruitment, consenting, sampling, and processing. YF, WJ, and VMH analyzed the data and wrote the manuscript. CCC edited the manuscript and provided a critical review. All authors read and approved the final manuscript.

DATA ACCESSIBILITY

Sequencing data have been deposited at the NCBI Sequence Read Archive (SRA accession SRP119289) with BioProject number PRJNA412953. All data supporting the conclusions of this article are included in Tables S1 and S2 (tabulated list of bacteria identified).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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