

Oral Microbiome Alterations Associated with Human Papillomavirus Infection in Edo State, Nigeria: A 16S rRNA Metagenomic Study

Kilsi BORGBARA
LCU/PG/005745

Being a MSc Thesis Submitted to the Department of Biological Sciences, Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan, Oyo State, Nigeria

In Partial Fulfillment of the Requirement for the Award of Master of Science Degree (MSc) in Molecular Biology and Genomics

2025

Certification

This is to certify that this research work was carried out by **Borgbara, Kils** with the matriculation number: LCU/PG/005745, under the supervision of Prof Rosemary Audu in the Department of Biological Sciences, Faculty of Basic Medical and Applied Sciences, Lead City University Ibadan, Oyo State. This is to certify that Borgbara, Kils with matriculation number LCU/PG/005745 carried out this research work titled “Oral Microbiome Alterations Associated with Human Papillomavirus Infection in Edo State, Nigeria: A 16S rRNA Metagenomic Study” Department of Biological Sciences, Faculty of Basic Medical and Applied Sciences, Lead City University Ibadan, Oyo State, for the award of MSc in Molecular Biology and Genomics and that this has not been previously submitted.

.....
Prof Rosemary Audu

.....
Date

Promoter

.....
Dr Omonike Bakare

.....
Date

Head of Department

Dedication

This research work is dedicated to God Almighty.

Lead City University Ibadan DO NOT COPY

Acknowledgement

My appreciation goes to the management of Lead City University, Ibadan, for the opportunity to conduct this study.

I would like to express my deepest gratitude to my supervisor, Prof Rosemary Audu, for her invaluable guidance, insightful feedback, and continuous support throughout this research project. Her expertise and encouragement were instrumental in shaping and completing this work. I am also grateful to Dr. Modupe Coker and the entire HOMINY study team for granting me access to the research samples used in this study.

Additionally, I extend my thanks to Inqaba Biotec for their technical support throughout this program.

Even though the aforementioned institutions and individuals have assisted in this research, I alone stand responsible for any errors or shortcomings in this work.

Abstract

Understanding the oral microbiome's interplay with Human Papillomavirus (HPV) and Human Immunodeficiency Virus (HIV) is crucial for elucidating HPV-related disease pathogenesis. However, over 90% of HPV–microbiome studies originate from high-income countries. A Nigerian-specific microbial signature for HPV-associated dysbiosis is lacking, limiting efforts to develop relevant biomarkers or understand unique disease progression in this population.

This study aimed to identify oral microbial signatures associated with HPV infection, hypothesizing distinct alterations due to immunomodulation, particularly when stratified by HIV status. A nested case-control design was employed within the prospective HOMINY cohort in Edo State, Nigeria. Twenty oral rinse samples (10 HPV-positive, 10 HPV-negative), stratified by HIV status, were analyzed via full-length 16S rRNA sequencing on a PacBio Revio platform. Bioinformatics analyses used QIIME 2 for diversity metrics, ANCOM-BC2 for differential abundance (adjusting for age, sex, and HIV status), and PICRUST2 for functional predictions. Oral HPV prevalence was higher and more diverse in HIV-positive individuals. Alpha diversity showed greater Chao1 richness ($\beta = 455.9$, $p = 0.050$) and Shannon diversity ($\beta = 1.53$, $p = 0.033$) in HIV-negative/HPV-positive individuals, suggesting microbial expansion. Beta diversity showed no significant community-wide differences. Taxonomic richness was highest in HIV-positive/HPV-positive individuals, with enrichment of low-abundance phyla (*Spirochaetota*, *Mycoplasmata*). *Bacillota* dominated overall, while *Bacteroidota* increased in HIV-negative/HPV-positive samples. HIV status modified HPV-related microbial shifts: HIV-positive/HPV-positive individuals showed moderate changes (e.g., enriched *Nanoperiomorbus*, depleted *Aggregatibacter*), whereas HIV-negative/HPV-positive individuals showed stronger effects (e.g., enriched *Nanosynbacter*, depleted *Moryella*). Functional predictions revealed nominal shifts, including enrichment of CRISPR-associated systems and polysaccharide degradation enzymes and depletion of nucleotide metabolism pathways. These findings highlight the context-dependent oral microbiome response to HPV infection and potential biomarkers for HPV-related oral pathologies.

Keywords: HPV, 16s rRNA Metagenomics, Oral microbiome, Oral HPV in Nigeria

Word Count: 275

Table of Contents

Content	Page
Certification	ii
Dedication	iii
Acknowledgement	iv
Abstract	v
Table of Contents	vi
List of Tables	xii
List of Figures	xiii
List of Acronyms	xiv
Chapter One: Introduction	1
1.1 Background to the Study	1
1.2 Statement of the Problem	5
1.3 Justification of the Study	5
1.4 Aim and Objectives of the Study	6
1.5 Research Questions	6
1.6 Hypotheses	7
1.7 Significance of the Study	8
1.8 Scope of the Study	8
1.9 Limitations of the Study	9
1.10 Operational Definition of Terms	10
Endnotes	14

Chapter Two: Literature Review	17
2.1 Conceptual Review	17
2.1.1 Genome of HPV	19
2.1.2 Classification Human of Papillomavirus	23
2.1.3 Transmission of HPV	24
2.1.4 Life Cycle of HPVs	28
2.1.5 Clinical manifestations of HPV	32
2.2 Diagnosis of HPV	35
2.2.1 Clinical Examination and Colposcopy	35
2.2.2 Conventional and Liquid-Based Cytology	35
2.2.3 Microscopy and Histopathology	36
2.2.4 Direct Nucleic Acid Detection	36
2.2.5 Signal Amplification Assays: The Hybrid Capture System	36
2.2.6 Polymerase Chain Reaction (PCR) and Genomic Amplification	37
2.2.7 Detection of Integrated HPV Genome and Viral Load Evaluation	37
2.2.8 <i>In Situ</i> Hybridization (ISH)	37
2.2.9. HPV Variant Analysis	38
2.2.10. HPV Serology	38
2.3 Epidemiology of Oral HPV Infections	38
2.4 Oral HPV and Head and Neck Cancers	40
2.5 Role of HPV in Oropharyngeal and Head and Neck Cancers	41

2.6 Comparison of HPV-Associated Cancers in Different Regions	43
2.7 The Oral Microbiome	46
2.7.1 Microbial Communities in the Oral Cavity	48
2.7.2 Key Bacterial Species and Their Functions	51
2.8 Impact of HPV on the Oral Microbiome	53
2.9 Oral Microbiota as a Biomarker for HPV-Related Cancers	58
2.10 Geographic and Population-Specific Gaps in Oral HPV Interaction Studies	61
2.11 16S rRNA Analysis in HPV-Microbiome Research	62
2.12 Research Gaps Specific to Nigeria and Sub-Saharan Africa	63
Endnotes	66
Chapter Three: Methodology	79
3.1 Research Design	79
3.2 Population of the Study	79
3.3 Study Area	80
3.4 Sampling Methods	80
3.5 Ethical Clearance	82
3.6 HIV Serostatus Determination	82
3.7 DNA Extraction	82
3.8 HPV Genotyping	83
3.9 16S rRNA Gene Sequencing	84
3.10 Bioinformatics Analysis	85

3.11 Statistical Analysis	86
Endnotes	87
Chapter Four: Results and Discussion of Findings	89
4.1 Results	89
4.1.1 Study Population	89
4.1.2 HPV Genotypes in Oral Infections	92
4.1.3 Alpha Diversity	92
4.1.3.1 Chao1 Richness Estimator	92
4.1.3.2 Shannon Diversity and HIV/HPV Status	94
4.1.3.3 Shannon Diversity and HIV/HPV Status	98
4.1.3.4 Faith's Phylogenetic Diversity	98
4.1.3.5 Pielou's Evenness	99
4.1.4 Beta Diversity	101
4.1.4.1 Beta Diversity Analysis using Bray–Curtis dissimilarity	101
4.1.4.2 Jaccard Index	101
4.1.4.3 Unweighted UniFrac Beta Diversity	104
4.1.4.4 Weighted UniFrac Beta Diversity	106
4.1.5 Differential Abundance Analysis	106
4.1.5.1 Taxonomic Richness Across HIV and HPV Infection Groups	106
4.1.5.2 Distribution of Low-Abundance Phyla by HIV/HPV Co-Infection Status	110
4.1.5.3 Taxonomic Composition at the Phylum Level in Relation to HIV and HPV Status	110

4.1.5.4 Differential Abundance of Bacterial Genera Associated with HPV	112
4.1.5.5 Differential Abundance of Bacterial Species Associated with HPV Status	115
4.1.5.6 Differential Abundance of KEGG Orthologs Associated with HPV Status	119
4.1.5.7 Enzyme Commission Feature Prediction and Differential Abundance Analysis	123
4.1.5.8 Differential Abundance of Predicted MetaCyc Pathways	125
4.2 Discussion of Findings	127
4.2.1 HPV Genotype Distribution and Oral HPV Prevalence	127
4.2.2 Alpha Diversity	129
4.2.3 Beta Diversity	130
4.2.4 Taxonomic Richness Across HIV and HPV Infection Groups	131
4.2.5 Differential Abundance in HIV-Positive Participants	132
4.2.6 Differential Abundance of KEGG Orthologs Associated with HPV Status	135
4.2.7 Differential Abundance of Enzyme Commission Features	136
4.2.8 Differential Abundance of Predicted MetaCyc Pathways	137
Endnotes	139
Chapter Five: Conclusion	143
5.1 Summary of Findings	143
5.1.1 HPV Genotype Distribution and Oral HPV Prevalence	143
5.1.2 Alpha Diversity	143
5.1.3 Beta Diversity	143
5.1.4 Taxonomic Richness and Composition	144

5.1.5 Differential Abundance of Bacterial Genera and Species	144
5.1.6 Differential Abundance of Enzyme Commission Features	145
5.1.7 Differential Abundance of Predicted MetaCyc Pathways	146
5.2 Conclusion	146
5.3 Recommendations	146
5.4 Contribution to Knowledge	147
5.5 Suggestion for Further Studies	148
Bibliography	151
Appendices	171
Bio-data	178
University Compliance Certification	181

List of Tables

Table	Title	Page
2.1	Papillomavirus Proteins and Functions	17
2.2	HPV Classification Criteria	23
4.1	Demographic Distribution of Participants by HIV Status and Oral HPV Infection, Highlighting Age and Sex Composition	85
4.2	Number of Phyla, Genera, and Species Identified Across Study Groups	102
4.3	Comparative Overview of HPV-Associated Genera by HIV Status	110
4.4	Summary of the Number of Kegg Orthologs Features Reaching Various Significance Thresholds	114

List of Figures

Figure	Title	Page
1.1	Structure of the HPV Particle Showing the L1 And L2 Proteins, the Genetic Material and the Histones	15
1.2	Papillomavirus Genera and Species	22
1.3	Replication Cycle of HPV	26
4.1	Distribution of HPV Genotype in Oral Infections by HIV Status	87
4.2	Unadjusted Chao1 richness Across HIV/HPV Groups	89
4.4	Unadjusted Shannon diversity Across HIV/HPV Groups	90
4.5	Unadjusted Faith's Phylogenetic Diversity Across HIV/HPV Groups	93
4.6	Principal Coordinates Analysis (PCoA) Based on Bray–Curtis Dissimilarity was used to Explore the Beta Diversity of Microbiomes in Oral Samples	96
4.7	The Variation in Oral Microbiomes (Beta Diversity) was Explored Through PCoA using the Jaccard Index as the Distance Measure	98
4.8	Principal Coordinates Analysis (PCoA) of Unweighted UniFrac Distances Showing Microbial Community Structure Across HIV/HPV Categories	100
4.9	Principal Coordinates Analysis (PCoA) Plot of Weighted Unifrac Distances Colored by HIV/HPV Category	101
4.10	Relative Abundance of Low-abundance Phyla (<1%) Across HIV/HPV Co-infection Categories	104
4.11	Stacked Bar Plot Showing Mean Relative Abundance of Dominant Phyla (>1%) in Each HIV/HPV Infection Category	106
4.12	Bar Plot HIV Positive Genera with $p < 0.05$, Colored by Direction of Change	107

4.13	Bar Plot of HIV Negative Genera with $p < 0.05$, Colored by Enrichment/Depletion in HPV+	109
4.14	Differential Abundance of Oral Microbiota in HPV+ Individuals Stratified by HIV Status	113
4.15	Volcano Plot Showing Log-Fold Changes (LFC) Versus $-\log_{10}(p\text{-Values})$ For 4,612 KO Features	115
4.16	Top 50 KO Features Ranked by Absolute Effect Size Among Features with $P < 0.05$	117
4.17	Log Fold Change Across Top 30 HPV Positive and Negative Groups	119
4.18	A Barplot Depicting the Top Discriminatory Pathways	121

List of Acronyms

Abbreviation	Full Meaning
16S rRNA	16S Ribosomal Ribonucleic Acid
AIDS	Acquired Immunodeficiency Syndrome
ANCOM-BC2	Analysis Of Composition of Microbiomes with Bias Correction 2
ASV	Amplicon Sequence Variant
BPVs	Bovine Papillomaviruses
Cas	CRISPR-Associated Proteins
CCS	Circular Consensus Sequences
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
CIN	Cervical Intraepithelial Neoplasia
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Df	Degrees of Freedom
DNA	Deoxyribonucleic Acid
EC	Enzyme Commission
eHOMD	Expanded Human Oral Microbiome Database
FDR	False Discovery Rate
FSWs	Female Sex Workers
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
ISH	In Situ Hybridization
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	Kegg Ortholog
LCR	Long Control Region
LFC	Log Fold Change

LMICs	Low- And Middle-Income Countries
MetaCyc	Metabolic Pathway Database
NF- κ B	Nuclear Factor-Kappa B
OPCs	Oropharyngeal Cancers
ORF	Open Reading Frame
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis
PCR	Polymerase Chain Reaction
PERMANOVA	Permutational Multivariate Analysis of Variance
	Phylogenetic Investigation of Communities by Reconstruction of
PICRUST2	Unobserved States 2
PVs	Papillomaviruses
QIIME2	Quantitative Insights into Microbial Ecology (Version 2)
RefSeq	Reference Sequence Database
RNA	Ribonucleic Acid
SSA	Sub-Saharan Africa
UCHIME	UCHIME Chimera Detection Algorithm
URR	Upstream Regulatory Region
VIA	Visual Inspection with Acetic Acid
VLPs	Virus-Like Particles
WHO	World Health Organization

Chapter One

Introduction

1.1 Background to the Study

The term "papilloma" is derived from the Latin words "papilla," meaning a small, rounded projection, and "oma," meaning an abnormal growth or tumor. This combination reflects the characteristic appearance of papillomas as small, wart-like growths. Papillomaviruses infect mucosal and cutaneous epithelia in a wide variety of higher vertebrates in a species-specific manner and induce cellular proliferation¹. Bovine Papillomaviruses (BPVs) 1 and 2 are the only BPVs known to infect mesenchymal tissues and show cross-species transmission. They have been documented to infect equids, buffaloes, and bison². Human papillomavirus (HPV) is a sexually transmitted infectious disease that is endemic around the world³. It is a double-stranded DNA virus, which belongs to the family of *Papillomaviridae*.

HPV infections can present with a variety of symptoms, ranging from no noticeable signs to benign growths, precancerous lesions, and invasive cancers. Although the majority of HPV infections are transient and tend to resolve on their own, a minority of infections can cause clinical diseases such as warts or cancers⁴. HPV encompasses over 200 variants, grouped into high-risk and low-risk categories based on their potential to cause cervical cancer. Fifteen types—including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82—are confirmed high-risk, while low-risk HPV types are associated with benign growths, such as warts, on the genitals and skin⁴. High-risk HPV types are linked to cancers in the oropharynx (oral, tonsil, and throat areas) and anogenital regions, including cervical, anal, vulvar, vaginal, and penile cancers⁴.

Human papillomavirus remains a leading cause of infection-related cancers globally, responsible for 5% of malignancies worldwide, including 90% of cervical cancers and 70%

of oropharyngeal cancers (OPCs)⁵⁻⁷. While cervical cancer remains the most studied HPV-associated malignancy, the rising incidence of HPV-driven OPCs in high-income countries has shifted global attention to oral HPV epidemiology. Globally, it is estimated that between 30.8% and 42.7% of oropharyngeal cancers are attributable to human papillomavirus (HPV) infection, indicating that approximately one-third to nearly half of these cancers are caused by HPV⁸. In the United States, HPV-positive OPCs now exceed cervical cancer cases, with a 3% annual increase in incidence since 2015⁹. However, this trend obscures a critical disparity: low- and middle-income countries (LMICs), particularly in sub-Saharan Africa (SSA), face a dual burden of high HPV prevalence and limited diagnostic infrastructure, leaving oral HPV's role in OPCs poorly understood¹⁰.

In Africa, where cervical cancer mortality rates exceed 90%, oral HPV remains understudied despite its potential contribution to head and neck cancers¹¹. A systematic review found no country-specific oral HPV prevalence data for most sub-Saharan Africa SSA nations¹². Limited studies suggest oral HPV rates range from 2.3% to 6.5% in African populations, lower than cervical rates but with significant variability¹². A 2022 study of female sex workers (FSWs) in Ibadan, Nigeria, reported 24% oral HPV prevalence—the highest recorded in SSA—with HPV35 as the dominant high-risk type⁵. HIV coinfection further amplifies risks: HIV-positive individuals exhibit higher oral HPV persistence and viral loads^{5,12}.

Nigeria's HPV burden is among the world's highest, yet oral HPV is underprioritized. A 2021 study of sexually active women in Ibadan found 16.1% oral HPV prevalence, with HPV35 and HPV16 as prevalent genotypes¹². Among heterosexual men, oral HPV rates are lower (7.8%), but penile HPV prevalence reaches 40.5%, highlighting site-specific transmission dynamics¹³. Risk factors include cohabiting with sexual partners (linked to

mucosal viral exchange), penile-anal HPV concordance and HIV coinfection, which triples high-risk HPV odds^{12,13}.

Southern Nigeria reports alarming cervical HPV rates (35.9% among unmarried women), driven by low vaccine uptake (6%) and high-risk behaviors like smoking¹⁴. However, oral HPV data are scarce. A 2023 study in the South-South geopolitical zone identified HIV positivity as a key predictor of cervical HPV, a trend likely mirrored in oral infections¹⁵. Structural barriers exacerbate risks, only 6 of 25 circulating HPV types are covered by available vaccines and OPCs are rarely included in cancer registries, masking true incidence¹².

Beyond viruses, research has implicated bacterial involvement in the pathogenesis of various human malignancies¹⁶. This association appears to be multifaceted, potentially involving chronic bacterial-induced inflammation in specific organs as a contributing factor to tumorigenesis. The oral cavity harbors one of the most diverse microbial communities in the human body, second only to the gut. Over 800 bacterial species colonize niches such as the tongue, gingival sulcus, and palatine tonsils, forming dynamic consortia that regulate immune homeostasis, nutrient metabolism, and pathogen defense¹⁷. Commensal bacteria like *Streptococcus salivarius* and *Rothia mucilaginosa* secrete antimicrobial peptides that inhibit viral replication, while others, such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, are pathobionts linked to chronic inflammation and carcinogenesis¹⁸.

Recent advances in metagenomic sequencing have revealed that dysbiosis—a disruption of microbial equilibrium—is associated with systemic diseases, including diabetes, cardiovascular disorders, and cancers¹⁹. In the oral cavity, dysbiotic shifts are hallmarked by reduced microbial diversity, depletion of health-associated taxa (e.g., *Lactobacillus*), and overgrowth of pro-inflammatory species. *Fusobacterium nucleatum* produces adhesins that activate nuclear factor-kappa B (NF-κB) signaling, driving the production of cytokines like

IL-6 and TNF- α , which promote tumor angiogenesis and immune evasion²⁰. Similarly, *Porphyromonas gingivalis* secretes proteases that degrade tumor suppressor proteins, facilitating genomic instability²¹. The emerging evidence from these findings points towards a crucial mechanism: the oral microbiome may function as a key mediator in the process of HPV-induced carcinogenesis. The interplay between HPV and microbial communities is a burgeoning area of research. Studies of the cervicovaginal microbiome have demonstrated that *Lactobacillus*-dominant ecosystems are associated with reduced HPV persistence, whereas dysbiotic communities enriched with *Gardnerella vaginalis* correlate with higher viral load and progression to cervical intraepithelial neoplasia²². Despite the availability of effective prophylactic HPV vaccines, equitable implementation remains a global challenge. Over 80% of cervical and oropharyngeal cancer cases occur in low- and middle-income countries (LMICs), where vaccine rollout is hindered by cost, logistical barriers, and sociocultural stigma²³. This systemic inequity leaves millions vulnerable to preventable malignancies, underscoring the urgent need for complementary strategies to mitigate HPV-related cancer risks in underserved populations.

This study bridges a critical gap in understanding how HPV and the oral microbiome interact to drive carcinogenesis. By characterizing microbial signatures in a high-risk Nigerian cohort—a population underrepresented in existing research—we aim to uncover novel biomarkers and therapeutic targets that reflect region-specific host-microbe-viral dynamics. These findings could inform interim prevention strategies (e.g., microbiome-modulating therapies or risk stratification tools) for populations lacking vaccine access, while simultaneously advocating for policy reforms to address the root causes of global health disparities.

1.2 Statement of the Problem

Geographic bias dominates HPV-microbiome research, with >90% of studies concentrated in high-income countries despite disproportionate disease burdens in sub-Saharan Africa. This region faces a dual challenge: HPV prevalence exceeds 24%—the highest globally—and oropharyngeal cancers (OPCs) are frequently underdiagnosed until advanced stages²⁴. Although persistent high-risk HPV infection drives OPC pathogenesis, the bidirectional relationship between HPV and the oral microbiome remains unresolved. A critical knowledge gap persists: Does HPV-induced immune modulation create dysbiotic microbial niches, or do pre-existing microbiome imbalances facilitate viral persistence and oncogenesis? This causal ambiguity undermines efforts to identify conserved microbial signatures predictive of malignancy, particularly in African populations where unique sociodemographic factors, endemic pathogens, and underrepresented HPV genotypes may influence host-microbe-viral dynamics. Focusing on a Nigerian cohort, this study investigates HPV-associated alterations in oral microbiome composition, addressing a key barrier to translational applications in regions bearing the highest HPV-attributable cancer burdens.

1.3 Justification of the Study

Most studies linking HPV to the microbiome have focused on cervical or gut or anal microbiota, with limited data on oral microbial dynamics in HPV infection. Furthermore, African populations—which face a disproportionate burden of HPV-associated cancers—are underrepresented in existing research. This study addresses these gaps by analyzing the oral microbiome of a Nigerian cohort using 16S rRNA sequencing. By comparing HPV-positive and HPV-negative individuals, we aim to identify microbiome patterns that may serve as biomarkers for HPV infection or persistence. Such insights could inform early detection strategies or microbiome-targeted interventions to mitigate HPV-related oral carcinogenesis in high-risk populations.

1.4 Aim and Objectives of the Study

To investigate whether HPV infection is associated with distinct alterations in the taxonomic composition and functional profiles of the oral microbiome among individuals in South-South Nigeria. This study will leverage 16S rRNA sequencing to analyze bacterial communities in oral rinse samples from 10 HPV-positive and 10 HPV-negative individuals. The goal is to identify microbial biomarkers, community shifts, and functional pathways that may contribute to HPV persistence and its potential role in carcinogenesis.

The objectives of the study are to:

- I. Perform 16S rRNA metagenomic sequencing on 20 oral rinse samples (10 HPV-positive, 10 HPV-negative) to profile bacterial communities.
- II. Analyze alpha/beta diversity metrics and differentially abundant taxa between HPV groups.
- III. Predict functional metabolic pathways.

1.5 Research Questions

- i. Does HPV infection influence the overall taxonomic composition of the oral microbiome in individuals from South-South Nigeria?
- ii. Are there differences in alpha and beta diversity indices (e.g., Shannon, Chao1, Faith's PD, UniFrac) between HPV-positive and HPV-negative individuals?
- iii. Which bacterial taxa are differentially abundant between HPV-positive and HPV-negative groups after adjusting for relevant covariates?
- iv. Does HPV infection correspond to distinct functional metabolic profiles in predicted pathways (e.g., KEGG Orthologs, MetaCyc pathways) inferred from PICRUST2?
- v. Can specific microbial signatures serve as potential biomarkers for HPV infection or persistence within this population?

1.6 Hypotheses

To address the research questions, the study proposes the following hypotheses:

Null Hypotheses (H_0)

- i. **H₀₁:** There is no significant difference in the taxonomic composition of the oral microbiome between HPV-positive and HPV-negative individuals.
- ii. **H₀₂:** Alpha and beta diversity metrics do not differ significantly between HPV-positive and HPV-negative groups.
- iii. **H₀₃:** No bacterial taxa are differentially abundant between HPV-positive and HPV-negative individuals after adjusting for age, sex, and HIV status.
- iv. **H₀₄:** HPV-positive and HPV-negative groups do not differ in predicted functional pathway profiles inferred from PICRUSt2.
- v. **H₀₅:** There are no microbial signatures associated with HPV infection or potential persistence.

Alternative Hypotheses (H_1)

- i. **H₁₁:** HPV infection is associated with distinct alterations in the oral microbiome's taxonomic composition.
- ii. **H₁₂:** HPV-positive individuals exhibit significantly different alpha and beta diversity metrics compared with HPV-negative individuals.
- iii. **H₁₃:** Certain bacterial taxa are differentially abundant between HPV-positive and HPV-negative groups, even after adjusting for confounders.
- iv. **H₁₄:** HPV positivity corresponds to distinct predicted functional pathways in the oral microbiome.

- v. **H₁₅:** Specific microbial signatures are associated with HPV infection and may serve as biomarkers for HPV persistence or carcinogenesis.

1.7 Significance of the Study

The oral microbiome likely plays a key role in HPV-driven carcinogenesis, but its importance has only recently been appreciated. Studies in the uterine cervix show that Lactobacillus-dominant microbiota are linked to HPV clearance, whereas dysbiotic communities (rich in *Gardnerella*, *Fusobacterium*, *Prevotella*, etc.) correlate with higher HPV persistence and progression. Similarly, oral dysbiosis has been strongly associated with head and neck cancers. For example, key oral pathogens (like *Fusobacterium nucleatum* or *Porphyromonas gingivalis*) promote inflammation and immune evasion, creating a tumor-favorable niche. Persistent oral HPV is the established primary risk factor for oropharyngeal cancer, but HPV infection alone is not sufficient for malignancy. By characterizing how the oral microbiome shifts in the presence of high-risk HPV, we can begin to uncover the additional co-factors that drive cancer. In summary, this study addresses a critical gap by exploring HPV–microbiome interactions in a high-risk African cohort, with the goal of identifying microbial signatures that may predict HPV persistence or carcinogenic progression.

1.8 Scope of the Study

This study analyzes oral microbiome differences between HPV-positive and HPV-negative individuals within the broader HOMINY cohort at the University of Benin Teaching Hospital (UBTH) in Benin City, Nigeria. It is limited to baseline, cross-sectional data: 20 oral rinse samples (10 HPV+ and 10 HPV–). All HPV status was determined by PCR (the Allplex™ HPV28 assay detecting 28 HPV genotypes). The microbial analysis is restricted to bacterial 16S rRNA gene sequencing using PacBio (full-length) and subsequent bioinformatic pipelines (QIIME2 with DADA2 and the eHOMD reference database) as described above.

No other viruses (beyond HPV), fungi, or host transcriptomic data are assessed. Functional predictions of the microbiome (KEGG, MetaCyc) come solely from PICRUST2 inference, not direct metagenomic or metatranscriptomic sequencing.

In practical terms, this means the study only examines bacteria in oral rinse samples, and only in these 20 participants (a nested case-control design within HOMINY). Analyses focus on taxonomic composition, alpha/beta diversity (Shannon, Chao1, Faith's PD, UniFrac, etc.), and differential abundance of taxa and predicted pathways between HPV groups. The scope does *not* include longitudinal changes (beyond baseline), other body sites, or direct functional/immune assays. All findings are specific to the HOMINY inclusion criteria (specific HIV-exposure strata) and should not be extrapolated to different populations without caution.

1.9 Limitations of the Study

Several methodological limitations should be acknowledged. Sample size is small (n=20), which limits statistical power and may preclude detection of subtle differences or control of all confounders (e.g. diet, oral hygiene). Cross-sectional design prevents any causal inference – this study can identify associations but cannot determine whether HPV causes microbiome shifts or vice versa. The study population is also specialized, so findings may not generalize to other age groups or regions.

PCR amplification can introduce errors, chimeras, and uneven amplification of certain taxa. The choice of 16S primers and full-length sequencing provides broader coverage, but some bacteria (especially rare or uncharacterized oral taxa) may still be missed or misclassified. Likewise, the Allplex HPV28 assay, while comprehensive for known types, cannot detect novel or uncharacterized HPV genotypes, possibly underestimating true infection prevalence.

The PacBio platform produces high accuracy reads, yet any sequencing platform has finite error rates and potential contaminants.

The use of predictive bioinformatics (DADA2 for ASV inference, PICRUSt2 for functional profiling) also has limits. ASV methods resolve sequences to single nucleotide differences, but multiple rRNA gene copies can exist within one bacterium, possibly inflating observed diversity. PICRUSt2 infers metabolic pathways from 16S data, which is inherently predictive (based on reference genomes) rather than a direct measure of gene expression or function; thus, pathway results should be interpreted cautiously. Finally, I adjust for age, sex, and HIV status in our models, but residual confounding (e.g. socioeconomic factors, recent diet, undetected antibiotics or probiotics) may still influence results.

1.10 Operational Definition of Terms

16S rRNA gene sequencing: A method of identifying bacteria by sequencing the 16S ribosomal RNA gene, which contains conserved and variable regions. Primers 27F and 1492R amplify the full 16S gene from each bacterial genome.

Amplicon Sequence Variant (ASV): A unique DNA sequence observed in the 16S data after denoising (using DADA2). Each ASV is assumed to represent a distinct bacterial strain or species-level taxon.

OTU (Operational Taxonomic Unit): A conventional term (not used here) referring to clusters of 16S sequences grouped by similarity (often 97%). In contrast, ASVs distinguish single-nucleotide differences.

Alpha diversity: A measure of microbial diversity *within* a single sample. It includes metrics like Chao1 (estimates species richness), Shannon index (accounts for both richness and evenness of species), and Faith's phylogenetic diversity (sums branch lengths of the

phylogenetic tree of observed taxa). High alpha diversity means many different, evenly distributed bacteria.

Beta diversity: A measure of diversity *between* samples. It quantifies how different the microbial communities are across individuals. Common metrics include Jaccard index (shared species presence/absence), UniFrac (phylogenetic distance; weighted UniFrac accounts for abundance), etc. In this study, we compare beta diversity between HPV+ and HPV– groups.

ANCOM-BC2: A statistical method (“Analysis of Compositions of Microbiomes with Bias Correction”) used to identify taxa or pathways that are differentially abundant between groups while accounting for compositional data bias and covariates.

PICRUSt2: A bioinformatics tool (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) that *predicts* functional gene content (KEGG Orthology, MetaCyc pathways) from 16S profiles by referencing known genome sequences. It provides an inferred functional profile, not a direct measurement.

HOMINY cohort: The parent study (“HPV, HIV, and Oral Microbiota in Nigerian Youth”) – a longitudinal cohort of Nigerian children/adolescents (9–18 years) and their mothers, stratified by HIV status. It examines oral HPV incidence and microbiome changes over time.

HPV genotyping: Detection of HPV DNA and identification of its type. The Allplex™ HPV28 assay uses PCR to identify 19 high-risk (oncogenic) and 9 low-risk HPV genotypes in each sample. A sample is HPV-positive if any HPV DNA is detected, and HPV-negative if none of the 28 types are found.

High-risk (HR) vs. low-risk (LR) HPV: HR HPV types (e.g., HPV16, 18, 31, etc.) are those epidemiologically linked to cancer development; LR types (e.g., HPV6, 11) typically cause

benign warts or lesions. In this study, HR HPV types are the primary focus regarding cancer risk.

Oral rinse sample: A method of collecting cells and microbes from the mouth. Participants swish and gargle saline or buffer, and the expectorated fluid (oral rinse) is used for DNA extraction, capturing bacteria from the oral cavity and oropharynx.

NanoDrop spectrophotometer: An instrument for measuring DNA concentration and purity (A260/280 ratios) using a small (1 μ L) sample. It ensures sufficient DNA quality before sequencing.

QIIME 2: A widely used, open-source bioinformatics platform for microbial community analysis. In this study, QIIME 2 (v2025.5.1 “Mariposa Orchid” release) handles diversity analyses (alpha/beta metrics) and interfaces with DADA2 for denoising and taxonomy assignment.

eHomD (expanded Human Oral Microbiome Database): A curated reference database of 16S sequences from human oral bacteria. We use its Version 16.02 taxonomy to assign taxonomy to ASVs at species/genus levels.

Chao1 index: An alpha diversity metric estimating total species richness by accounting for rare taxa (based on number of singletons and doubletons).

Shannon index: An alpha diversity metric combining richness and evenness; higher values mean more diversity and more evenly distributed taxa.

Faith’s phylogenetic diversity: An alpha diversity measure that sums the lengths of all branches in a phylogenetic tree covered by the sample’s taxa (i.e., evolutionary diversity).

Pielou’s evenness: An alpha diversity measure (Shannon/ $\ln(\text{richness})$) indicating how uniformly individuals are distributed across observed species (1 = perfectly even).

Jaccard index: A beta diversity measure based on presence/absence of species; it calculates the fraction of shared species between two samples.

UniFrac distance: A beta diversity measure incorporating phylogeny; unweighted UniFrac uses presence/absence of lineages, while weighted UniFrac also accounts for their relative abundance.

Regularization (ANCOM-BC2): In ANCOM-BC2, a method to stabilize variance and handle sparse data in differential abundance tests.

Lead City University Ibadan DO NOT COPY

Endnotes

1. K. Zheng, N. Egawa, A. Shiraz, M. Katakuse, M. Okamura, H. M. Griffin, and J. Doorbar, *The Reservoir of Persistent Human Papillomavirus Infection; Strategies for Elimination Using Anti-Viral Therapies*, **Viruses**, 14(2), 2022, 214.
3. R. E. Plotzker, A. Vaidya, U. Pokharel, and E. A. Stier, *Sexually Transmitted Human Papillomavirus: Update in Epidemiology, Prevention, and Management*, **Infectious Disease Clinics of North America**, 37(2), 2023, 289–310.
4. S. Karmakar, R. Dutta, and S. Moulik, *A Transdisciplinary Analysis on Human Papilloma Virus and Cervical Cancer*, **Discover Medicine**, 2(1), 2025, 35.
5. I. O. Morhason-Bello, K. Baisley, M. A. Pavon, I. F. Adewole, R. A. Bakare, S. De Sanjosé, S. C. Francis, and D. Watson-Jones, *Oral, Genital and Anal Human Papillomavirus Infections among Female Sex Workers in Ibadan, Nigeria*, **PLOS ONE**, 17(3), 2022, e0265269.
6. J.-M. Kutz, P. Rausche, T. Gheit, D. I. Puradiredja, and D. Fusco, *Barriers and Facilitators of HPV Vaccination in Sub-Saharan Africa: A Systematic Review*, **BMC Public Health**, 23(1), 2023, 974.
7. P. Economopoulou, I. Kotsantis, and A. Psyrri, *Special Issue about Head and Neck Cancers: HPV Positive Cancers*, **International Journal of Molecular Sciences**, 21(9), 2020, 3388.
8. Y. Lu, Z. Xie, G. Luo, H. Yan, H.-Z. Qian, L. Fu, B. Wang, R. Huang, F. Cao, H. Lin, R. You, L. Tan, T. Yu, M. Chen, C. Li, X. Liu, W. Lei, and H. Zou, *Global Burden of Oropharyngeal Cancer Attributable to Human Papillomavirus by Anatomical Subsite and Geographic Region*, **Cancer Epidemiology**, 78(1), 2022, 102-140.
9. B. R. Roman, and A. Aragones, *Epidemiology and Incidence of HPV-Related Cancers of the Head and Neck*, **Journal of Surgical Oncology**, 124(6), 2021, 920–922.
10. L. Bruni, G. Albero, J. Rowley, L. Alemany, M. Arbyn, A. R. Giuliano, L. E. Markowitz, N. Broutet, and M. Taylor, *Global and Regional Estimates of Genital Human Papillomavirus Prevalence among Men: A Systematic Review and Meta-Analysis*, **The Lancet Global Health**, 11(9), 2023.
11. D. Singh, J. Vignat, V. Lorenzoni, M. Eslahi, O. Ginsburg, B. Lauby-Secretan, M. Arbyn, P. Basu, F. Bray, and S. Vaccarella, *Global Estimates of Incidence and Mortality of Cervical Cancer in 2020: A Baseline Analysis of the WHO Global Cervical Cancer Elimination Initiative*, **The Lancet Global Health**, 11(2), 2023, 43-50.
12. I. O. Morhason-Bello, K. Baisley, M. A. Pavon, I. F. Adewole, R. Bakare, S. De Sanjosé, S. C. Francis, and D. Watson-Jones, *Prevalence and Genotype Specific Concordance of Oro-Genital and Anal Human Papillomavirus Infections among Sexually Active Nigerian Women*, **Infectious Agents and Cancer**, 16(1), 2021, 59.
13. I. O. Morhason-Bello, K. Baisley, M. A. Pavon, I. F. Adewole, R. Bakare, S. A. Adebayo, S. De Sanjosé, S. C. Francis, and D. Watson-Jones, *Prevalence and Concordance of Penile*,

Anal, and Oral Human Papillomavirus Infections among Sexually Active Heterosexual Men in Ibadan, Nigeria, **Cancer Causes & Control**, 36(1), 2025, 51–66.

14. A. G. Ohihoin, P. A. Okwuraiwe, A. Z. Musa, G. Olorunfemi, C. K. Onwuamah, F. Ige, O. S. Amoo, R. Audu, F. Okogbo, B. Daniyan, T. Swende, G. C. Onyemelukwe, H. Daru, H. Usman, O. Shittu, J. Musa, O. C. Ezechi, and I. A. Ujah, *Prevalence and Predictors of High-Risk HPV in Nigeria*, **Advances in Infectious Diseases**, 12(4), 2022, 745–757.

15. C. K. Onwuamah, N. Feng, A. E. Momoh, M. Uwandu, R. A. Ahmed, I. Idigbe, G. D. Vincent, C. A. Ogbu, N. Okonkwo, J. Sokei, B. S. Abimbola, T. Ojopagogo, L. C. Okoli, M. Adesina, P. N. Ezemelue, O. Sowunmi, J. Okwuzu, O. H. Labo–Popoola, J. O. Shaibu, G. A. Ohihoin, E. Nzeribe, A. David, O. Olaleye, I. Ofotokun, X. Dong, and O. C. Ezechi, *Prevalence and Risk Factors for High-Risk Human Papillomavirus Infection among Women from Three Southern Geopolitical Zones of Nigeria*, **Frontiers in Oncology**, 13(1), 2023, 1254304.

16. Q. Xia, and S. Pierson, *HPV Infection and Oral Microbiota: Interactions and Future Implications*, **International Journal of Molecular Sciences**, 26(4), 2025, 1424.

17. J. L. Baker, J. L. Mark Welch, K. M. Kauffman, J. S. McLean, and X. He, *The Oral Microbiome: Diversity, Biogeography and Human Health*, **Nature reviews Microbiology**, 22(2), 2024, 89–104.

18. G. W. Lawrence, N. McCarthy, C. J. Walsh, T. M. Kunyoshi, E. M. Lawton, P. M. O'Connor, M. Begley, P. D. Cotter, and C. M. Guinane, *Effect of a Bacteriocin-Producing Streptococcus Salivarius on the Pathogen Fusobacterium Nucleatum in a Model of the Human Distal Colon*, **Gut Microbes**, 14(1), 2022, 2100203.

19. A. J. Kombe Kombe, B. Li, A. Zahid, H. M. Mengist, G.-A. Bounda, Y. Zhou, and T. Jin, *Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation*, **Frontiers in Public Health**, 8(1), 2020, 1-19.

20. L. Galasso, F. Termite, I. Mignini, G. Esposto, R. Borriello, F. Vitale, A. Nicoletti, M. Paratore, M. E. Ainora, A. Gasbarrini, and M. A. Zocco, *Unraveling the Role of Fusobacterium Nucleatum in Colorectal Cancer: Molecular Mechanisms and Pathogenic Insights*, **Cancers**, 17(3), 2025, 368.

21. R. J. Lamont, Z. R. Fitzsimonds, H. Wang, and S. Gao, *Role of Porphyromonas Gingivalis in Oral and Orodigestive Squamous Cell Carcinoma*, **Periodontology** 2000, 89(1), 2022, 154–165.

22. P. Łaniewski, Z. E. İlhan, and M. M. Herbst-Kralovetz, *The Microbiome and Gynaecological Cancer Development, Prevention and Therapy*, **Nature Reviews Urology**, 17(4), 2020, 232–250.

23. G. R. Do Nascimento, A. C. Da Silva Santos, N. N. T. Silva, N. S. Guilmarães, A. A. Lima, and W. Coura-Vital, *Prevalence of Non-Vaccine High-Risk HPV Cervical Infections in Vaccinated Women: A Systematic Review and Meta-Analysis*, **BMC Infectious Diseases**, 25(1), 2025, 131.

24. N. H. Wood, K. S. Makua, R. L. Lebelo, N. Redzic, I. Benoy, O. M. Vanderveken, and J. Bogers, *Human Papillomavirus Prevalence in Oral and Oropharyngeal Rinse and Gargle*

Specimens of Dental Patients and of an HIV-Positive Cohort from Pretoria, South Africa,
Advances in Virology, 20(1), 2020, 239-249.

Lead City University Ibadan DO NOT COPY

Chapter Two

Literature Review

2.1 Conceptual Review

The human papillomavirus targets epithelial cells and needs to reach the basal layer of the epithelium to start a detailed series of processes. This infection process also depends on particular host responses and interactions to effectively infect the basal keratinocyte¹. Infection with high-risk (HR) HPV subtypes is well-established as a factor that significantly increases the risk of developing cancers such as cervical, anal, penile, and oropharyngeal carcinoma².

Papillomaviruses are small, spherical DNA viruses that lack an outer envelope. They have a diameter of approximately 52-55 nanometers. The virus's genetic material is a circular double-stranded DNA molecule, which accounts for 10–13% of the virion mass (Figure 1.1). The Papillomavirus structure does not contain any enzymes, lipids or saccharides. The virus exhibits stability within a pH range of 3 to 7 but becomes inactivated at temperatures of 70°C. Exposure to temperatures exceeding 50°C for 30 minutes or more results in the virus being killed. Additionally, the virus demonstrates resistance to solvents, acids and X-ray radiation³. The viral particles are made up of a single double-stranded DNA molecule that is associated with cellular histones and enclosed within a protein capsid consisting of 72 pentameric capsomers. This capsid is composed of two structural proteins: late protein L1 (55 kDa, accounting for 80% of the total viral protein) and L2 (70 kDa), both of which are encoded by the virus. The complete virion exhibits a density of 1.34 g/mL when analyzed in cesium chloride and has a sedimentation coefficient (S_{20, W}) of 300⁴⁵.

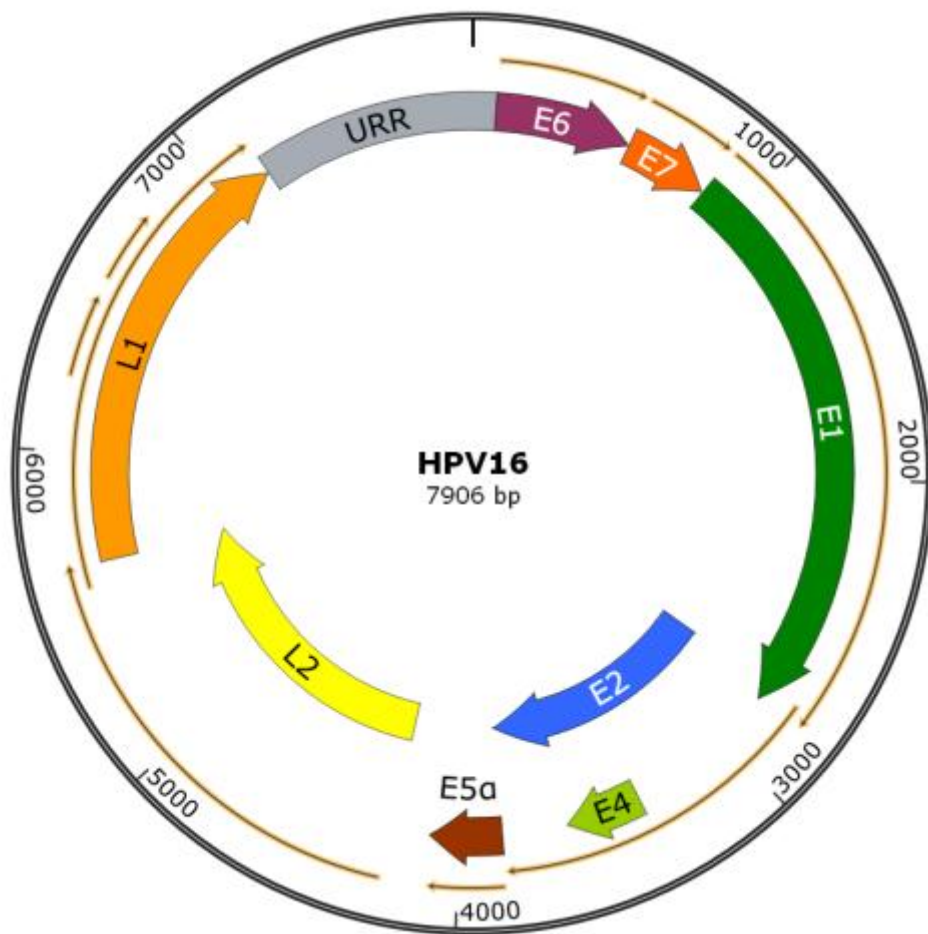


Figure 2.1: Structure of the HPV particle showing the L1 and L2 proteins, the genetic material and the histones⁶

2.1.1 Genome of HPV

The HPV genome is approximately 8kb in size and contains around eight ORFs, all transcribed from a single DNA strand. The HPV genome is structured into three main regions:

1. a long control region (LCR)/upstream regulatory region (URR), that contains transcription factor-binding sites and regulates gene expression.
2. an early region that encodes six genes (E1–E7) responsible for various functions (Table 2.1), including viral replication and cell transformation
3. late region that encodes the L1 and L2 capsid proteins, which self-assemble to form the virion.

Common features shared by all papillomaviruses include the presence of an URR, the early proteins E1 and E2 (with the E4 gene possibly nested within E2), and the late proteins L1 and L2⁷.

a) E1 Protein

This protein is essential for viral DNA replication. Additionally, it acts as a repressor in transcription and inhibits DNA replication, helping to maintain a consistent number of episomal copies within the cell⁹.

b) E2 protein

Protein E2 plays a crucial role in the DNA replication process by partnering with the E1 protein to initiate replication, particularly in HPV types 6, 11, and 16. It is responsible for coding proteins that regulate viral DNA transcription. Additionally, E2 is significant in cell transformation, where it can initiate or inhibit apoptosis, regulate transcription, and influence the immortalization and transformation potential of HPV. When E2 is inactivated, it can lead to the development of tumor lesions by promoting the expression of the oncogenes E6 and E7.

Table 2.1: Papillomavirus Proteins and Functions⁸

Viral Protein	Function	Host Protein Interactions
E1	Helicase, interacts with PV E2.	DNA polymerase alpha and RPA.
E2	Transcriptional repressor, transcriptional enhancer.	Brd4, TopBP1.
E4	Association with the cellular keratin network. A likely role in virus release and transmission.	Cellular keratins?
E5	Hydrophobic protein that can dimerize growth-factor receptors, deregulate the autophagic process, modulate epithelial-mesenchymal transition and reduce some host microRNA.	Vacuolar ATPase, PDGF, zinc transporter ZnT1, protocadherin 1 (PCDH1), and AHNAK/desmoyokin, YIP1 family member 4, calpactin I.
E6	Viral oncogene. A negative regulator of UV-DNA damage repair (betaPVs).	p53 (alphaPVs), E6AP (alphaPVs), histone acetyltransferase p300/CBP (betaPVs), MAML1 (betaPVs), and proteins containing PDZ domains. 153 cellular proteins.
E7	Viral oncogene.	pRB, CENP-C.

Table 2.1 Continued

L1	Major capsid protein.	HSPG, L332, cyclophilins.
L2	Minor capsid protein.	PATZ, PLINP, PMSP, dynein.
E8	E5-like protein in several animal papillomaviruses. Not similar to the E8 protein fragment of many HPVs.	Zinc transporter ZnT1, protocadherin 1 (PCDH1), and AHNAK/desmoyokin.
E8 [^] E2C; E9 [^] E2C; E8 [^] E1; E1M [^] E2C E6 [^] E7	Transcriptional repressor. (E9 [^] E2C represents the equivalent spliced product in the CRPV genome).	NCoR1 and TBLR1.

Conversely, active E2 suppresses the transcription of E6 and E7, resulting in increased expression of p53 and enhanced apoptosis in infected cells.

c) E4 Protein

The E4 protein is a cytoplasmic protein that disrupts the structural integrity of keratin. Although it is primarily cytoplasmic, it can occasionally be found in the cell nucleus and plays a role in the formation of nodules triggered by HPV-1. It may also have a role in regulating the cell cycle. The activity of the E4 protein leads to the thickening of the spinous and cornified layers of the epidermis, as well as koilocytosis. E4 protein is expressed at higher levels in cells that support viral genome amplification, and its presence in lesions suggests its involvement in disease progression. This study confirms the previously suggested importance of the E4 biomarker in diagnosing and staging the disease, expanding its application to confirm HPV causality. E4 proteins are observed during the later stages of infection, coinciding with the initiation of genome amplification. Despite diversity at the primary amino acid sequence level, E4 proteins from various papillomaviruses share a recognizable modular structure and can accumulate in high concentrations in the upper epithelial layers, where viral particles gather. Any analysis of E4 function should consider this. Additionally, it has been suggested that E4's ability to disrupt the keratin network and contribute to cornified envelope formation may facilitate viral release and/or transmission.

d) L1 and L2

L1 and L2 proteins are the capsid proteins of the virus, with L1 serving as the major protein and L2 as the minor capsid protein. L1 is composed of 360 copies arranged into 72 pentamers on a T=7 icosahedral lattice, while L2 is present in smaller amounts. The interaction between L1 and L2 is primarily hydrophobic and involves specific domains that facilitate their assembly into virus-like particles (VLPs). L2 is crucial for efficient DNA packaging and virion assembly, and it is synthesized before L1, being imported into the nucleus where L1

proteins self-assemble into pentameric capsomeres. The L1 and L2 proteins interact closely, with L2 likely incorporated into the centers of the capsomeres during assembly. This interaction is essential for the proper formation and stability of the viral capsid.

2.1.2 Classification Human of Papillomavirus

Research on the classification and evolutionary history of papillomaviruses (PVs) indicates that the diversification of new PV types from a primordial ancestor began with the emergence of hair and skin glands in early mammals over 200 million years ago¹⁰. Currently, species-specific PVs are present in most mammals, birds, and several reptiles, including turtles and snakes. In humans, more than 150 types of PVs have been completely sequenced, with an additional 200 HPV genotypes partially sequenced, and many more likely still to be identified (Figure 2.2).

Papillomaviruses are initially categorized based on their target sites of infection as cutaneous or mucosal types. They are further classified into three main genera: *Alphapapillomaviruses* (mostly mucosotropic), *Betapapillomaviruses* (mostly cutaneous), and *Gammapapillomaviruses* (which can infect both cutaneous and mucosal sites). Additionally, there are other genera, such as *Mupapillomaviruses*, which include types genetically related to papillomaviruses found in animals. Within these groups, HPV types are further classified as high-risk, probably high-risk, or low-risk based on epidemiological data about the lesions they cause, with 15 high-risk, three possibly high-risk, and 12 low-risk genotypes identified.

The L1 open reading frame (ORF) is utilized to identify new HPV types due to its high conservation among the eight ORFs in the genome. If the DNA sequences of the L1 ORFs vary by more than 10 percent from the nearest known HPV type, it is classified as a new genotype. These genotypes are numbered according to the sequence of their discovery and

characterization. Researchers have identified over 120 distinct HPV types based on the isolation and sequencing of complete genomes. Furthermore, by comparing nucleotide sequences of L1, the evolutionary relationships among papillomavirus types can be illustrated through phylogenetic trees at three taxonomic levels: genus, species, and type. A new papillomavirus is considered different from existing ones if its entire genetic code has been sequenced and the gene for the L1 protein is more than 10% different from the most similar known type. If the difference is between 2% and 10%, it's considered a subtype, and if it's less than 2%, it's considered a variant (Table 2.2).

2.1.3 Transmission of HPV

Human papillomavirus (HPV) infections are thought to initiate following injuries to the epithelial layer, which allow the virus to access basal epithelial cells and the components of the basement membrane. This process is facilitated by the presence of micro-wounds or abrasions in the skin or mucous membranes, creating opportunities for infectious virions to penetrate deeper layers. Experimental studies have confirmed that wounding enhances infection rates in preclinical models.

Once the virus breaches the epithelial barrier, it can bind to specific receptors, such as heparan sulfate proteoglycans, on the surface of basal cells. This interaction is crucial for viral entry and subsequent infection. The virus then replicates within the basal epithelial cells, and the lesions formed are maintained through the persistence of these infected cells. As the infected basal cells undergo differentiation, they contribute to the mass of the lesions, leading to the formation of warts in the upper layers of the epithelium.

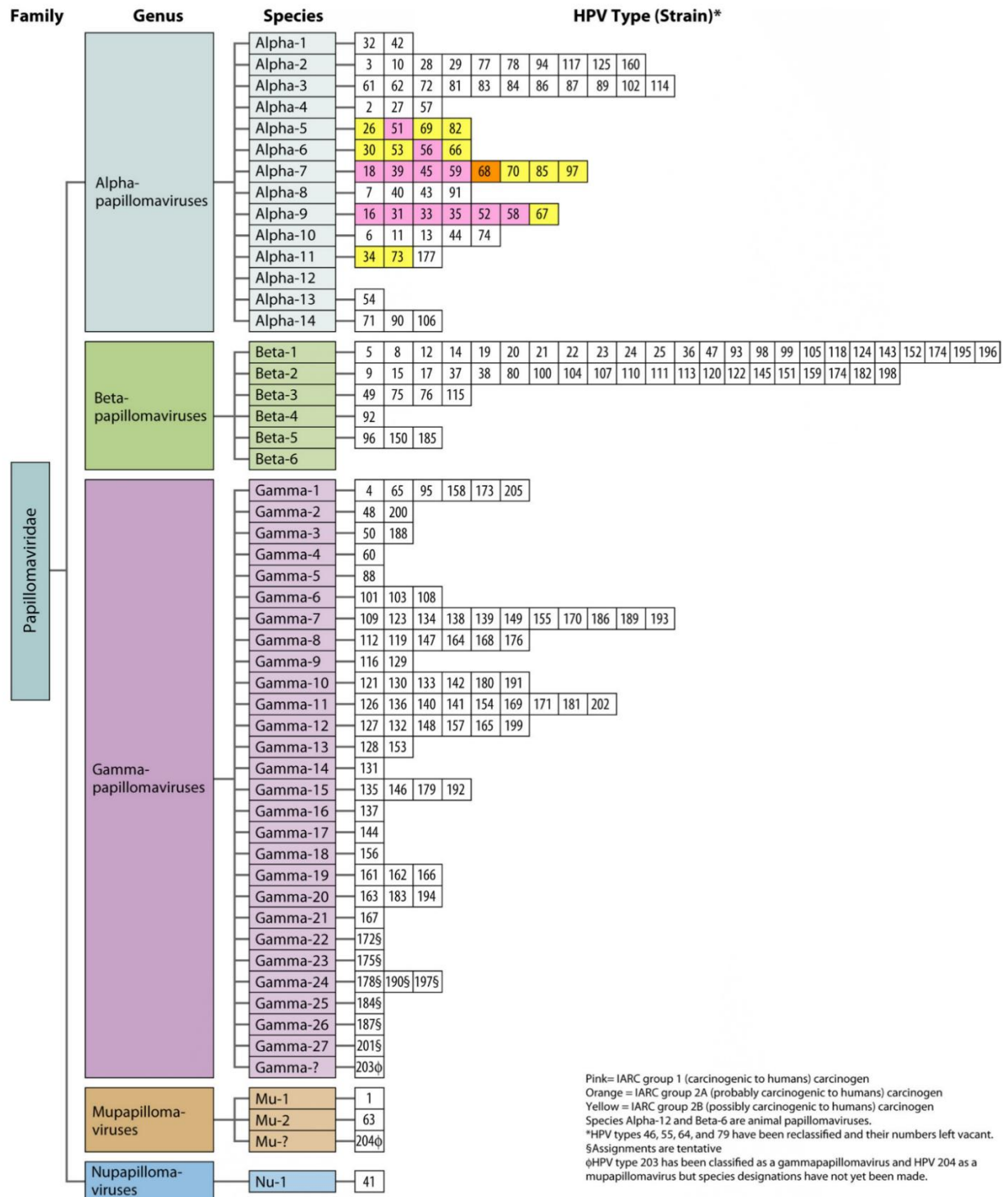


Figure 2.2: Papillomavirus Genera and Species¹¹

Table 2.2: HPV Classification Criteria¹²

Classification	Criteria
A new HPV type	<90% similarity with any known HPV strain.
Genus	>60% similarity with other strains in the same genus (e.g., α , β , γ , μ , ν).
Species	>70% similarity with other strains in the same species (e.g., $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$).
Sub-Type	90% to 98% similarity with other strains in the same sub-type.
Variants	<2% difference in similarity with other variants.

Natural transmission of cutaneous HPV infections likely occurs through direct contact, where the upper keratinized layers of warts come into contact with normal skin, generating micro-abrasions that allow virus-laden squames to be shed into the wounded areas. Environmental contact between these virus-containing shed squames and skin surface wounds also serves as a potential transmission mechanism.

In mucosal infections, mechanical wounding during sexual intercourse can facilitate the entry of the virus, particularly in vulnerable areas such as the cervix and anal canal, known as transition zones. These sites are particularly susceptible to persistent HPV infections, which can lead to malignant progression. Research suggests that virions may also access basal cells through a retro-transport mechanism, where they bind to cellular filopodia and are transported significant distances, potentially bypassing the need for direct mechanical damage.

HPV infections are primarily transmitted through close contact, with sexual contact being the most common route. However, the virus can also spread vertically, such as during passage through the cervical canal during delivery, and through autoinoculation. Research has detected HPV in placental (4.5% positivity) and cord blood samples (3.5%), indicating a potential risk for newborns to become carriers of oral HPV at birth¹³.

Horizontal transmission among family members can occur later in childhood, with the mother playing a more significant role than the father in this transmission. A mother's persistent HPV infection may increase the infant's risk of developing oral HPV, with an odds ratio of 5.7 (95% confidence interval: 1.5-21.3)¹⁴.

In addition to sexual contact, HPV can be transmitted through non-sexual means, including skin-to-skin contact, hand-to-genital contact, and contaminated objects. Autoinoculation is

also a documented route, particularly in cases involving children with genital warts who have no history of sexual abuse.

The virus is resilient and can survive on surfaces, making it possible for transmission through fomites and skin contact. Vertical transmission can occur through amniotic fluid, the placenta, or contact with maternal genital mucosa during natural birth.

2.1.4 Life Cycle of HPVs

The HPV life cycle is somehow coupled to the host cell's differentiation program, but the precise nature of this relationship remains to be elucidated (Figure 1.3).

a) Entry into Basal Epithelial Cells

The life cycle of HPV begins with the virus targeting basal epithelial cells, which are the proliferating cells located at the base of stratified epithelia, including the skin, anogenital tract, and oral cavity. These basal cells are typically exposed through minor injuries or abrasions. The initial step involves the attachment of the viral particles to heparan sulfate proteoglycans (HSPGs) on the basal membrane. This interaction facilitates the transfer of the virions to specific receptors on keratinocytes, allowing the virus to enter the cells. The entry process is crucial because it ensures that the virus targets the proliferating basal cells, which are the only cells capable of division in the epithelium.

b) Uptake and Endocytosis

Once attached, the virus undergoes endocytosis, a process where the cell membrane engulfs the virus, bringing it into the cytoplasm within a vesicle. The exact mechanisms of this uptake are not fully understood, but it is believed that the virus may exploit cellular pathways involved in wound healing to facilitate its entry.

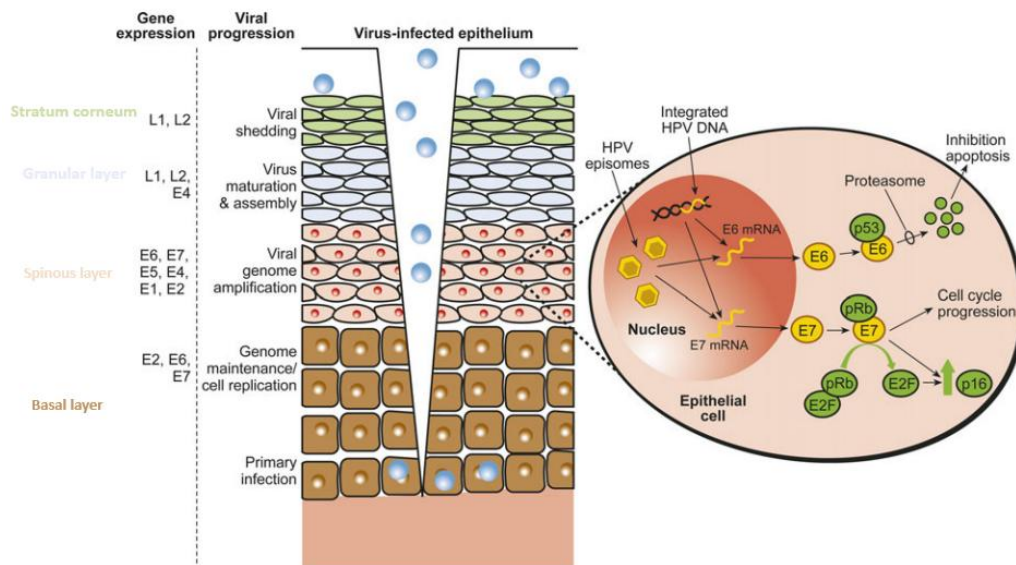


Figure 2.3: Replication cycle of HPV²

c) Transport to the Nucleus and Uncoating

After endocytosis, the viral particles are transported to the nucleus of the host cell. This transport is critical for the release of the viral DNA into the nucleus, where it can be established as an episome. Uncoating refers to the process where the viral capsid is removed, allowing the viral DNA to enter the nucleus and initiate the next steps of the life cycle.

d) E-Region Transcription

In the nucleus, the early region (E-region) of the viral genome is transcribed. This transcription is tightly regulated and occurs in the basal cells and the infected suprabasal epithelial cells. The early genes, including E1, E2, E6, and E7, are expressed, setting the stage for viral replication and manipulation of the host cell cycle.

e) Translation of E Proteins

Upon entering the nucleus, the viral DNA adopts a circular, plasmid-like form. The viral genome then begins the transcription of early genes located in the E-region. The early proteins are translated from the transcribed mRNA. These proteins play crucial roles in regulating the host cell's machinery to favor viral replication. E6 and E7 proteins interfere with the host's cell cycle control mechanisms, promoting cell proliferation and survival, which is essential for the virus's lifecycle.

f) Steady-State Viral DNA Replication

The viral DNA is maintained at a low copy number in the basal cells, replicating alongside the host's cellular DNA during cell division. This maintenance phase allows the virus to persist in the host without triggering a strong immune response.

g) Differentiation and Activation of the Productive Phase

As the infected basal cells divide, one daughter cell remains in the basal layer while the other moves upward, initiating differentiation. This differentiation triggers the productive phase of

the viral life cycle. In the terminally differentiated keratinocytes, the virus amplifies its genome significantly, producing thousands of copies per cell.

h) Vegetative Viral DNA Replication

In the differentiating keratinocytes, vegetative viral DNA replication occurs, leading to the amplification of the viral genome. This phase is characterized by a dramatic increase in the number of viral genomes, which prepares the virus for assembly and release.

i) Transcription of the L Region

Following the productive phase, the late region (L region) of the viral genome is transcribed. This region encodes the structural proteins L1 and L2, which are essential for the formation of the viral capsid.

j) Production of Capsid Proteins L1 and L2

The capsid proteins L1 and L2 are synthesized in the upper layers of the epithelium. L1 is the major capsid protein, while L2 is the minor one. Their expression is crucial for the assembly of new virion particles.

k) Assembly of Virion Particles

Once sufficient capsid proteins are produced, they assemble into virion particles. This assembly occurs in the terminally differentiated keratinocytes, where the viral DNA is encapsulated by the L1 and L2 proteins.

l) Nuclear Breakdown

As the virion assembly is completed, the host cell undergoes nuclear breakdown, allowing the newly formed virions to be released from the cell.

m) Release of Virus

Finally, the mature virions are released from the infected keratinocytes, typically through desquamation, where the cornified cells of the epithelium are shed. This release allows the virus to spread to new hosts, continuing the cycle of infection.

2.1.5 Clinical manifestations of HPV

The clinical features of Human Papillomavirus (HPV) infections exhibit significant variability, influenced by the specific HPV type, the site of infection, and the host's immune response. HPV is implicated in a spectrum of clinical manifestations, ranging from asymptomatic infections to benign lesions, and in certain instances, malignant transformations.

a) Asymptomatic Infections

A substantial proportion of HPV infections remain asymptomatic, particularly in their initial stages. It is estimated that most individuals infected with HPV do not exhibit symptoms or clinical disease, as the virus can persist in a latent state within the basal layer of the epithelium, remaining undetected for extended periods, sometimes years¹⁵. Asymptomatic infections are notably more prevalent with high-risk HPV types, such as HPV 16 and HPV 18, which are correlated with an increased risk of progression to malignancy¹⁶.

b) Cutaneous Warts

HPV is responsible for various types of warts, which are benign proliferative lesions affecting the skin and mucous membranes. Common warts (*Verruca vulgaris*) are primarily caused by HPV types 1, 2, 4, 27, and 57, presenting as small, rough lesions on the hands, fingers, and knees¹⁷. Plantar warts (*Verruca plantaris*), associated with HPV types 1, 2, and 4, typically develop on the soles of the feet and can be painful under pressure^{18,19}. Flat warts (*Verruca plana*), caused by HPV types 3, 10, 28, and 49, are smaller and smoother, often appearing on

the face and neck^{20,21}. Filiform warts, characterized by long, narrow projections, are commonly found on the face and neck^{22,23}.

c) Genital Warts

Genital warts, or condylomata acuminata, are among the most recognized manifestations of HPV, primarily caused by low-risk HPV types 6 and 11^{24,25}. These warts typically present as soft, flesh-colored, or slightly pigmented growths in the genital and perianal regions, varying in size and morphology. Although generally benign, genital warts can lead to significant discomfort, itching, and psychosocial distress²⁶.

d) Respiratory Papillomatosis

Respiratory papillomatosis, a rare condition characterized by the proliferation of HPV-induced papillomas in the respiratory tract, is most commonly associated with HPV types 6 and 11²⁷. This condition can manifest in both juvenile-onset and adult-onset forms, with clinical features including hoarseness, chronic cough, and respiratory distress due to airway obstruction. Severe cases may necessitate repeated surgical interventions to manage obstructive papillomas²⁸.

e) Cervical Intraepithelial Neoplasia (CIN)

Cervical intraepithelial neoplasia (CIN) represents premalignant changes in the cervical epithelium due to persistent infection with high-risk HPV types, particularly HPV 16 and 18²⁹. CIN is categorized into three grades based on the severity of epithelial dysplasia: CIN 1 (low-grade), CIN 2 (moderate-grade), and CIN 3 (high-grade), with the latter having the highest risk of progression to invasive cervical cancer²⁹. While many cases of CIN are asymptomatic, advanced stages may present with abnormal vaginal bleeding, discharge, and pelvic pain³⁰.

f) Cervical Cancer

Cervical cancer is a type of cancer that originates in the cervix, the lower part of the uterus that connects to the vagina. It typically develops slowly over time, often starting with

precancerous changes in the cervical cells known as dysplasia. If these abnormal cells are not detected and treated, they can progress to cancer. Persistent infection with high-risk HPV types, especially HPV 16 and 18, is the leading cause of cervical cancer³¹. The progression from CIN to cervical cancer can span years to decades, with squamous cell carcinoma being the most prevalent type, followed by adenocarcinoma. Early clinical features of cervical cancer may include postcoital bleeding, intermenstrual bleeding, and foul-smelling vaginal discharge, while advanced disease may present with pelvic pain, weight loss, and urinary or rectal symptoms³².

g) Other HPV-associated Malignancies

High-risk HPV types are also linked to several other malignancies, including oropharyngeal cancer, anal cancer, penile cancer, and vulvar and vaginal cancers³³. HPV 16 is a significant contributor to oropharyngeal cancers, with symptoms such as persistent sore throat and difficulty swallowing. Anal cancer is primarily associated with HPV 16, presenting with rectal bleeding and pain. Similarly, HPV types 16 and 18 are implicated in penile cancer, which may manifest as growths or sores on the penis. Vulvar and vaginal cancers, although less common, can present with persistent itching, pain, or ulceration.

h) Cutaneous Squamous Cell Carcinoma

Beta HPV types have been associated with the development of cutaneous squamous cell carcinoma (cSCC), particularly in immunocompromised individuals, such as organ transplant recipients³⁴. These carcinomas typically arise on sun-exposed skin and may present as non-healing ulcers, scaly patches, or nodules.

i) HPV-related Complications in Immunocompromised Individuals

Individuals with compromised immune systems, such as those with HIV or those undergoing immunosuppressive therapy, face a heightened risk of developing HPV-related complications.

These patients may experience more aggressive and recurrent warts, a higher incidence of high-grade CIN, and an increased risk of HPV-associated cancers.

2.2 Diagnosis of HPV

The natural progression of cervical cancer is well documented, typically featuring a detectable preinvasive stage that can be identified through screening. In contrast, this is not true for HPV-associated oropharyngeal cancer (HPV-AOC). As a result, routine screening for HPV-AOC is not conducted, and patients often come forward with clinical symptoms, usually at an advanced stage of the disease. Because HPV has specific tissue and species requirements, its complete viral cycle and infectivity are confined to human epithelial tissues, making it impossible to culture *in vitro*. As a result, the detection of HPV has mainly depended on serological, histological, and molecular biological techniques^{35,36}.

2.2.1 Clinical Examination and Colposcopy

Clinical examination alone is insufficiently sensitive for detecting latent or subclinical HPV infections. Colposcopy, particularly when combined with acetic acid application, enhances the visualization of HPV-induced cervical lesions. However, colposcopy is both time-consuming and costly³⁷. Even colposcopy-directed biopsies may miss high-grade lesions in up to 3% of women with abnormal cytological findings³⁸. Therefore, while colposcopy plays a role in the evaluation of abnormal smears, it is not an optimal primary screening tool.

2.2.2 Conventional and Liquid-Based Cytology

The Papanicolaou (Pap) smear remains the cornerstone of cervical cancer screening. Conventional cytology identifies cellular abnormalities suggestive of HPV infection, yet its sensitivity can vary considerably—from 29% to 80%—necessitating repeated testing³⁹. Liquid-based cytology (LBC) has improved detection rates, particularly for high-grade squamous intraepithelial lesions (HSIL), with reported sensitivities around 80%⁴⁰.

2.2.3 Microscopy and Histopathology

Microscopic evaluation for koilocytosis—characterized by perinuclear clearing, nuclear atypia, hyperchromasia, and occasional double nucleation—provides evidence of productive HPV infection. However, the subtlety and variability of these changes lead to reduced inter- and intraobserver reproducibility compared with molecular methods. Moreover, the sensitivity of microscopic detection is lower than that of nucleic acid–based assays, limiting its role to an adjunctive diagnostic modality.

2.2.4 Direct Nucleic Acid Detection

Early direct hybridization techniques, including Southern blot and dot blot assays, once considered the gold standard for HPV research, have largely been supplanted by more sensitive molecular methods. Direct hybridization is labor-intensive, requires large amounts of sample DNA, and suffers from issues of interlaboratory variability⁴¹.

2.2.5 Signal Amplification Assays: The Hybrid Capture System

Signal amplification methods such as the Hybrid Capture II (HC-2) assay have revolutionized HPV detection. HC-2 employs a cocktail of RNA probes to detect a spectrum of high-risk (and low-risk) HPV types, offering high sensitivity (approximately 90% for high-grade lesions) and reproducibility with an intra-assay variation below 5%. Although HC-2 does not offer genotyping, its user-friendly format, resistance to interfering substances, and capability to process exfoliated cervical cells make it a mainstay in clinical laboratories. Future iterations, such as the third-generation HC-3 system, promise improved specificity by incorporating biotin-labeled oligonucleotide probes and blocker probes to minimize background noise.

2.2.6 Polymerase Chain Reaction (PCR) and Genomic Amplification

PCR has become the gold standard for HPV research, offering remarkable sensitivity and specificity by amplifying even minute quantities of viral DNA. Consensus PCR methods—using primer sets such as GP5+/GP6+, MY09/MY11, PGMY09/PGMY11, and SPF1/SPF2—enable the detection of a broad spectrum of HPV genotypes, including novel and variant strains. Although PCR-based techniques may be more prone to contamination and require strict quality control measures, they also allow for subsequent variant analysis and quantitative viral load evaluation. Real-time PCR methods not only quantify HPV-DNA but also assess the ratio of episomal to integrated viral genomes—a parameter that may correlate with lesion grade and oncogenic potential⁴².

2.2.7 Detection of Integrated HPV Genome and Viral Load Evaluation

The integration of HPV DNA into the host genome is a critical event in cervical carcinogenesis. Techniques such as Southern blotting and real-time PCR assays (targeting the E2 and E6 genes) can help distinguish between episomal and integrated viral states. Moreover, quantitative real-time PCR allows for the estimation of viral load, providing insights into disease progression. However, accurate viral load measurement must account for cellular content to avoid sampling bias.

2.2.8 *In Situ* Hybridization (ISH)

ISH techniques offer the advantage of correlating the presence of HPV-DNA with histopathological changes directly on tissue sections. Although conventional ISH methods may lack sensitivity—detecting only when more than 25 copies of HPV-DNA are present per cell—advances in PCR in situ hybridization are showing promise in detecting lower viral loads.

2.2.9. HPV Variant Analysis

The intratypic diversity among HPV isolates can be significant. Variant analysis—using methods such as single-strand conformation polymorphism (SSCP) or direct PCR sequencing—can elucidate differences in oncogenic potential. Variants of HPV-16, have been shown to influence immune recognition and may confer a higher risk for progression to high-grade lesions.

2.2.10. HPV Serology

Serological assays based on virus-like particles (VLPs) detect antibodies against HPV capsid proteins, offering insight into both current and past infections. Although serology is useful for epidemiological studies and vaccine trials, its sensitivity is generally lower (approximately 50–60%) than that of nucleic acid-based methods, and seroconversion may be delayed relative to DNA detection. Additionally, crossreactivity between certain HPV types (notably HPV-6 and HPV-11) can complicate type-specific interpretations.

2.3 Epidemiology of Oral HPV Infections

Human papillomavirus (HPV) infection is an established etiological factor in a subset of oropharyngeal carcinomas. However, epidemiological data regarding oral HPV infection remains limited, although research in this area is emerging. The more extensively researched field of genital HPV infection provides a valuable framework for understanding the dynamics of oral HPV infection.

a) Global Trends

Oral HPV infections are a growing public health concern, with an estimated global prevalence of 7.5% in adults⁴³. Although the global prevalence of oral HPV infections varies widely, with studies indicating rates from approximately 2.4% to 7.5% in different populations. A study by Giuliano and colleagues reported a prevalence of 7.0% for any oral

HPV among a healthy adult population in the U.S., with higher rates observed in older adults⁴⁴. This increase in prevalence with age may be attributed to cumulative exposure to HPV, potential loss of immune control, and reactivation of latent infections⁴⁴. Additionally, D'Souza found that oncogenic oral HPV infections often precede the diagnosis of oropharyngeal cancer (OPC) by several years, emphasizing the importance of early detection⁴⁵. Moreover, the prevalence of oral HPV is notably higher among men, particularly those who engage in high-risk sexual behaviors. A study by Mistry highlighted that men who have sex with men (MSM) exhibit significantly higher rates of oral HPV, primarily due to practices such as oral sex and rimming⁴⁶. This aligns with findings of Bettampadi, which indicated that clinical periodontitis may facilitate the acquisition and persistence of oral HPV infections, further complicating the epidemiological landscape⁴⁷.

In high-income countries, HPV-driven oropharyngeal cancers (OPCs) now surpass cervical cancers in some regions, with HPV-16 accounting for 90% of HPV+ OPCs^{43,48}. The U.S. NHANES study reported a 6.9% oral HPV prevalence, with high-risk HPV-16 detected in 3.8% of adults⁴³. In contrast, Asian countries like Japan report lower rates (~2.4%) due to differences in sexual behaviors and vaccine coverage. Notably, HPV vaccination programs in countries like Australia have reduced cervical HPV prevalence by 77%, but oral HPV data remain limited^{43,48}.

b) Regional Disparities in Sub-Saharan Africa and Nigeria

Sub-Saharan Africa (SSA) bears the highest cervical cancer burden globally, yet oral HPV data are sparse. A 2023 Nigerian study using next-generation sequencing (NGS) identified 44 HPV types in cervical samples, with high-risk HPV-16 and HPV-82 prevalent in 16% and 15% of cases, respectively⁴⁹. However, oral HPV prevalence in Nigeria is understudied. A 2023 meta-analysis reported a pooled HPV prevalence of 32% in Nigerian women, but

regional disparities were stark: South South Nigeria had the lowest prevalence (4.9%) compared to 71% in the South East⁵⁰. This gap highlights the need for region-specific studies, particularly in South South Nigeria, where socio-cultural practices and healthcare access differ^{50,51}.

In Nigeria, the trends in oral HPV prevalence reflect a complex interplay of socio-cultural factors, sexual behaviors, and health conditions. Recent studies have reported varying prevalence rates of oral HPV among different populations. A study conducted in Ibadan found that the prevalence of oral HPV infections was significantly higher among female sex workers compared to the general population, indicating that high-risk sexual behaviors contribute to increased HPV transmission⁵². Additionally, a study by Faneye and colleagues reported a prevalence of 13.2% among dental clinic attendees, highlighting the importance of oral health in the context of HPV infections⁵³. The high prevalence of HPV among HIV-positive individuals in Nigeria is particularly concerning. A study indicated that the prevalence of oral HPV was significantly higher in HIV-positive women compared to their HIV-negative counterparts, with rates of 44.9% and 11.2%, respectively⁵⁴.

2.4 Oral HPV and Head and Neck Cancers

Human papillomavirus (HPV) is a significant etiological agent in head and neck cancers (HNCs), particularly oropharyngeal squamous cell carcinoma (OPSCC). Oropharyngeal squamous cell carcinoma (OPSCC) is a type of head and neck cancer that arises in the oropharynx, which includes the base of the tongue, tonsils, soft palate, and the walls of the pharynx. It is a subset of squamous cell carcinoma (SCC), which refers to malignancies originating from the squamous epithelial cells lining these tissues⁵⁵. OPSCC typically manifests with symptoms such as persistent sore throat, difficulty swallowing (dysphagia), ear pain, voice changes, and, in advanced cases, a visible lump in the neck due to metastatic lymph node involvement. Unlike traditional OPSCC cases linked to heavy tobacco and

alcohol use, a significant proportion of modern OPSCC cases are driven by persistent infection with high-risk human papillomavirus (HPV), particularly HPV-16⁵⁶. HPV-associated HNCs now surpass cervical cancer as the most common HPV-related malignancy in high-income countries, with HPV-16 accounting for 80–90% of cases^{57,58}. Oral HPV infections are sexually transmitted through oral-genital or oral-anal contact, with risk factors including multiple sexual partners, early sexual debut, and lack of HPV vaccination^{56,59}. Unlike cervical HPV infections, oral HPV infections are often transient, but persistent high-risk HPV (HR-HPV) can lead to malignant transformation, particularly in the tonsillar crypts and base of tongue, which have single-layered epithelia prone to viral entry^{59,60}.

Globally, HPV is detected in 10% of oral cavity squamous cell carcinomas (OSCCs) and 42% of OPSCCs, with significant regional variation. Japan reports the highest HPV prevalence in OSCC (20–30%), while Finland and Sweden lead in OPSCC (up to 89% HPV-16 positivity)⁵⁷. The rising incidence of HPV+ OPSCC is linked to changing sexual behaviors and insufficient vaccination coverage in males^{56,58}. Clinically, HPV+ HNCs present with smaller primary tumors but advanced nodal metastases, often diagnosed through p16 immunohistochemistry (IHC) or HPV DNA/RNA testing^{58,61}.

Emerging non-invasive biomarkers, such as saliva-based HPV DNA detection and circulating tumor HPV DNA, are under investigation for early diagnosis^{59,61}. However, challenges remain in differentiating HPV-driven tumors from HPV-passenger infections, necessitating dual testing for HPV DNA and E6/E7 mRNA to confirm transcriptional activity^{58,62}.

2.5 Role of HPV in Oropharyngeal and Head and Neck Cancers

HPV-positive OPSCC has distinct clinical and molecular characteristics compared to HPV-negative OPSCC. The role of HPV in the pathogenesis of oropharyngeal and head and neck cancers is multifaceted, involving complex interactions between viral oncogenes and host

cellular mechanisms. HPV oncoproteins E6 and E7 drive carcinogenesis by degrading tumor suppressors p53 and retinoblastoma (Rb), respectively, leading to uncontrolled cell proliferation, genomic instability, and immune evasion^{58,59}. HPV integration into the host genome occurs in 50–70% of cases, often disrupting the E2 gene and dysregulating E6/E7 expression⁵⁸. Episomal HPV may also contribute to carcinogenesis through epigenetic modifications, such as promoter hypermethylation of tumor suppressor genes (e.g., *RASSF1A*, *PITX2*)^{58,61}. Recent studies have elucidated the epigenetic modifications induced by HPV that contribute to its oncogenic potential. Modifications in DNA and histone have been implicated in the regulation of viral gene expression and the host cellular environment^{63,64}. Furthermore, the immune response to HPV-infected cells is also a crucial factor; HPV-positive tumors often exhibit a unique immune profile, which may influence treatment responses and overall survival^{64–66}.

HPV+ OPSCC patients exhibit superior survival rates (80–90% 5-year survival) compared to HPV-negative counterparts (40–60%), attributed to enhanced radiation sensitivity and robust immune responses^{56,58}. This contrasts sharply with HPV-negative cancers, which are frequently linked to traditional risk factors such as tobacco and alcohol use⁶⁷. The 8th edition AJCC staging system now classifies HPV+ OPSCC separately, reflecting its unique prognosis⁵⁶. However, treatment de-escalation trials (e.g., reduced radiation doses or cisplatin substitution) have shown mixed results, underscoring the need for risk stratification biomarkers⁵⁸. Studies have shown that tumors with intact E2 or low PD-L1 expression may resist de-intensified therapies⁵⁸.

The tumor microenvironment in HPV+ OPSCC is characterized by immune cell infiltration (e.g., CD8+ T cells) and elevated PD-L1 expression, making immunotherapy a promising adjunct. Pembrolizumab and nivolumab are FDA-approved for recurrent/metastatic HNSCC, with ongoing trials exploring therapeutic HPV vaccines targeting E6/E7^{56,58}.

2.6 Comparison of HPV-Associated Cancers in Different Regions

The global landscape of human papillomavirus (HPV)-associated cancers reveals significant regional variations in prevalence, types, and associated risk factors. HPV is a well-established causative agent of cervical cancer, but its role extends to various other malignancies, including oropharyngeal and anal cancers. The prevalence of HPV infection varies widely across continents, with studies indicating that regions in Africa and Asia report higher rates compared to Europe and North America. A recent systematic review highlighted that HPV prevalence is notably high in Asia and Africa, with the highest rates of HPV-associated cancers observed in these regions⁶⁸.

In high-income countries, the introduction of HPV vaccination programs has led to a decline in cervical cancer rates, while HPV-related oropharyngeal cancers have seen an increase, particularly among men⁶⁹. Conversely, in low- and middle-income countries, the burden of HPV-related cancers remains significant, exacerbated by limited access to healthcare and vaccination. The World Health Organization (WHO) reports that HPV is responsible for approximately 14.2% of all cancers in sub-Saharan Africa, underscoring the critical need for enhanced prevention and screening strategies in these regions⁷⁰.

The epidemiological data suggest that while HPV-16 and HPV-18 are the most common high-risk types globally, other types such as HPV-35 and HPV-45 show higher prevalence in specific regions, particularly in sub-Saharan Africa⁷¹. This regional heterogeneity in HPV type distribution has implications for vaccine development and cancer prevention strategies, emphasizing the need for tailored approaches based on local epidemiological data.

a) HPV-Associated Cancers in Africa

In Africa, the burden of HPV-associated cancers is particularly pronounced, with cervical cancer being the most common malignancy among women. Studies indicate that the

prevalence of HPV in African women ranges from 7% to 60%, with significant regional variations influenced by factors such as sexual behavior, access to healthcare, and the presence of co-factors like HIV. A meta-analysis revealed that HPV-35 is among the top five high-risk HPV genotypes in women with invasive cervical cancer in Africa, a trend not observed in other continents.

The high prevalence of HPV in sub-Saharan Africa is compounded by the high rates of HIV co-infection, which significantly increases the risk of developing cervical cancer. HIV-positive women are more likely to harbor multiple HPV infections and are at a greater risk of persistent infections with high-risk HPV types^{70,71}. This relationship highlights the critical intersection of HPV and HIV in cancer epidemiology in Africa, necessitating integrated approaches for prevention and treatment.

Moreover, the lack of effective screening programs and vaccination coverage in many African countries contributes to the high incidence of cervical cancer. Studies in South Africa have shown that HPV prevalence among women without cytological abnormalities is alarmingly high, indicating a need for improved screening and vaccination efforts. The introduction of HPV vaccination programs has shown promise in reducing cervical cancer rates, but disparities in access and awareness remain significant.

b) HPV-Associated Cancers in Nigeria

In Nigeria, the prevalence of HPV and its association with cervical cancer is a pressing public health concern. Research indicates that the age-specific prevalence of HPV infection is highest among younger women, with a notable decline as age increases⁵⁰. This pattern reflects broader sexual behavior trends and highlights the importance of targeted interventions for younger populations.

A study conducted in Nigeria found that HPV-16 and HPV-18 are the most prevalent high-risk HPV types among women with cervical lesions, consistent with findings from other regions⁷². However, the presence of other high-risk types, such as HPV-35, is also significant, contributing to the overall burden of HPV-associated cancers in the country⁷². The high prevalence of HPV in Nigeria is further complicated by the high rates of HIV infection, which exacerbate the risk of cervical cancer among women^{70,71}.

Despite the high burden of HPV-associated cancers, Nigeria faces challenges in implementing effective screening and vaccination programs. The lack of awareness and access to healthcare services significantly hampers efforts to reduce the incidence of cervical cancer^{50,72}. Furthermore, cultural factors and stigma associated with sexual health can impede the uptake of HPV vaccination and screening services, underscoring the need for comprehensive public health strategies that address these barriers⁷³.

The contrast between high-income countries (HICs) and low and medium-income countries (LMICs) highlights urgent priorities for global health equity. In HICs, declining cervical cancer rates reflect successful vaccination programs (e.g., Australia's 90% coverage) and advanced diagnostics. Conversely, LMICs like Nigeria are making progress through recent initiatives, such as the October 2023 introduction of HPV vaccination for girls aged 9–14 into the national immunization program, with phased rollouts across 16 states aiming for nationwide coverage⁷⁴. Despite this critical step, scalable solutions remain essential to address persistent gaps—including expanding single-dose HPV vaccine regimens and integrating low-cost screening methods like visual inspection with acetic acid (VIA) in underserved regions. Regional research must also address rising non-cervical HPV cancers, increasingly relevant as survival rates improve for HIV-positive populations⁷⁵. By 2030, targeted interventions in SSA could avert 1.4 million cervical cancer deaths, aligning with the WHO's elimination targets⁷⁶. Bridging these disparities demands not only funding but also

culturally sensitive strategies to overcome stigma and misinformation, particularly in regions where HPV remains shrouded in taboo.

2.7 The Oral Microbiome

The oral microbiome refers to the diverse community of microorganisms residing in the oral cavity, including bacteria, viruses, fungi, and protozoa^{77,78}. Historically, the study of oral microbes began with Antony van Leeuwenhoek's observations of "animalcules" in dental plaque, but modern advancements in sequencing technologies, such as 16S rRNA profiling and metagenomics, have revolutionized our understanding of its diversity and function⁷⁹. This complex ecosystem plays a crucial role in maintaining oral health and influencing systemic health. The oral cavity is one of the most densely populated microbial habitats in the human body, with estimates suggesting that it harbors over 700 distinct microbial species, although only a fraction of these have been cultured and characterized^{77,80}. The composition of the oral microbiome is influenced by various factors, including age, diet, oral hygiene practices, and the presence of systemic diseases^{81,82}.

The oral microbiome can be divided into several niches, including the tongue, buccal mucosa, teeth, and saliva, each hosting distinct microbial communities^{77,79}. The dynamic interactions among these microbial populations and their host contribute to a balanced oral environment. However, disruptions to this balance, often referred to as dysbiosis, can lead to oral diseases such as dental caries, periodontal disease, and even systemic conditions like cardiovascular disease and diabetes^{82,83}. Recent studies have highlighted the importance of the oral microbiome in the pathogenesis of various diseases, including its role in the development of oral squamous cell carcinoma (OSCC) and its association with human papillomavirus (HPV) infections^{82,84}.

The oral microbiome's composition is not static; it evolves throughout life, influenced by factors such as diet, lifestyle, and environmental exposures^{81,85}. The transition from a milk-based diet to a more diverse diet in early childhood is associated with significant changes in the oral microbiome⁸⁵. Furthermore, the use of antibiotics and other medications can alter microbial diversity, potentially leading to opportunistic infections or the overgrowth of pathogenic species⁸². Understanding the oral microbiome's composition and function is essential for developing strategies to prevent and manage oral diseases and their systemic implications^{78,82}.

Non-bacterial members of the oral microbiome including fungi like *Candida* and archaea such as *Methanobrevibacter*, contribute to this ecosystem's metabolic and immunological interactions^{79,82}. Viruses, particularly bacteriophages, further modulate microbial dynamics through predation and horizontal gene transfer⁸². A key feature of the oral microbiome is its biogeography—spatial organization shaped by microenvironmental gradients in pH, oxygen levels, and nutrient availability. This explains why anaerobic taxa like *Porphyromonas gingivalis* dominate subgingival crevices, while oxygen-tolerant *Streptococcus* species prevail on the tongue dorsum^{79,85}.

The oral microbiome's resilience—its ability to resist and recover from perturbations like antibiotic use—stems from functional redundancy, where phylogenetically distinct taxa perform overlapping metabolic roles. It has been shown that nitrate reduction, critical for cardiovascular health, is mediated by *Actinomyces*, *Rothia*, and *Neisseria*⁷⁹. Dysbiosis, or microbial imbalance, disrupts this equilibrium, fostering pathologies such as periodontitis and dental caries while exacerbating systemic diseases like cardiovascular disorders and diabetes⁷⁹. Recent studies highlight the role of candidate phyla radiation (CPR) bacteria, such as *Saccharibacteria* (TM7), which exhibit parasitic lifestyles dependent on host bacteria, influencing biofilm stability and inflammatory responses^{79,85}.

Microorganisms can inhabit both living and nonliving surfaces in the oral cavity, such as teeth, gums, dental implants, and restorative materials. Over time, these microbes aggregate into biofilms—structured microbial communities embedded within a matrix of extracellular polymeric substances⁸⁶. This matrix shields the microbes from threats like antimicrobial treatments, environmental challenges, and the host's immune defenses. While bacteria are the main constituents of biofilms, fungi, viruses, and archaea may also be present. Under normal conditions, oral biofilms contribute to a balanced microbiome, promoting oral health by preventing the overgrowth of harmful pathogens. However, dysbiosis (microbial imbalance) can shift the biofilm composition toward dominance by disease-causing bacteria, triggering chronic inflammation and compromising the biofilm's beneficial functions. Such pathogenic biofilms are central to the development of oral conditions like periodontal disease, tooth decay, and oral infections.

Technological innovations, including single-cell genomics and multi-omics integration, are unraveling the oral microbiome's functional potential. Metatranscriptomics reveals active metabolic pathways in caries-associated biofilms, such as upregulated sugar metabolism in *Streptococcus mutans*, while metaproteomics identifies biomarkers like arginine deiminase in health-associated communities^{82,85}. These advances underscore the microbiome's dual role as a mediator of disease and a reservoir for therapeutic targets.

2.7.1 Microbial Communities in the Oral Cavity

The oral cavity hosts a mosaic of microbial communities, each adapted to specific anatomical niches. These include the supragingival plaque, subgingival crevice, tongue dorsum, buccal mucosa, and saliva, each exhibiting unique taxonomic and functional profiles. The Human Microbiome Project (HMP) identified Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Fusobacteria, and Spirochaetes as the dominant bacterial phyla, collectively constituting 80–99.9% of oral taxa⁷⁹. At the genus

level, *Streptococcus*, *Veillonella*, *Prevotella*, *Fusobacterium*, and *Neisseria* are prevalent, though their abundances vary across sites⁸⁵.

Biofilms, the structural backbone of these communities, are organized into spatially stratified consortia. Early colonizers like *Streptococcus sanguinis* adhere to enamel pellicle proteins, facilitating subsequent adhesion of late colonizers such as *Porphyromonas gingivalis* through coaggregation^{79,85}. These interactions are mediated by adhesins, extracellular polysaccharides, and quorum-sensing molecules, creating a scaffold for polymicrobial synergy or competition. *Fusobacterium nucleatum* for instance acts as a bridging species, binding both early and late colonizers via its Fap2 lectin⁸⁵.

Saliva serves as a critical medium for microbial transmission and nutrient exchange. It contains antimicrobial peptides (e.g., defensins) and immunoglobulin A (IgA), which shape community composition by suppressing pathogens while nurturing symbionts⁷⁹. Despite this regulatory role, saliva's enzymatic activity, such as amylase-mediated starch breakdown, supplies fermentable carbohydrates that fuel acidogenic taxa like *S. mutans*, linking dietary habits to dysbiosis⁸².

The concept of a “core microbiome” highlights taxa conserved across individuals, such as *Streptococcus mitis* and *Rothia mucilaginosa*, which contribute to baseline metabolic functions like nitrate reduction and hydrogen peroxide production⁷⁹. In contrast, the “variable microbiome” reflects host-specific factors, including genetics, diet, and hygiene practices. Smoking, has been shown to enrich *Prevotella* and reduces *Neisseria*, altering pH and redox potential^{82,85}. Emerging evidence also implicates host immune responses, such as interleukin-1 β secretion, in shaping microbial landscapes by promoting inflammation-tolerant taxa⁷⁹.

Spatial mapping using fluorescence *in situ* hybridization (FISH) and Raman spectroscopy has revealed microniches within biofilms, such as oxygen-depleted zones favoring *Treponema*

denticola in periodontal pockets⁸⁵. These techniques, combined with metabolomics, illustrate how localized metabolite exchange (e.g., lactate cross-feeding between *Streptococcus* and *Veillonella*) sustains community stability^{79,85}.

In individuals with human papillomavirus (HPV) infection, emerging evidence indicates that the oral microbial community undergoes measurable alterations. Studies employing high-resolution sequencing have observed shifts in beta diversity—that is, differences in community composition between individuals—when comparing HPV-positive with HPV-negative subjects. Such compositional changes include not only a reordering of the relative abundance of dominant phyla but also an enrichment or depletion of specific functional pathways, like those related to immune modulation and inflammatory responses. These differences may be critical because the altered microbial landscape can affect the local immune environment, potentially influencing HPV acquisition, persistence, and even the early stages of viral carcinogenesis⁸⁷.

Technical advances over the past few years have allowed researchers to probe deeper into the community-level interactions that characterize the oral cavity. Novel bioinformatic tools now enable the reconstruction of microbial networks and the prediction of metabolic interactions between species, thus providing insights into how interbacterial communication may regulate biofilm homeostasis. Network analyses have revealed that cooperative interactions among certain commensals enhance resistance against pathogens, while disruption of these networks—often through poor oral hygiene or environmental stress—can predispose to dysbiosis. This dysbiotic state not only increases susceptibility to oral diseases such as periodontitis and dental caries but may also contribute to a permissive environment for HPV persistence by modifying epithelial barrier function and local cytokine profiles⁴⁸.

2.7.2 Key Bacterial Species and Their Functions

Within the diverse oral microbiome, a subset of bacterial species plays pivotal roles in maintaining both health and disease states. Among the key players, several genera have emerged as particularly influential due to their metabolic functions, interactions with host tissues, and potential to modulate immune responses. Certain *Streptococcus* species (including *S. mitis* and *S. sanguinis*) act as early colonizers that not only establish the initial biofilm but also produce enzymes and signaling molecules that facilitate the attachment of later colonizers. Their production of extracellular polysaccharides is critical for the structural integrity of dental plaque, while secreted metabolic by-products can alter the local pH, thereby influencing the growth of aciduric bacteria implicated in dental caries⁸⁸.

Fusobacterium nucleatum, another frequently encountered species in the oral cavity, has garnered significant attention in recent studies due to its dual role as both a commensal and a potential pathobiont. As a Gram-negative, anaerobic bacterium, *F. nucleatum* is known for its strong coaggregation abilities, which allow it to serve as a “bridge” organism linking early and late colonizers in the biofilm. Its cell-surface adhesins facilitate binding to epithelial cells and other bacterial species, and its metabolic activity leads to the production of pro-inflammatory mediators. These properties are particularly significant in the context of HPV-related carcinogenesis, as chronic inflammation is a known risk factor for malignant transformation in the oropharynx⁸⁷. Recent evidence suggests that an increased abundance of *F. nucleatum* may promote an inflammatory microenvironment that, in concert with HPV infection, could predispose to neoplastic progression⁴⁸.

Prevotella species, such as *Prevotella melaninogenica*, are also notable for their role in the oral ecosystem. These Gram-negative anaerobes contribute to the degradation of complex carbohydrates and the production of short-chain fatty acids, which can modulate local immune responses. In HPV-positive individuals, shifts in the relative abundance of

Prevotella have been observed, suggesting that these bacteria may influence viral persistence through the modulation of epithelial barrier function and cytokine secretion. Their ability to ferment dietary substrates and produce metabolic end-products has implications for both oral health and systemic conditions, reinforcing the concept that metabolic cross-talk between microbes and the host is a critical determinant of disease risk⁸⁹.

Actinomyces species are another important group in the oral microbiome, known for their filamentous growth and role in the initial colonization of the dental surface. These Gram-positive bacteria are involved in the formation of complex multispecies biofilms and are capable of producing extracellular polymeric substances that contribute to plaque stability. Recent investigations have highlighted that certain *Actinomyces* may interact synergistically with HPV, possibly by influencing local immune surveillance or by creating micro-niches that favor viral persistence. Their metabolic versatility—ranging from carbohydrate fermentation to the synthesis of bioactive peptides—positions them as key modulators of the oral ecosystem⁸⁸.

Other taxa, such as *Veillonella*, contribute by utilizing lactate produced by *Streptococcus* species, thereby helping to regulate the pH of the biofilm. This interspecies metabolic cooperation is essential in preventing the excessive acidification of the oral cavity, which is a risk factor for both dental caries and inflammatory conditions. The delicate balance maintained by these microbial interactions is often disrupted in dysbiotic states, which may then have downstream effects on HPV infection dynamics. In the context of HPV, a disrupted balance among these key bacterial species might influence the epithelial cell turnover and local cytokine milieu, thereby altering the susceptibility of the mucosa to viral infection and subsequent transformation⁴⁸.

2.8 Impact of HPV on the Oral Microbiome

Human Papillomavirus (HPV) has been increasingly recognized for its role not only in cervical cancer but also in various head and neck cancers, particularly oral squamous cell carcinoma (OSCC). Recent studies have highlighted the intricate relationship between HPV infection and the oral microbiome, suggesting that HPV may induce significant alterations in microbial composition, which in turn could influence disease progression and immune responses. The oral cavity, as a complex ecosystem, hosts a diverse range of microorganisms that interact with each other and with the host, playing crucial roles in maintaining oral health and preventing disease.

Research indicates that HPV-positive individuals exhibit distinct microbial profiles compared to their HPV-negative counterparts. Studies have shown that the presence of HPV is associated with an increase in pathogenic bacteria such as *Fusobacterium nucleatum*, which is known for its role in periodontal disease and has been implicated in the progression of OSCC^{90,91}. Notably, increased abundance of *Fusobacterium* species has been linked to HPV-related cancers across multiple sites, including the oral cavity, vagina, and anus⁹²⁻⁹⁴. Furthermore, the dysbiosis observed in HPV-positive individuals may contribute to an inflammatory microenvironment conducive to cancer development⁹¹. The alteration of the oral microbiome in response to HPV infection can be attributed to several factors, including changes in local immune responses and the production of viral proteins that may modulate microbial interactions and the formation of pathogenic biofilms that shield HPV from immune surveillance^{95,96}.

The mechanisms by which HPV alters the oral microbiome are multifaceted. HPV can influence the host's immune system, leading to a shift in microbial composition. The viral infection may induce a state of chronic inflammation, which can disrupt the balance of the oral microbiome, favoring the growth of pathogenic species while suppressing beneficial

ones^{97,98}. Chronic inflammation driven by pro-inflammatory bacteria like *Prevotella* spp., *Porphyromonas* spp., and *Fusobacterium* spp. leads to the release of cytokines (IL-1, IL-6, TNF- α , IFN- γ), activating signaling pathways that generate reactive oxygen species (ROS), contributing to tumor migration and aggressive phenotypes^{99,100}. Additionally, HPV's interaction with host cells can result in the secretion of cytokines that further modulate microbial communities, creating a feedback loop that perpetuates dysbiosis¹⁰¹. Persistent inflammation depletes antioxidants, increasing oxidative damage and genetic mutations, thereby promoting malignancy.

HPV infections can exist in a latent state and reactivate under immunosuppression or microbiota dysbiosis, increasing cancer risk. This dysbiotic state is not merely a byproduct of HPV infection but may actively contribute to the oncogenic process by promoting an environment that facilitates tumor growth and metastasis^{90,91}. Pathogenic oral bacteria, such as *P. gingivalis* and *F. nucleatum*, impair immune responses by interfering with macrophages and dendritic cells, facilitating HPV persistence and malignant transformation¹⁰².

The oral microbiome plays a critical role in maintaining oral health and modulating systemic immunity. A healthy oral microbiome is characterized by a diverse array of microbial species that work synergistically to prevent the colonization of pathogenic organisms. This balance is essential for the integrity of the oral mucosal barrier and the overall immune response¹⁰³. Beneficial genera like *Corynebacterium* and *Kingella* are associated with a decreased risk of HNSCC, highlighting their potential protective role. Dysbiosis, often characterized by a reduction in microbial diversity, can lead to various oral diseases, including periodontitis and dental caries, and has been linked to systemic conditions such as cardiovascular disease and diabetes^{104,105}.

Moreover, the oral microbiome interacts with host tissues through various mechanisms, including the production of metabolites that can influence local immune responses¹⁰⁶. Short-chain fatty acids produced by beneficial bacteria can enhance epithelial barrier function and modulate inflammatory responses, thereby contributing to oral and systemic health^{95,103}. Conversely, pathogenic bacteria can produce virulence factors that disrupt epithelial integrity and promote inflammation, further exacerbating dysbiosis and disease progression^{90,97}. Pathogens like *S. salivarius*, *S. mitis*, and *P. aeruginosa* generate carcinogenic metabolites such as nitrite, NO, and acetaldehyde (ACH), which are linked to oral carcinogenesis^{107–110}.

i. **Changes in Microbial Composition in HPV-Positive Individuals**

The microbial composition of the oral cavity in HPV-positive individuals has been shown to differ significantly from that of HPV-negative individuals. This shift in microbial diversity is characterized by an increase in pathogenic bacteria and a decrease in beneficial species, leading to a state of dysbiosis that may facilitate the development of oral diseases, including OSCC. Recent studies have utilized advanced sequencing techniques to elucidate these compositional changes, revealing specific microbial signatures associated with HPV infection.

One of the most notable findings is the increased abundance of *Fusobacterium nucleatum* in HPV-positive individuals. This bacterium is not only a key player in periodontal disease but has also been implicated in the pathogenesis of OSCC^{90,91}. Its association with HPV-related cancers across multiple anatomical sites underscores its oncogenic potential^{92–94}. The presence of *F. nucleatum* has been linked to inflammatory responses that can promote tumor progression, suggesting that its overrepresentation in HPV-positive individuals may contribute to the oncogenic process⁹⁸. Additionally, other pathogenic species such as *Porphyromonas gingivalis* and *Treponema denticola* have also been found to be more prevalent in HPV-positive oral microbiomes, further indicating a shift towards a more pathogenic microbial community¹¹¹.

Conversely, beneficial bacteria such as *Streptococcus* and *Lactobacillus*, which are known to play protective roles in oral health, are often found in reduced abundance in HPV-positive individuals¹⁰³. Protective genera like *Corynebacterium* and *Kingella* are also diminished, increasing susceptibility to HNSCC¹⁰². This loss of beneficial microbes can compromise the oral microbiome's ability to maintain homeostasis and protect against pathogenic invasion, thereby increasing the risk of disease^{104,105}. The interplay between HPV and the oral microbiome is complex, as the viral infection may not only alter microbial composition but also influence the functional capabilities of the microbial community.

The functional implications of these compositional changes are significant. Dysbiosis in HPV-positive individuals is associated with increased inflammatory markers and altered immune responses, which can further exacerbate the risk of oral diseases^{97,101}. The presence of pathogenic bacteria can trigger the release of pro-inflammatory cytokines, creating a microenvironment that is conducive to cancer development^{103,104}. *P. gingivalis* promotes tumor progression by recruiting myeloid-derived suppressor cells via chemokines like CCL2 and CXCL2, and cytokines like IL-6 and IL-8¹¹². Moreover, the altered microbial community may produce different metabolic byproducts that can influence host health, potentially leading to systemic effects beyond the oral cavity^{103,104}.

ii. Mechanisms of Microbiome Alteration by HPV

The mechanisms by which HPV alters the oral microbiome involve interactions between the virus, host immune responses, and microbial communities. HPV can influence the oral microbiome through several pathways, including the modulation of local immune responses, the production of viral proteins, and the alteration of the oral environment.

One of the primary mechanisms by which HPV affects the oral microbiome is through the induction of chronic inflammation. HPV infection can lead to the activation of the host

immune system, resulting in the release of pro-inflammatory cytokines that can disrupt the balance of the oral microbiome^{97,101}. Chronic inflammation driven by pathogens like *P. gingivalis* and *F. nucleatum* generates ROS, which deplete antioxidants, cause DNA damage, and disrupt cell cycle regulation, promoting malignancy. This inflammatory response may favor the growth of pathogenic bacteria while inhibiting beneficial species, leading to a state of dysbiosis^{95,113}. Studies have shown that inflammatory cytokines such as IL-6 and TNF- α can promote the proliferation of *Fusobacterium nucleatum*, further contributing to the dysbiotic state observed in HPV-positive individuals^{90,91}.

Additionally, HPV can produce viral proteins that directly influence microbial interactions, the E6 and E7 oncoproteins of HPV have been shown to modulate host cell signaling pathways, which can impact the local microbial environment^{90,91}. These viral proteins may alter the expression of host receptors involved in microbial adhesion and colonization, thereby facilitating the establishment of pathogenic bacteria while hindering the growth of commensal species^{95,113}. This manipulation of host-microbe interactions underscores the role of HPV as an active participant in shaping the oral microbiome. HPV integration into the host genome, a key step in carcinogenesis, may be facilitated by biofilms that shield the virus from immune detection.

Moreover, HPV infection can lead to changes in the oral environment that favor the growth of specific microbial communities. As a matter of fact, the presence of HPV may alter the pH and oxygen levels in the oral cavity, creating conditions that are more conducive to the proliferation of pathogenic bacteria^{97,101}. Fungal co-infections, such as *Candida albicans*, exacerbate this process by producing cytolytic toxins like candida lysin, which damage epithelial barriers and facilitate HPV infection¹¹⁴. This environmental shift can further exacerbate dysbiosis and promote the development of oral diseases, including OSCC^{90,91}.

Co-infections with viruses like Epstein–Barr virus (EBV) and herpes simplex virus (HSV) may synergize with HPV to promote carcinogenesis. EBV, has been found to enhance cellular invasion in HPV-infected cells, as demonstrated in *in vitro* models¹¹⁵. Additionally, microbial dysbiosis influences cancer treatment outcomes; for example, *Pseudomonas viridiflava* is linked to improved survival in HNSCC patients, while *Yersinia pseudotuberculosis* correlates with poorer prognosis¹¹⁶.

2.9 Oral Microbiota as a Biomarker for HPV-Related Cancers

Oral squamous cell carcinoma (OSCC) is the predominant form of oral cancer, accounting for roughly 90% of cases. The prognosis for advanced OSCC is grim—with five-year survival rates reported between 30% and 41%—yet early detection can push these rates above 85%^{117–119}. Given that nearly half of OSCC cases are diagnosed at advanced stages, there is a pressing need for reliable, non-invasive biomarkers that can both detect early disease and help predict its progression, particularly in cancers driven by human papillomavirus (HPV)¹²⁰.

Historically, clinicians have relied on biomarkers such as HPV DNA, overexpression of the p16 protein, and antibodies against viral oncogenes like E6 and E7 to assess cancer risk^{121,122}. Recent developments, however, suggest that the composition of the oral microbiota could provide additional, valuable insights into the presence and progression of HPV-associated cancers. Saliva, which mirrors the complex ecosystem of the oral cavity, has become a focus for these studies. By analyzing salivary samples, researchers can now detect subtle shifts in microbial communities that occur during the early stages of dysbiosis associated with HPV infection^{123,124}.

Investigations into the oral microbiome have revealed that while overall microbial richness (alpha diversity) may not differ drastically between healthy individuals and those with HPV-related cancers, the overall community composition (beta diversity) does. In HPV-positive

patients, there is a notable enrichment of certain bacterial families—such as *Actinomycetaceae*, *Prevotellaceae*, *Veillonellaceae*, and *Campylobacteraceae*—as well as a higher relative abundance of *Bacteroidetes*. Conversely, beneficial or commensal groups, including members of *Gemellaceae*, *Neisseria*, and *Lactobacillus*, are less common in these patients^{125,126}. Additional analyses have further highlighted that specific genera (like *Actinomyces*, *Granulicatella*, *Oribacterium*, and *Campylobacter*) and species like *Veillonella dispar*, *Rothia mucilaginosa*, and *Haemophilus parainfluenzae* tend to be elevated in HPV-associated cancers, whereas others such as *Streptococcus anginosus* and *Mycoplasma* are diminished. These distinct microbial signatures not only differentiate tumor sites from normal mucosa but also appear to evolve with disease progression and treatment¹²⁷.

The clinical promise of these microbial markers is multifold. First, by pairing traditional HPV biomarkers with oral microbiota profiling, clinicians could develop more comprehensive screening protocols that improve early detection. The use of salivary sampling is particularly appealing due to its non-invasive nature and its ability to capture dynamic changes in the microbial ecosystem. Advances in next-generation sequencing and metagenomic techniques have made it feasible to detect even subtle microbial shifts, offering the potential for predictive models that indicate whether an HPV infection may clear or progress to malignancy⁴⁸.

Beyond detection, alterations in the oral microbiome are thought to actively contribute to carcinogenesis. An imbalance in the microbial community—commonly termed dysbiosis—can lead to chronic inflammation, which in turn promotes oxidative stress and DNA damage. Pathogenic bacteria that flourish during dysbiosis may secrete pro-inflammatory mediators, such as interleukin-6 (IL-6), and produce reactive oxygen species that damage host DNA. In animal models, co-infection with periodontopathic bacteria like *Porphyromonas gingivalis*

and *Fusobacterium nucleatum* has been shown to worsen tumor severity, suggesting that these pathogens may work together with HPV to drive cellular transformation¹¹¹.

Several hypotheses have been proposed to explain how shifts in the oral microbiota might facilitate HPV persistence and the onset of malignancy. One idea is that a loss of protective, commensal bacteria weakens mucosal immunity, allowing HPV to evade immune clearance. Additionally, dysbiotic communities may alter the metabolic landscape—reducing the production of beneficial compounds such as short-chain fatty acids while increasing the generation of DNA-damaging substances. Furthermore, the formation of bacterial biofilms in the oral cavity might create physical niches that protect the virus from host immune responses, thereby prolonging its residence time and increasing the risk of viral integration into the host genome⁴⁸.

The interaction between the oral microbiota and the host immune system is also a key factor in this process. Bacterial components are recognized by pattern recognition receptors like Toll-like receptors (TLRs) on epithelial and immune cells, triggering the release of cytokines and other mediators. While such activation is essential for fighting off infections, persistent stimulation in a dysbiotic environment can lead to chronic inflammation, impair effective antiviral responses, and ultimately facilitate HPV persistence and oncogenesis¹¹⁹.

Recent research suggests that the oral microbiota offers a promising and non-invasive biomarker for HPV-related oral cancers^{122–124}. Changes in the microbial community—both in composition and metabolic function—mirror the presence of oncogenic HPV and actively contribute to the inflammatory and immunological processes that drive cancer development. Future longitudinal and multi-omics studies will be critical to validate these microbial signatures and to integrate them into clinical practice, potentially leading to earlier detection and more tailored therapeutic approaches for patients with HPV-associated oral malignancies.

2.10 Geographic and Population-Specific Gaps in Oral HPV-Microbiome Interaction

Studies

The interplay between oral human papillomavirus (HPV) infection and the oral microbiome is a critical yet understudied area, particularly in sub-Saharan Africa (SSA). Recent studies highlight that oral HPV infections are linked to oropharyngeal cancers and are influenced by microbial dysbiosis, with shifts in bacterial taxa such as *Prevotellaceae*, *Veillonellaceae*, and *Campylobacteraceae* associated with HPV persistence¹²⁵. However, most data derive from high-income countries, leaving significant gaps in understanding region-specific microbial-HPV dynamics in SSA, where HPV prevalence is disproportionately high and cervical cancer remains a leading cause of mortality^{71,128}.

In SSA, HIV co-infection amplifies HPV susceptibility, with studies reporting a 3.2-fold higher prevalence of high-risk HPV (hrHPV) among HIV-positive women compared to HIV-negative individuals⁷¹. This intersection of HIV and HPV underscores the need to investigate how immunosuppression alters oral and cervicovaginal microbiomes, potentially exacerbating HPV oncogenicity. It has been found that HIV-positive individuals in SSA exhibit higher rates of non-vaccine hrHPV genotypes (e.g., HPV-56, HPV-31), which may correlate with unique microbial biomarkers not yet identified in regional studies⁷¹. Furthermore, oral microbiome diversity in HPV-positive individuals shows reduced xenobiotic biodegradation pathways, suggesting impaired metabolic detoxification that could heighten cancer risk¹²⁵. These findings emphasize the need for region-specific studies to unravel how local microbial communities modulate HPV persistence in immunocompromised populations.

The oral microbiome's role as a biomarker for HPV-related cancers is another under-explored frontier in SSA. *Fusobacterium* and *Porphyromonas*—pathogens linked to periodontal disease—are enriched in oral and vaginal microbiomes of cervical cancer patients, indicating

systemic microbial crosstalk¹²⁹. However, African microbiomes are distinct due to genetic, dietary, and environmental factors, as highlighted by the limited representation of African datasets in global microbiome repositories¹³⁰. A 2024 study in China demonstrated that oral microbial markers (e.g., *Streptococcus*, *Lactobacillus*) could predict cervical cancer with high accuracy, suggesting similar potential in SSA if regionally validated¹²⁹. Without localized data, public health strategies cannot leverage microbial biomarkers for early cancer detection or probiotic interventions tailored to African populations.

Finally, the lack of longitudinal studies in SSA limits understanding of microbiome-HPV temporal dynamics. Cross-sectional data from Nigeria reveal high oro-genital HPV concordance (10% of women harbor HPV in four anatomic sites) but fail to track how microbial shifts precede or follow HPV acquisition⁷². Such insights are critical for designing microbiome-modulating therapies. Regional research must prioritize cohort studies integrating multi-omics (metagenomics, metabolomics) to map microbial pathways influencing HPV clearance or persistence.

2.11 16S rRNA Analysis in HPV-Microbiome Research

16S ribosomal RNA (rRNA) gene sequencing remains a gold standard for microbiome profiling due to its cost-effectiveness, scalability, and ability to resolve bacterial communities at the genus and species levels. Recent advancements in full-length 16S sequencing using third-generation platforms like Oxford Nanopore Technology (ONT) have enhanced taxonomic resolution, enabling discrimination of closely related species such as *Streptococcus oralis* versus *S. mitis*, which are misclassified by short-read Illumina sequencing¹³¹. This precision is critical for HPV research, where specific taxa like *Veillonellaceae* (enriched in HPV-positive individuals) may serve as biomarkers¹²⁵.

The choice of hypervariable regions and reference databases significantly impacts results. For oral microbiome studies, the V3-V4 and V4 regions paired with Greengenes2 achieve optimal genus-level accuracy, while the V1-V2 region with the Human Oral Microbiome Database (HOMD) improves species-level resolution¹³². These technical considerations are vital in HPV studies, where subtle microbial shifts (e.g., reduced *Gemellaceae* abundance) correlate with viral persistence¹²⁹. Additionally, bioinformatics pipelines like Emu, combined with HOMD, reduce false positives in noisy Nanopore data, ensuring reliable identification of low-abundance taxa¹³¹.

16S sequencing also facilitates functional pathway prediction via tools like PICRUST2, revealing metabolic shifts in HPV-associated microbiomes. Interestingly, HPV-positive individuals exhibit decreased xenobiotic biodegradation pathways, suggesting impaired detoxification mechanisms that may synergize with viral oncogenesis¹²⁹. Such insights are unattainable with culture-based methods or shotgun metagenomics, which are prohibitively expensive for large cohorts. In resource-limited SSA settings, 16S analysis offers a pragmatic balance between depth and cost, enabling population-scale studies to identify microbial signatures of HPV progression.

However, methodological standardization is critical. A 2024 study highlighted that DNA extraction protocols and primer biases can skew microbial profiles, particularly in saliva samples¹³¹. It has been reported that robot-extracted DNA from biobanks may underrepresent gram-positive bacteria due to inefficient lysis, necessitating protocol optimization for oral samples¹³¹. These nuances underscore the importance of adhering to validated workflows, such as those outlined in the Human Microbiome Project, to ensure cross-study comparability—a gap noted in SSA research¹³⁰.

2.12 Research Gaps Specific to Nigeria and Sub-Saharan Africa

Despite bearing the highest global burden of HPV-related cancers, SSA remains underrepresented in microbiome and HPV research. In Nigeria, a 2021 study found 68% prevalence of genital HPV and 16% oral HPV among women, with HPV-35 as the dominant hrHPV genotype—distinct from the global HPV-16/18 paradigm⁷². This genotype divergence suggests region-specific viral evolution and microbiome interactions that are poorly characterized. Furthermore, HIV co-infection in Nigeria alters HPV genotype distribution, with HPV-56 and HPV-51 more prevalent in HIV-positive women, yet no studies have explored how this affects microbial communities^{71,72}.

A critical gap is the lack of multi-site microbiome-HPV studies. While cervical microbiome dysbiosis (e.g., *Lactobacillus* depletion) is linked to HPV persistence, oral and anal microbiomes are rarely analyzed concurrently in SSA populations^{72,129}. A 2024 Chinese study identified synergistic vaginal-oral microbial networks in cervical cancer patients, but similar data are absent in African cohorts⁷². This limits the development of holistic screening tools leveraging microbial biomarkers across anatomic sites.

Additionally, socioeconomic and structural barriers impede HPV-microbiome research in SSA. Vaccine hesitancy, inadequate healthcare infrastructure, and competing infectious disease priorities (e.g., HIV, malaria) divert attention from microbiome studies¹²⁸. Only 12% of SSA countries have implemented national HPV vaccination programs, and fewer have integrated microbiome profiling into cancer screening¹²⁸. Cultural stigma around sexual health further complicates participant recruitment, as seen in Nigeria, where oral HPV studies face low enrollment due to misconceptions about transmission routes⁷².

Finally, there is a paucity of translational research. While probiotic interventions (e.g., *Lactobacillus crispatus*) show promise in enhancing HPV clearance in Western cohorts, their efficacy in African microbiomes—which harbor unique taxa

like *Prevotella* and *Bacteroides*—remains untested ^{119,129}. Addressing these gaps requires collaborative frameworks to build regional biobanks, standardize 16S protocols, and prioritize microbiome-centric interventions in national cancer control plans.

Lead City University Ibadan DO NOT COPY

Endnotes

1. T. Letian, and Z. Tianyu, *Cellular Receptor Binding and Entry of Human Papillomavirus*, **Virology Journal**, 7(1), 2020, 20-25.
2. S. Syrjänen, *Oral manifestations of Human Papillomavirus Infections*, **European Journal of Oral Sciences**, 126(1), 2018, 49–66.
3. K. Morshed, D. Polz-Gruszka, M. Szymański, and M. Polz-Dacewicz, *Human Papillomavirus (HPV) - Structure, Epidemiology and Pathogenesis*, **The Polish Otolaryngology**, 68(5), 2024, 213–219.
4. R. Kirnbauer, F. Booy, N. Cheng, D. R. Lowy, and J. T. Schiller, *Papillomavirus L1 Major Capsid Protein Self-Assembles into Virus-Like Particles that are Highly Immunogenic.*, **Proceedings of the National Academy of Sciences**, 89(24), 1992, 12180–12184.
5. M. E. Hagensee, N. Yaegashi, and D. A. Galloway, *Self-assembly of Human Papillomavirus Type 1 Capsids by Expression of the L1 Protein Alone or by Co-Expression of the L1 and L2 Capsid Proteins*, **Journal of Virology**, 67(1), 1993, 315–322.
6. F. Han, X. Guo, M. Jiang, N. Xia, Y. Gu, and S. Li, *Structural Biology of the Human Papillomavirus*, **Structure**, 32(11), 2024, 1877–1892.
7. G. V. Santiago, A. Alonso, and B. Ignacio, *Papillomaviruses: Different Genes Have Different Histories*, **Trends in Microbiology**, 13(11), 2025, 514-21 <https://pubmed.ncbi.nlm.nih.gov/16181783/>.
8. D. Balaji, I. B. Kalarani, V. Mohammed, and R. Veerabathiran, *Potential Role of Human Papillomavirus Proteins Associated with the Development of Cancer*, **Virus Disease**, 33(3), 2022, 322–333.
9. M. Gnanamony, A. Peedicayil, and P. Abraham, *An Overview of Human Papillomaviruses and Current Vaccine Strategies*, **Indian Journal of Medical Microbiology**, 25(1), 2007, 10–17.
10. I. G. Bravo, and M. Félez-Sánchez, *Papillomaviruses: Viral Evolution, Cancer and Evolutionary Medicine*, **Evolution, Medicine, and Public Health**, 2015(1), 2015, 32–51.
11. E. D. S. Foro Ramos, R. Da Silva Couto, T. R. Tozetto-Mendoza, P. Bortoletto, E. M. G. Barbosa, N. E. Ferreira, I. M. Linhares, S. D. Spandorfer, A. C. Da Costa, E. Leal, M. C. Mendes-Correa, and S. S. Witkin, *Characterization of Multiple Human Papillomavirus Types in the Human Vagina Following Ovarian Hormonal Stimulation*, **Virology Journal**, 21(1), 2024, 22-29.
12. L. S. Arroyo Mühr, C. Lagheden, S. S. Hassan, C. Eklund, and J. Dillner, *The International Human Papillomavirus Reference Center: Standardization, collaboration, and quality assurance in HPV research and diagnostics*, **Journal of Medical Virology**, 95(12), 2023, 293-332.
13. M. E. Sarkola, S. E. Grénman, M. A. M. Rintala, K. J. Syrjänen, and S. M. Syrjänen, *Human Papillomavirus in the Placenta and Umbilical Cord Blood*, **Acta Obstetricia Et Gynecologica Scandinavica**, 87(11), 2023, 1181–1188.

14. S. Syrjänen, *Current Concepts on Human Papillomavirus Infections in Children*, **Journal of Pathology, Microbiology and Immunology**, 118(6-7), 2010, 494-509.
15. J. Doorbar. *Host control of human papillomavirus infection and disease*, **Best Practice & Research Clinical Obstetrics & Gynaecology**, 47(1), 2018, 27–41.
16. Z. Z. Szalai, "Human Papillomavirus Infection". In. *Harper's Textbook of Pediatric Dermatology* (Alan D. Irvine, Peter H. Hoeger, Albert C. Yan), **Wiley**, 2011, 1-20.
17. A. M. Al Aboud, and P. K. Nigam. "Wart". In. *StatPearlsTreasure Island (FL): StatPearls Publishing*, 2024.
18. D. J. Witchey, N. B. Witchey, M. M. Roth-Kauffman, and M. K. Kauffman, *Plantar Warts: Epidemiology, Pathophysiology, and Clinical Management*, **The Journal of the American Osteopathic Association**, 118(2), 2018, 92-100.
19. E. de Planell-Mas, B. Martínez-Garriga, A. J. Zalacain, T. Vinuesa, and M. Viñas, *Human Papillomaviruses Genotyping in Plantar Warts*, **Journal of Medical Virology**, 89(5), 2017, 902–907.
20. T. Maity, *Homoeopathic Simillimum in Treatment of Verruca Plana (Flat Wart): A Case Report*, **Homœopathic Links**, 35, 2022, 191–197.
21. H. Yoo, S.-S. Won, H.-C. Choi, T.-J. Yoon, S.-K. Ye, T.-K. Park, and K.-H. Lee . *Detection and Identification of Human Papillomavirus Types Isolated from Korean Patients with Flat Warts*, **Microbiology and Immunology**, 49(7), 2025, 633–638.
22. A. Dutta, *Filiform Warts of 2 Years' Duration in a Child Treated by Homoeopathy*, **Homœopathic Links**, 37, 2024, 117–118.
23. M. M. Lipke, *An Armamentarium of Wart Treatments*, **Clinical Medicine and Research**, 4(4), 2006, 273–293.
24. N. Dupin, *Genital Warts*, **Clinics in Dermatology**, 22(6), 2004, 481–486.
25. Z.-Y. Luo, Q. Chen, H. Yang, M. Lin, C.-Y. Chen, C. Yang, and L.-Y. Yang, *The Prevalence and Genotype of Human Papillomavirus from Patients with Genital Warts in Eastern Guangdong Province*, **Asian Pacific Journal of Cancer Prevention: APJCP**, 16(14), 2015, 5675–5679.
26. S. Richards, *An Overview of Genital Warts*, **Nursing Standard (Royal College of Nursing)**, 28(24), 2014, 46–50.
27. M. Peñaloza-Plascencia, H. Montoya-Fuentes, S. E. Flores-Martínez, F. J. Fierro-Velasco, J. M. Peñaloza-González, and J. Sánchez-Corona, *Molecular Identification Of 7 Human Papillomavirus Types in Recurrent Respiratory Papillomatosis*, **Archives of Otolaryngology--Head & Neck Surgery**, 126(9.), 2000, 1119–1123.
28. R. Y. Seedat, C. E. Combrinck, and F. J. Burt, *HPV Associated with Recurrent Respiratory Papillomatosis*, **Future Virology**, 8(5), 2023, 477–492.

29. K. K. Susanne, F. Kirsten, M. Christian, and I. Thomas. *Long-Term Absolute Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse Following Human Papillomavirus Infection: Role of Persistence*, **Journal of the National Cancer Institute**, 102(19), 2010 Available Online: <https://pubmed.ncbi.nlm.nih.gov/20841605/>.
30. Z. Khan, F. Appleton, and J. Turner, *Is Cervical Intra-Epithelial Neoplasia Symptomatic?* **Journal of Obstetrics and Gynaecology: The Journal of the Institute of Obstetrics and Gynaecology**, 28(3), 2008, 336–337.
31. K. S. Okunade, *Human Papillomavirus and Cervical Cancer*, **Journal of Obstetrics and Gynaecology: The Journal of the Institute of Obstetrics and Gynaecology**, 40(5), 2020, 602–608.
32. M. A. Ijaiya, A. P. Aboyeji, A. W. O. Olatinwo, and M. O. Buhari, *Clinico-Pathological Presentation of Primary Cervical Cancer Seen in Ilorin, Nigeria*, **Nigerian Journal of Surgical Research**, 4(3), 2002, 89–93.
33. C. de Martel, D. Georges, F. Bray, J. Ferlay, and G. M. Clifford, *Global Burden of Cancer Attributable to Infections in 2018: A Worldwide Incidence Analysis*, **The Lancet. Global Health**, 8(2), 2020, 180–190.
34. R. Chockalingam, C. Downing, and S. K. Tying, *Cutaneous Squamous Cell Carcinomas in Organ Transplant Recipients*, **Journal of Clinical Medicine**, 4(6), 2015, 122-129.
35. E. Leung, K. Han, J. Zou, Z. Zhao, Y. Zheng, T. T. Wang, A. Rostami, L. L. Siu, T. J. Pugh, and S. V. Bratman, *HPV Sequencing Facilitates Ultrasensitive Detection of HPV Circulating Tumor DNA*, **Clinical Cancer Research**, 27(21), 2021, 5857–5868.
36. L. Wu, W. Wang, J. Zhang, X. Wu, Y. Chen, X. Gu, H. Shao, H. Li, and W. Liu, *Detection of Five Types of HPV Genotypes Causing Anogenital Warts (Condyloma Acuminatum) Using PCR-Tm Analysis Technology*, **Frontiers in Microbiology**, 13(1), 2022, 857-860.
37. S. Wang, L. Dang, S. Liu, R. Rezhake, H. Yan, X. Duan, L. Zhang, L. Zhang, L. Zhang, M. Su, F. Guo, C. Yan, M. Liu, X. Cao, M. Sun, Y. Qiao, and F. Zhao, *Cervical Cancer Screening via Visual Inspection With Acetic Acid and Lugol Iodine for Triage of HPV-Positive Women*, **JAMA Network Open**, 7(3), 2024, 244-290.
38. J. Valls, A. Baena, G. Venegas, M. Celis, M. González, C. Sosa, J. L. Santin, M. Ortega, A. Soilán, E. Turcios, J. Figueroa, M. Rodríguez De La Peña, A. Figueredo, A. V. Beracochea, N. Pérez, J. Martínez-Better, O. Lora, J. Y. Jiménez, D. Giménez, L. Fleider, Y. Salgado, S. Martínez, Y. Bellido-Fuentes, B. Flores, S. Tatti, V. Villagra, A. Cruz-Valdez, C. Terán, G. I. Sánchez, G. Rodríguez, M. A. Picconi, A. Ferrera, L. Mendoza, A. Calderón, R. Murillo, C. Wiesner, N. Broutet, S. Luciani, C. Pérez, T. M. Darragh, J. Jerónimo, R. Herrero, and M. Almonte . *Performance Of Standardized Colposcopy to Detect Cervical Precancer and Cancer for Triage of Women Testing Positive for Human Papillomavirus: Results from the ESTAMPA Multicentric Screening Study*, **The Lancet Global Health**, 11 (3), 2023, 350–360.
39. A. Mremi, B. Mchome, J. Mlay, D. Schledermann, J. Blaakær, and V. Rasch, *Performance of HPV testing, Pap smear and VIA in Women Attending Cervical Cancer*

Screening in Kilimanjaro Region, Northern Tanzania: A Cross-Sectional Study Nested in a Cohort, **BMJ Open**, 12(10), 2022, 643-651.

40. Y. Maheshwari, U. Handa, P. Aggarwal, and B. Goel, *Comparative Analysis of Conventional Cytology and Liquid-Based Cytology in the Detection of Carcinoma Cervix and its Precursor Lesions*, **Journal of Cytology**, 40(3), 2023, 114–118.

41. J. Jin, S. Li, H. Huang, J. Li, Y. Lyu, Y. Ran, H. Chang, and X. Zhao, *Development of Human Papillomavirus and its Detection Methods (Review)*, **Experimental and Therapeutic Medicine**, 28(4), 2024, 382.

42. Z. S. M. Al-Hadeithi, S. A. Jasim, and O. D. Salahdina, *Detection and Genotyping of Papillomavirus by Real-Time PCR in Iraqi Patients*, **Archives of Clinical Infectious Diseases**, 17(3), 2022, e121143.

43. T. Malagón, E. L. Franco, R. Tejada, and S. Vaccarella, *Epidemiology of HPV-Associated Cancers Past, Present and Future: Towards Prevention and Elimination*, **Nature Reviews Clinical Oncology**, 21(7), 2024, 522–538.

44. A. R. Giuliano, M. Felsher, T. Waterboer, H. Mirghani, H. Mehanna, C. Roberts, Y.-T. Chen, M. Lynam, M. Pedrós, E. Sanchez, B. Sirak, S. Surati, L. Alemany, E. Morais, and M. A. Pavón, *Oral Human Papillomavirus Prevalence and Genotyping Among a Healthy Adult Population in the US*, **JAMA Otolaryngology–Head & Neck Surgery**, 149(9), 2023, 78-83.

45. G. D'Souza, G. Clemens, H. D. Strickler, D. J. Wiley, T. Troy, L. Struijk, M. Gillison, and C. Fakhry, *Long-term Persistence of Oral HPV Over 7 Years of Follow-up*, **JNCI Cancer Spectrum**, 4(5), 2020, 47-50.

46. H. B. Mistry, R. L. Lebelo, F. Matshonyonge, M. Nchabeleng, M. Mathebula, J.-P. Bogers, N. H. Wood, Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa, HIV and Hepatitis Research Unit, National Health Laboratory service, Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa, Department of Microbiology, Sefako Makgatho Health Sciences University, Pretoria, South Africa, Mecru Clinical Research Unit, Sefako Makgatho Health Sciences University, Pretoria, South Africa, and Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), Laboratory of Cell Biology and Histology, University of Antwerp, Belgium . *Oral and Oropharyngeal High-risk HPV Prevalence, HIV Status, and Risk Behaviours in a Cohort of South African Men who have Sex with Men*, **AIMS Public Health**, 9(1), 2021, 129–141.

47. D. Bettampadi, B. Dickey, M. Abrahamsen, B. Sirak, M. L. Baggio, L. Galan, R. C. Silva, L. Villa, E. L. Ponce, and A. R. Giuliano, *Differences in Factors Associated with High- and Low-Risk Oral Human Papillomavirus Genotypes in Men*, **The Journal of Infectious Diseases**, 223(12), 2021, 2099–2107.

48. M. Constantin, M. C. Chifiriuc, G. Mihaescu, C. O. Vrancianu, E.-G. Dobre, R.-E. Cristian, C. Bleotu, S. V. Bertesteanu, R. Grigore, B. Serban, and C. Cirstoiu, *Implications of Oral Dysbiosis and HPV Infection in Head and Neck Cancer: From Molecular and Cellular Mechanisms of Early Diagnosis and Therapy*, **Frontiers in Oncology**, 13(1), 2023, 1273-516.

49. N. Dom-Chima, Y. A. Ajang, C. I. Dom-Chima, E. Biswas-Fiss, M. Aminu, and S. B. Biswas, *Human Papillomavirus Spectrum of HPV-Infected Women in Nigeria: An Analysis by Next Generation Sequencing and Type-Specific PCR*, **Virology Journal**, 20(1), 2023, 14-24.
50. O. Anoruo, C. Bristow, N. Mody, and J. D. Klausner, *Estimated Prevalence of Human Papillomavirus Among Nigerian Women: A Systematic Review and Meta-Analysis*, **African Journal of Reproductive Health**, 26(6), 2022, 89–96.
51. O. Ezechi, F. Akinsolu, A. Salako, O. Abodunrin, I. Adewole, M. Olagunju, H. Okunbor, R. Sanni-Adeniyi, E. Zamba, D. W. Njuguna, and G. Eleje, *High-Risk Human Papillomavirus Infection Among Nigerian Women: A Systematic Review and Meta-Analysis*, **The Journal of International Medical Research**, 51(7), 2023, 30-34.
52. I. O. Morhason-Bello, K. Baisley, M. A. Pavon, I. F. Adewole, R. A. Bakare, S. De Sanjosé, S. C. Francis, and D. Watson-Jones, *Oral, Genital and Anal Human Papillomavirus Infections Among Female Sex Workers in Ibadan, Nigeria*, **PLOS ONE**, 17(3), 2022, 265-269.
53. A. O. Faneye, O. S. Babalola, G. N. Odaibo, J. Arotiba, and O. D. Olaleye, *Oral Human Papilloma Virus Infection Among Dental Clinic Attendees in Ibadan, Nigeria*, **African Journal of Laboratory Medicine**, 11(1), 2022, 15-25.
54. E. Onu, E. Onu, C. Ejikeugwu, C. Azi, J. Ugwu, and O. Ogbu, *Seroprevalence and Molecular Investigation of Human Papillomavirus (HPV) Type-16 in HIV Positive Women in Abakaliki, Nigeria*, **International Journal of Virology**, 19(1), 2023, 1–5.
55. N. Rahadiani, M. Habiburrahman, M. Stephanie, D. R. Handjari, and E. Krisnuhoni, *Estimated projection of Oral Squamous Cell Carcinoma Annual Incidence from Twenty Years Registry Data: A Retrospective Cross-Sectional Study in Indonesia*, **PeerJ**, 11(1), 2023, 159-161.
56. M. Lechner, J. Liu, L. Masterson, and T. R. Fenton, *HPV-Associated Oropharyngeal Cancer: Epidemiology, Molecular Biology and Clinical Management*, **Nature Reviews. Clinical Oncology**, 19(5), 2022, 306–327.
57. T. C. Fonsêca, L. A. Jural, G. A. Marañón-Vásquez, M. B. Magno, A. L. O. C. Roza, D. M. T. P. Ferreira, L. C. Maia, M. J. Romañach, M. Agostini, and A. C. Abrahão, *Global Prevalence of Human Papillomavirus-Related Oral and Oropharyngeal Squamous Cell Carcinomas: A Systematic Review and Meta-Analysis*, **Clinical Oral Investigations**, 28(1), 2023, 62-69.
58. Y. X. Lim, M. L. Mierzwa, M. A. Sartor, and N. J. D'Silva, *Clinical, Morphologic and Molecular Heterogeneity of HPV-Associated Oropharyngeal Cancer*, **Oncogene**, 42(40), 2023, 2939–2955.
59. Y. X. Lim, and N. J. D'Silva, *HPV-Associated Oropharyngeal Cancer: In Search of Surrogate Biomarkers for Early Lesions*, **Oncogene**, 43(8), 2024, 543–554.
60. M. E. Sabatini, and S. Chiocca, *Human Papillomavirus as a Driver of Head and Neck Cancers*, **British Journal of Cancer**, 122(3), 2020, 306–314.

61. S. Gougousis, E. Mouchtaropoulou, I. Besli, P. Vrochidis, I. Skoumpas, and I. Constantinidis, *HPV-Related Oropharyngeal Cancer and Biomarkers Based on Epigenetics and Microbiome Profile*, **Frontiers in Cell and Developmental Biology**, 8(1), 2021, 625-630.
62. H. Shigeishi, *Association Between Human Papillomavirus and Oral Cancer: A Literature Review*, **International Journal of Clinical Oncology**, 28(8), 2023, 982–989.
63. M. Mac, and C. A. Moody, *Epigenetic Regulation of the Human Papillomavirus Life Cycle*, **Pathogens**, 9(6), 2020, 48-53.
64. M. Saliba, R. Ghossein, and B. Xu, *HPV-Related Head and Neck Cancers: Pathology and Biology*, **Journal of Surgical Oncology**, 124(6), 2021, 923–930.
65. N. Benzerdjeb, A. Traverse-Glehen, P. Philouze, J. Bishop, and M. Devouassoux-Shisheboran, *Poorly Differentiated Neuroendocrine Carcinoma of the Head and Neck: Human Papillomavirus Tumour Status/P16 Status and Impact on Overall Survival*, **Histopathology**, 76(4), 2020, 581–591.
66. N. C. Schmitt, *HPV in Non-Oropharyngeal Head and Neck Cancer: Does it Matter* **Annals of Translational Medicine**, 8(18), 2020, 1120–1120.
67. D. Nishikawa, H. Suzuki, S. Beppu, H. Terada, M. Sawabe, and N. Hanai, *Near-Infrared Photoimmunotherapy for Oropharyngeal Cancer*, **Cancers**, 14(22), 2022, 56-62.
68. A. Satanova, D. Kaidarova, E. Kukubassov, R. Bolatbekova, D. Kaldybekov, and O. Bertleuov, *The Global Prevalence of Human Papillomavirus Causing Cervical Cancer: A Literature Review*, **Oncologia Radiologia Kazakhstan**, 65(3), 2022, 42–46.
69. M. Goto, N. Hanai, D. Nishikawa, T. Nagao, and Y. Hasegawa, *Prognosis of HPV-Positive Oral Squamous Carcinoma: A Cohort Study from Japan*, **Journal of Hard Tissue Biology**, 32(1), 2023, 77–82.
70. C. Klein, C. Kahesa, J. Mwaiselage, J. T. West, C. Wood, and P. C. Angeletti, *How the Cervical Microbiota Contributes to Cervical Cancer Risk in Sub-Saharan Africa*, **Frontiers in Cellular and Infection Microbiology**, 10(1), 2020, 23-30.
71. J. O. Okoye, C. A. Ofodile, O. K. Adeleke, and O. Obioma, *Prevalence of High-risk HPV Genotypes in Sub-Saharan Africa According to HIV status: A 20-year Systematic Review*, **Epidemiology and Health**, 43(1), 2021, 202-239.
72. I. O. Morhason-Bello, K. Baisley, M. A. Pavon, I. F. Adewole, R. Bakare, S. De Sanjosé, S. C. Francis, and D. Watson-Jones, *Prevalence and Genotype Specific Concordance of Oro-Genital and Anal Human Papillomavirus Infections Among Sexually Active Nigerian Women*, **Infectious Agents and Cancer**, 16(1), 2021, 59-64.
73. J. O. Okoye, S. I. Okekpa, C. F. Chukwukelu, I. N. Onyekachi-Umeh, and A. A. Ngokere. *Racial Disparities in the Prevalence of Vaccine and Non-Vaccine HPV Types and Multiple HPV Infection between Asia and Africa: A Systematic Review*, **Asian Pacific Journal of Cancer Prevention**, 22(9), 2021, 2729–2741.

74. National Primary Health Care Development Agency. Speech by the Executive Director/CEO of National Primary Health Care Development Agency (NPHCDA) at the National Flag-Off of Human Papilloma Virus Vaccine Introduction (HPV-Vi) on the 24th Of October 2023 – NPHCDA, Accessed June, 2025.
75. Joint United Nations Programme on HIV/AIDS. *The Path That Ends AIDS: UNAIDS Global AIDS Update 2023*. Geneva: **Joint United Nations Programme on HIV/AIDS**, 2023.
76. L. Zhou, Y. Li, H. Wang, R. Qin, Z. Han, and R. Li, *Global Cervical Cancer Elimination: Quantifying the Status, Progress, and Gaps*, **BMC Medicine**, 23(1), 2025, 6-7.
77. E. Caselli, C. Fabbri, M. D'Accolti, I. Soffritti, C. Bassi, S. Mazzacane, and M. Franchi, *Defining the Oral Microbiome by Whole-Genome Sequencing and Resistome Analysis: The Complexity of The Healthy Picture*, **BMC Microbiology**, 20(1), 2020, 120-125.
78. J. R. Willis, and T. Gabaldón, *The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems*, **Microorganisms**, 8(2), 2020, 30-38.
79. J. L. Baker, J. L. Mark Welch, K. M. Kauffman, J. S. McLean, and X. He, *The Oral Microbiome: Diversity, Biogeography and Human Health*, **Nature reviews. Microbiology**, 22(2), 2024, 89–104.
80. W. Li, H. Liang, X. Lin, T. Hu, Z. Wu, W. He, M. Wang, J. Zhang, Z. Jie, X. Jin, X. Xu, J. Wang, H. Yang, W. Zhang, K. Kristiansen, L. Xiao, and Y. Zou, *A Catalog of Bacterial Reference Genomes from Cultivated Human Oral Bacteria*, **Biofilms and Microbiomes**, 9(1), 2023, 4-8.
81. L. Guo, J. Zhou, F. Xie, Q. Lang, Y. Xu, L. Chen, Z. Xue, Y. Mao, and R. Wang, *The Profile of Oral Microbiome in Chinese Elderly Population Associated with Aging and Systemic Health Status*, **BMC Oral Health**, 24(1), 2024, 89-95.
82. J. J. Rajasekaran, H. K. Krishnamurthy, J. Bosco, V. Jayaraman, K. Krishna, T. Wang, and K. Bei, *Oral Microbiome: A Review of Its Impact on Oral and Systemic Health*, **Microorganisms**, 12(9), 2024, 1797.
83. Q. Xu, W. Wang, Y. Li, J. Cui, M. Zhu, Y. Liu, and Y. Liu, *The Oral-Gut Microbiota Axis: A Link in Cardiometabolic Diseases*, **NPJ Biofilms and Microbiomes**, 11(1), 2025, 11-30.
84. Z. Han, Y. Hu, X. Lin, H. Cheng, B. Dong, X. Liu, B. Wu, and Z. Z. Xu, *Systematic Analyses Uncover Robust Salivary Microbial Signatures and Host-Microbiome Perturbations in Oral Squamous Cell Carcinoma*, **American Society for Microbiology Journals**, 10(2), 2025, 124-129.
85. Y. Lin, X. Liang, Z. Li, T. Gong, B. Ren, Y. Li, and X. Peng, *Omics for Deciphering Oral Microecology*, **International Journal of Oral Science**, 16(1), 2024, 23-40.
86. M. Bertolini, R. C. Costa, V. A. R. Barão, C. Cunha Villar, B. Retamal-Valdes, M. Feres, and J. G. Silva Souza, *Oral Microorganisms and Biofilms: New Insights to Defeat the Main Etiologic Factor of Oral Diseases*, **Microorganisms**, 10(12), 2022, 24-33.

87. R. S. Chung, S. Wong, D. Lin, N. C. Kokot, U. K. Sinha, and A. Y. Han, *Mechanisms of Crosstalk Between the Oropharyngeal Microbiome and Human Papillomavirus in Oropharyngeal Carcinogenesis: A Mini Review*, **Frontiers in Oncology**, 14(1), 2024, 1425-1545.
88. L. Sedghi, V. DiMassa, A. Harrington, S. V. Lynch, and Y. L. Kapila, *The Oral Microbiome: Role of Key Organisms and Complex Networks in Oral Health and Disease*, **Periodontology** **2000**, 87(1), 2021, 107–131.
89. X. Feng, E. U. Patel, J. L. White, S. Li, X. Zhu, N. Zhao, J. Shi, D. E. Park, C. M. Liu, R. Kaul, J. L. Prodder, T. C. Quinn, M. K. Grabowski, and A. A. R. Tobian, *Association of Oral Microbiome with Oral Human Papillomavirus Infection: A Population Study of the National Health and Nutrition Examination Survey, 2009-2012*, **The Journal of Infectious Diseases**, 230(3), 2024, 726–735.
90. R. Li, L. Xiao, T. Gong, J. Liu, Y. Li, X. Zhou, Y. Li, and X. Zheng, *Role of Oral Microbiome in Oral Oncogenesis, Tumor Progression, and Metastasis*, **Molecular Oral Microbiology**, 38(1), 2023, 9–22.
91. Z. Li, R. Fu, X. Wen, Q. Wang, X. Huang, and L. Zhang, *The Significant Clinical Correlation of the Intratumor Oral Microbiome in Oral Squamous Cell Carcinoma Based on Tissue-Derived Sequencing*, **Frontiers in Physiology**, 13(1), 2023, 1089-1539.
92. C. Guo, W. Dai, Q. Zhou, L. Gui, H. Cai, D. Wu, J. Hou, C. Li, S. Li, H. Du, and R. Wu, *Cervicovaginal Microbiota Significantly Changed For HPV-Positive Women with High-Grade Squamous Intraepithelial Lesion*, **Frontiers in Cellular and Infection Microbiology**, 12(1), 2022, 973-975.
93. M. Oliva, P. H. H. Schneeberger, V. Rey, M. Cho, R. Taylor, A. R. Hansen, K. Taylor, A. Hosni, A. Bayley, A. J. Hope, S. V. Bratman, J. Ringash, S. Singh, I. Weinreb, B. Perez-Ordoñez, D. Chepeha, J. Waldron, W. Xu, D. Guttman, L. L. Siu, B. Coburn, and A. Spreafico, *Transitions in Oral and Gut Microbiome of HPV+ Oropharyngeal Squamous Cell Carcinoma Following Definitive Chemoradiotherapy (ROMA LA-OPSCC Study)*, **British Journal of Cancer**, 124(9), 2021, 1543–1551.
94. J. H. Elnaggar, V. O. Huynh, D. Lin, R. T. Hillman, C. O. Abana, M. B. El Alam, K. C. Tomasic, T. V. Karpinets, R. Kouzy, J. L. Phan, J. Wargo, E. B. Holliday, P. Das, M. P. Mezzari, N. J. Ajami, E. J. Lynn, B. D. Minsky, V. K. Morris, A. Milbourne, C. A. Messick, A. H. Klopp, P. A. Futreal, C. M. Taniguchi, K. M. Schmeler, and L. E. Colbert . *HPV-Related Anal Cancer is Associated with Changes in the Anorectal Microbiome During Cancer Development*, **Frontiers in Immunology**, 14(1), 2023, 1405-1431.
95. X. Chen, H. Huang, C. Guo, X. Zhu, J. Chen, J. Liang, R. Yang, D. Shao, F. Chen, B. Shi, C. Yang, K. W. Leong, and L. Zhao, *Controlling Alveolar Bone Loss by Hydrogel-Based Mitigation of Oral Dysbiosis and Bacteria-Triggered Proinflammatory Immune Response*, **Advanced Functional Materials**, 35(3), 2025, 2409-2421.
96. S. L. Shiao, K. M. Kershaw, J. J. Limon, S. You, J. Yoon, E. Y. Ko, J. Guarnerio, A. A. Potdar, D. P. B. McGovern, S. Bose, T. B. Dar, P. Noe, J. Lee, Y. Kubota, V. I. Maymi, M. J. Davis, R. M. Henson, R. Y. Choi, W. Yang, J. Tang, M. Gargus, A. D. Prince, Z. S. Zumsteg,

and D. M. Underhill . *Commensal Bacteria and Fungi Differentially Regulate Tumor Responses to Radiation Therapy*, **Cancer Cell**, 39(9), 2021, 1202-1213.

97. S. E. Kleinstein, K. E. Nelson, and M. Freire, *Inflammatory Networks Linking Oral Microbiome with Systemic Health and Disease*, **Journal of Dental Research**, 99(10), 2020, 1131–1139.

98. A. Radaic, S. Ganther, P. Kamarajan, J. Grandis, S. S. Yom, and Y. L. Kapila, *Paradigm Shift in the Pathogenesis and Treatment of Oral Cancer and other Cancers Focused on the Oralome and Antimicrobial-Based Therapeutics*, **Periodontology** 2000, 87(1), 2021, 76–93.

99. J. Sun, Q. Tang, S. Yu, M. Xie, Y. Xie, G. Chen, and L. Chen, *Role of The Oral Microbiota in Cancer Evolution and Progression*, **Cancer Medicine**, 9(17), 2020, 6306–6321.

100. H. Tuominen, and J. Rautava, *Oral Microbiota and Cancer Development*, **Pathobiology: Journal of Immunopathology, Molecular and Cellular Biology**, 88(2), 2021, 116–126.

101. H. Alqedari, K. Altabtbaei, J. L. Espinoza, S. Bin-Hasan, M. Alghounaim, A. Alawady, A. Altabtabae, S. AlJamaan, S. Devarajan, T. AlShammari, M. Ben Eid, M. Matsuoka, H. Jang, C. L. Dupont, and M. Freire, *Host-Microbiome Associations in Saliva Predict COVID-19 Severity*, **The Preprint Server for Biology**, 3(2), 2023, 30-36.

102. H. S. Kim, C. G. Kim, W. K. Kim, K.-A. Kim, J. Yoo, B. S. Min, S. Paik, S. J. Shin, H. Lee, K. Lee, H. Kim, E.-C. Shin, T.-M. Kim, and J. B. Ahn, *Fusobacterium nucleatum Induces a Tumor Microenvironment with Diminished Adaptive Immunity Against Colorectal Cancers*, **Frontiers in Cellular and Infection Microbiology**, 13(1), 2023, 1101-1291.

103. T. Maier, *Oral Microbiome in Health and Disease: Maintaining a Healthy, Balanced Ecosystem and Reversing Dysbiosis*, **Microorganisms**, 11(6), 2023, 14-53.

104. X. Peng, L. Cheng, Y. You, C. Tang, B. Ren, Y. Li, X. Xu, and X. Zhou, *Oral Microbiota in Human Systematic Diseases*, **International Journal of Oral Science**, 14(1), 2022, 14-23.

105. B. Khor, M. Snow, E. Herrman, N. Ray, K. Mansukhani, K. A. Patel, N. Said-Al-Naief, T. Maier, and C. A. Machida, *Interconnections between the Oral and Gut Microbiomes: Reversal of Microbial Dysbiosis and the Balance between Systemic Health and Disease*, **Microorganisms**, 9(3), 2021, 49-56.

106. E. Abbasi, and E. Akçay. *Host Control and Species Interactions Jointly Determine Microbiome Community Structure*, **Theoretical Population Biology**, 258(2), 2024, 185-194.

107. N. S. Bryan, M. C. Burleigh, and C. Easton, *The Oral Microbiome, Nitric Oxide and Exercise Performance*, **Nitric Oxide: Biology and Chemistry**, 125(1), 2022, 23–30.

108. M. Z. Kakabadze, T. Paresishvili, L. Karalashvili, D. Chakhunashvili, and Z. Kakabadze, *Oral microbiota and Oral Cancer: Review*, **Oncology Reviews**, 14(2), 2020, 47-56.

109. A. Amer, A. Whelan, N. N. Al-Hebshi, C. M. Healy, and G. P. Moran, *Acetaldehyde Production by Rothia mucilaginosa Isolates from Patients with Oral Leukoplakia*, **Journal of Oral Microbiology**, 12(1), 2020, 1743-1766.

110. A. Smędra, and J. Berent, *The Influence of the Oral Microbiome on Oral Cancer: A Literature Review and a New Approach*, **Biomolecules**, 13(5), 2023, 81-85.
111. P. Kamarajan, I. Ateia, J. M. Shin, J. C. Fenno, C. Le, L. Zhan, A. Chang, R. Darveau, and Y. L. Kapila, *Periodontal Pathogens Promote Cancer Aggressivity Via TLR/Myd88 Triggered Activation of Integrin/FAK Signaling that is Therapeutically Reversible by a Probiotic Bacteriocin*, **PLOS Pathogens**, 16(10), 2020, 1001-1011.
112. L. Wen, W. Mu, H. Lu, X. Wang, J. Fang, Y. Jia, Q. Li, D. Wang, S. Wen, J. Guo, W. Dai, X. Ren, J. Cui, G. Zeng, J. Gao, Z. Wang, and B. Cheng, *Porphyromonas gingivalis Promotes Oral Squamous Cell Carcinoma Progression in an Immune Microenvironment*, **Journal of Dental Research**, 99(6), 2020, 666–675.
113. M. Pisano, F. Giordano, G. Sangiovanni, N. Capuano, A. Acerra, and F. D'Ambrosio, *The Interaction between the Oral Microbiome and Systemic Diseases: A Narrative Review*, **Microbiology Research**, 14(4), 2023, 1862–1878.
114. Q. Du, B. Ren, J. He, X. Peng, Q. Guo, L. Zheng, J. Li, H. Dai, V. Chen, L. Zhang, X. Zhou, and X. Xu, *Candida albicans Promotes Tooth Decay by Inducing Oral Microbial Dysbiosis*, **The ISME journal**, 15(3), 2021, 894–908.
115. M. G. McKeon, J.-N. Gallant, Y. J. Kim, and S. R. Das, *It Takes Two to Tango: A Review of Oncogenic Virus and Host Microbiome Associated Inflammation in Head and Neck Cancer*, **Cancers**, 14(13), 2022, 31-40.
116. R. Upadhyay, A. Dhakal, C. Wheeler, R. Hoyd, M. Jagjit Singh, V. Karivedu, P. Bhateja, M. Bonomi, S. Valentin, M. E. Gamez, D. J. Konieczkowski, S. Baliga, J. C. Grecula, D. M. Blakaj, E. Gogineni, D. L. Mitchell, N. C. Denko, D. Spakowicz, and S. R. Jhawar, *Comparative Analysis of the Tumor Microbiome, Molecular Profiles, and Immune Cell Abundances by HPV Status in Mucosal Head and Neck Cancers and their Impact on Survival*, **Cancer Biology & Therapy**, 25(1), 2024, 235-249.
117. J. Yang, K. Guo, A. Zhang, Y. Zhu, W. Li, J. Yu, and P. Wang, *Survival Analysis of Age-Related Oral Squamous Cell Carcinoma: A Population Study Based on SEER*, **European Journal of Medical Research**, 28(1), 2023, 41-53.
118. B. S. Sheena, L. Hiebert, H. Han, H. Ippolito, M. Abbasi-Kangevari, Z. Abbasi-Kangevari, H. Abbastabar, A. Abdoli, H. Abubaker Ali, M. M. Adane, O. A. Adegboye, Q. E. S. Adnani, S. M. Advani, M. S. Afzal, S. Afzal, M. Aghaie Meybodi, B. Ahadinezhad, B. O. Ahinkorah, S. Ahmad, T. Ahmad, S. Ahmadi, H. Ahmed, M. B. Ahmed, T. Ahmed Rashid, G. T. Akalu, A. Aklilu, T. Akram, H. Al Hamad, F. Alahdab, A. Z. Alem, D. T. Alem, F. A. N. Alhalaiqa, R. K. Alhassan, L. Ali, M. A. Ali, Y. Alimohamadi, V. Alipour, M. Alkhayyat, S. Almustanyir, R. M. Al-Raddadi, H. Altawalrah, S. Amini, H. Amu, R. Ancuceanu, C. L. Andrei, T. Andrei, A. Anoushiravani, A. Ansar, A. E. Anyasodor, J. Arabloo, M. Arab-Zozani, A. M. Argaw, Z. G. Argaw, M. Arshad, A. A. Artamonov, T. Ashraf, D. Atlaw, F. Ausloos, M. Ausloos, S. Azadnajafabad, M. Azangou-Khyavy, A. Azari Jafari, G. Azarian, S. Bagheri, S. Bahadory, A. A. Baig, M. Banach, N. Barati, A. Barrow, A.-M. M. Batiha, D. F. Bejarano Ramirez, U. I. Belgaumi, A. Y. Berhie, D. S. Bhagat, N. Bhardwaj, P. Bhardwaj, K. Bhattacharyya, V. S. Bhojaraja, A. Bijani, A. Biondi, B. B. A. Bodicha, H. A. Bojia, A. Bloor, C. Bosetti, D. Braithwaite, N. I. Briko, Z. A. Butt, L. A. Cámara, R. C. Chakinala, P. A. Chakraborty, J. Charan, S. Chen, J.-Y. J. Choi, S. G. Choudhari, F. R. Chowdhury, D.-T.

Chu, S.-C. Chung, P. A. Cortesi, B. C. Cowie, G. T. Culbreth, O. Dadras, X. Dai, L. Dandona, R. Dandona, F. P. De La Hoz, S. A. Debela, M. G. Dedefo, F. M. Demeke, T. G. G. Demie, G. D. Demissie, M. Derbew Molla, A. A. Desta, D. Dhamnetiya, M. L. Dhimal, M. Dhimal, M. Didehdar, L. P. Doan, F. Dorostkar, T. M. Drake, F. Eghbalian, M. Ekholuenetale, I. El Sayed, M. El Sayed Zaki, M. Elhadi, M. A. Elmonem, A. Elsharkawy, S. Enany, D. B. Enyew, R. Erkhembayar, S. Eskandarieh, F. Esmaeilzadeh, S. Ezzikouri, H. Farrokhpour, G. Fetensa, F. Fischer, M. Foroutan, M. M. Gad, A. M. Gaidhane, S. Gaidhane, N. C. Galles, S. Gallus, T. G. Gebremeskel, E. A. Gebreyohannes, K. Ghadiri, K. Ghaffari, M. Ghafourifard, S.-H. Ghamari, A. Ghashghaee, A. Gholami, A. Gholizadeh, A. Gilani, A. Goel, M. Golechha, P. Goleij, D. Golinelli, G. Gorini, Y. A. Goshu, M. G. Griswold, M. I. M. Gubari, B. Gupta, S. Gupta, V. B. Gupta, V. K. Gupta, R. Haddadi, R. Halwani, S. S. Hamid, S. Hamidi, A. Hanif, S. Haque, H. Harapan, A. Hargono, S. Hariri, A. I. Hasaballah, S. M. M. Hasan, S. Hassanipour, H. Hassankhani, S. I. Hay, K. Hayat, G. Heidari, C. Herteliu, D. Z. Heyi, K. Hezam, R. Holla, M.-S. Hosseini, M. Hosseini, M. Hosseinzadeh, M. Hostiuc, M. Househ, J. Huang, N. R. Hussein, I. Iavicoli, S. E. Ibitoye, O. S. Ilesanmi, I. M. Ilic, M. D. Ilic, L. M. Irham, J. Y. Islam, N. E. Ismail, K. H. Jacobsen, F. Jadidi-Niaragh, A. Javadi Mamaghani, S. Jayaram, R. Jayawardena, R. Jebai, R. P. Jha, N. Joseph, F. Joukar, B. Kaambwa, A. Kabir, Z. Kabir, R. Kalhor, H. Kandel, T. K. T. Kanko, R. S. Kantar, I. M. Karaye, B. G. Kassa, P. M. Kemp Bohan, M. Keykhahi, Y. S. Khader, H. Khajuria, G. Khan, I. A. Khan, J. Khan, M. A. Khan, J. Khanali, A. M. Khater, M. N. Khatib, M. Khodadost, A. T. Khoja, O. Khosravizadeh, J. Khubchandani, G. R. Kim, H. Kim, M. S. Kim, Y. J. Kim, J. M. Kocarnik, A.-A. Kolahi, R. Koteeswaran, G. A. Kumar, C. La Vecchia, D. K. Lal, I. Landires, S. Lasrado, J. V. Lazarus, C. Ledda, D. W. Lee, S. Lee, Y. Y. Lee, M. Levi, J. Li, S. S. Lim, S. W. Lobo, P. D. Lopukhov, J. A. Loureiro, J. H. MacLachlan, H. Magdy Abd El Razek, M. Magdy Abd El Razek, A. Majeed, A. Makki, M.-R. Malekpour, R. Malekzadeh, A. A. Malik, F. Mansour-Ghanaei, M. A. Mansournia, F. R. Martins-Melo, P. C. Matthews, W. Mendoza, R. G. Menezes, T. J. Meretoja, A. G. Mersha, T. Mestrovic, T. R. Miller, L. H. N. Minh, A. Mirica, S. Mirmoeeni, E. M. Mirzakhimov, S. Misra, P. Mithra, B. Moazen, A. Mohamadkhani, M. Mohammadi, S. Mohammed, N. Moka, A. H. Mokdad, J. Moludi, S. Momtazmanesh, L. Monasta, G. Moradi, M. Moradzadeh, R. Moradzadeh, P. Moraga, E. Mostafavi, S. Mubarik, M. Muniyandi, C. J. L. Murray, M. Naghavi, M. D. Naimzada, S. Narasimha Swamy, Z. S. Natto, B. P. Nayak, J. Nazari, I. Negoii, S. M. Negru, S. A. Nejadghaderi, S. Neupane Kandel, H. L. T. Nguyen, C. H. Ngwa, R. K. Niazi, C. A. Nnaji, J. J. Noubiap, A. Nowroozi, V. Nuñez-Samudio, B. Oancea, C. Ochir, O. O. Odukoya, I.-H. Oh, A. T. Olagunju, B. O. Olakunde, A. Omar Bali, E. Omer, S. S. Otstavnov, B. Oumer, J. R. Padubidri, A. Pana, A. Pandey, E.-C. Park, F. Pashazadeh Kan, U. K. Patel, U. Paudel, I.-R. Petcu, Z. Z. Piracha, R. C. G. Pollok, M. J. Postma, A. Pourshams, H. Poustchi, M. Rabiee, N. Rabiee, A. Rafiei, S. Rafiei, P. M. Raghuram, M. Rahman, A. M. Rahmani, S. Rahmawaty, A. Rajesh, P. Ranasinghe, C. R. Rao, S. J. Rao, M. Rashidi, M.-M. Rashidi, D. L. Rawaf, S. Rawaf, R. Rawassizadeh, N. Rezaei, A. Rezapour, S. Rezazadeh-Khadem, J. A. B. Rodriguez, G. M. Rwegerera, S. Sabour, B. Saddik, M. R. Saeb, U. Saeed, A. Sahebkar, K. Saif-Ur-Rahman, S. Salahi, H. Salimzadeh, C. Sampath, A. M. Samy, J. Sanabria, F. Sanmarchi, M. M. Santric-Milicevic, A. Sarveazad, B. Sathian, M. Sawhney, A.-A. Seidu, S. G. Sepanlou, A. Seylani, S. Shahabi, M. A. Shaikh, E. Shaker, M. Z. Shakhmardanov, M. Shannawaz, S. M. Shenoy, J. K. Shetty, P. H. Shetty, K. Shibuya, J. I. Shin, P. Shobeiri, M. M. Sibhat, A. D. Singh, J. A. Singh, S. Singh, V. Y. Skryabin, A. A. Skryabina, A. A. Sohrabpour, S. Song, S. P. Tabaeian, E. G. Tadesse, M. Taheri, M. Tampa, K.-K. Tan, A. Tavakoli, A. Tbakhi, B. N. Tefera, A. Tehrani-Banihashemi, H. M. Tesfaw, R. Thapar, A. Thavamani, S. A. Tohidast, D. N. Tolloso, M. E. Tosti, M. R. Tovani-Palone, E. Traini, M. T. N. Tran, I. Trihandini, B. S. Tusa, I. Ullah, M. Vacante, S. Valadan Tahbaz, P. R. Valdez, S. B. Varthya, B. Vo, Y.

Waheed, A. B. Weldesenbet, M. Woldemariam, S. Xu, S. H. Yahyazadeh Jabbari, M. Yaseri, Y. Yeshaw, V. Yiğit, B. W. Yirdaw, N. Yonemoto, C. Yu, I. Yunusa, M. Zahir, L. Zaki, M. Zamani, M. Zamanian, M. S. Zastrozhin, T. Vos, J. W. Ward, and M. A. Dirac, *Global, Regional, and National Burden of Hepatitis B, 1990–2019: A Systematic Analysis for the Global Burden of Disease Study 2019*, **The Lancet Gastroenterology & Hepatology**, 7(9), 2022, 796–829.

119. Q. Xia, and S. Pierson, *HPV Infection and Oral Microbiota: Interactions and Future Implications*, **International Journal of Molecular Sciences**, 26(4), 2025, 14-24.

120. C. Bramati, S. Abati, S. Bondi, A. Lissoni, G. Arrigoni, F. Filipello, and M. Trimarchi, *Early Diagnosis of Oral Squamous Cell Carcinoma May Ensure Better Prognosis: A Case Series*, **Clinical Case Reports**, 9(10), 2021, 50-54.

121. A. D. Colevas, *HPV DNA as a Biomarker in Oropharyngeal Cancer: A Step in the Right Direction*, **Clinical Cancer Research: An Official Journal of the American Association for Cancer Research**, 28(19), 2022, 4171–4172.

122. C. Ekanayake Weeramange, Z. Liu, G. Hartel, Y. Li, S. Vasani, J. Langton-Lockton, L. Kenny, L. Morris, I. Frazer, K. D. Tang, and C. Punyadeera, *Salivary High-Risk Human Papillomavirus (HPV) DNA as a Biomarker for HPV-Driven Head and Neck Cancers*, **The Journal of Molecular Diagnostics**, 23(10), 2021, 1334–1342.

123. P. Kumar, S. Gupta, and B. C. Das, *Saliva as a Potential Non-Invasive Liquid Biopsy for Early and Easy Diagnosis/Prognosis of Head and Neck Cancer*, **Translational Oncology**, 40(1), 2024, 101-127.

124. F. Di Spirito, M. P. Di Palo, V. Folliero, D. Cannatà, G. Franci, S. Martina, and M. Amato, *Oral Bacteria, Virus and Fungi in Saliva and Tissue Samples from Adult Subjects with Oral Squamous Cell Carcinoma: An Umbrella Review*, **Cancers**, 15(23), 2023, 55-60.

125. Y. Zhang, G. D'Souza, C. Fakhry, E. O. Bigelow, M. Usyk, R. D. Burk, and N. Zhao, *Oral Human Papillomavirus Associated with Differences in Oral Microbiota Beta Diversity and Microbiota Abundance*, **The Journal of Infectious Diseases**, 226(6), 2022, 1098–1108.

126. K. R. Dahlstrom, A. G. Sikora, Y. Liu, C.-C. Chang, P. Wei, E. M. Sturgis, and G. Li, *Characterization of the Oral Microbiota Among Middle-Aged Men with and Without Human Papillomavirus Infection*, **Oral Oncology**, 142(1), 2023, 106-111.

127. D. Börnigen, B. Ren, R. Pickard, J. Li, E. Ozer, E. M. Hartmann, W. Xiao, T. Tickle, J. Rider, D. Gevers, E. A. Franzosa, M. E. Davey, M. L. Gillison, and C. Huttenhower, *Alterations in Oral Bacterial Communities are Associated with Risk Factors for Oral and Oropharyngeal Cancer*, **Scientific Reports**, 7(1), 2020, 176-186.

128. J.-M. Kutz, P. Rausche, T. Gheit, D. I. Puradiredja, and D. Fusco, *Barriers and Facilitators of HPV Vaccination in Sub-Saharan Africa: A Systematic Review*, **BMC Public Health**, 23(1), 2023, 97-104.

129. W. Zhang, Y. Yin, Y. Jiang, Y. Yang, W. Wang, X. Wang, Y. Ge, B. Liu, and L. Yao, *Relationship Between Vaginal and Oral Microbiome in Patients of Human Papillomavirus (HPV) Infection and Cervical Cancer*, **Journal of Translational Medicine**, 22(1), 2024, 39-46.

130. T. P. Makhalanyane, O. K. I. Bezuidt, R. E. Pierneef, E. Mizrachi, A. Zeze, R. K. Fossou, C. G. Kouadjo, S. Duodu, C. B. Chikere, O. O. Babalola, A. Klein, M. Keyster, M. Du Plessis, N. S. Yorou, M. Hijri, T. Rossouw, C. N. Kamutando, S. Venter, L. N. Moleleki, and C. Murrell, *African Microbiomes Matter*, **Nature Reviews Microbiology**, 21(8), 2023, 479–481
131. A. Esberg, N. Fries, S. Haworth, and I. Johansson, *Saliva Microbiome Profiling by Full-Gene 16S rRNA Oxford Nanopore Technology versus Illumina MiSeq Sequencing*, **NPJ Biofilms and Microbiomes**, 10(1), 2024, 149-150.
132. T. Nagai, T. Shiba, K. Komatsu, T. Watanabe, T. Nemoto, S. Maekawa, R. Kobayashi, S. Matsumura, Y. Ohsugi, S. Katagiri, Y. Takeuchi, and T. Iwata, *Optimal 16S rRNA Gene Amplicon Sequencing Analysis for Oral Microbiota to Avoid the Potential Bias Introduced by Trimming Length, Primer, and Database*, **Microbiology Spectrum**, 12(12), 2024, 351-363.

Chapter Three

Methodology

3.1 Research Design

This study examines baseline cross-sectional differences in oral microbiota between HPV positive and negative individuals within the HOMINY framework conducted at the University of Benin Teaching Hospital (UBTH), Nigeria¹. The HOMINY study investigates oral HPV incidence, persistence, and microbiome interactions in children/adolescents living with HIV (CALHIV), HIV-exposed uninfected (HEU), and HIV-unexposed uninfected (HUU) groups. From this cohort, 20 oral rinse samples were selected to compare taxonomic and functional microbiome differences between HPV-infected and uninfected individuals. The design aligns with the parent study's longitudinal framework but focuses on cross-sectional microbiome profiling at baseline.

3.2 Population of the Study

The study population derives from the HOMINY cohort¹, which enrolled mother-child pairs (HIV-infected [HI], HEU, HUU) in South-South Nigeria. Inclusion criteria for youth participants:

- i. **Age:** 9–18 years.
- ii. **HIV status:** Confirmed via PCR (HI/HEU) or rapid testing (HUU).
- iii. **Exclusion:** Recent antibiotic use (within 3 months), immunocompromised status (non-HIV), or severe systemic illness.

These participants, recruited from UBTH's specialized HIV care and general outpatient clinics, represent a diverse sample of urban and semiurban residents in South–South Nigeria. HIV status is a known significant modulator of the oral immune environment and

stratification is necessary to isolate the independent effect of HPV infection on the microbiome.

3.3 Study Area

The study was conducted at UBTH, Benin City, Nigeria, a tertiary hospital serving the South-South region. This area is characterized by high HIV prevalence, tropical climate, and diverse ethnic groups. Environmental and socio-cultural factors (e.g., oral hygiene practices, diet) were documented in the parent study and controlled for in analyses. The study area benefits from a stable patient referral network and offers a diverse demographic mix, which is critical for understanding regional differences in oral HPV epidemiology and microbiome composition.

3.4 Sampling Methods

3.4.1 Parent Cohort Recruitment

Participants were consecutively recruited at UBTH's dental, specialized HIV care, and general practice clinics between October 2022 and May 2023. The recruitment strategy included participants exposed to HIV perinatally but uninfected (HEU), participants infected with HIV and on highly active antiretroviral therapy (HI), HIV uninfected (HU) and HIV-unexposed and uninfected (HUU) participants. Sampling involved;

- i. Screening of children aged 9–18 years (and their mothers) for eligibility.
- ii. Age-matching and sex-matching of HI and HEU subjects with HUU controls (± 6 months).
- iii. Obtaining written informed consent/assent before enrollment.

3.4.2 Sub-Study Sample Selection

After baseline oral rinse specimens were collected and tested for HPV, 10 HPV-positive samples were identified. To form the control group, 10 HPV-negative samples were randomly selected from the remaining pool using simple random sampling. This approach minimizes selection bias and ensures the control group is representative of the HPV-negative segment within the larger cohort. Oral rinse samples were stored at -80 degrees, for transport, samples were stored in DNA/RNA Shield (Zymo Research, Irvine, USA) to stabilize the nucleic acid and transported on dry ice.

3.4.3 Sample Size Determination

A formal prospective sample-size calculation for high-dimensional microbiome outcomes (e.g., alpha/beta diversity metrics and feature-wise differential abundance) requires precise assumptions about expected effect sizes, variance and multiple-testing correction that are not well-established for oral HPV-associated microbiome shifts in this population. Therefore, this investigation was conducted as a nested pilot sub-study within the HOMINY cohort, with selection driven primarily by the number of HPV-positive oral rinse specimens available at baseline and feasibility considerations. The study population is highly specific, consisting of adolescents (ages 9–18) in South-South Nigeria with defined HIV and HPV co-infection statuses. This population is inherently difficult to recruit, and research on this group in Sub-Saharan Africa is scarce.

This sample size is therefore exploratory and intended to provide effect-size estimates and identify candidate taxa and functional features for future, adequately powered studies. Prior microbiome investigations of HPV- and cancer-associated oral microbiomes have been successfully reported from pilot datasets of similar or slightly larger sizes, supporting the utility of small, hypothesis-generating studies in this field¹⁸.

Where possible, statistical analyses were chosen or interpreted with awareness of limited statistical power. Results are presented primarily as exploratory and hypothesis-generating; p-

values are reported with effect sizes and, where appropriate, false-discovery-rate adjusted q-values. Findings that are nominally significant but do not survive multiple testing are interpreted cautiously and recommended for validation in larger cohorts.

3.5 Ethical Clearance

This study was approved by the Ethics Review Committee of the University of Benin Teaching Hospital (ADM/E22/A/VOL. VII/14813674). Written informed consent/assent was obtained from parents/guardians and participants. Data anonymized per Nigerian Health Research Ethics guidelines.

3.6 HIV Serostatus Determination

Status of HIV infection among adolescents in this cohort had been determined at infancy for all the HIV-infected (HI) and HIV exposed uninfected (HEU) participants in compliance with the World Health Organization (WHO) and Nigeria's Federal Ministry of Health (FMoH) Early Infant Diagnosis (EID) guidelines. For this research, adolescents and their mothers were reconfirmed for HIV serostatus in accordance with the Nigerian FMoH national HIV testing algorithm, which follows a tiered approach of rapid testing. A total of three rapid test kits were used in a sequence; the Determine™ (Abbott Diagnostics, Japan) was used as the first tier screening assay and was followed by a second line confirmation with the Uni-Gold™ (Trinity Biotech, Ireland) and the third test as the Stat-Pak® (ChemBio Diagnostics, USA) used for confirmation of discordant results.

3.7 DNA Extraction

DNA was extracted from oral rinse samples using Quick-DNA™ Miniprep Plus kit (Zymo Research; Cat. No. D4068). Briefly, 2 ml of each sample was centrifuged at 3,000 x g for 15 minutes at room temperature. The supernatant was discarded the pellet resuspended in 200 µl of DNA Elution Buffer and then treated with proteinase K. The digested samples were bound to Zymo-Spin™ IIC-XLR Columns, washed and then eluted in 50 µl DNA Elution. The

quality and quantity of the extracted DNA were measured using a NanoDrop (Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer). The system was blanked using 1ul of DNA Elution Buffer. Afterwards 1ul of the DNA was placed on the pedestal and measured. The concentration (ng/ul), A260/280 ratio and A260/230 ratio of the sample was subsequently recorded¹⁴.

3.8 HPV Genotyping

HPV genotyping was performed at Inqaba Biotec West Africa (Ibadan, Nigeria) using the Allplex™ HPV28 Detection assay (Seegene Inc., South Korea), a multiplex real-time PCR kit that detects and differentiates 28 human papillomavirus genotypes (19 high-risk types and 9 low-risk types) in a single assay. The Allplex™ HPV28 Detection assay consists of two multiplex primer-mix reactions (A-MOM and B-MOM). A-MOM targets 14 high-risk HPV (HR-HPV) types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. B-MOM targets 5 additional HR-HPV types (26, 53, 69, 73, 82) and 9 low-risk HPV (LR-HPV) types (6, 11, 40, 42, 43, 44, 54, 61, 70). An internal control (IC), based on a human housekeeping gene, is included to monitor DNA extraction efficacy, specimen adequacy, and the presence of PCR inhibitors. For each sample, aliquots of extracted 5 µL DNA were combined with the A-MOM or B-MOM master mix, enzyme mix (EM4, which contains DNA polymerase and uracil-DNA glycosylase to avoid carryover contamination), buffer, dNTPs, primers and probes, to the final assay volume of 20 µL as per manufacturer's protocol. Each run included positive controls (PC1, PC2, PC3 provided in the kit) and a no-template negative control to detect contamination. Amplification and detection were carried out on a Bio-Rad CFX96 (Bio-Rad, Hercules, California, USA) real-time PCR instrument. Fluorescence signals were monitored across multiple channels (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) depending on the target probes. Raw fluorescence data and Ct values were analyzed using

Seegene Viewer software (Seoul, South Korea). Genotype calls were reported for each HPV type detected.

3.9 16S rRNA Gene Sequencing

The genomic DNA was sent to Inqaba Biotechnical Industries, a commercial NGS service provider, for sequencing. The full-length 16S rRNA gene was amplified via PCR with the primer pair 27F (AGRGTTTGATYNTGGCTCAG) and 1492R (TASGGHTACCTTGTTASGACTT), with each primer modified to include sample-specific PacBio barcodes. This barcode tagging facilitates the multiplexing of samples during sequencing. After PCR, the individual amplicons were quantified and pooled in equal proportions to create a composite sample for library preparation.

SMRTbell libraries were constructed from the pooled, barcoded amplicons using the SMRTbell Express Template Prep Kit 2.0, following the manufacturer's guidelines. The prepared libraries were sequenced on a PacBio Revio platform using the binding kit 3.2. Raw subreads obtained from the sequencing run were processed by generating circular consensus sequences (CCS) with SMRT Link version 8.0, which enhances the accuracy of the reads.

Following sequencing, the CCS reads were demultiplexed using Lima software (version 1.7.0) by assigning them to their corresponding samples based on the barcode information. Primer sequences were subsequently trimmed from the reads using Cutadapt software (version 1.9.1), with length filtering applied to retain only the desired amplicon regions. Finally, any chimeric sequences that may have been generated during PCR were identified and removed using UCHIME (version 8.1), resulting in a final set of high-quality, non-chimeric CCS sequences ready for further bioinformatic analysis⁴.

3.10 Bioinformatics Analysis

High-quality circular consensus sequences (CCS) generated from the PacBio Revio platform were processed using the *HiFi-16S-workflow* developed by Pacific Biosciences². Raw CCS reads were subjected to quality filtering to remove low-quality sequences, followed by primer sequence removal using Cutadapt to retain only the target full-length 16S rRNA gene regions³. Chimeric sequences—artefacts introduced during PCR—were detected and removed with UCHIME, producing a curated set of non-chimeric CCS reads⁴. Amplicon sequence variants (ASVs) were inferred using the DADA2 algorithm within the QIIME 2 framework, preserving single-nucleotide resolution and avoiding the loss of fine-scale sequence variation inherent in OTU clustering. Taxonomic classification was performed using the expanded Human Oral Microbiome Database (eHOMD) 16S rRNA RefSeq Version 16.02 taxonomy file in conjunction with the corresponding reference sequence database, enabling accurate taxonomic resolution of oral microbial communities at the phylum, genus, and species levels⁷.

Diversity analyses were conducted in QIIME2 (Posit Software, PBC, version 2025.5.1.513, release *Mariposa Orchid*)⁵. Alpha diversity was quantified using indices such as Chao1, Shannon, Faith's phylogeny and Pielou's evenness. Beta diversity was assessed using Jaccard index and both weighted and unweighted UniFrac distances to compare microbial community structure between samples, particularly between HPV-positive and HPV-negative groups.

Differential abundance analyses were performed in RStudio (v4.5.1) using the ANCOM-BC2 package, with models adjusted for potential confounders (age and sex) and, where applicable, stratified by HIV status. Taxonomic richness of various groups, low (below 1%) and high (above 1%) abundance distribution of various phyla were determined. Functional potential was inferred using PICRUST2 (run via the Galaxy platform), with KEGG Orthology (KO),

Enzyme Commission (EC), and MetaCyc pathway predictions annotated for biological interpretation^{10–12}. All downstream visualization, statistical modelling, and figure preparation were carried out in R using *tidyverse*, *ggplot2*, and related packages^{13,14}.

3.11 Statistical Analysis

Statistical analyses were performed in RStudio (v4.5.1). Alpha diversity differences between HPV-positive and HPV-negative groups were assessed using the Mann–Whitney U test. Beta diversity differences were evaluated via PERMANOVA, applied to Bray–Curtis and UniFrac distance matrices.

Differential abundance testing was conducted with ANCOM-BC2, incorporating univariable and multivariable models⁹. Univariable analyses were used for initial screening, followed by multivariable models adjusting for age, sex, and—where relevant—HIV status. False Discovery Rate (FDR) correction was applied to control for multiple testing, and statistical significance was set at $p < 0.05$ (two-sided).

Functional profiling results from PICRUST2 were analyzed in R to identify significantly different KOs, ECs, and MetaCyc pathways between study groups. KEGG pathway mapping and annotation were integrated for biological interpretation. Data visualization included bar plots of relative abundance, volcano plots for effect size vs. significance, and heatmaps to depict patterns of functional enrichment¹¹.

Endnotes

1. E. Osagie, P. Akhigbe, N. Idemudia, O. Obuekwe, R. Adebisi, N. Schlecht, J. Liu, Y. Bromberg, F. E. Eki-Udoko, N. Osazuwa-Peters, and M. O. Coker, *Human Papillomavirus, Human Immunodeficiency Virus, and Oral Microbiota Interplay in Nigerian Youth (HOMINY): A Prospective Cohort Study Protocol*, **BMJ Open**, 15(2), 2025, 10-17.
2. K. P. Chua, and F. Ripp, *HiFi-16S-workflow: Nextflow Pipeline to Analyze Pacbio HiFi Full-Length 16S Data*, **Pacific Biosciences**, 2(1), 2024, 44-47.
3. M. Martin, *Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads*. **EMBnet Journal** 17(1), 2011, 10.
4. R. C. Edgar, B. J. Haas, J. C. Clemente, C. Quince, and R. Knight, *UCHIME Improves Sensitivity and Speed of Chimera Detection*, **Bioinformatics**, 27(16), 2011, 2194–2200.
5. E. Bolyen, J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciulek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. Van Der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. Von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso, *Reproducible, Interactive, Scalable and Extensible Microbiome Data Science using QIIME 2*, **Nature Biotechnology**, 37(8), 2019, 852–857.
6. B. J. Callahan, P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes, *DADA2: High-Resolution Sample Inference from Illumina Amplicon Data*, **Nature Methods**, 13(7), 2016, 581–583.
7. I. F. Escapa, Y. Huang, T. Chen, M. Lin, A. Kokaras, F. E. Dewhirst, and K. P. Lemon, *Construction of habitat-specific training sets to achieve species-level assignment in 16S rRNA gene datasets*, **Microbiome**, 8(1), 2020, 65.
8. Posit team, *RStudio: Integrated Development Environment for R*. Boston, **Posit Software, PBC**, 3(2), 2025, 1-29.
9. H. Lin, and S. D. Peddada, *Analysis of Compositions of Microbiomes with Bias Correction*. **Nature Communications**, 11(1), 2020, 35-44.

10. G. M. Douglas, V. J. Maffei, J. R. Zaneveld, S. N. Yurgel, J. R. Brown, C. M. Taylor, C. Huttenhower, and M. G. I. Langille, *PICRUSt2 for Prediction of Metagenome Functions*, **Nature Biotechnology**, 38(6), 2020, 685–688.
11. M. Kanehisa, S. Goto, Y. Sato, M. Furumichi, and M. Tanabe, *KEGG for Integration And Interpretation of Large-Scale Molecular Data Sets*, **Nucleic Acids Research**, 40(1), 2012, 109–114.
12. L. A. L. Abueg, E. Afgan, O. Allart, A. H. Awan, W. A. Bacon, D. Baker, M. Bassetti, B. Batut, M. Bernt, D. Blankenberg, A. Bombarely, A. Bretaudeau, C. J. Bromhead, M. L. Burke, P. K. Capon, M. Čech, M. Chavero-Díez, J. M. Chilton, T. J. Collins, F. Coppens, N. Coraor, G. Cuccuru, F. Cumbo, J. Davis, P. F. De Geest, W. De Koning, M. Demko, A. DeSanto, J. M. D. Begines, M. A. Doyle, B. Driesbeke, A. Erxleben-Eggenhofer, M. C. Föll, G. Formenti, A. Fouilloux, R. Gangazhe, T. Genthon, J. Goecks, A. N. G. Beltran, N. A. Goonasekera, N. Goué, T. J. Griffin, B. A. Grüning, A. Guerler, S. Gundersen, O. J. R. Gustafsson, C. Hall, T. W. Harrop, H. Hecht, A. Heidari, T. Heisner, F. Heyl, S. Hiltemann, H.-R. Hotz, C. J. Hyde, P. D. Jagtap, J. Jakiela, J. E. Johnson, J. Joshi, M. Jossé, K. Jum'ah, M. Kalaš, K. Kamieniecka, T. Kayikcioglu, M. Konkol, L. Kostykin, N. Kucher, A. Kumar, M. Kuntz, D. Lariviere, R. Lazarus, Y. L. Bras, G. L. Corguillé, J. Lee, S. Leo, L. Liborio, R. Libouban, D. L. Taberner, L. Lopez-Delisle, L. S. Los, A. Mahmoud, I. Makunin, P. Marin, S. Mehta, W. Mok, P. A. Moreno, F. Morier-Genoud, S. Mosher, T. Müller, E. Nasr, A. Nekrutenko, T. M. Nelson, A. J. Oba, A. Ostrovsky, P. V. Polunina, K. Poterlowicz, E. J. Price, G. R. Price, H. Rasche, B. Raubenolt, C. Royaux, L. Sargent, M. T. Savage, V. Savchenko, D. Savchenko, M. C. Schatz, P. Seguinéau, B. Serrano-Solano, N. Soranzo, S. K. Srikakulam, K. Suderman, A. E. Syme, M. A. Tangaro, J. A. Tedds, M. Tekman, W. Cheng (Mike) Thang, A. S. Thanki, M. Uhl, M. Van Den Beek, D. Varshney, J. Vessio, P. Videm, G. Von Kuster, G. R. Watson, N. Whitaker-Allen, U. Winter, M. Wolstencroft, F. Zambelli, P. Zierep, and R. Zoabi, *The Galaxy Platform for Accessible, Reproducible, and Collaborative Data Analyses: 2024 update*, **Nucleic Acids Research** 52(1), 2024, 83–94.
13. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis*, **Springer-Verlag New York**, 3(2), 2016, 67–87.
14. H. Wickham, M. Averick, J. Bryan, W. Chang, L. D. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. L. Pedersen, E. Miller, S. M. Bache, K. Müller, J. Ooms, D. Robinson, D. P. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, and H. Yutani, *Welcome to the Tidyverse*, **Journal of Open-Source Software**, 4(43), 2019, 76–86.
15. P. Desjardins, D. Conklin, *NanoDrop Microvolume Quantitation of Nucleic Acids*, **Journal of Visualized Experiments**, 1(45), 2020, 25–65.
16. M. Zakrzewski, O. M. Gannon, B. J. Panizza, N. A. Saunders, and A. Antonsson, *Human Papillomavirus Infection and Tumor Microenvironment are Associated with the Microbiota in Patients with Oropharyngeal Cancers—Pilot Study*, **Head and Neck**, 43(11), 2021, 3324–3330.

Chapter Four

Results and Discussion of Findings

4.1 Results

4.1.1 Study Population

The study included 21 individuals, with samples collected from oral sites to assess HPV infection. Of these, 14 individuals (66.7%) were living with HIV (HIV+), and 7 (33.3%) were HIV-negative (HIV-), including HIV-uninfected (HUU), HIV-unexposed (HU), and HIV-exposed uninfected (HEU) individuals. Among the 14 HIV+ individuals, 7 harbored HPV infections (HIV+/HPV+), including high-risk and low-risk HPV genotypes, while the remaining 7 were HPV-negative (HIV+/HPV-), serving as controls. Of the 7 HIV- individuals, 3 had oral HPV infections (HIV-/HPV+), and 4 were HPV-negative (HIV-/HPV-), representing the respective controls. The characteristics of the four study groups, stratified by HIV status and oral HPV infection, are summarized in Table 4.1. Among the HIV+ individuals, the HIV+/HPV+ group (n=7) included 4 children (Under 18, 3 males, 1 female) and 3 mothers (Over 18, all female), while the HIV+/HPV- group (n=7) comprised 5 children (Under 18, 4 males, 1 female) and 2 mothers (Over 18, all female). Among the HIV- individuals, the HIV-/HPV+ group (n=3) consisted entirely of children (Under 18, all female), and the HIV-/HPV- group (n=4) included 2 children (Under 18, 1 male, 1 female) and 2 mothers (Over 18, all female). The study population was predominantly female among mothers (Over 18, n=6, all female), reflecting the mother-child pair design, with a mix of males (n=9) and females (n=6) among children (Under 18). Most HIV+ individuals were under 18 (9/14) and HIV- individuals split between children (5/7) and mothers (2/7). The absence of males in the Over 18 group (mothers) is a noted limitation of the study design. Additional clinical variables, such as cART status, CD4+ T-cell counts, or behavioral factors,

were not available for this analysis but would be relevant for further characterizing group differences.

Table 4.1: Demographic Distribution of Participants by HIV Status and Oral HPV Infection, Highlighting Age and Sex Composition

Characteristic	HIV+/HPV+ (n=7)	HIV+/HPV- (n=7)	HIV-/HPV+ (n=3)	HIV-/HPV- (n=4)
Age Category				
Under 18 (Children)	4 (57.1%)	6 (85.7%)	3 (100.0%)	2 (50.0%)
Male (Children)	3 (42.9%)	5 (71.4%)	0 (0.0%)	1 (25.0%)
Female (Children)	1 (14.3%)	1 (14.3%)	3 (100.0%)	3 (75.0%)
Over 18 (Mothers)	3 (42.9%)	1 (14.3%)	0 (0.0%)	2 (50.0%)

Laboratory Work, 2025

Key:

HIV+ / HIV-: Indicates participants who tested positive or negative for HIV.

HPV+ / HPV-: Indicates participants who tested positive or negative for HPV

Under 18 (Children): Participants aged below 18 years.

Over 18 (Mothers): Adult female participants aged 18 years and above.

Male (Children): Male participants below 18 years of age.

Female (Children): Female participants below 18 years of age.

n: Number of participants in each subgroup.

Values in parentheses (%): Represent the proportion of individuals within each subgroup relative to the total number in that category.

Lead City University Ibadan DO NOT COPY

4.1.2 HPV Genotypes in Oral Infections

Among the 7 HIV+/HPV+ subjects, the following HPV genotypes were detected in oral samples: HPV16 (1, 14.3%), HPV39 (1, 14.3%), HPV59 (1, 14.3%), HPV35 (1, 14.3%), HPV52 (1, 14.3%), HPV53 (1, 14.3%), HPV40 (1, 14.3%), HPV42 (1, 14.3%), and HPV82 (1, 14.3%) (Figure 4.1). Of these, high-risk HPV genotypes (HPV16, HPV35, HPV39, HPV52, HPV53, HPV59) were present in 6 subjects (85.7%), with one subject harboring both high-risk and low-risk genotypes (HPV35 and HPV82). Among the 3 HIV-/HPV+ individuals, the following genotypes were found: HPV51 (1, 33.3%), HPV40 (1, 33.3%), and HPV61 (1, 33.3%). Of these, high-risk HPV (HPV51) was detected in 1 subject (33.3%), with the remaining 2 subjects harboring low-risk genotypes (HPV40, HPV61).

4.1.3 Alpha Diversity

4.1.3.1 Chao1 Richness Estimator

Chao1 richness estimates were compared across HIV/HPV categories using a linear model adjusted for age and sex (Figure 4.2). The model explained ~40% of the variation in richness ($R^2 = 0.398$), though this was not statistically significant ($F(5,14) = 1.85$, $p = 0.168$). The HIV-/HPV+ group had the highest adjusted mean richness (emmean = 896.0, 95% CI: 510.1–1281.9), followed by HIV+/HPV- (676.98, 95% CI: 503.9–850.0), HIV+/HPV+ (664.1, 95% CI: 506.3–822.0), and the HIV-/HPV- reference group (440.1, 95% CI: 205.4–674.8). The HIV-/HPV+ group had significantly higher Chao1 richness compared to the HIV-/HPV- group ($\beta = 455.9$, 95% CI: 0.98–911, $p = 0.050$). The differences observed for the HIV+/HPV- ($\beta = 236.9$, $p = 0.11$) and HIV+/HPV+ ($\beta = 224.0$, $p = 0.11$) groups were not statistically significant.

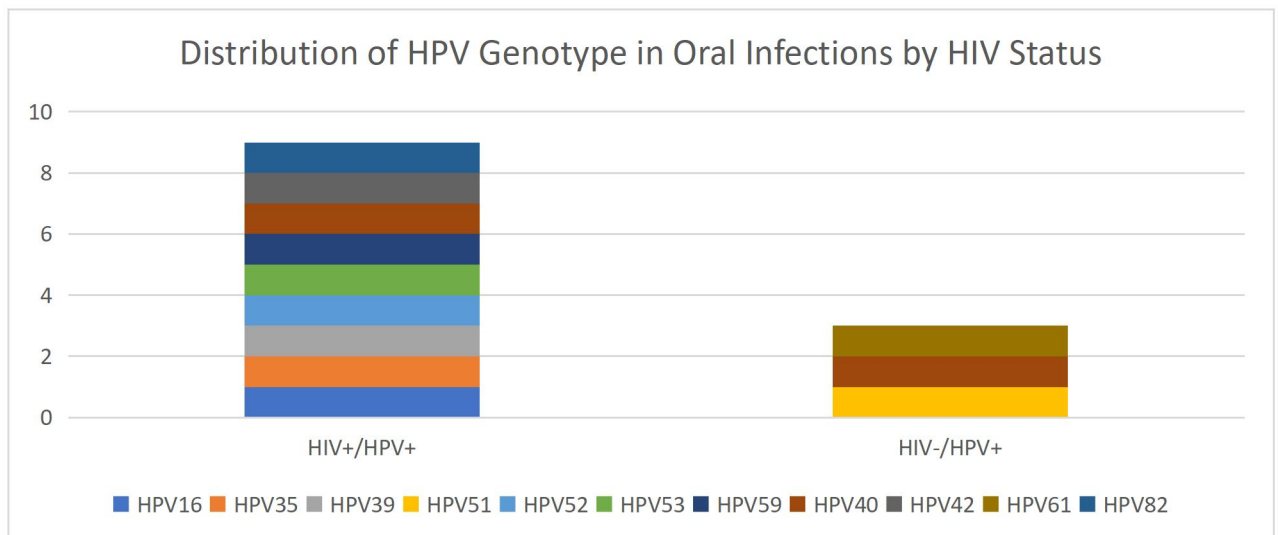


Figure 4.1: Distribution of HPV Genotype in Oral Infections by HIV Status

Author's Laboratory Work, 2025

Participants under 18 years had lower Chao1 richness compared to those over 18 ($\beta = -328.5$, 95% CI: -671 to 14 , $p = 0.059$), approaching statistical significance. No significant association was observed for sex ($p = 0.63$). Tukey-adjusted pairwise comparisons showed no statistically significant differences between group pairs ($p > 0.18$ for all). Likewise, a Kruskal–Wallis test found no overall significant difference across groups ($\chi^2 = 1.97$, $df = 3$, $p = 0.580$).

4.1.3.2 Shannon Diversity and HIV/HPV Status

Model-estimated mean Shannon diversity was highest in the HIV⁻/HPV⁺ group (8.13, 95% CI: 6.95–9.32), followed by HIV⁺/HPV⁺ (7.21, 95% CI: 6.73–7.70), HIV⁺/HPV⁻ (7.15, 95% CI: 6.62–7.68), and lowest in the HIV⁻/HPV⁻ group (6.60, 95% CI: 5.88–7.32). After adjusting for age and sex, individuals in the HIV⁻/HPV⁺ group had significantly higher Shannon diversity compared to the HIV⁻/HPV⁻ reference group ($\beta = 1.53$, 95% CI: 0.14–2.9, $p = 0.033$). No statistically significant differences were observed between the HIV⁻/HPV⁻ group and either the HIV⁺/HPV⁻ ($\beta = 0.55$, 95% CI: -0.37 – 1.5 , $p = 0.22$) or HIV⁺/HPV⁺ groups ($\beta = 0.61$, 95% CI: -0.26 – 1.5 , $p = 0.15$).

Neither age category nor sex was significantly associated with Shannon diversity. However, individuals under 18 years had slightly lower diversity ($\beta = -0.76$, 95% CI: -1.8 – 0.28 , $p = 0.14$), and males had slightly higher diversity ($\beta = 0.25$, 95% CI: -0.71 – 1.2 , $p = 0.58$), though these effects were not statistically significant (Figure 4.3).

Pairwise comparisons between HIV/HPV groups showed no statistically significant differences after multiple testing correction. The largest adjusted mean difference was observed between the HIV⁻/HPV⁺ and HIV⁻/HPV⁻ groups ($p = 0.13$), but this did not reach statistical significance after Tukey adjustment. A non-parametric Kruskal–Wallis test

supported the overall model trend but was not statistically significant ($\chi^2 = 4.36$, $df = 3$, $p = 0.225$).

Lead City University Ibadan DO NOT COPY

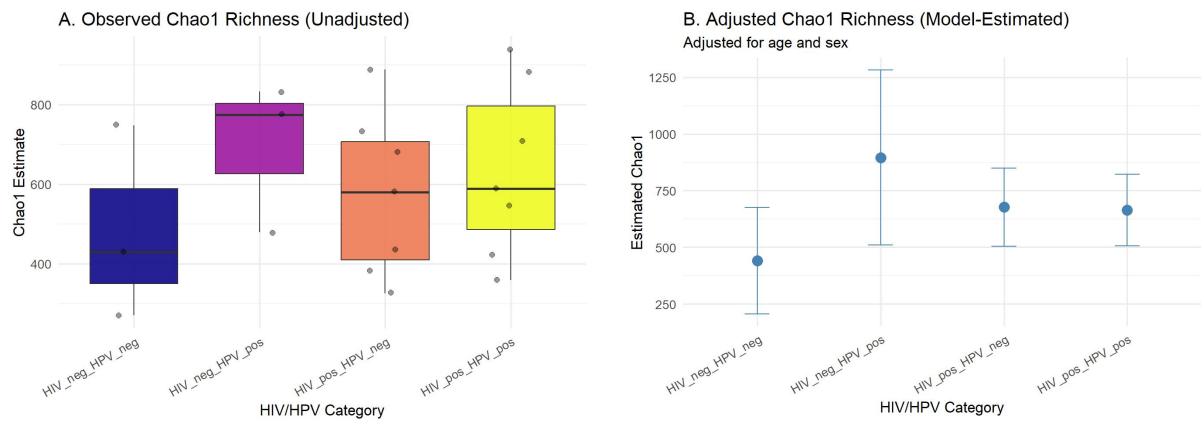


Figure 4.2: Panel A Shows Unadjusted Chao1 Richness Across HIV/HPV Groups. Panel B Presents Model-Estimated Means Adjusted for Age and Sex, with 95% Confidence Intervals
Author's Laboratory Work, 2025

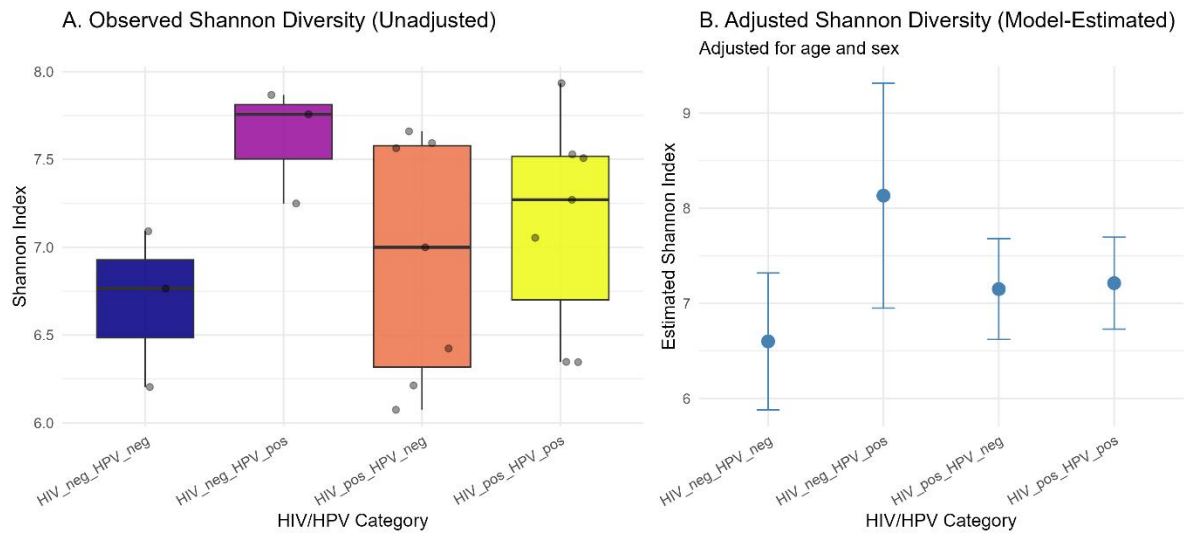


Figure 4.3: Panel A Shows Unadjusted Shannon Diversity Across HIV/HPV Groups. Panel B Presents Model-Estimated Means Adjusted for Age and Sex, with 95% Confidence Intervals
Author's Laboratory Work 2025

4.1.3.3 Shannon Diversity and HIV/HPV Status

Model-estimated mean Shannon diversity was highest in the HIV⁻/HPV⁺ group (8.13, 95% CI: 6.95–9.32), followed by HIV⁺/HPV⁺ (7.21, 95% CI: 6.73–7.70), HIV⁺/HPV⁻ (7.15, 95% CI: 6.62–7.68), and lowest in the HIV⁻/HPV⁻ group (6.60, 95% CI: 5.88–7.32). After adjusting for age and sex, individuals in the HIV⁻/HPV⁺ group had significantly higher Shannon diversity compared to the HIV⁻/HPV⁻ reference group ($\beta = 1.53$, 95% CI: 0.14–2.9, $p = 0.033$). No statistically significant differences were observed between the HIV⁻/HPV⁻ group and either the HIV⁺/HPV⁻ ($\beta = 0.55$, 95% CI: -0.37–1.5, $p = 0.22$) or HIV⁺/HPV⁺ groups ($\beta = 0.61$, 95% CI: -0.26–1.5, $p = 0.15$).

Neither age category nor sex was significantly associated with Shannon diversity. However, individuals under 18 years had slightly lower diversity ($\beta = -0.76$, 95% CI: -1.8–0.28, $p = 0.14$), and males had slightly higher diversity ($\beta = 0.25$, 95% CI: -0.71–1.2, $p = 0.58$), though these effects were not statistically significant (Figure 4.3).

Pairwise comparisons between HIV/HPV groups showed no statistically significant differences after multiple testing correction. The largest adjusted mean difference was observed between the HIV⁻/HPV⁺ and HIV⁻/HPV⁻ groups ($p = 0.13$), but this did not reach statistical significance after Tukey adjustment. A non-parametric Kruskal–Wallis test supported the overall model trend but was not statistically significant ($\chi^2 = 4.36$, $df = 3$, $p = 0.225$).

4.1.3.4 Faith's Phylogenetic Diversity

Faith's Phylogenetic Diversity (PD) was evaluated across four HIV/HPV co-infection categories, using a linear regression model adjusted for age and sex (Figure 4.4). The model explained approximately 42% of the variance in Faith's PD ($R^2 = 0.42$), though the overall effect was not statistically significant ($F(5,14) = 2.06$, $p = 0.13$).

The HIV⁻/HPV⁺ group exhibited the highest estimated mean Faith's PD (emmean = 24.78, 95% CI: 16.90–32.66), followed by HIV⁺/HPV⁺ (17.87, 95% CI: 14.65–21.10), HIV⁺/HPV⁻ (17.65, 95% CI: 14.11–21.18), and HIV⁻/HPV⁻ (15.84, 95% CI: 11.05–20.63). Compared to the reference group (HIV⁻/HPV⁻), the HIV⁻/HPV⁺ group had a notably higher PD ($\beta = 8.94$, 95% CI: -0.35 to 18.0, $p = 0.058$), which approached statistical significance. Differences for the HIV⁺/HPV⁺ ($\beta = 2.03$, $p = 0.46$) and HIV⁺/HPV⁻ ($\beta = 1.81$, $p = 0.54$) groups were smaller and not statistically significant. Among the covariates, age showed a significant inverse association with diversity: participants under 18 years had significantly lower Faith's PD than those over 18 ($\beta = -7.91$, 95% CI: -15.0 to -0.92, $p = 0.029$). No significant effect of sex was observed ($\beta = 2.77$, $p = 0.37$). Pairwise comparisons using Tukey-adjusted emmeans did not identify any statistically significant differences between groups ($p > 0.20$ for all comparisons), although the contrast between HIV⁻/HPV⁺ and HIV⁻/HPV⁻ showed the largest difference in magnitude ($\Delta = 8.94$, $p = 0.21$). A non-parametric Kruskal–Wallis test supported the lack of overall group differences ($\chi^2 = 1.96$, $df = 3$, $p = 0.58$).

4.1.3.5 Pielou's Evenness

Pielou's Evenness, a measure of community evenness, was assessed across four HIV/HPV status categories using linear regression adjusted for age and sex (Figure 4.5). The model explained approximately 21% of the variability in evenness ($R^2 = 0.21$), but the overall model was not statistically significant ($F(5,14) = 0.76$, $p = 0.593$). Adjusted mean evenness estimates indicated the highest value in the HIV⁻/HPV⁺ group (emmean = 0.837, 95% CI: 0.751–0.923), followed by HIV⁺/HPV⁺ (0.786, 95% CI: 0.751–0.821), HIV⁺/HPV⁻ (0.774, 95% CI: 0.736–0.813), and HIV⁻/HPV⁻ (0.768, 95% CI: 0.715–0.820). Although the HIV⁻/HPV⁺ group showed a trend toward higher evenness compared to the reference group (HIV⁻/HPV⁻), the difference was not statistically significant ($\beta = 0.069$, 95% CI: -0.03 to 0.17, $p = 0.166$). None of the other comparisons reached statistical significance.

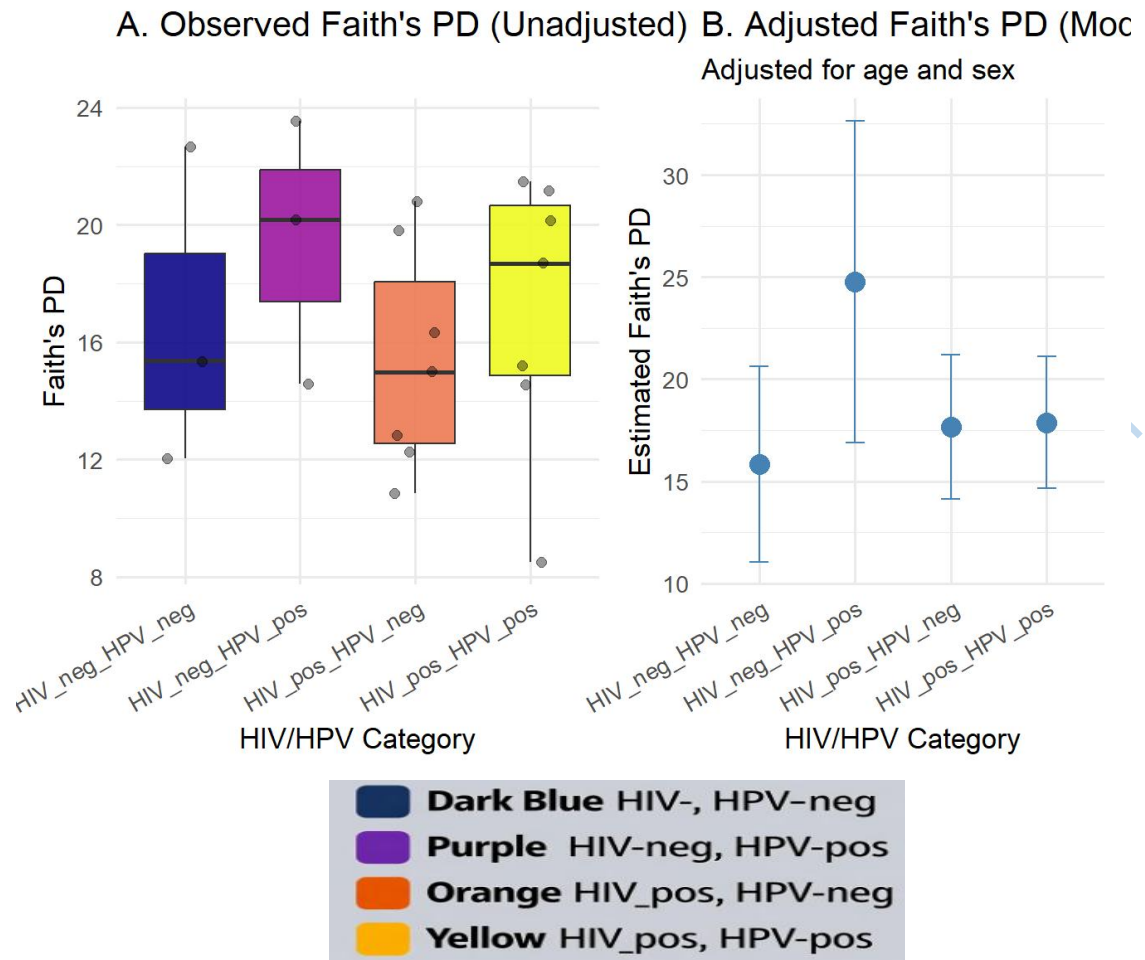


Figure 4.4: Panel A Shows Unadjusted Faith's Phylogenetic Diversity Across HIV/HPV Groups. Panel B Presents Model-Estimated Means Adjusted for Age and Sex, with 95% Confidence Intervals

Author's Laboratory Work, 2025

Neither age category nor sex had a significant impact on evenness. Participants under 18 years had slightly lower evenness compared to those over 18 ($\beta = -0.022$, $p = 0.552$), and males had slightly higher evenness compared to females ($\beta = 0.013$, $p = 0.708$), but both effects were minimal and non-significant. Pairwise comparisons among groups using Tukey adjustment showed no statistically significant differences ($p > 0.42$ for all contrasts). Similarly, a non-parametric Kruskal–Wallis test revealed no significant difference in Pielou’s Evenness across groups ($\chi^2 = 1.64$, $df = 3$, $p = 0.651$).

4.1.4 Beta Diversity

4.1.4.1 Beta Diversity Analysis using Bray–Curtis dissimilarity

To assess differences in overall microbial community composition across HIV/HPV categories, beta diversity was analyzed using Bray–Curtis dissimilarity. A permutational multivariate analysis of variance (PERMANOVA), adjusted for age and sex, revealed no statistically significant differences between HIV/HPV categories ($R^2 = 0.154$, $F = 0.97$, $p = 0.658$). Similarly, neither age ($R^2 = 0.053$, $p = 0.491$) nor sex ($R^2 = 0.055$, $p = 0.345$) were significantly associated with differences in community composition. The model explained approximately 26% of the variance in beta diversity, with the majority (74%) attributed to residual variation. Principal Coordinates Analysis (PCoA) was used to visualize Bray–Curtis dissimilarity across samples (Figure 4.6). The first two axes explained a moderate proportion of the total variance (PCoA1: 8.6% %, PCoA2: 8.2%). Although some group-wise clustering was observed, 95% confidence ellipses showed considerable overlap between HIV/HPV categories, consistent with the non-significant PERMANOVA results.

4.1.4.2 Jaccard Index

To evaluate microbial community composition based on presence–absence data, beta diversity was analyzed using the Jaccard distance metric.

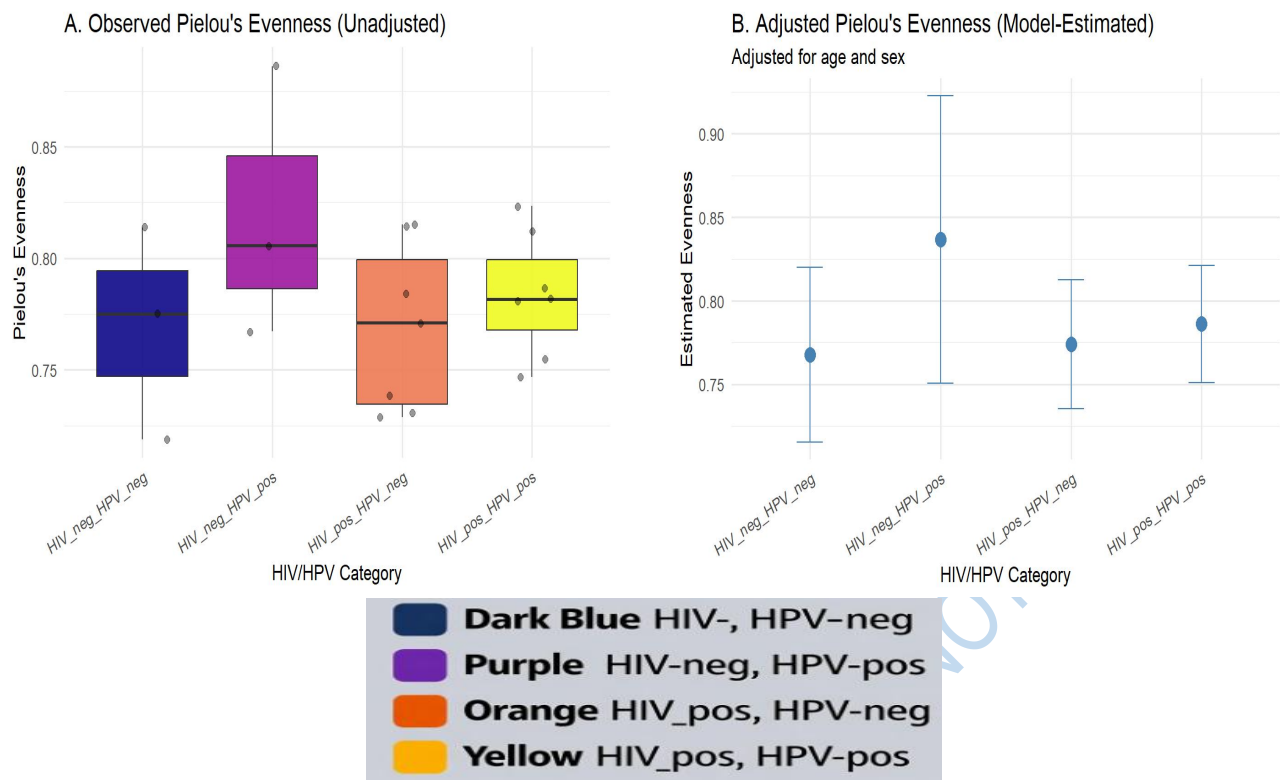


Figure 4.5: Panel A Shows Unadjusted Pielou's Evenness Across HIV/HPV Groups. Panel B Presents Model-Estimated Means Adjusted for Age and Sex, with 95% Confidence Intervals
Author's Laboratory Work, 2025

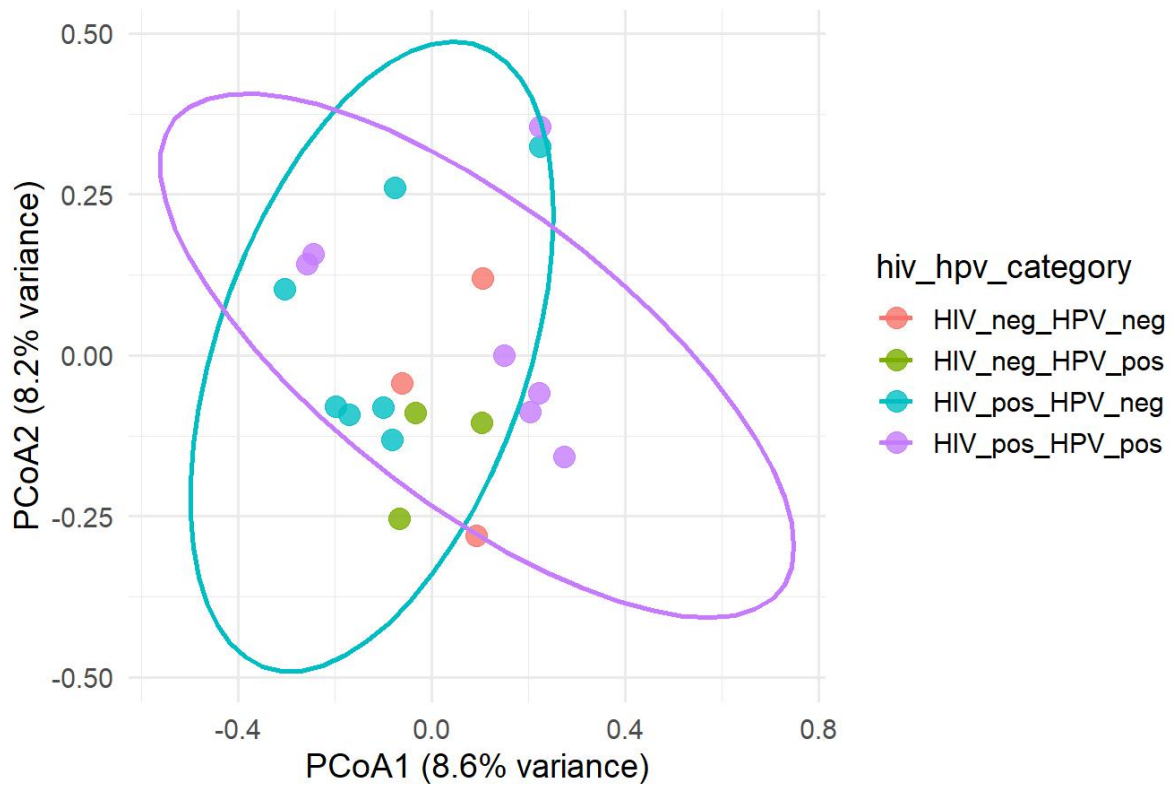


Figure 4.6: Principal Coordinates Analysis (Pcoa) Based on Bray–Curtis Dissimilarity was used to Explore the Beta Diversity of Microbiomes in Oral Samples
Author's Laboratory Work, 2025

A permutational multivariate analysis of variance (PERMANOVA) was performed to assess differences between HIV/HPV categories while adjusting for age and sex. The analysis revealed no statistically significant differences in community structure among HIV/HPV groups ($R^2 = 0.155$, $F = 0.98$, $p = 0.819$). Similarly, neither age category ($R^2 = 0.053$, $p = 0.424$) nor sex ($R^2 = 0.053$, $p = 0.421$) were significantly associated with variation in beta diversity. The model accounted for approximately 26% of the total variance, with the majority (74%) attributed to unexplained residual variation (Figure 4.7).

To visualize community patterns, Principal Coordinates Analysis (PCoA) was conducted on the Jaccard distance matrix. The first two principal coordinate axes explained a moderate portion of the variation in community composition (PCoA1: 7%, PCoA2: 6.4%). Although slight visual clustering of samples was observed, 95% confidence ellipses showed substantial overlap between HIV/HPV categories, consistent with the non-significant PERMANOVA findings.

4.1.4.3 Unweighted UniFrac Beta Diversity

To assess phylogenetic-based community composition using presence/absence data, beta diversity was computed using unweighted UniFrac distance. A PERMANOVA test adjusting for age and sex showed no significant differences in microbial community structure across HIV/HPV categories ($R^2 = 0.127$, $F = 0.76$, $p = 0.933$). Neither age ($R^2 = 0.052$, $p = 0.517$) nor sex ($R^2 = 0.040$, $p = 0.872$) significantly contributed to variation in beta diversity (Figure 4.8). The model explained approximately 22% of the total variance, with most variation (78%) attributed to residual effects.

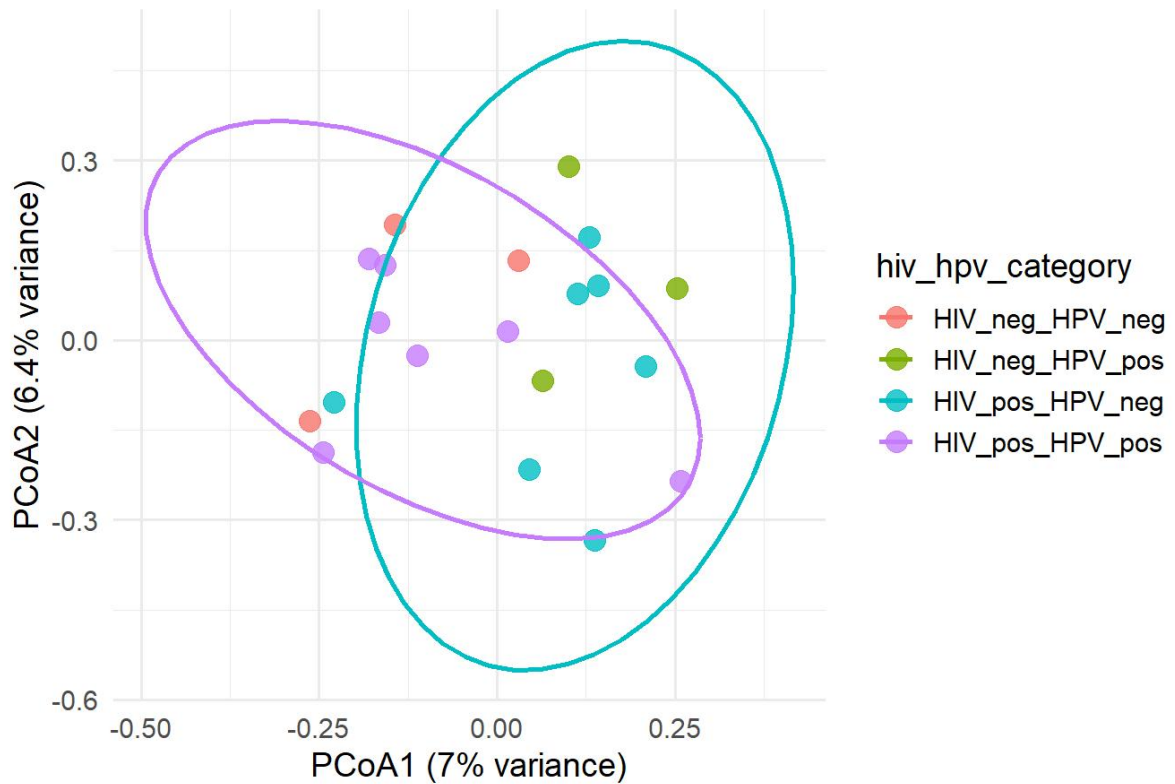


Figure 4.7: The Variation in Oral Microbiomes (Beta Diversity) was Explored Through PCoA using the Jaccard Index as the Distance Measure (95% Confidence Interval)
 Author's Laboratory Work, 2025

Principal Coordinates Analysis (PCoA) based on the unweighted UniFrac distance matrix revealed moderate separation between groups on the first two principal coordinate axes, which explained 20.6% and 12.7% of the variance, respectively. However, 95% confidence ellipses displayed substantial overlap among HIV/HPV categories, consistent with the non-significant PERMANOVA findings.

4.1.4.4 Weighted UniFrac Beta Diversity

Beta diversity analysis using weighted UniFrac distance, which incorporates both taxonomic abundance and phylogenetic relatedness, did not reveal significant differences in microbial community composition across HIV/HPV groups. PERMANOVA results adjusting for age and sex showed no statistically significant effect of HIV/HPV category ($R^2 = 0.14$, $F = 0.85$, $p = 0.539$), age category ($R^2 = 0.05$, $p = 0.351$), or sex ($R^2 = 0.03$, $p = 0.631$). Principal Coordinates Analysis (PCoA) indicated that the first two axes explained 63.0% and 12.1% of the variation, respectively. Visual inspection of the PCoA plot, with 95% confidence ellipses overlaid, showed substantial overlap between HIV/HPV categories, supporting the PERMANOVA findings and suggesting no distinct clustering among the groups based on phylogenetic community structure weighted by abundance (Figure 4.9).

4.1.5 Differential Abundance Analysis

4.1.5.1 Taxonomic Richness Across HIV and HPV Infection Groups

Across the four study groups, variations in microbial richness were observed at all taxonomic levels (Table 4.2). At the species level, the highest number of observed taxa was found in the HIV⁺/HPV⁺ group ($n = 360$ species), followed by HIV⁺/HPV⁻ ($n = 335$), HIV⁻/HPV⁺ ($n = 299$), and HIV⁻/HPV⁻ ($n = 272$). Similarly, genus-level richness ranged from 112 (HIV⁻/HPV⁺) to 126 (HIV⁺/HPV⁺), while phylum-level richness ranged from 10 to 12. Taxonomic richness was highest in the HIV⁺/HPV⁺ group at all levels except phylum.

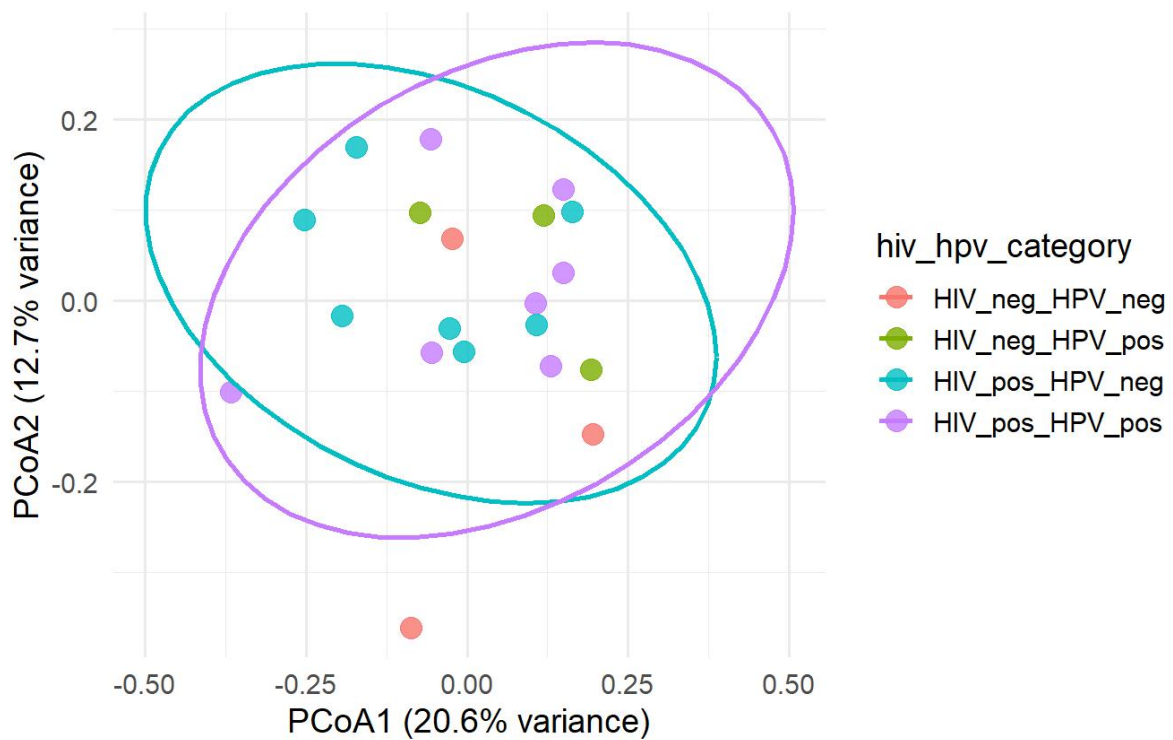


Figure 4.8: Principal Coordinates Analysis (PCoA) of Unweighted Unifrac Distances Showing Microbial Community Structure Across HIV/HPV Categories. Each Point Represents a Sample, Colored by Group. Confidence Ellipses Represent the 95% Interval for Each Group. The First and Second Principal Coordinates (PCoA1 And PCoA2) Explain 20.6% and 12.7% of the Variation, respectively.
Author's Laboratory Work, 2025

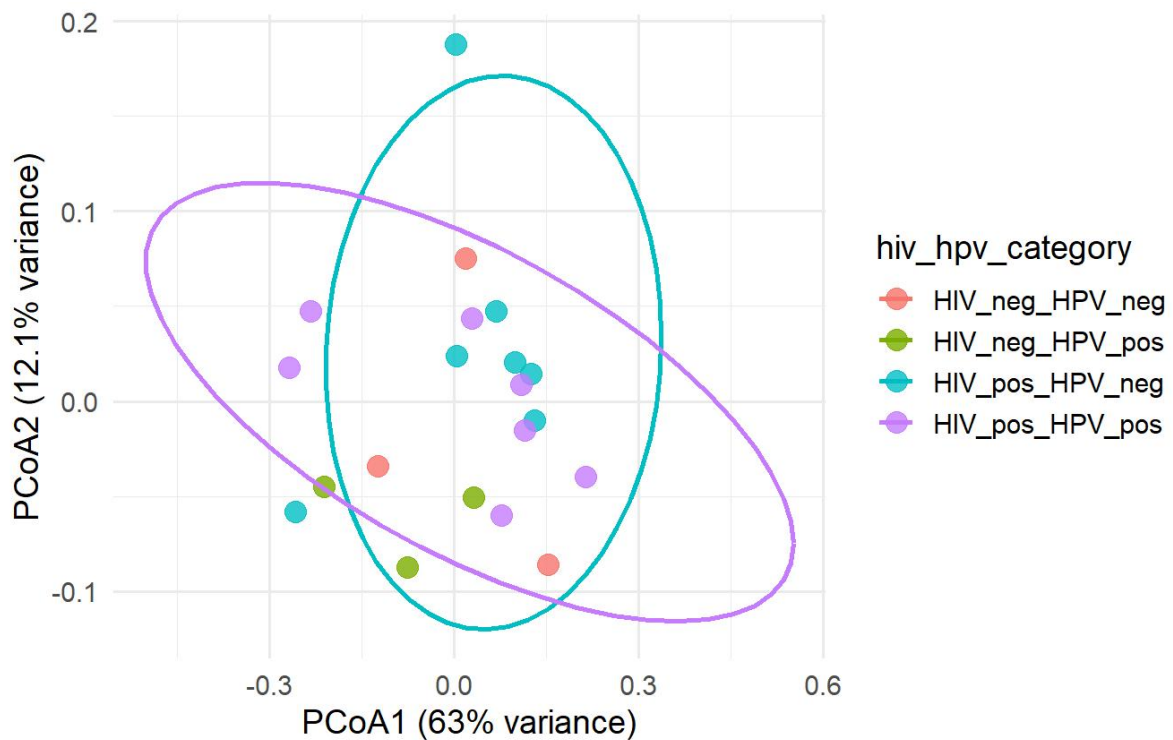


Figure 4.9: Principal Coordinates Analysis (PCoA) Plot of Weighted Unifrac Distances Colored By HIV/HPV Category. Each Point Represents a Sample, and Ellipses Denote 95% Confidence Intervals for Each Group. The X- and Y-Axes Represent the First two Principal Coordinates, which Explain 63.0% and 12.1% of the Total Variation, respectively. No Significant Clustering was Observed Between Groups, Consistent with PERMANOVA Results ($P > 0.05$).

Author's Laboratory Work, 2025

Table 4.2: Number of Phyla, Genera, and Species Identified Across Study Groups

HIV/HPV Category	No. of Phyla	No. of Genera	No. of Species
HIV ⁻ / HPV ⁻	12	118	272
HIV ⁻ / HPV ⁺	10	112	299
HIV ⁺ / HPV ⁻	11	115	335
HIV ⁺ / HPV ⁺	11	126	360

Author's Laboratory Work, 2025

Lead City University Ibadan DO NOT COPY

4.1.5.2 Distribution of Low-Abundance Phyla by HIV/HPV Co-Infection Status

Analysis of 42 oral bacterial phyla identified six taxa with mean relative abundance <1% in either HIV⁺ or HIV⁻ groups: *Chloroflexota*, *Mycoplasmata*, *Spirochaetota*, *Synergistota*, unclassified Bacteria, and *Verrucomicrobiota* (Figure 4.10). These low-abundance phyla exhibited distinct distribution patterns across HIV/HPV co-infection categories. *Spirochaetota* demonstrated the highest overall abundance among low-abundance phyla (0.13% in HIV⁺/HPV⁺), with a 2.5-fold enrichment in HIV⁺/HPV⁺ women (0.13%) compared to HIV⁺/HPV⁻ (0.05%), HIV⁻/HPV⁺ (0.05%), and HIV⁻/HPV⁻ (0.03%). *Mycoplasmata* showed marked elevation exclusively in HIV⁺/HPV⁺ samples (0.020%), exceeding other categories by 2.7–14.4 fold (HIV⁺/HPV⁻: 0.0014%; HIV⁻/HPV⁺: 0.0071%; HIV⁻/HPV⁻: 0.0088%). Unclassified bacteria were uniquely detected in HIV⁻/HPV⁻ women (0.11%), with complete absence in all other groups. *Synergistota* exhibited minimal variation across groups, maintaining consistently low abundance (0.0049–0.0072%) without category-specific trends (Figure 4.10). *Chloroflexota* showed marginally higher abundance in HIV⁻/HPV⁻ women (0.0065%) compared to other categories (0.0018–0.0045%), though all values were <0.01%. *Verrucomicrobiota* was undetectable in HIV⁻/HPV⁺ samples, with peak abundance in HIV⁻/HPV⁻ women (0.0053%) and trace levels in HIV⁺ groups (0.0002–0.0009%).

4.1.5.3 Taxonomic Composition at the Phylum Level in Relation to HIV and HPV Status

Six phyla exceeded 1% mean relative abundance threshold in at least one group and were included in the analysis: *Bacillota*, *Actinomycetota*, *Bacteroidota*, *Fusobacteriota*, *Patescibacteria*, and *Pseudomonadota*. Across all groups, *Bacillota* was the most dominant phylum, representing 71.3–75.4% of the oral microbiome (Figure 4.11).

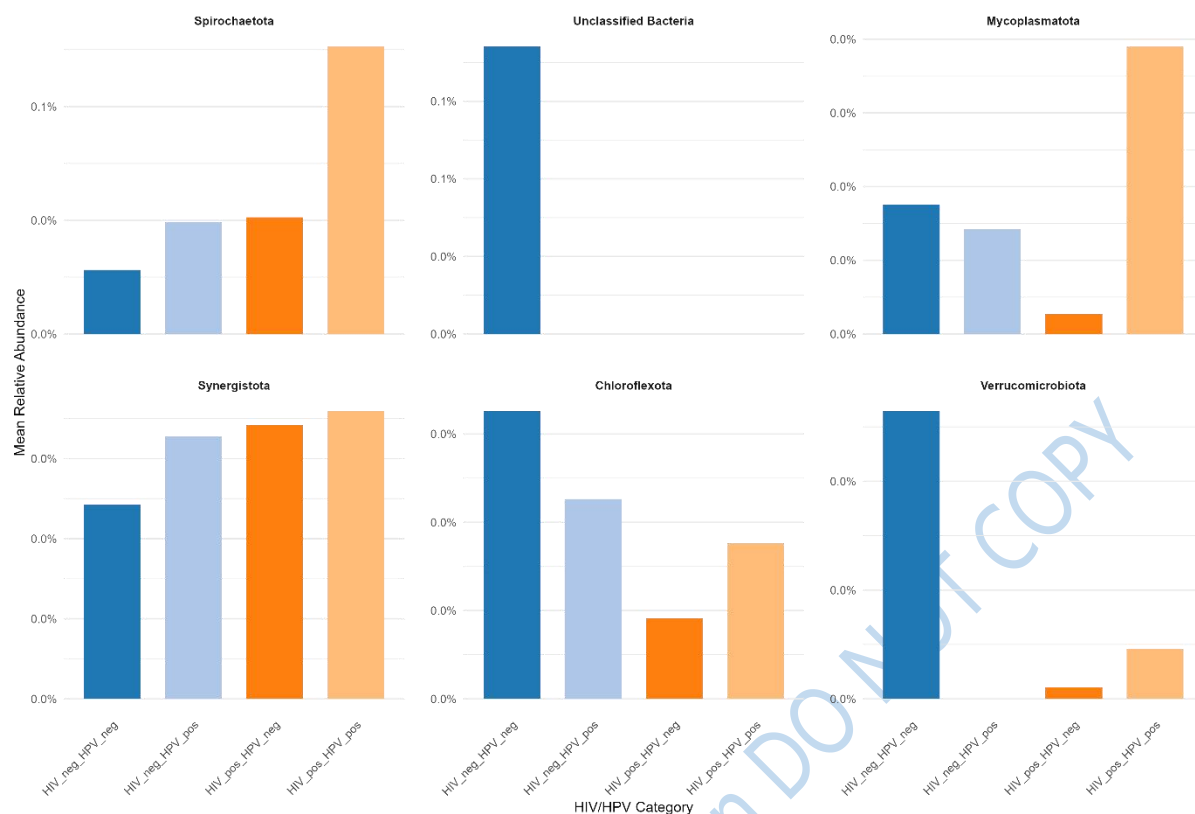


Figure 4.10. Relative Abundance of Low-Abundance Phyla (<1%) Across HIV/HPV Co-Infection Categories. Each Panel Shows One Phylum; Bar Heights Indicate Mean Relative Abundance (Log-Scale, %), Colored by Co-Infection Group. Unclassified Bacteria Absent in All Non-HIV⁻/HPV⁻ Groups; *Spirochaetota* and *Mycoplasmatota* Peak in HIV⁺/HPV⁺. Author's Laboratory Work, 2025

The relative abundance of *Actinomycetota* was highest in the HIV⁺/HPV⁻ group (16.8%) and lowest in the HIV⁻/HPV⁺ group (9.3%). A notable increase in *Bacteroidota* was observed in the HIV⁻/HPV⁺ group (14.1%), compared to 5.4–6.9% in the other groups. *Fusobacteriota*, *Patescibacteria*, and *Pseudomonadota* were present in lower abundances (<3%), with minor variations across infection.

4.1.5.4 Differential Abundance of Bacterial Genera Associated with HPV, Stratified by HIV Status

Genus-level differential abundance analysis was conducted to assess changes in the oral microbiome associated with HPV infection among HIV-positive individuals. A total of 70 genera were detected in this group. No genera passed the FDR-corrected significance threshold ($q < 0.1$), indicating the absence of statistically robust associations after multiple testing correction. However, eight genera showed significant differences in abundance at a nominal $p < 0.05$ threshold, with two of these passing a more stringent $p < 0.01$ level (Fig. 4.12).

Among the significantly altered genera, *Nanoperiomorbus* ($\log_2FC = 2.71$, $p = 0.004$) was more abundant in HPV⁺ individuals compared to HPV⁻ controls. In contrast, *Aggregatibacter* showed significantly lower abundance in the HPV⁺ group ($\log_2FC = -3.16$, $p = 0.0059$), suggesting potential depletion with HPV infection. Other genera significantly reduced in HPV⁺ individuals included *Nanogingivalis*, *Abiotrophia*, *Desulfobulbus*, and *Veillonella*, while *Selenomonas* and *Shuttleworthia* were more abundant among those with HPV infection. All eight genera exhibited nominal significance ($p < 0.05$), although their corresponding FDR-adjusted q -values were above 0.3. Genus-level differential abundance analysis was performed to assess the association between oral bacterial composition and HPV status among HIV-negative individuals. A total of 56 genera were detected across the

samples in this stratum. No genera passed the FDR-adjusted significance threshold of $q < 0.05$, indicating no statistically robust findings after multiple testing correction.

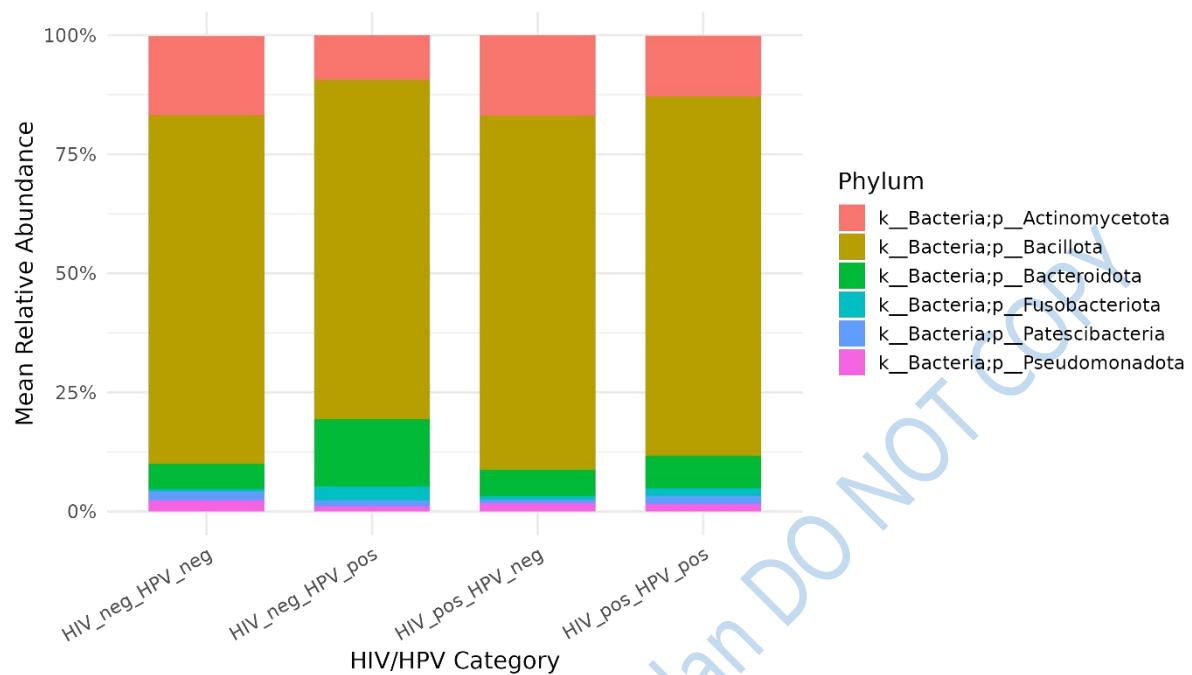


Figure 4.11: Stacked Bar Plot Showing Mean Relative Abundance of Dominant Phyla (>1%) in Each HIV/HPV Infection Category.
Author's Laboratory Work, 2025

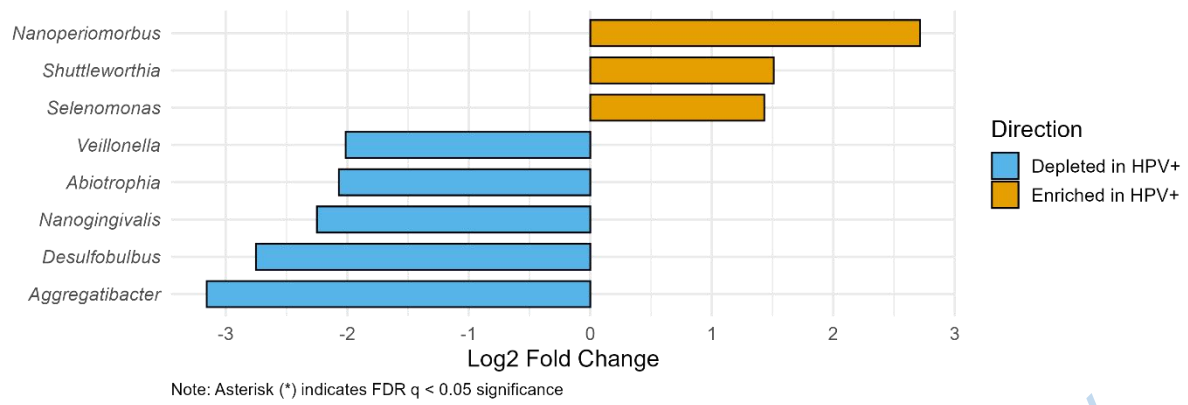


Figure 4.12. Bar Plot HIV Positive Genera with $p < 0.05$, Colored by Direction of Change.
Author's Laboratory Work, 2025

Nevertheless, 20 genera demonstrated significant differences in relative abundance between HPV+ and HPV- individuals at a nominal $p < 0.05$ threshold (Figure 4.13), with 4 of these passing the more stringent $p < 0.01$ level. Among the genera significantly enriched in HPV+ individuals were *Nanosynbacter*, *Mogibacterium*, *Peptostreptococcus*, *Oribacterium*, and *Parvisynbacter*, the majority of which are members of the Firmicutes and Actinobacteria phyla. Notably, *Nanosynbacter* exhibited the highest effect size (log₂ fold change: 11.0; $p = 0.0028$), suggesting a substantial increase in HPV+ individuals. In contrast, genera such as *Moryella*, *Lachnospiraceae* [G3], *Absconditococcaceae* [G1], *Shuttleworthia*, *Rothia*, and *Haemophilus* were significantly depleted in HPV+ individuals relative to HPV- controls. These depleted genera also predominantly belonged to the *Firmicutes* phylum.

Analysis of the HIV-positive stratum revealed 8 genera with nominal significance ($p < 0.05$), including 2 strongly associated genera ($p < 0.01$). *Nanoperiomorbus* (log₂-FC = 2.71, $p = 0.0044$) was enriched in HPV+ individuals, while *Aggregatibacter* (log₂-FC = -3.16, $p = 0.0059$) was depleted (Table 4.3). No genera reached FDR-corrected significance ($q < 0.05$). In contrast, the HIV-negative stratum showed more pronounced differences, with 20 genera at $p < 0.05$ and 4 at $p < 0.01$. Notably, *Nanosynbacter* (log₂-FC = 11.0, $p = 0.0028$) and *Moryella* (log₂-FC = -6.15, $p = 0.0096$) exhibited large effect sizes. *Shuttleworthia* showed divergent trends, it was enriched in HPV+ (HIV+: log₂-FC = 1.51, $p = 0.034$) but depleted in HPV- (HIV-: log₂-FC = -10.5, $p = 0.034$).

4.1.5.5 Differential Abundance of Bacterial Species Associated with HPV Status

After merging taxonomy with feature tables, a total of 143 microbial species were detected. Among the 143 bacterial species identified, marked differences in both the magnitude and direction of associations when stratifying by HIV status was observed. In HIV-positive individuals, thirty-one species demonstrated nominally significant associations with HPV status ($p < 0.05$), though none survived false discovery rate correction.

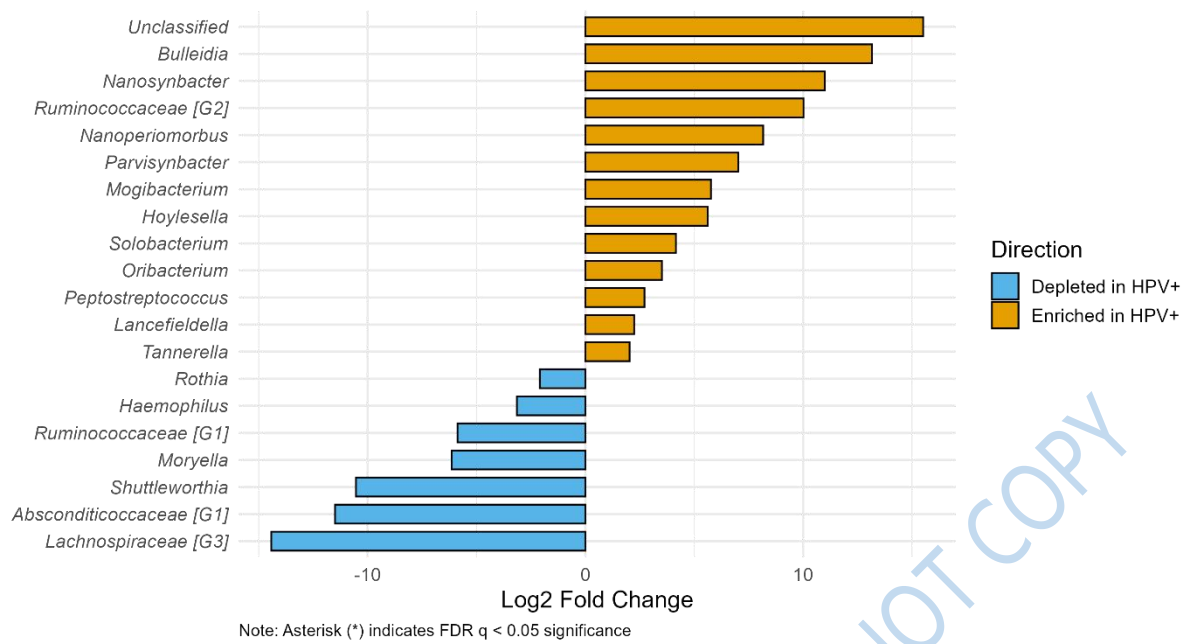


Figure 4.13: Bar Plot of HIV Negative Genera with $p < 0.05$, Colored by Enrichment/Depletion in HPV+.
Author's Laboratory Work, 2025

Table 4.3: Comparative Overview of HPV-Associated Genera by HIV Status.

Metric	HIV-Negative Cohort	HIV-Positive Cohort
Genera ($p < 0.05$)	20	8
Genera ($p < 0.01$)	4	2
Largest log ₂ -FC	<i>Nanosynbacter</i> (+11.0)	<i>Nanoperiomorbus</i> (+2.71)
<i>Shuttleworthia</i>	Depleted in HPV+	Enriched in HPV+
Author's Laboratory Work, 2025		

The most prominent increases among HPV-positive participants include *Streptococcus koreensis* (log2 fold change [LFC] = 3.85, $p = 0.00044$), *Lachnoanaerobaculum saburreum* (LFC = 2.84, $p = 0.00048$), and *Nanoperiomorbus periodonticus* (LFC = 3.35, $p = 0.0017$). Conversely, *Veillonella rogosae* (LFC = -5.22, $p = 0.0029$) and an unclassified *Porphyromonas* species (LFC = -5.84, $p = 0.016$) showed the most substantial decreases in HPV-positive individuals.

The HIV-negative stratum exhibited a contrasting profile, with thirty-seven species reaching nominal significance. Notably, this group demonstrated more extreme fold-changes, including a 12.7-fold increase in *Nanosynbacter* sp. ($p = 0.0016$) and a 21.9-fold increase in *Hoylesella shahii* ($p = 0.014$) among HPV-positive individuals. Several streptococcal species showed divergent associations by HIV status: while *Streptococcus sinensis* increased in HPV-positive/HIV-positive participants (LFC = 3.35), *Streptococcus intermedius* (LFC = -6.79) and *Streptococcus australis* (LFC = -10.2) were markedly depleted in HPV-positive/HIV-negative individuals.

Visualization of effect sizes revealed fundamental differences between HIV strata (Figure 4.14). HIV-positive participants predominantly showed moderate fold-changes ($|LFC| < 4$), whereas HIV-negative individuals exhibited several species with dramatic effect magnitudes ($|LFC| > 10$). This pattern persisted when examining abundance-significance relationships: in HIV-positive individuals, significant taxa typically demonstrated moderate relative abundances (0.1–1%), while HIV-negative participants showed large effect sizes even among low-abundance species ($<0.5\%$). The volcano plots further highlighted this dichotomy, with HIV-negative individuals displaying a broader distribution of effect sizes compared to the more concentrated cluster observed in HIV-positive participants.

Notably, some taxa demonstrated HIV-dependent reversal of association directions. *Shuttleworthia satelles* increased in HPV-positive/HIV-positive individuals (LFC = 1.81) but decreased in HPV-positive/HIV-negative participants (LFC = -10.2). Similarly, *Nanoperiomorbus periodonticus* showed positive associations in both strata but with markedly different effect sizes (HIV+: LFC = 3.35; HIV-: LFC = 8.50). These findings underscore the importance of HIV status as an effect modifier in HPV-related microbial dysbiosis.

The abundance-filtered analysis (mean relative abundance > 0.1%) preserved these overall patterns while removing potentially spurious signals from ultra-low-abundance taxa. Among these more prevalent species, the HIV-negative group maintained stronger effect magnitudes, suggesting that the observed differences are not merely artifacts of differing microbial biomass or sequencing depth between strata.

4.1.5.6 Differential Abundance of KEGG Orthologs Associated with HPV Status

A total of 4,612 KEGG Ortholog (KO) terms were analyzed for differential abundance between HPV-positive and HPV-negative samples after adjusting for age and sex. No KO terms met the stringent false discovery rate (FDR) threshold of $q < 0.05$. However, 31 KO terms exhibited marginal significance ($q < 0.1$), and 458 KO terms showed nominal significance at the $p < 0.05$ level, including 200 that passed the $p < 0.01$ threshold (Table 4.3).

A volcano plot was used to visualize the distribution of KO-level associations with HPV status (Fig. 4.15). While most KO terms clustered around zero, several features demonstrated large effect sizes. The estimated log-fold changes (LFC) for HPV-positive versus HPV-negative groups ranged from -5.12 to +3.18, indicating both enriched and depleted functions in the HPV-positive microbiome.

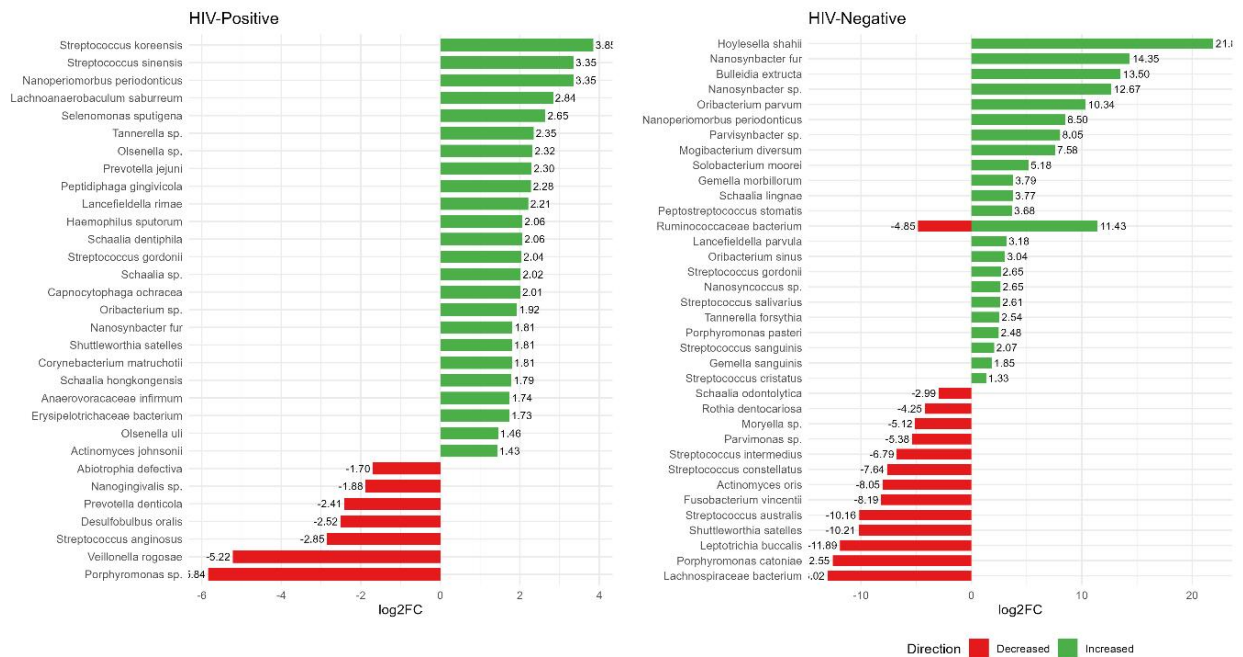


Figure 4.14: Differential Abundance of Oral Microbiota in HPV+ Individuals Stratified by HIV Status ($p < 0.05$)

HIV+ Panel: Shows 15 Species with Strongest Increase in *Streptococcus Koreensis* (+3.85) and Decrease in *Veillonella Rogosae* (-5.22). HIV- Panel: Displays 12 Species Featuring *Nanosynbacter* Sp. (+12.7) and *Streptococcus Australis* (-10.2)
 Author's Laboratory Work, 2025

Table 4.4: Summary of the Number of KO Features Reaching Various Significance Thresholds

Significance Threshold	Number of KOs
$q < 0.05$	0
$q < 0.1$	31
$p < 0.01$	200
$p < 0.05$	458
$p < 0.1$	681
Author's Laboratory Work, 2025	

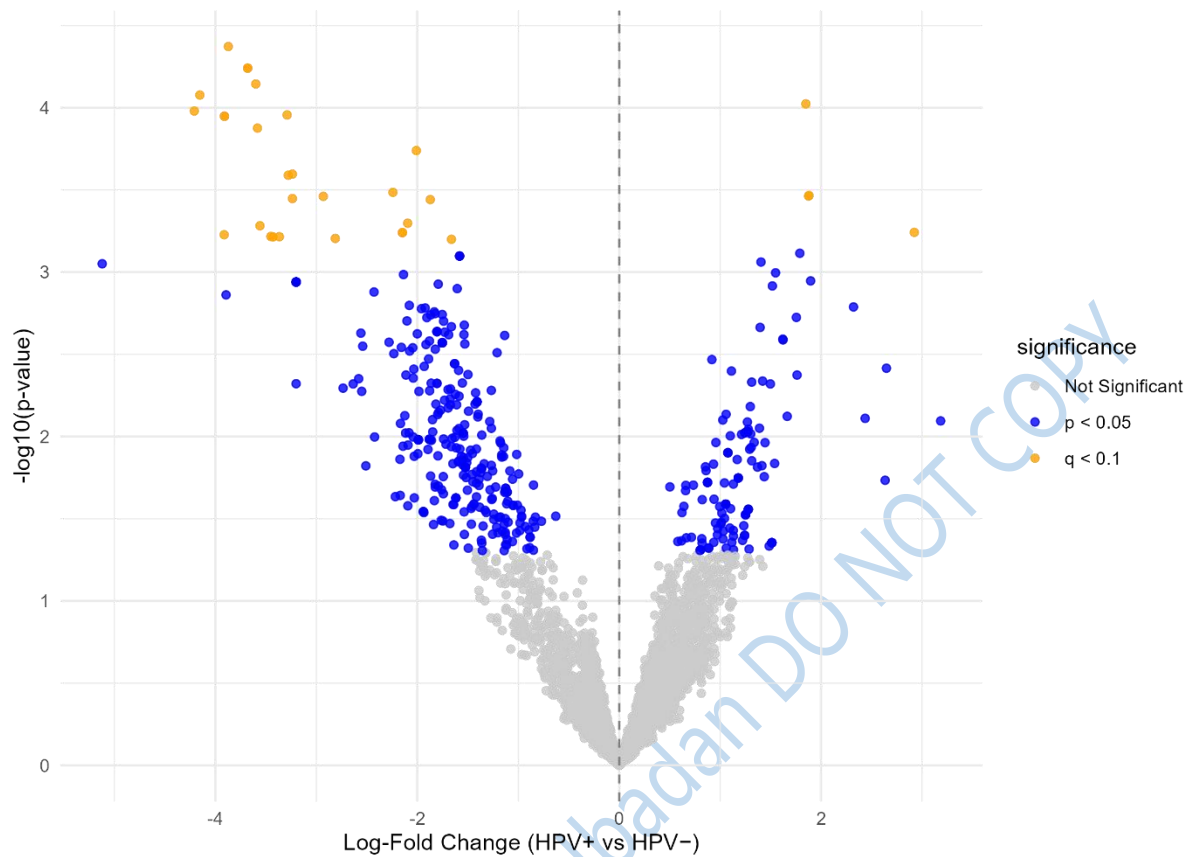


Figure 4.15: Volcano Plot Showing Log-Fold Changes (LFC) Versus $-\log_{10}(\text{p-values})$ for 4,612 KO Features. KO Terms with $Q < 0.05$ are Shown in Red, $q < 0.1$ in Orange, $P < 0.05$ in Blue, and Non-Significant Features in Grey.

Author's Laboratory Work, 2025

The top 20 KO features with $p < 0.05$ and the largest absolute effect sizes are shown in Figure 4.16. Among the KO terms enriched in HPV-positive samples were K01729 (poly(β -D-mannuronate) lyase), K09957 (uncharacterised protein), and K05375 (MbtH protein), with log-fold changes of +3.18, +2.92, and +2.65, respectively. Several components of CRISPR-associated systems (e.g., K07016, K19138) and transport systems (e.g., K10201, K10200) were also more abundant in HPV-positive individuals (Figure 4.16).

Conversely, 319 KO terms were more abundant in HPV-negative samples at $p < 0.05$. These included enzymes involved in carbon and nitrogen metabolism such as K01483 (ureidoglycolate lyase), K14581 (naphthalene dioxygenase reductase), and K10710 (fructoselysine 6-kinase), with log-fold changes ranging from -0.63 to -3.58 (Figure 4.16). Notably, transcriptional regulators (e.g., K07663, K07641) and two-component signaling proteins were frequently depleted in the HPV-positive group.

4.1.5.7 Enzyme Commission (EC) Feature Prediction and Differential Abundance

Analysis

Analysis of 1,575 Enzyme Commission (EC) features revealed significant differences in microbial metabolic potential between HPV-positive (HPV+) and HPV-negative (HPV-) groups. While no features met the threshold for statistical significance after multiple testing correction ($q < 0.05$), 141 EC features showed nominally significant differential abundance ($p < 0.05$), with 61 features surviving a more stringent threshold ($p < 0.01$). The effect sizes for these associations ranged from -3.33 to $+3.17$ log-fold change (LFC), indicating substantial shifts in enzymatic pathway abundance related to HPV status (Figure 4.17).

The most pronounced differences were observed in carbohydrate metabolism and nucleotide processing pathways.

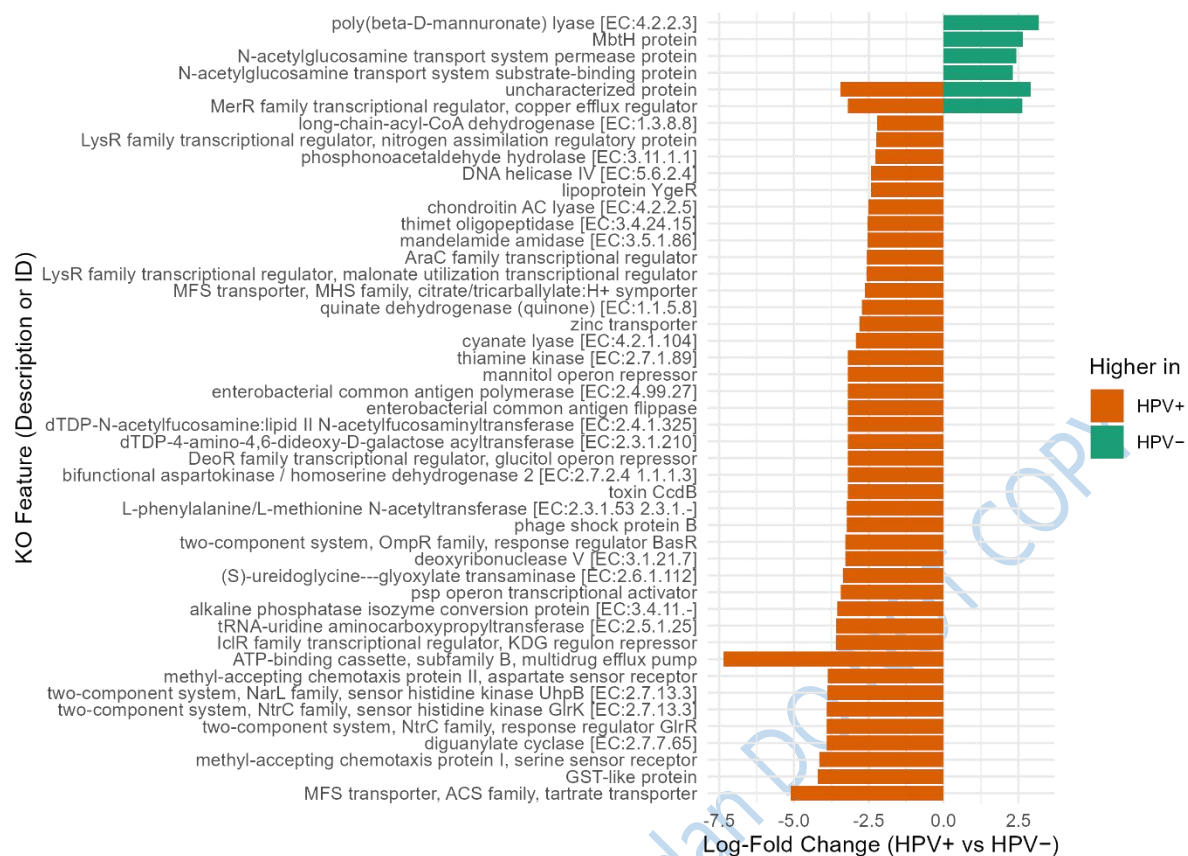


Figure 4.16: Top 50 KO Features Ranked by Absolute Effect Size Among Features with $P < 0.05$. Positive Values Indicate Enrichment in HPV-Positive Samples; Negative Values Indicate Depletion.

Author's Laboratory Work, 2025

Among the top differentially abundant features, polysaccharide degradation enzymes, including mannuronate-specific alginate lyase (EC:4.2.2.3; LFC = +3.17, $p = 0.008$) and β -1,4-mannosyl-glycoprotein 4- β -N-acetylglucosaminyltransferase (EC:2.4.1.144; LFC = +1.89, $p = 0.0003$), were significantly enriched in HPV+ samples. Conversely, enzymes involved in nucleotide metabolism such as deoxyribonuclease V (EC:3.1.21.7; LFC = -3.33, $p = 0.0001$) and cyanase (EC:4.2.1.104; LFC = -2.95, $p = 0.0003$) were depleted in HPV+ samples, indicating these pathways are more prominent in HPV- microbiomes.

Notable functional patterns emerged from these associations. HPV+ samples demonstrated enrichment of glycosyltransferases and carbohydrate-active enzymes, suggesting altered glycan metabolism in these microbial communities. In contrast, HPV- samples showed higher abundance of enzymes related to cofactor biosynthesis and detoxification pathways, including thiamine kinase (EC:2.7.1.89; LFC = -3.20, $p = 0.001$) and phosphonoacetaldehyde hydrolase (EC:3.11.1.1; LFC = -2.26, $p = 0.003$). These differential patterns suggest distinct metabolic priorities in the microbiomes associated with each HPV status group.

A focused analysis of the top 30 most discriminatory features (by absolute effect size at $p < 0.05$) further illustrated the contrasting enrichment patterns between groups. The directional consistency of these effects, with carbohydrate metabolism enzymes consistently elevated in HPV+ and nucleotide processing enzymes in HPV- samples, supports biologically meaningful differences in microbial community function.

4.1.5.8 Differential Abundance of Predicted MetaCyc Pathways

A total of 316 MetaCyc pathways were assessed using ANCOM-BC2, no pathways reached statistical significance at a false discovery rate (FDR) threshold of $q < 0.05$. 26 pathways showed nominal significance at $p < 0.10$, of which 18 pathways were significant at $p < 0.05$, and 6 pathways met a more stringent cutoff of $p < 0.01$.

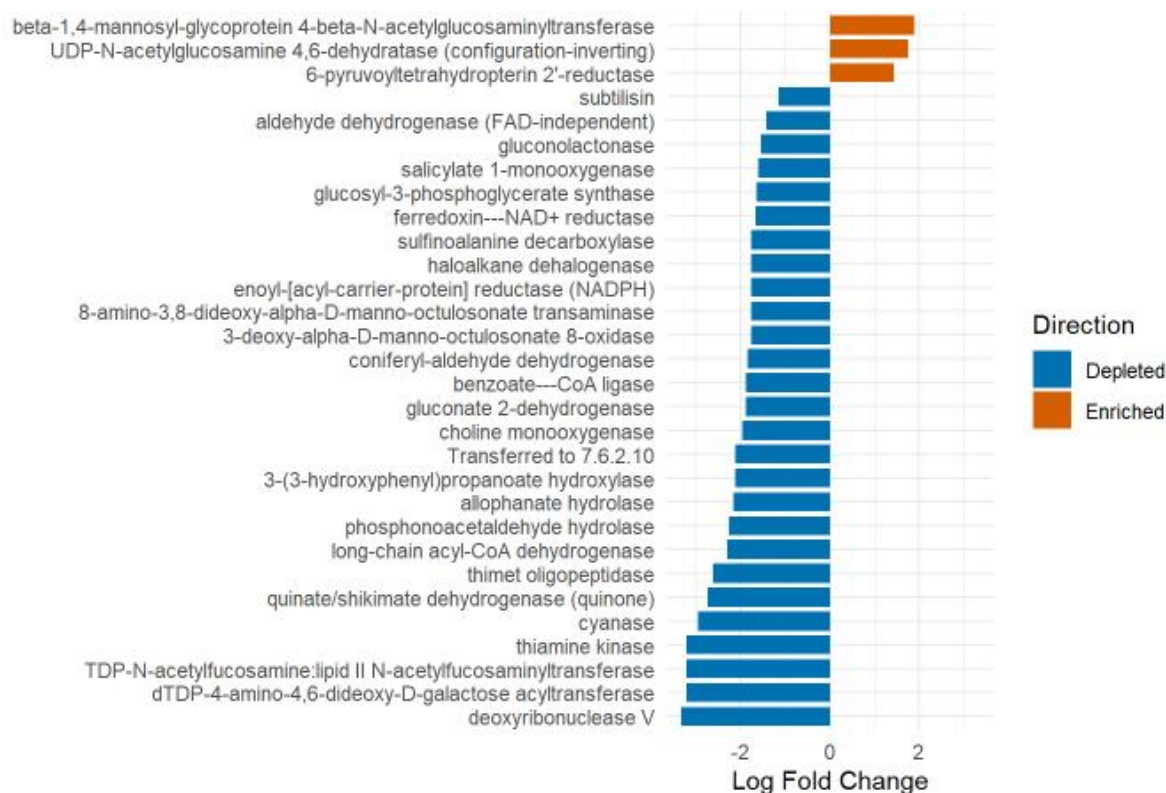


Figure 4.17: Log Fold Change Across Top 30 HPV Positive and Negative Groups
Author's Laboratory Work, 2025

The estimated log-fold changes (logFC) for pathway abundance between HPV-positive and HPV-negative participants ranged from -3.19 to $+1.66$ (Figure 4.18). Among pathways with $p < 0.05$, 11 pathways were enriched in HPV-positive individuals, while 7 pathways were more abundant in HPV-negative individuals. Notably, pathways enriched in the HPV-positive group include toluene degradation I and II (aerobic), Peptidoglycan biosynthesis II (staphylococci), protein N-glycosylation (bacterial) Pyrimidine deoxyribonucleotide biosynthesis (de novo and from CTP), CMP-pseudamate biosynthesis, UDP-N-acetylglucosamine-derived o-antigen biosynthesis, dTDP-N-acetylviosamine biosynthesis, Demethylmenaquinol-6 biosynthesis and mannan degradation (Figure 4.18). Conversely, pathways depleted in HPV-positive individuals (i.e., more abundant in HPV-negative participants) include 4-hydroxyphenylacetate degradation, enterobacterial common antigen biosynthesis, ketogluconate metabolism, 3-phenylpropanoate degradation, methyl ketone biosynthesis, methylphosphonate degradation I, and superpathway of L-threonine metabolism.

4.2 Discussion of Findings

4.2.1 HPV Genotype Distribution and Oral HPV Prevalence

This study examined oral microbiome and HPV genotype profiles across four distinct groups—HIV+/HPV+, HIV+/HPV–, HIV–/HPV+, and HIV–/HPV—within a mother–child pair framework. The design reflects a strong female representation among mothers (over 18), and a balanced mix among children. A limitation remains the absence of adult males, potentially affecting generalizability across age–sex strata. In HIV+ individuals ($n=7$), we detected a wide array of HPV genotypes: HPV16, 35, 39, 52, 53, 59, 40, 42, and 82—six being high-risk types (85.7%), and one individual carrying both high- and low-risk types (35 + 82). Among HIV– participants, three had HPV: one with high-risk HPV51 and two with low-risk types (40, 61).

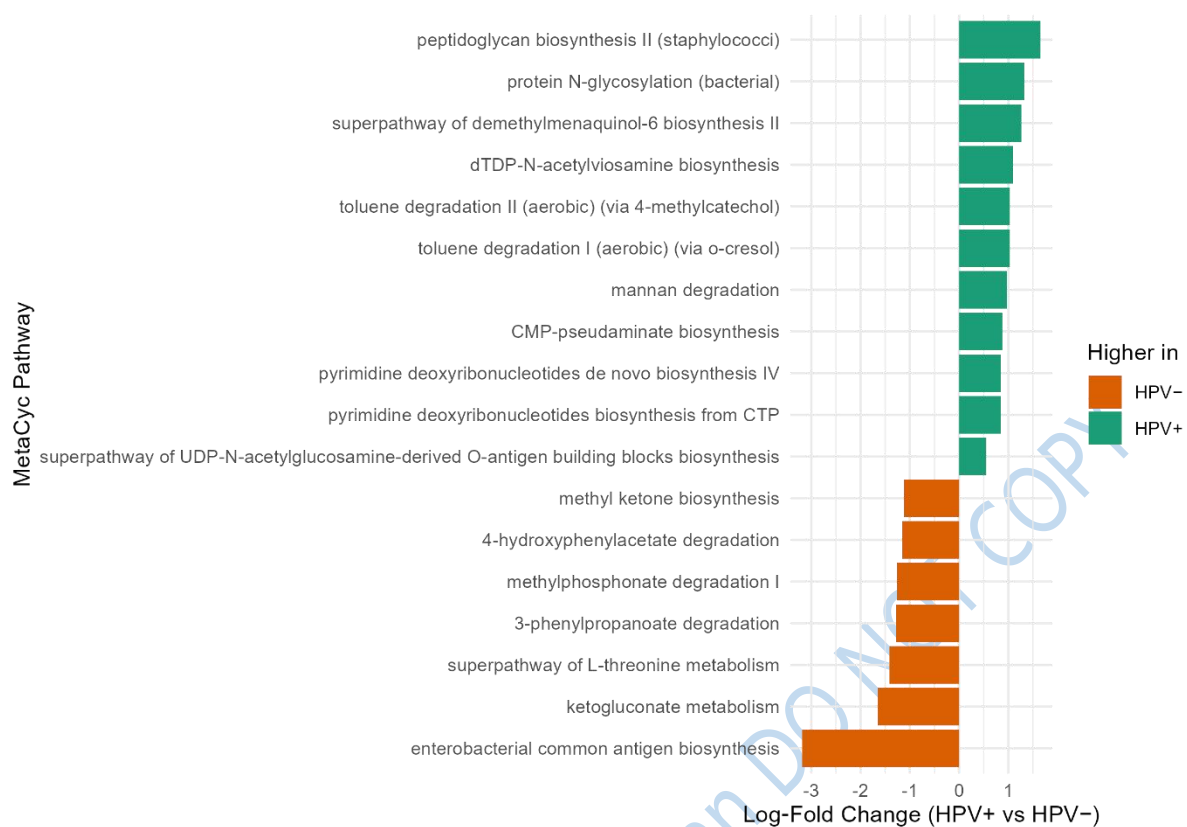


Figure 4.18: A Barplot Depicting the Top Discriminatory Pathways ($p < 0.05$)

Author's Laboratory Work, 2025

This distribution underscores increased oral HPV prevalence and diversity in HIV+ individuals, resonating with prior observations of elevated oral HPV carriage among people living with HIV (PLWH) and especially those with high-risk sexual behaviors¹.

4.2.2 Alpha Diversity

Our study assessed alpha diversity of the oral microbiome across HIV and HPV status using four complementary metrics—Chao1 richness, Shannon diversity, Faith's Phylogenetic Diversity (PD), and Pielou's Evenness. Consistently, the HIV⁻/HPV⁺ group exhibited the highest diversity estimates, followed by HIV⁺/HPV⁺ and HIV⁺/HPV⁻ individuals, with the lowest diversity observed in HIV⁻/HPV⁻ controls. While most differences did not achieve statistical significance, likely due to the modest sample size, the trends observed provide important biological insights. Chao1 richness and Shannon diversity revealed significantly higher microbial diversity in HIV⁻/HPV⁺ individuals compared to HIV⁻/HPV⁻ controls, suggesting that oral HPV infection may be associated with an expansion of microbial taxa ($\beta = 455.9$, $p = 0.050$; $\beta = 1.53$, $p = 0.033$, respectively). Faith's PD further supported this trend, with HIV⁻/HPV⁺ participants showing greater phylogenetic breadth, approaching significance ($p = 0.058$).

These findings resonate with studies in the cervical and vaginal microbiome, where HPV infection has been linked to increased richness and evenness, often reflecting a shift away from *Lactobacillus* dominance toward more diverse, polymicrobial communities²⁻⁶. In particular, Lee and colleagues observed that HPV-positive women in a Korean twin cohort exhibited higher alpha diversity compared to HPV-negative individuals, consistent with our oral data³. Interestingly, Zhang and colleagues found similar alpha diversity between HPV positive and HPV negative participants⁷.

4.2.3 Beta Diversity

In this study, we assessed oral microbial β -diversity using Jaccard, unweighted UniFrac, and weighted UniFrac distance metrics, thereby capturing both taxonomic presence–absence and phylogenetic abundance-weighted information. Across all three measures, we observed no statistically significant differences in community structure between HIV/HPV groups, nor were age or sex significant contributors to variation. These findings indicate that, while HPV status appeared to influence α -diversity, broader community-level compositional shifts were not evident at the β -diversity level.

My results partly align with previous studies but also highlight inconsistencies across different cohorts and analytical approaches. Zhang and colleagues reported significant, though modest, differences between HPV-positive and HPV-negative oral rinse samples using Bray–Curtis and weighted UniFrac metrics (Bray–Curtis $P = 0.009$, $R^2 = 0.5\%$; weighted UniFrac $P = 0.05$, $R^2 = 0.5\%$), whereas no differences were detected with unweighted UniFrac, consistent with our findings⁷. Conversely, Escobar Marcillo and colleagues, found no significant differences between hrHPV and HPV-unaffected groups using Jaccard and weighted UniFrac, but observed significant separation with unweighted UniFrac².

Several factors may explain these discrepancies. First, methodological differences—including sample type (oral rinse versus site-specific swabs), sequencing depth, and diversity metric selection—may influence the detection of community-level differences. Second, the effect sizes reported in prior studies were small, suggesting that HPV-associated alterations in the oral microbiome are subtle and not consistently detectable across cohorts. Finally, our relatively modest sample size may have limited statistical power to detect subtle shifts in β -diversity.

4.2.4 Taxonomic Richness Across HIV and HPV Infection Groups

At all taxonomic levels, richness was consistently highest among HIV⁺/HPV⁺ individuals, suggesting that co-infection may promote expansion of microbial diversity. Notably, species-level richness increased progressively from HIV⁻/HPV⁻ (n = 272) to HIV⁺/HPV⁺ (n = 360), with intermediate richness in the singly infected groups. This pattern aligns with prior observations that HIV infection, through chronic immune suppression and systemic inflammation, destabilizes mucosal microbial communities and promotes colonization by a broader range of taxa^{8,9}. The additional burden of oral HPV infection may exacerbate this effect by altering local epithelial immunity and facilitating microbial niche diversification¹⁰.

The enrichment of low-abundance phyla in the HIV⁺/HPV⁺ group further supports the idea that co-infection may open ecological space for opportunistic or rare taxa. *Spirochaetota* and *Mycoplasmata* were most enriched in HIV⁺/HPV⁺ samples, with 2.5-fold and >2.7-fold increases, respectively, compared to other groups. Both phyla include organisms linked to mucosal inflammation and dysbiosis: *Spirochaetota* members have been implicated in periodontal disease and epithelial barrier disruption¹¹, while *Mycoplasmata* are known for immune evasion and pathogenic synergy in co-infected hosts¹². Interestingly, Escobar Marcillo and his colleagues also reported significant modulation of less abundant phyla with hrHPV infection, noting reductions in *Saccharibacteria* (TM7) and enrichment of *Gracilibacteria* (GN02), particularly in HIV-negative individuals². The parallel observation that HPV infection impacts rare phyla across studies reinforces the importance of low-abundance taxa as sensitive markers of viral–microbial interactions.

At the phylum level, the oral microbiome in our study was dominated by *Bacillota*, consistent with the core microbiome structure reported by both Zhang and Escobar Marcillo where *Firmicutes* (≈34–37%), *Bacteroidetes* (≈28–33%), *Proteobacteria* (≈14–21%), *Actinobacteria* (≈8–11%), and *Fusobacteria* (≈3–6%) consistently comprised the majority of

oral communities^{2,7}. Despite these similarities, subtle group-specific shifts were apparent. In our dataset, *Actinomycetota* peaked in HIV⁺/HPV⁻ individuals (16.8%), echoing Zhang and colleagues' (2022) report of higher abundance of *Actinomycetaceae* and *Actinomyces* in HPV-positive oral rinse samples. Similarly, my observation of elevated *Bacteroidota* in HIV⁻/HPV⁺ individuals (14.1%) is consistent with findings from both Zhang and Escobar-Marcillo who reported enrichment of *Prevotellaceae*, *Lachnospiraceae*, and multiple *Bacteroidetes* species in oral HPV-positive individuals^{2,7}. The convergence across studies suggests that *Bacteroidota* taxa may represent a shared ecological signature of oral HPV infection, potentially driven by their ability to thrive in inflamed or immunologically altered mucosa.

4.2.5 Differential Abundance in HIV-Positive Participants

In HIV-positive individuals, the analysis revealed nominally significant associations between HPV status and the abundance of eight genera ($p < 0.05$), with two reaching $p < 0.01$, though none passed the False Discovery Rate-corrected significance threshold. Among these, *Nanoperiomorbus* was notably enriched in HPV⁺ individuals, while *Aggregatibacter* showed a significant depletion. Other genera such as *Selenomonas* and *Shuttleworthia* were more abundant, whereas *Nanogingivalis*, *Abiotrophia*, *Desulfobulbus*, and *Veillonella* were reduced. At the species level, 31 nominally significant associations were identified. Prominent increases in HPV-positive HIV-positive individuals included *Streptococcus koreensis*, *Lachnoanferobaculum saburreum*, and *Nanoperiomorbus periodonticus*. Conversely, *Veillonella rogosae* and an unclassified *Porphyromonas* species exhibited the most substantial decreases.

These findings align with the broader understanding that HIV infection significantly impacts the oral microbiome, leading to distinct microbial compositions compared to HIV-negative individuals^{13,14}. The observed shifts, even if moderate in fold-change, suggest a specific

microbial perturbation in HIV-positive individuals co-infected with HPV. While direct comparisons for some genera like *Nanoperiomorbus* in this specific context are limited in existing literature, the depletion of *Aggregatibacter* is noteworthy given its known roles in periodontitis and host interactions^{15,16}. Previous research on oral HPV has also indicated a general dysbiosis characterized by higher abundance of certain bacterial families (e.g., Actinomycetaceae, Prevotellaceae, Veillonellaceae, Campylobacteraceae, Bacteroidetes) and lower abundance of commensals like Gemellaceae in HPV+⁷. Our results in HIV-positive individuals, though specific to genera and species, generally fit this pattern of altered community structure, potentially driven by the compromised immune environment in HIV.

In contrast, the HIV-negative cohort exhibited more pronounced alterations in oral microbial composition associated with HPV status. While no genera reached FDR-corrected significance, 20 genera showed nominal significance ($p < 0.05$), with four demonstrating $p < 0.01$. Genera significantly enriched in HPV+ HIV-negative individuals included *Nanosynbacter*, *Mogibacterium*, *Peptostreptococcus*, *Oribacterium*, and *Parvisynbacter*, primarily members of the Firmicutes and Actinobacteria phyla. Notably, *Nanosynbacter* exhibited a particularly high effect size (12.7-fold increase for *Nanosynbacter* sp.). Conversely, genera like *Moryella*, *Lachnospiraceae* [G3], *Absconditococcaceae* [G1], *Shuttleworthia*, *Rothia*, and *Haemophilus* were significantly depleted. The substantial effect magnitudes observed in HIV-negative participants suggest a more robust and discernible microbial dysbiosis linked to HPV infection in an immunocompetent setting.

These patterns resonate with findings from other studies on oral HPV, which consistently report a shift in microbial community structure and composition^{7,17,18}. For instance, the general trend of certain anaerobic or pro-inflammatory bacteria increasing, and commensals decreasing, is a recurring theme in HPV-associated oral dysbiosis⁷. The strong enrichment of *Nanosynbacter* in this cohort represents a significant finding, warranting further investigation

into its potential role in HPV pathogenesis in HIV-negative individuals. The depletion of *Haemophilus* is also interesting, as some studies have identified specific *Haemophilus* species as part of multi-marker signatures in HPV-positive head and neck cancer¹⁹. The pronounced shifts observed in this group suggest that, in the absence of HIV-induced immunomodulation, the HPV infection might exert a more direct and potent influence on the oral microbial environment.

A pivotal finding of this study is the compelling demonstration that HIV status acts as a critical effect modifier in HPV-related oral microbial dysbiosis. This is exemplified by taxa like *Shuttleworthia satelles*, which showed divergent trends, being enriched in HPV-positive HIV-positive individuals but significantly depleted in HPV-positive HIV-negative participants. Similarly, *Nanoperiomorbus periodonticus* showed positive associations in both strata but with markedly different effect sizes (HIV+: LFC = 3.35; HIV-: LFC = 8.50). These findings explicitly highlight how the host's immune status, particularly as influenced by HIV infection, profoundly shapes the oral microbiome's response to HPV.

This observation is consistent with the established understanding that HIV infection leads to significant immunological changes that can alter mucosal environments and microbial communities^{14,20}. The differential responses of specific bacteria underscore that the pathogenesis of HPV infection in the oral cavity is not uniform but is instead heavily influenced by co-morbidities like HIV. This suggests that the mechanisms by which the oral microbiome contributes to or is affected by HPV infection may differ substantially between immunocompetent and immunocompromised individuals.

The distinct microbial signatures identified in HPV-positive individuals, stratified by HIV status, offer valuable insights into the complex pathogenesis of HPV-related oral diseases. The presence of specific enriched genera and species in each cohort could serve as potential

biomarkers for HPV infection or progression within these specific populations, particularly given the known link between oral dysbiosis and head and neck pathologies^{21,22}. Identifying these distinct profiles could inform targeted prophylactic strategies or adjunctive therapies tailored to the specific immune status of the individual. A primary limitation of this analysis is that many of the observed associations reached only nominal significance ($p < 0.05$) and did not withstand FDR-correction, especially at the genus level across both strata, and for some species within the HIV-positive group. This indicates that while strong trends are apparent, the statistical robustness for many individual taxa requires further validation in larger, more diverse cohorts.

4.2.6 Differential Abundance of KEGG Orthologs Associated with HPV Status

The analysis of 4,612 KEGG Ortholog terms revealed 458 nominally significant associations ($p < 0.05$), with 200 reaching $p < 0.01$. Key KOs enriched in HPV-positive samples included K01729 (poly(β -D-mannuronate) lyase), K09957 (uncharacterized protein), and K05375, along with components of CRISPR-associated systems (e.g., K07016, K19138) and transport systems (e.g., K10201, K10200). Conversely, KOs more abundant in HPV-negative samples included enzymes involved in carbon and nitrogen metabolism (e.g., K01483: ureidoglycolate lyase; K14581: naphthalene dioxygenase reductase; K10710: fructoselysine 6-kinase), transcriptional regulators, and two-component signaling proteins.

The enrichment of CRISPR-associated systems in HPV-positive individuals is particularly intriguing. Clustered Regularly Interspaced Short Palindromic Repeats-Cas systems are bacterial adaptive immune systems that defend against foreign genetic elements, including phages and plasmids²³. The oral cavity is known to harbor a high diversity of prokaryotic species with active CRISPR-Cas systems and oral habitats show a high CRISPR load compared to other body sites^{23,24}. Increased abundance of CRISPR-related KOs might suggest a heightened bacterial defense response, potentially against increased phage activity

or other invading genetic elements, which could include interactions with HPV or other viruses. Furthermore, CRISPR-Cas systems in oral pathogens have been implicated in modulating virulence, suggesting a potential link to disease progression²⁵.

The enrichment of poly(β -D-mannuronate) lyase, an enzyme involved in polysaccharide degradation, aligns with the broader understanding of altered carbohydrate metabolism in dysbiotic oral microbiomes²⁶. Polysaccharides play diverse roles in bacterial adhesion, biofilm formation, and host interaction. Changes in their degradation or synthesis pathways could impact the structural integrity of the oral biofilm and its interaction with host epithelial cells. For instance, sulfated polysaccharides have been shown to interfere with HPV's binding to host cells, and enzymes breaking down such compounds could therefore be relevant in the context of HPV infection²⁷.

The depletion of KOs related to carbon and nitrogen metabolism, transcriptional regulation, and two-component signaling in HPV-positive samples suggests a functional suppression or shift away from these fundamental bacterial processes. These systems are crucial for bacterial growth, adaptation, and response to environmental cues. Their reduced abundance could reflect a less metabolically diverse or active microbial community in HPV-positive environments, potentially linked to altered nutrient availability or host-mediated immune responses characteristic of a dysbiotic state^{28,29}.

4.2.7 Differential Abundance of Enzyme Commission Features

Analysis of 1,575 EC features further delineated metabolic potential shifts, with 141 nominally significant associations ($p < 0.05$). HPV-positive samples showed enriched polysaccharide degradation enzymes, including mannuronate-specific alginate lyase (EC:4.2.2.3) and β -1,4-mannosyl-glycoprotein 4- β -N-acetylglucosaminyltransferase (EC:2.4.1.144), as well as general glycosyltransferases and carbohydrate-active enzymes.

This enrichment suggests altered glycan metabolism, possibly favoring the breakdown of host or microbial carbohydrates. Carbohydrate-active enzymes are critical for microbial adaptation and nutrient acquisition, and their increased activity can contribute to changes in the oral environment that favor certain bacterial populations²⁶. This is consistent with previous findings indicating shifts in functional potential and metabolic pathways in oral microbiomes associated with HPV and oral carcinogenesis^{7,30,31}. Specifically, studies on oral cancer have indicated that microbiota can metabolically reprogram the tumor microenvironment through onco-metabolites, linking microbial metabolism to carcinogenesis^{32,33}.

Conversely, enzymes involved in nucleotide metabolism, such as deoxyribonuclease V (EC:3.1.21.7) and cyanase (EC:4.2.1.104), were depleted in HPV-positive samples, alongside those related to cofactor biosynthesis and detoxification (e.g., thiamine kinase (EC:2.7.1.89) and phosphonoacetaldehyde hydrolase (EC:3.11.1.1)) this is in line with other studies²⁷. The depletion of nucleotide metabolism enzymes could indicate reduced bacterial proliferation or a shift toward dormancy in the HPV-positive microenvironment, or a dominance of bacteria with different metabolic priorities. This functional shift might reflect a microbial community less geared towards rapid growth and replication, or one where specific metabolic pathways are inhibited.

4.2.8 Differential Abundance of Predicted MetaCyc Pathways

Investigation of 316 MetaCyc pathways revealed 18 nominally significant associations ($p < 0.05$). Pathways enriched in HPV-positive individuals included toluene degradation I and II (aerobic), Peptidoglycan biosynthesis II (staphylococci), protein N-glycosylation (bacterial), pyrimidine deoxyribonucleotide biosynthesis, and mannan degradation. The enrichment of peptidoglycan biosynthesis pathways suggests an increased bacterial cell wall synthesis activity in HPV-positive individuals, potentially driven by the proliferation of specific bacterial taxa, such as staphylococci, which are known commensals that can become

opportunistic pathogens and are involved in biofilm formation^{34,35}. The presence of toluene degradation pathways, while not extensively studied in the context of oral HPV, indicates the potential for microbial xenobiotic metabolism within the oral cavity. Some studies have noted a decrease in xenobiotic biodegradation pathways in oral HPV+ individuals, suggesting a complex and potentially varied landscape of metabolic responses⁷.

Conversely, pathways depleted in HPV-positive individuals included 4-hydroxyphenylacetate degradation, enterobacterial common antigen biosynthesis, and methylphosphonate degradation I. The depletion of enterobacterial common antigen biosynthesis pathways is notable. ECA is a surface antigen characteristic of Enterobacteriaceae, a group of Gram-negative bacteria that are typically transient in the oral cavity but can be associated with inflammatory conditions³⁶⁻³⁸. Its reduced presence might indicate a shift in the composition or metabolic activity of certain Gram-negative bacteria in the HPV-positive oral environment.

The distinct functional signatures identified in HPV-positive individuals offer valuable insights into the complex pathogenesis of HPV-related oral diseases. The observed shifts in metabolic potential, particularly those related to carbohydrate metabolism, bacterial defense mechanisms, and general bacterial growth processes, suggest a microenvironment conducive to or resulting from HPV infection. These functional changes in the oral microbiome could contribute to the altered host-microbe interactions that may influence HPV persistence and potentially progression to oral pathologies^{39,40}. A significant limitation of this functional analysis is that none of the identified KOs, EC features, or MetaCyc pathways reached statistical significance after FDR-correction. This indicates that while there are discernible trends and biologically plausible shifts, their statistical robustness at a highly corrected level is not met within this dataset, highlighting the inherent variability and complexity of microbiome data and the need for larger studies. Despite this, the presence of numerous nominally significant features with considerable effect sizes warrants further investigation.

Endnotes

1. J. Riddell, A. F. Brouwer, H. M. Walline, L. P. Campredon, R. Meza, M. C. Eisenberg, E. C. Andrus, R. L. Delinger, M. L. Yost, J. K. McCloskey, T. B. Thomas, S. Huang, R. L. Ferris, D. M. Shin, C. Fakhry, T. Ow, D. Li, A. Berlot, T. E. Carey, and N. F. Schlecht, *Oral Human Papillomavirus Prevalence, Persistence, and Risk-Factors in HIV-Positive and HIV-Negative Adults*, **Tumour Virus Research**, 13(1), 2022, 200-237
2. D. I. Escobar Marcillo, G. F. Privitera, F. Rollo, A. Latini, E. Giuliani, M. Benevolo, M. Giuliani, B. Pichi, R. Pellini, and M. G. Donà, *Microbiome Analysis in Individuals with Human Papillomavirus Oral Infection*, **Scientific Reports**, 15(1), 2025, 2953
3. J. E. Lee, S. Lee, H. Lee, Y.-M. Song, K. Lee, M. J. Han, J. Sung, and G. Ko, *Association of the Vaginal Microbiota with Human Papillomavirus Infection in a Korean Twin Cohort*, **PLoS ONE**, 8(5), 2013, 635-644
4. H. Onywera, A.-L. Williamson, Z. Z. A. Mbulawa, D. Coetzee, and T. L. Meiring, *Factors Associated with The Composition and Diversity of the Cervical Microbiota of Reproductive-Age Black South African Women: A Retrospective Cross-Sectional Study*, **Peer Reviewed Journal**, 7(1), 2019, 74-88
5. L. L. Reimers, S. D. Mehta, L. S. Massad, R. D. Burk, X. Xie, J. Ravel, M. H. Cohen, J. M. Palefsky, K. M. Weber, X. Xue, K. Anastos, H. Minkoff, J. Atrio, G. D'Souza, Q. Ye, C. Colie, C. P. Zolnik, G. T. Spear, and H. D. Strickler, *The Cervicovaginal Microbiota and Its Associations With Human Papillomavirus Detection in HIV-Infected and HIV-Uninfected Women*, **Journal of Infectious Diseases**, 214(9), 2016, 1361–1369
6. H. Tuominen, and J. Rautava, *Oral Microbiota and Cancer Development*, **Pathobiology: Journal of Immunopathology, Molecular and Cellular Biology**, 88(2), 2021, 116–126
7. Y. Zhang, G. D'Souza, C. Fakhry, E. O. Bigelow, M. Usyk, R. D. Burk, and N. Zhao, *Oral Human Papillomavirus Associated with Differences in Oral Microbiota Beta Diversity and Microbiota Abundance*, **The Journal of Infectious Diseases**, 226(6), 2022, 1098–1108
8. A. L. Griffen, Z. A. Thompson, C. J. Beall, E. A. Lilly, C. Granada, K. D. Treas, K. R. DuBois, S. B. Hashmi, C. Mukherjee, A. E. Gilliland, J. A. Vazquez, M. E. Hagensee, E. J. Leys, and P. L. Fidel, *Significant Effect of HIV/HAART on Oral Microbiota Using Multivariate Analysis*, **Scientific Reports**, 9(1), 2019, 199-206
9. Z. Pan, N. Wu, and C. Jin, *Intestinal Microbiota Dysbiosis Promotes Mucosal Barrier Damage and Immune Injury in HIV-Infected Patients*, **The Canadian Journal of Infectious Diseases & Medical Microbiology**, 28(1), 2023, 308-320
10. R. Upadhyay, A. Dhakal, C. Wheeler, R. Hoyd, M. Jagjit Singh, V. Karivedu, P. Bhateja, M. Bonomi, S. Valentin, M. E. Gamez, D. J. Konieczkowski, S. Baliga, J. C. Grecula, D. M. Blakaj, E. Gogineni, D. L. Mitchell, N. C. Denko, D. Spakowicz, and S. R. Jhavar, *Comparative Analysis of the Tumor Microbiome, Molecular Profiles, and Immune Cell Abundances by HPV Status in Mucosal Head and Neck Cancers and their Impact on Survival*, **Cancer Biology & Therapy**, 25(1), 2024, 235-249

11. L. Yousefi, H. E. Leylabadlo, T. Poulak, H. Eslami, S. Taghizadeh, K. Ganbarov, M. Yousefi, A. Tanomand, B. Yousefi, and H. S. Kafil, *Oral spirochetes: Pathogenic mechanisms in Periodontal Disease*, **Microbial Pathogenesis**, 144(1), 2020, 104-193
12. J. Hu, Y. Ye, X. Chen, L. Xiong, W. Xie, and P. Liu, *Insight into the Pathogenic Mechanism of Mycoplasma pneumoniae*, **Current Microbiology**, 80(1), 2022, 14-30
13. A. E. Mann, L. M. O'Connell, E. Osagie, P. Akhigbe, O. Obuekwe, A. Omoigberale, C. Kelly, the DOMHaIN Study Team, M. O. Coker, and V. P. Richards, *Impact of HIV on the Oral Microbiome of Children Living in Sub-Saharan Africa, Determined by Using an rpoC Gene Fragment Metataxonomic Approach*, **Microbiology Spectrum**, 11(4), 2023, 11-23
14. E. Perez Rosero, S. Heron, J. Jovel, C. R. O'Neil, S. L. Turvey, P. Parashar, and S. Elahi, *Differential Signature of the Microbiome and Neutrophils in the Oral Cavity of HIV-Infected Individuals*, **Frontiers in Immunology**, 12(1), 2021, 78-91
15. K. Bao, J. Oscarsson, P. Gehring, J. Grossmann, G. N. Belibasakis, and N. Bostanci, *Aggregatibacter aphrophilus T6SS Effectors in Host-Bacterial Interactions*, **Journal of Dental Research**, 1(1), 2025, 00220345251337745
16. M. P. D. Silva, V. D. O. Silva, S. Pasetto, E. S. Ando-Suguimoto, D. Kawamoto, G. M. S. C. Mata, R. M. Murata, M. P. A. Mayer, and C. Chen, *Aggregatibacter actinomycetemcomitans Outer Membrane Proteins 29 and 29 Parologue Induce Evasion of Immune Response*, **Frontiers in Oral Health**, 3(1), 2022, 835-902
17. P. Sarkar, S. Malik, S. Laha, S. Das, S. Bunk, J. G. Ray, R. Chatterjee, and A. Saha, *Dysbiosis of Oral Microbiota During Oral Squamous Cell Carcinoma Development*, **Frontiers in Oncology**, 11(1), 2021, 614-648
18. M. Zakrzewski, O. M. Gannon, B. J. Panizza, N. A. Saunders, and A. Antonsson, *Human Papillomavirus Infection and Tumor Microenvironment are Associated with the Microbiota in Patients with Oropharyngeal Cancers—Pilot Study*, **Head and Neck**, 43(11), 2021, 3324–3330
19. J.-L. C. Mougeot, M. F. Beckman, H. C. Langdon, R. V. Lalla, M. T. Brennan, and F. K. Bahrani-Mougeot, *Haemophilus pittmaniae and Leptotrichia spp. Constitute a Multi-Marker Signature in a Cohort of Human Papillomavirus-Positive Head and Neck Cancer Patients*, **Frontiers in Microbiology**, 12(1), 2021, 794-796
20. G. Zilberman-Schapira, N. Zmora, S. Itav, S. Bashirdes, H. Elinav, and E. Elinav, *The Gut Microbiome in Human Immunodeficiency Virus Infection*, **BMC Medicine**, 14(1), 2016, 83-85
21. A. Herreros-Pomares, C. Llorens, B. Soriano, F. Zhang, S. Gallach, L. Bagan, J. Murillo, E. Jantus-Lewintre, and J. Bagan, *Oral microbiome in Proliferative Verrucous Leukoplakia Exhibits Loss of Diversity and Enrichment of Pathogens*, **Oral Oncology**, 120(1), 2021, 105-14
22. J. Makarewicz, K. Kaźmierczak-Siedlecka, B. K. Sobocki, I. T. Dobrucki, L. Kalinowski, and E. Stachowska, *Anti-cancer management of head and neck cancers and oral microbiome—what can we clinically obtain*, **Frontiers in Cellular and Infection Microbiology**, 14(1), 2024, 132-157

23. T. Gong, J. Zeng, B. Tang, X. Zhou, and Y. Li, *CRISPR-Cas Systems in Oral Microbiome: From Immune Defense to Physiological Regulation*, **Molecular Oral Microbiology**, 35(2), 2020, 41–48
24. P. C. Münch, E. A. Franzosa, B. Stecher, A. C. McHardy, and C. Huttenhower, *Identification of Natural CRISPR Systems and Targets in the Human Microbiome*, **Cell Host and Microbe**, 29(1), 2021, 94-106
25. M. N. Kedlaya, L. Puzhankara, R. Prasad, and A. Raj, *Periodontal Disease Pathogens, Pathogenesis, and Therapeutics: The CRISPR-Cas Effect*, **The CRISPR Journal**, 6(2), 2023, 90–98
26. S. O. Onyango, J. Juma, K. De Paepe, and T. Van De Wiele, *Oral and Gut Microbial Carbohydrate-Active Enzymes Landscape in Health and Disease*, **Frontiers in Microbiology** 12(1), 2021, 653-668
27. S. Wang, Z. Lu, S. Wang, W. Liu, J. Gao, L. Tian, L. Wang, X. Zhang, X. Zhao, W. Wang, and C. Li, *The Inhibitory Effects and Mechanisms of Polymannuroguluronate Sulfate Against Human Papillomavirus Infection In Vitro And In Vivo*, **Carbohydrate Polymers**, 241(1), 2020, 116-125
28. Z. Min, L. Yang, Y. Hu, and R. Huang, *Oral Microbiota Dysbiosis Accelerates the Development and Onset of Mucositis and Oral Ulcers*, **Frontiers in Microbiology**, 14(1), 2023, 106-122
29. J. Talapko, S. Erić, T. Meštrović, M. M. Stipetić, M. Juzbašić, D. Katalinić, S. Bekić, D. Muršić, J. Flam, D. Belić, D. Lešić, R. Fureš, M. Markanović, and I. Škrlec, *The Impact of Oral Microbiome Dysbiosis on the Aetiology, Pathogenesis, and Development of Oral Cancer*, **Cancers**, 16(17), 2024, 29-37
30. J. W. Chen, J. H. Wu, W. F. Chiang, Y. L. Chen, W. S. Wu, and L. W. Wu, *Taxonomic and Functional Dysregulation in Salivary Microbiomes During Oral Carcinogenesis*, **Frontiers in Cellular and Infection Microbiology**, 11(1), 2021, 663-668
31. Q. Lan, C. Zhang, H. Hua, and X. Hu, *Compositional and Functional Changes in the Salivary Microbiota Related to Oral Leukoplakia and Oral Squamous Cell Carcinoma: A Case Control Study*, **BMC Oral Health**, 23(1), 2023, 10-21
32. K. Wei, Y. Ma, J. Xu, H. Zheng, L. Xue, Y. Chu, Y. Shi, Z. Sun, and Q. Sun, *Potential Changes in Microorganisms and Metabolites Associated with Oral Cancer: A Preliminary Stud*, **BMC Cancer**, 25(1), 2025, 6-11
33. J. Zhou, Z. Hu, L. Wang, Q. Hu, Z. Chen, T. Lin, R. Zhou, Y. Cai, Z. Wu, Z. Zhang, Y. Yang, C. Zhang, G. Li, L. Zeng, K. Su, H. Li, Q. Su, G. Zeng, B. Cheng, and T. Wu, *Tumor-Colonized Streptococcus Mutans Metabolically Reprograms Tumor Microenvironment and Promotes Oral Squamous Cell Carcinoma*, **Microbiome**, 12(1), 2024, 19-30
34. A. P. V. Colombo, R. M. Do Souto, L. L. Araújo, L. C. P. Espíndola, F. A. R. R. Hartenbach, C. B. Magalhães, G. Da Silva Oliveira Alves, T. G. B. Lourenço, and C. M. Da Silva-Boghossian, *Antimicrobial Resistance and Virulence of Subgingival Staphylococci Isolated from Periodontal Health and Diseases*, **Scientific Reports**, 13(1), 2023, 116-223

35. E. Kwapisz, K. Garbacz, M. Kosecka-Strojek, J. Schubert, J. Bania, and J. Międzobrodzki, *Presence of Egc-Positive Major Clones ST 45, 30 And 22 Among Methicillin-Resistant and Methicillin-Susceptible Oral Staphylococcus Aureus Strains*, **Scientific Reports**, 10(1), 2020, 188-199
36. H.-M. Kuhn, U. Meier-Dieter, and H. Mayer, *ECA, the Enterobacterial Common Antigen*, **FEMS Microbiology Letters**, 54(3), 1988, 195–222
37. A. K. Rai, and A. M. Mitchell, *Enterobacterial Common Antigen: Synthesis and Function of an Enigmatic Molecule*, **Molecular Biology**, 11(4), 2020, e01914-20
38. A. Basic, S. Blomqvist, G. Charalampakis, and G. Dahlén, *Antibiotic Resistance Among Aerobic Gram-Negative Bacilli Isolated from Patients with Oral Inflammatory Dysbiotic Conditions—A Retrospective Study*, **Frontiers in Dental Medicine**, 5(1), 2024, 1293-1302
39. R. A. Arthur, R. Dos Santos Bezerra, J. P. B. Ximenez, B. L. Merlin, R. De Andrade Morraye, J. V. Neto, N. M. N. Fava, D. L. A. Figueiredo, C. A. O. De Biagi, Summer Course 2020 group, M. J. Montibeller, J. B. Guimarães, E. G. Alves, M. Schreiner, T. S. Da Costa, C. F. L. Da Silva, J. M. Malheiros, L. H. B. Da Silva, G. T. Ribas, D. O. Achallma, C. M. Braga, K. F. A. Andrade, V. Do Carmo Alves Martins, G. V. N. Dos Santos, C. F. Granatto, U. C. Terin, I. H. Sanches, D. E. Ramos, H. M. Garay-Malpartida, G. M. P. De Souza, S. N. Slavov, and W. A. Silva. *Microbiome And Oral Squamous Cell Carcinoma: A Possible Interplay on Iron Metabolism and its Impact on Tumor Microenvironment*, **Brazilian Journal of Microbiology**, 52(3), 2021, 1287–1302
40. K. Wu, B. Xu, X. Zhou, H. Guo, G. Du, C. Zhang, F. Chen, and X. Chen, *Microbiota and Metabolite Profiles of Saliva, Oral Swab and Cancer Tissue from Patients with Oral Squamous Cell Carcinoma (OSCC)*, **Infectious Agents and Cancer**, 20(1), 2025, 32-40

Chapter Five

Conclusion

5.1 Summary of Findings

This study investigated the complex relationship between the oral microbiome, Human Papillomavirus infection, and Human Immunodeficiency Virus status within a specific cohort from Edo State, Nigeria. Our comprehensive analysis, spanning taxonomic composition, diversity, and functional potential, revealed several key findings.

5.1.1 HPV Genotype Distribution and Oral HPV Prevalence

The study found a diverse array of HPV genotypes in oral samples, with high-risk types present in both HIV-positive and HIV-negative individuals. Notably, there was an increased prevalence and diversity of oral HPV genotypes in HIV-positive individuals, consistent with global observations of elevated HPV carriage in this population.

5.1.2 Alpha Diversity

Across all alpha diversity metrics (Chao1 richness, Shannon diversity, Faith's Phylogenetic Diversity, and Pielou's Evenness), the HIV⁻/HPV⁺ group consistently exhibited the highest diversity estimates. Specifically, the HIV⁻/HPV⁺ group showed significantly higher Chao1 richness ($\beta = 455.9$, $p = 0.050$) and Shannon diversity ($\beta = 1.53$, $p = 0.033$) compared to HIV⁻/HPV⁻ controls, suggesting an association between oral HPV infection and an expansion of microbial taxa in immunocompetent individuals. Faith's Phylogenetic Diversity also trended higher in this group, approaching statistical significance ($p = 0.058$).

5.1.3 Beta Diversity

Despite observed trends in alpha diversity, beta diversity analyses using Bray–Curtis dissimilarity, Jaccard index, unweighted UniFrac, and weighted UniFrac distances did not reveal statistically significant differences in overall microbial community structure across

HIV/HPV categories. This indicates that while HPV status might influence the breadth and evenness of microbial communities (alpha diversity), broader compositional shifts at the community level were not statistically distinct in this cohort, possibly due to sample size or the subtle nature of these shifts.

5.1.4 Taxonomic Richness and Composition

Taxonomic richness (number of phyla, genera, and species) was consistently highest at all levels among HIV⁺/HPV⁺ individuals, with species-level richness showing a progressive increase from HIV⁻/HPV⁻ to HIV⁺/HPV⁺ groups. This suggests that co-infection with HIV and HPV may create an ecological space that promotes the expansion of microbial diversity. Low-abundance phyla, such as Spirochaetota and Mycoplasmatota, were notably enriched in HIV⁺/HPV⁺ samples. At the phylum level, *Bacillota* dominated the oral microbiome across all groups. *Actinomycetota* peaked in HIV⁺/HPV⁻ individuals, and a notable increase in *Bacteroidota* was observed in the HIV⁻/HPV⁺ group, suggesting that these phyla may represent shared ecological signatures of oral HPV infection.

5.1.5 Differential Abundance of Bacterial Genera and Species

Stratification by HIV status revealed distinct patterns of differential abundance. In HIV-positive individuals, *Nanoperiomorbus* was enriched in HPV⁺ individuals, while *Aggregatibacter* was depleted, alongside reductions in *Nanogingivalis*, *Abiotrophia*, *Desulfobulbus*, and *Veillonella*. Prominent species-level increases included *Streptococcus koreensis*, *Lachnoanaerobaculum saburreum*, and *Nanoperiomorbus periodonticus*, while *Veillonella rogosae* and an unclassified *Porphyromonas* species decreased. These shifts were generally of moderate fold-change. In HIV-negative individuals, more pronounced alterations were observed. Genera such as *Nanosynbacter*, *Mogibacterium*, *Peptostreptococcus*, *Oribacterium*, and *Parvisynbacter* were significantly enriched in HPV⁺ individuals, with *Nanosynbacter* showing a particularly high

effect size. Conversely, *Moryella*, *Lachnospiraceae*, *Absconditococcaceae*, *Shuttleworthia*, *Rothia*, and *Haemophilus* were depleted. Species-level changes included dramatic increases in *Nanosynbacter* sp. and *Hoylesella shahii*. Crucially, HIV status acted as a significant effect modifier, as exemplified by *Shuttleworthia satellites* and *Nanoperiomorbus periodonticus*, which showed divergent or markedly different trends depending on the HIV status of the individual.

5.1.6 Differential Abundance of KEGG Orthologs

Functional analysis of KOs revealed nominal, but not FDR-corrected, significance for several features. Enriched KOs in HPV-positive samples included poly(β -D-mannuronate) lyase, uncharacterized proteins, and components of CRISPR-associated systems and transport systems. Depleted KOs in HPV-positive samples included enzymes involved in carbon and nitrogen metabolism, transcriptional regulators, and two-component signaling proteins. The enrichment of CRISPR-associated systems is particularly noteworthy, suggesting an active bacterial defense response.

5.1.6 Differential Abundance of Enzyme Commission Features

Similar to KOs, EC feature analysis showed nominal, but not FDR-corrected, significance. HPV-positive samples demonstrated enrichment of polysaccharide degradation enzymes (e.g., mannuronate-specific alginate lyase, β -1,4-mannosyl-glycoprotein 4- β -N-acetylglucosaminyltransferase) and other carbohydrate-active enzymes, suggesting altered glycan metabolism. Conversely, enzymes involved in nucleotide metabolism (e.g., deoxyribonuclease V, cyanase) and cofactor biosynthesis/detoxification were depleted in HPV-positive samples.

5.1.7 Differential Abundance of Predicted MetaCyc Pathways

MetaCyc pathway analysis also yielded nominally significant associations. Pathways enriched in HPV-positive individuals included toluene degradation, Peptidoglycan biosynthesis II (staphylococci), bacterial protein N-glycosylation, pyrimidine deoxyribonucleotide biosynthesis, and mannan degradation. Pathways depleted in HPV-positive individuals included 4-hydroxyphenylacetate degradation, enterobacterial common antigen biosynthesis, and methylphosphonate degradation I.

5.2 Conclusion

This study provides a nuanced understanding of oral microbiome alterations in response to HPV infection, critically highlighting the modifying effect of HIV status. Our findings demonstrate that while oral HPV infection is associated with an expansion of microbial diversity, particularly in HIV-negative individuals, the specific taxonomic and functional shifts within the microbial community are significantly shaped by the host's HIV status. The distinct microbial signatures and metabolic pathway dysregulations observed in HPV-positive individuals, stratified by HIV, underscore that the interplay between the oral microbiome and HPV infection is highly context-dependent. These results point towards specific microbial taxa and functional capabilities that may contribute to HPV persistence or progression in diverse immune environments.

5.3 Recommendations

Based on the findings of this study, the following recommendations are put forth:

1. **Validate Findings in Larger Cohorts:** The nominal significance of many findings necessitates validation in larger, independent cohorts to confirm the statistical robustness of the observed microbial and functional shifts.

2. **Focus on Specific Taxa and Pathways:** Future research should prioritize mechanistic investigations into the roles of specific enriched/depleted genera (e.g., *Nanosynbacter*, *Aggregatibacter*, *Nanoperiomorbus*) and functional pathways (e.g., CRISPR-associated systems, polysaccharide degradation, nucleotide metabolism, peptidoglycan biosynthesis) in HPV pathogenesis.
3. **Consider HIV Status as a Critical Modifier:** All future studies examining the oral microbiome in the context of HPV should rigorously account for HIV status, recognizing its profound influence as an effect modifier on microbial communities.
4. **Explore Biomarker Potential:** The identified distinct microbial and functional signatures hold potential as biomarkers for oral HPV infection, progression, or even therapeutic response. Further research should explore their utility in diagnostic or prognostic applications.
5. **Inform Targeted Interventions:** Understanding the specific microbial shifts associated with HPV in different immune contexts could pave the way for targeted prophylactic strategies or adjunctive therapies that modulate the oral microbiome to influence HPV natural history.

5.4 Contribution to Knowledge

This study makes several significant contributions to the existing body of knowledge regarding the oral microbiome and HPV infection:

1. **First Stratified Functional Analysis:** To our knowledge, this is one of the first studies to provide a detailed functional metagenomic analysis of the oral microbiome in HPV-positive individuals, specifically stratified by HIV status in Edo State, Nigeria. This provides a granular view beyond taxonomic composition.

2. **Elucidation of HIV as an Effect Modifier:** The study explicitly demonstrates and quantitatively highlights the critical role of HIV status as a profound effect modifier on the oral microbiome's response to HPV infection, revealing distinct microbial and functional signatures that would be obscured in non-stratified analyses.
3. **Identification of Novel Microbial and Functional Associations:** We identified specific genera (e.g., *Nanosynbacter*, *Nanoperiomorbus*) and functional capabilities (e.g., enriched CRISPR-associated systems, specific polysaccharide degradation enzymes, depleted nucleotide metabolism) associated with HPV status, with varying magnitudes and directions dependent on HIV co-infection.
4. **Contextualization within a Unique Population:** By focusing on a cohort from Edo State, Nigeria, the study contributes to understanding regional differences in oral HPV epidemiology and microbiome composition, adding valuable data from a population that is often underrepresented in global microbiome research.
5. **Foundation for Mechanistic Studies:** The comprehensive taxonomic and functional profiling provides a robust foundation for future mechanistic studies aimed at elucidating the causal roles of specific microbial elements in HPV persistence and disease progression.

5.5 Suggestion for Further Studies

To build upon the findings of this research and address its inherent limitations, we suggest the following avenues for further studies:

1. **Longitudinal Cohort Studies:** Conduct longitudinal studies with larger sample sizes to establish temporal relationships and potential causality between specific microbial shifts and HPV acquisition, persistence, clearance, or progression to oral lesions.

2. **Multi-Omics Integration:** Integrate multi-omics approaches (e.g., metatranscriptomics, metaproteomics, metabolomics) to directly assess gene expression, protein production, and metabolic activities of the oral microbiome. This would provide a more dynamic and mechanistic understanding of the microbial functions in relation to HPV and HIV.
3. **Targeted Mechanistic Investigations:** Perform *in vitro* and *in vivo* studies to experimentally validate the functional roles of specific identified genera (e.g., *Nanosynbacter*, *Aggregatibacter*) and pathways (e.g., CRISPR-Cas systems, polysaccharide degradation) in modulating HPV infection and host immune responses.
4. **Host-Microbe Interaction Studies:** Investigate the specific molecular mechanisms of interaction between key differentially abundant oral bacteria and HPV, as well as with host epithelial cells and immune cells, particularly in the context of HIV-mediated immunomodulation.
5. **Impact of Antiretroviral Therapy:** Future studies in HIV-positive cohorts should incorporate detailed ART regimen and adherence data, as ART is known to impact the microbiome and could further modify HPV-microbiome interactions.
6. **Virome and Fungi Analysis:** Expand the scope to include virome and mycobiome analysis, as these components of the oral ecosystem also interact with both bacteria and host immunity and could play significant roles in HPV infection.
7. **Geographical and Ethnic Diversity:** Conduct similar studies in diverse geographical regions and ethnic populations to assess the generalizability of these findings and identify population-specific microbial signatures.

8. **Clinical Correlation:** Directly correlate microbial and functional profiles with clinical outcomes, such as the development of oral pre-malignant lesions or oral squamous cell carcinoma, to identify predictive biomarkers.

Lead City University Ibadan DO NOT COPY

Bibliography

Books

Al Aboud, A. M. and Nigam, P. K., *Wart*, (1st Edition), **StatPearls Publishing**, 2025, 33-67. Available Online: <http://www.ncbi.nlm.nih.gov/books/NBK431047/>.

Joint United Nations Programme on HIV/AIDS, *The Path that Ends AIDS: UNAIDS Global AIDS Update 2023*, (1st Edition), **Geneva: Joint United Nations Programme on HIV/AIDS**, 2023, 100-120.

Szalai Z. Z., *Human Papillomavirus Infection*, Harper's Textbook of Pediatric Dermatology, (Irvine AD, Hoeger PH, Yan AC, eds), (1st Edition), **Wiley-Blackwell**, 2011, 208-247, DOI: 10.1002/9781444345384.ch47.

Wickham, H., *GGplot2: Elegant Graphics for Data Analysis*, (1st Edition,) **Springer-Verlag New York**, 2016, 30-38.

Journal Articles

Abbasi, E. and E. Akçay, *Host Control and Species Interactions Jointly Determine Microbiome Community Structure*, **Theoretical Population Biology**, 158(3), 2024, 185-194.

Al-Hadeithi, Z. S. M., Jasim, S. A., and Salahldina, O. D., *Detection and Genotyping of Papillomavirus by Real-Time PCR in Iraqi Patients*, **Archives of Clinical Infectious Diseases**, 17(3), 2022, 242-250.

Alqedari, H., Altabtbaei, K., Espinoza, J. L., Bin-Hasan, S., Alghounaim, M., Alawady, A., Altabtabae, A., AlJamaan, S., Devarajan, S., AlShammari, T., Ben Eid, M., Matsuoka, M., Jang, H., Dupont, C. L., and Freire, M., *Host-Microbiome Associations in Saliva Predict COVID-19 Severity*, **PNAS Nexus**, 3(4), 2024, 126-130.

Amer, A., Whelan, A., Al-Hebshi, N. N., Healy, C. M., and Moran, G. P., *Acetaldehyde Production by Rothia Mucilaginosa Isolates from Patients with Oral Leukoplakia*, **Journal of Oral Microbiology**, 12(1), 2020, 174-180.

Anoruo, O., Bristow, C., Mody, N., and Klausner, J. D., *Estimated Prevalence of Human Papillomavirus among Nigerian Women: A Systematic Review and Meta-Analysis*, **African Journal of Reproductive Health**, 26(6), 2022, 89–96.

Arroyo Mühr, L. S., Lagheden, C., Hassan, S. S., Eklund, C., and Dillner, J., *The International Human Papillomavirus Reference Center: Standardization, Collaboration, and Quality Assurance in HPV Research and Diagnostics*, **Journal of Medical Virology**, 95(12), 2023, 29-32.

Arthur, R. A., Dos Santos Bezerra, R., Ximenez, J. P. B., Merlin, B. L., De Andrade Morraye, R., Neto, J. V., Fava, N. M. N., Figueiredo, D. L. A., De Biagi, C. A. O., Summer Course 2020 group, Montibeller, M. J., Guimarães, J. B., Alves, E. G., Schreiner, M., Da Costa, T. S., Da Silva, C. F. L., Malheiros, J. M., Da Silva, L. H. B., Ribas, G. T., Achallma, D. O., Braga, C. M., Andrade, K. F. A., Do Carmo Alves Martins, V., Dos Santos, G. V. N., Granatto, C. F., Terin, U. C., Sanches, I. H., Ramos, D. E., Garay-Malpartida, H. M., De Souza, G. M. P., Slavov, S. N., and Silva, W. A., *Microbiome and Oral Squamous Cell Carcinoma: A*

Possible Interplay on Iron Metabolism and Its Impact on Tumor Microenvironment, **Brazilian Journal of Microbiology**, 52(3), 2021, 1287–1302.

Baker, J. L., Mark Welch, J. L., Kauffman, K. M., McLean, J. S., and He, X., *The Oral Microbiome: Diversity, Biogeography and Human Health*, **Nature Reviews Microbiology**, 22(2), 2024, 89–104.

Balaji, D., Kalarani, I. B., Mohammed, V., and Veerabathiran, R., *Potential Role of Human Papillomavirus Proteins Associated with the Development of Cancer*, **Virus Disease**, 33(3), 2022, 322–333.

Bao, K., Oscarsson, J., Gehring, P., Grossmann, J., Belibasakis, G. N., and Bostanci, N., *Aggregatibacter Aphrophilus T6SS Effectors in Host–Bacterial Interactions*, **Journal of Dental Research**, 104(13), 2025, 37–45.

Basic, A., Blomqvist, S., Charalampakis, G., and Dahlén, G., *Antibiotic Resistance among Aerobic Gram-Negative Bacilli Isolated from Patients with Oral Inflammatory Dysbiotic Conditions—a Retrospective Study*, **Frontiers in Dental Medicine**, 5, 2024, 129–202.

Benzerdjeb, N., Traverse-Glehen, A., Philouze, P., Bishop, J., and Devouassoux-Shisheboran, M., *Poorly Differentiated Neuroendocrine Carcinoma of the Head and Neck: Human Papillomavirus Tumour Status/P16 Status and Impact on Overall Survival*, **Histopathology**, 76(4), 2020, 581–591.

Bertolini, M., Costa, R. C., Barão, V. A. R., Cunha Villar, C., Retamal-Valdes, B., Feres, M., and Silva Souza, J. G., *Oral Microorganisms and Biofilms: New Insights to Defeat the Main Etiologic Factor of Oral Diseases*, **Microorganisms**, 10(12), 2022, 24–33.

Bettampadi, D., Dickey, B., Abrahamsen, M., Sirak, B., Baggio, M. L., Galan, L., Silva, R. C., Villa, L., Ponce, E. L., and Giuliano, A. R., *Differences in Factors Associated with High- and Low-Risk Oral Human Papillomavirus Genotypes in Men*, **The Journal of Infectious Diseases**, 223(12), 2021, 2099–2107.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciulek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Priesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., Van Der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., Von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu,

Q., Knight, R., and Caporaso, J. G., *Reproducible, Interactive, Scalable and Extensible Microbiome Data Science Using QIIME 2*, **Nature Biotechnology**, 37(8), 2019, 852–857.

Börnigen, D., Ren, B., Pickard, R., Li, J., Ozer, E., Hartmann, E. M., Xiao, W., Tickle, T., Rider, J., Gevers, D., Franzosa, E. A., Davey, M. E., Gillison, M. L., and Huttenhower, C., *Alterations in Oral Bacterial Communities Are Associated with Risk Factors for Oral and Oropharyngeal Cancer*, **Scientific Reports**, 7(1), 2017, 176-186.

Bramati, C., Abati, S., Bondi, S., Lissoni, A., Arrigoni, G., Filipello, F., and Trimarchi, M., *Early Diagnosis of Oral Squamous Cell Carcinoma May Ensure Better Prognosis: A Case Series*, **Clinical Case Reports**, 9(10), 2021, 50-64.

Bravo, I. G. and Félez-Sánchez, M., *Papillomaviruses: Viral Evolution, Cancer and Evolutionary Medicine*, **Evolution, Medicine, and Public Health**, 2015(1), 2015, 32–51.

Bruni, L., Albero, G., Rowley, J., Alemany, L., Arbyn, M., Giuliano, A. R., Markowitz, L. E., Broutet, N., and Taylor, M., *Global and Regional Estimates of Genital Human Papillomavirus Prevalence among Men: A Systematic Review and Meta-Analysis*, **The Lancet Global Health**, 11(9), 2023, e1345–e1362.

Bryan, N. S., Burleigh, M. C., and Easton, C., *The Oral Microbiome, Nitric Oxide and Exercise Performance*, **Nitric Oxide: Biology and Chemistry**, 125(1), 2022, 23–30.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P., *DADA2: High-Resolution Sample Inference from Illumina Amplicon Data*, **Nature Methods**, 13(7), 2016, 581–583.

Caselli, E., Fabbri, C., D'Accolti, M., Soffritti, I., Bassi, C., Mazzacane, S., and Franchi, M., *Defining the Oral Microbiome by Whole-Genome Sequencing and Resistome Analysis: The Complexity of the Healthy Picture*, **BMC Microbiology**, 20(1), 2020, 120-127.

Chen, J.-W., Wu, J.-H., Chiang, W.-F., Chen, Y.-L., Wu, W.-S., and Wu, L.-W., *Taxonomic and Functional Dysregulation in Salivary Microbiomes During Oral Carcinogenesis*, **Frontiers in Cellular and Infection Microbiology**, 11(1), 2021, 663-668.

Chen, X., Huang, H., Guo, C., Zhu, X., Chen, J., Liang, J., Yang, R., Shao, D., Chen, F., Shi, B., Yang, C., Leong, K. W., and Zhao, L., *Controlling Alveolar Bone Loss by Hydrogel-Based Mitigation of Oral Dysbiosis and Bacteria-Triggered Proinflammatory Immune Response*, **Advanced Functional Materials**, 35(3), 2025, 240-251.

Chockalingam, R., Downing, C., and Tying, S. K., *Cutaneous Squamous Cell Carcinomas in Organ Transplant Recipients*, **Journal of Clinical Medicine**, 4(6), 2015, 12-29.

Chung, R. S., Wong, S., Lin, D., Kokot, N. C., Sinha, U. K., and Han, A. Y., *Mechanisms of Crosstalk between the Oropharyngeal Microbiome and Human Papillomavirus in Oropharyngeal Carcinogenesis: A Mini Review*, **Frontiers in Oncology**, 14(1), 2024, 142-145.

Colevas, A. D., *HPV DNA as a Biomarker in Oropharyngeal Cancer: A Step in the Right Direction*, **Clinical Cancer Research**, 28(19), 2022, 4171–4172.

Colombo, A. P. V., Do Souto, R. M., Araújo, L. L., Espíndola, L. C. P., Hartenbach, F. A. R. R., Magalhães, C. B., Da Silva Oliveira Alves, G., Lourenço, T. G. B., and Da Silva-Boghossian, C. M., *Antimicrobial Resistance and Virulence of Subgingival Staphylococci Isolated from Periodontal Health and Diseases*, **Scientific Reports**, 13(1), 2023, 11-19.

Constantin, M., Chifiriuc, M. C., Mihaescu, G., Vrancianu, C. O., Dobre, E.-G., Cristian, R.-E., Bleotu, C., Bertesteanu, S. V., Grigore, R., Serban, B., and Cirstoiu, C., *Implications of Oral Dysbiosis and HPV Infection in Head and Neck Cancer: From Molecular and Cellular Mechanisms to Early Diagnosis and Therapy*, **Frontiers in Oncology**, 13(1), 2023, 127-136.

Dahlstrom, K. R., Sikora, A. G., Liu, Y., Chang, C.-C., Wei, P., Sturgis, E. M., and Li, G., *Characterization of the Oral Microbiota among Middle-Aged Men with and without Human Papillomavirus Infection*, **Oral Oncology**, 142(1), 2023, 106-120.

Di Spirito, F., Di Palo, M. P., Folliero, V., Cannatà, D., Franci, G., Martina, S., and Amato, M., *Oral Bacteria, Virus and Fungi in Saliva and Tissue Samples from Adult Subjects with Oral Squamous Cell Carcinoma: An Umbrella Review*, **Cancers**, 15(23), 2023, 55-60.

Do Nascimento, G. R., Da Silva Santos, A. C., Silva, N. N. T., Guilmarães, N. S., Lima, A. A., and Coura-Vital, W., *Prevalence of Non-Vaccine High-Risk HPV Cervical Infections in Vaccinated Women: A Systematic Review and Meta-Analysis*, **BMC Infectious Diseases**, 25(1), 2025, 13-21.

Dom-Chima, N., Ajang, Y. A., Dom-Chima, C. I., Biswas-Fiss, E., Aminu, M., and Biswas, S. B., *Human Papillomavirus Spectrum of HPV-Infected Women in Nigeria: An Analysis by Next-Generation Sequencing and Type-Specific PCR*, **Virology Journal**, 20(1), 2023, 14-24.

Doorbar, J., *Host Control of Human Papillomavirus Infection and Disease*, **Best Practice & Research Clinical Obstetrics & Gynaecology**, 47(1), 2018, 27-41.

Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., Huttenhower, C., and Langille, M. G. I., *PICRUSt2 for Prediction of Metagenome Functions*, **Nature Biotechnology**, 38(6), 2020, 685-688.

D'Souza, G., Clemens, G., Strickler, H. D., Wiley, D. J., Troy, T., Struijk, L., Gillison, M., and Fakhry, C., *Long-Term Persistence of Oral HPV Over 7 Years of Follow-Up*, **JNCI Cancer Spectrum**, 4(5), 2020, 47-53.

Du, Q., Ren, B., He, J., Peng, X., Guo, Q., Zheng, L., Li, J., Dai, H., Chen, V., Zhang, L., Zhou, X., and Xu, X., *Candida Albicans Promotes Tooth Decay by Inducing Oral Microbial Dysbiosis*, **The ISME Journal**, 15(3), 2021, 894-908.

Dupin, N., *Genital Warts*, **Clinics in Dermatology**, 22(6), 2004, 481-486.

Dutta, A., *Filiform Warts of 2 Years' Duration in a Child Treated by Homoeopathy*, **Homœopathic Links**, 37(1), 2024, 117-118.

Economopoulou, P., Kotsantis, I., and Psyrris, A., *Special Issue about Head and Neck Cancers: HPV Positive Cancers*, **International Journal of Molecular Sciences**, 21(9), 2020, 73-88.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., and Knight, R., *UCHIME Improves Sensitivity and Speed of Chimera Detection*, **Bioinformatics**, 27(16), 2011, 2194-2200.

Ekanayake Weeramange, C., Liu, Z., Hartel, G., Li, Y., Vasani, S., Langton-Lockton, J., Kenny, L., Morris, L., Frazer, I., Tang, K. D., and Punyadeera, C., *Salivary High-Risk Human Papillomavirus (HPV) DNA as a Biomarker for HPV-Driven Head and Neck Cancers*, **The Journal of Molecular Diagnostics: JMD**, 23(10), 2021, 1334–1342.

Elnaggar, J. H., Huynh, V. O., Lin, D., Hillman, R. T., Abana, C. O., El Alam, M. B., Tomasic, K. C., Karpinets, T. V., Kouzy, R., Phan, J. L., Wargo, J., Holliday, E. B., Das, P., Mezzari, M. P., Ajami, N. J., Lynn, E. J., Minsky, B. D., Morris, V. K., Milbourne, A., Messick, C. A., Klopp, A. H., Futreal, P. A., Taniguchi, C. M., Schmeler, K. M., and Colbert, L. E., *HPV-Related Anal Cancer Is Associated with Changes in the Anorectal Microbiome during Cancer Development*, **Frontiers in Immunology**, 14(1), 2023, 1051-1101.

Esberg, A., Fries, N., Haworth, S., and Johansson, I., *Saliva Microbiome Profiling by Full-Gene 16S rRNA Oxford Nanopore Technology versus Illumina MiSeq Sequencing*, **Biofilms and Microbiomes**, 10(1), 2024, 149-155.

Escobar Marcillo, D. I., Privitera, G. F., Rollo, F., Latini, A., Giuliani, E., Benevolo, M., Giuliani, M., Pichi, B., Pellini, R., and Donà, M. G., *Microbiome Analysis in Individuals with Human Papillomavirus Oral Infection*, **Scientific Reports**, 15(1), 2025, 49-53.

Ezechi, O., Akinsolu, F., Salako, A., Abodunrin, O., Adewole, I., Olagunju, M., Okunbor, H., Sanni-Adeniyi, R., Zamba, E., Njuguna, D. W., and Eleje, G., *High-Risk Human Papillomavirus Infection among Nigerian Women: A Systematic Review and Meta-Analysis*, **The Journal of International Medical Research**, 51(7), 2023, 30-41.

Escapa, F. I., Huang, Y., Chen, T., Lin, M., Kokaras, A., Dewhirst, F. E., and Lemon, K. P., *Construction of Habitat-Specific Training Sets to Achieve Species-Level Assignment in 16S rRNA Gene Datasets*, **Microbiome**, 8(1), 2020, 65-78.

Faney, A. O., Babalola, O. S., Odaibo, G. N., Arotiba, J., and Olaleye, O. D., *Oral Human Papilloma Virus Infection among Dental Clinic Attendees in Ibadan, Nigeria*, **African Journal of Laboratory Medicine**, 11(1), 2022, 1555-1556.

Feng, X., Patel, E. U., White, J. L., Li, S., Zhu, X., Zhao, N., Shi, J., Park, D. E., Liu, C. M., Kaul, R., Prodder, J. L., Quinn, T. C., Grabowski, M. K., and Tobian, A. A. R., *Association of Oral Microbiome With Oral Human Papillomavirus Infection: A Population Study of the National Health and Nutrition Examination Survey, 2009-2012*, **The Journal of Infectious Diseases**, 230(3), 2024, 726–735.

Fonsêca, T. C., Jural, L. A., Marañón-Vásquez, G. A., Magno, M. B., Roza, A. L. O. C., Ferreira, D. M. T. P., Maia, L. C., Romañach, M. J., Agostini, M., and Abrahão, A. C., *Global Prevalence of Human Papillomavirus-Related Oral and Oropharyngeal Squamous Cell Carcinomas: A Systematic Review and Meta-Analysis*, **Clinical Oral Investigations**, 28(1), 2023, 62-69.

Foro Ramos, E. D. S., Da Silva Couto, R., Tozetto-Mendoza, T. R., Bortoletto, P., Barbosa, E. M. G., Ferreira, N. E., Linhares, I. M., Spandorfer, S. D., Da Costa, A. C., Leal, E., Mendes-Correa, M. C., and Witkin, S. S., *Characterization of Multiple Human Papillomavirus Types in the Human Vagina Following Ovarian Hormonal Stimulation*, **Virology Journal**, 21(1), 2024, 22-29.

Galasso, L., Termite, F., Mignini, I., Esposto, G., Borriello, R., Vitale, F., Nicoletti, A., Paratore, M., Ainora, M. E., Gasbarrini, A., and Zocco, M. A., *Unraveling the Role of Fusobacterium Nucleatum in Colorectal Cancer: Molecular Mechanisms and Pathogenic Insights*, **Cancers**, 17(3), 2025, 36-38.

Giuliano, A. R., Felsher, M., Waterboer, T., Mirghani, H., Mehanna, H., Roberts, C., Chen, Y.-T., Lynam, M., Pedrós, M., Sanchez, E., Sirak, B., Surati, S., Alemany, L., Morais, E., and Pavón, M. A., *Oral Human Papillomavirus Prevalence and Genotyping Among a Healthy Adult Population in the US*, **JAMA Otolaryngology–Head & Neck Surgery**, 149(9), 2023, 78-83.

Gnanamony, M., Peedicayil, A., and Abraham, P., *An Overview of Human Papillomaviruses and Current Vaccine Strategies*, **Indian Journal of Medical Microbiology**, 25(1), 2007, 10–17.

Gong, T., Zeng, J., Tang, B., Zhou, X., and Li, Y., *CRISPR-Cas Systems in Oral Microbiome: From Immune Defense to Physiological Regulation*, **Molecular Oral Microbiology**, 35(2), 2020, 41–48.

Goto, M., Hanai, N., Nishikawa, D., Nagao, T., and Hasegawa, Y., *Prognosis of HPV-Positive Oral Squamous Carcinoma: A Cohort Study from Japan*, **Journal of Hard Tissue Biology**, 32(1), 2023, 77–82.

Gougousis, S., Mouchtaropoulou, E., Besli, I., Vrochidis, P., Skoumpas, I., and Constantinidis, I., *HPV-Related Oropharyngeal Cancer and Biomarkers Based on Epigenetics and Microbiome Profile*, **Frontiers in Cell and Developmental Biology**, 8(1), 2021, 625-630.

Griffen, A. L., Thompson, Z. A., Beall, C. J., Lilly, E. A., Granada, C., Treas, K. D., DuBois, K. R., Hashmi, S. B., Mukherjee, C., Gilliland, A. E., Vazquez, J. A., Hagensee, M. E., Leys, E. J., and Fidel, P. L., *Significant Effect of HIV/HAART on Oral Microbiota Using Multivariate Analysis*, **Scientific Reports**, 9(1), 2019, 39-46.

Guo, C., Dai, W., Zhou, Q., Gui, L., Cai, H., Wu, D., Hou, J., Li, C., Li, S., Du, H., and Wu, R., *Cervicovaginal Microbiota Significantly Changed for HPV-Positive Women with High-Grade Squamous Intraepithelial Lesion*, **Frontiers in Cellular and Infection Microbiology**, 12, 2022, 973-985.

Guo, L., Zhou, J., Xie, F., Lang, Q., Xu, Y., Chen, L., Xue, Z., Mao, Y., and Wang, R., *The Profile of Oral Microbiome in Chinese Elderly Population Associated with Aging and Systemic Health Status*, **BMC Oral Health**, 24(1), 2024, 89-95.

Hagensee, M. E., Yaegashi, N., and Galloway, D. A., *Self-Assembly of Human Papillomavirus Type 1 Capsids by Expression of the L1 Protein Alone or by Coexpression of the L1 and L2 Capsid Proteins*, **Journal of Virology**, 67(1), 1993, 315–322.

Han, F., Guo, X., Jiang, M., Xia, N., Gu, Y., and Li, S., *Structural Biology of the Human Papillomavirus*, **Structure**, 32(11), 2024, 1877–1892.

Han, Z., Hu, Y., Lin, X., Cheng, H., Dong, B., Liu, X., Wu, B., and Xu, Z. Z., *Systematic Analyses Uncover Robust Salivary Microbial Signatures and Host-Microbiome Perturbations*

in Oral Squamous Cell Carcinoma, **American Society for Microbiology Journals**, 10(2) 2025, 124-134.

Herreros-Pomares, A., Llorens, C., Soriano, B., Zhang, F., Gallach, S., Bagan, L., Murillo, J., Jantus-Lewintre, E., and Bagan, J., *Oral Microbiome in Proliferative Verrucous Leukoplakia Exhibits Loss of Diversity and Enrichment of Pathogens*, **Oral Oncology**, 120(1), 2021, 105-114.

Hu, J., Ye, Y., Chen, X., Xiong, L., Xie, W., and Liu, P., *Insight into the Pathogenic Mechanism of Mycoplasma Pneumoniae*, **Current Microbiology**, 80(1), 2022, 14-20.

Ijaiya, M. A., Aboyeji, A. P., Olatinwo, A. W. O., and Buhari, M. O., *Clinico-Pathological Presentation of Primary Cervical Cancer Seen in Ilorin, Nigeria*, **Nigerian Journal of Surgical Research**, 4(3), 2002, 89–93.

Jin, J., Li, S., Huang, H., Li, J., Lyu, Y., Ran, Y., Chang, H., and Zhao, X., *Development of Human Papillomavirus and Its Detection Methods (Review)*, **Experimental and Therapeutic Medicine**, 28(4), 2024, 382-389.

Kakabadze, M. Z., Paresishvili, T., Karalashvili, L., Chakhunashvili, D., and Kakabadze, Z., *Oral Microbiota and Oral Cancer: Review*, **Oncology Reviews**, 14(2), 2020, 476-477.

Kamarajan, P., Ateia, I., Shin, J. M., Fenno, J. C., Le, C., Zhan, L., Chang, A., Darveau, R., and Kapila, Y. L., *Periodontal Pathogens Promote Cancer Aggressivity via TLR/MyD88 Triggered Activation of Integrin/FAK Signaling That Is Therapeutically Reversible by a Probiotic Bacteriocin*, **PLOS Pathogens**, 16(10), 2020, 100-110.

Kanehisa, M., Goto, S., Sato, Y., Furumichi, M., and Tanabe, M., *KEGG for Integration and Interpretation of Large-Scale Molecular Data Sets*, **Nucleic Acids Research**, 40(1), 2012, 109–114.

Karmakar, S., Dutta, R., and Moulik, S., *A Transdisciplinary Analysis on Human Papilloma Virus and Cervical Cancer*, **Discover Medicine**, 2(1), 2025, 35-36.

Kedlaya, M. N., Puzhankara, L., Prasad, R., and Raj, A., *Periodontal Disease Pathogens, Pathogenesis, and Therapeutics: The CRISPR-Cas Effect*, **The CRISPR Journal**, 6(2), 2023, 90–98.

Khan, Z., Appleton, F., and Turner, J., *Is Cervical Intra-Epithelial Neoplasia Symptomatic*, **Journal of Obstetrics and Gynaecology**, 28(3), 2008, 336–337.

Khor, B., Snow, M., Herrman, E., Ray, N., Mansukhani, K., Patel, K. A., Said-Al-Naief, N., Maier, T., and Machida, C. A., *Interconnections between the Oral and Gut Microbiomes: Reversal of Microbial Dysbiosis and the Balance between Systemic Health and Disease*, **Microorganisms**, 9(3), 2021, 496-497.

Kim, H. S., Kim, C. G., Kim, W. K., Kim, K.-A., Yoo, J., Min, B. S., Paik, S., Shin, S. J., Lee, H., Lee, K., Kim, H., Shin, E.-C., Kim, T.-M., and Ahn, J. B., *Fusobacterium Nucleatum Induces a Tumor Microenvironment with Diminished Adaptive Immunity against Colorectal Cancers*, **Frontiers in Cellular and Infection Microbiology**, 13(1), 2023, 110-129.

- Kirnbauer, R., Booy, F., Cheng, N., Lowy, D. R., and Schiller, J. T., *Papillomavirus L1 Major Capsid Protein Self-Assembles into Virus-like Particles That Are Highly Immunogenic.*, **Proceedings of the National Academy of Sciences**, 89(24), 1992, 12180–12184.
- Klein, C., Kahesa, C., Mwaiselage, J., West, J. T., Wood, C., and Angeletti, P. C., *How the Cervical Microbiota Contributes to Cervical Cancer Risk in Sub-Saharan Africa*, **Frontiers in Cellular and Infection Microbiology**, 10(1), 2020, 23-24.
- Kleinstein, S. E., Nelson, K. E., and Freire, M., *Inflammatory Networks Linking Oral Microbiome with Systemic Health and Disease*, **Journal of Dental Research**, 99(10), 2020, 1131–1139.
- Kombe, A. J., Li, B., Zahid, A., Mengist, H. M., Bounda, G.-A., Zhou, Y., and Jin, T., *Epidemiology and Burden of Human Papillomavirus and Related Diseases, Molecular Pathogenesis, and Vaccine Evaluation*, **Frontiers in Public Health**, 8(1), 2020, 552-558.
- Kuhn, H.-M., Meier-Dieter, U., and Mayer, H., *ECA, the Enterobacterial Common Antigen*, **FEMS Microbiology Letters**, 54(3), 1988, 195–222.
- Kumar, P., Gupta, S., and Das, B. C., *Saliva as a Potential Non-Invasive Liquid Biopsy for Early and Easy Diagnosis/Prognosis of Head and Neck Cancer*, **Translational Oncology**, 40(1), 2024, 101-127.
- Kutz, J.-M., Rausche, P., Gheit, T., Puradiredja, D. I., and Fusco, D., *Barriers and Facilitators of HPV Vaccination in Sub-Saharan Africa: A Systematic Review*, **BMC Public Health**, 23(1), 2023, 974-975.
- Kwapisz, E., Garbacz, K., Kosecka-Strojek, M., Schubert, J., Bania, J., and Międzobrodzki, J., *Presence of Egc-Positive Major Clones ST 45, 30 and 22 among Methicillin-Resistant and Methicillin-Susceptible Oral Staphylococcus Aureus Strains*, **Scientific Reports**, 10(1), 2020, 188-189.
- Lamont, R. J., Fitzsimonds, Z. R., Wang, H., and Gao, S., *Role of Porphyromonas Gingivalis in Oral and Orodigestive Squamous Cell Carcinoma*, **Periodontology 2000**, 89(1), 2022, 154–165.
- Lan, Q., Zhang, C., Hua, H., and Hu, X., *Compositional and Functional Changes in the Salivary Microbiota Related to Oral Leukoplakia and Oral Squamous Cell Carcinoma: A Case Control Study*, **BMC Oral Health**, 23(1), 2023, 10-21.
- Łaniewski, P., İlhan, Z. E., and Herbst-Kralovetz, M. M., *The Microbiome and Gynaecological Cancer Development, Prevention and Therapy*, **Nature Reviews. Urology**, 17(4), 2020, 232–250.
- Lawrence, G. W., McCarthy, N., Walsh, C. J., Kunyoshi, T. M., Lawton, E. M., O'Connor, P. M., Begley, M., Cotter, P. D., and Guinane, C. M., *Effect of a Bacteriocin-Producing Streptococcus Salivarius on the Pathogen Fusobacterium Nucleatum in a Model of the Human Distal Colon*, **Gut Microbes**, 14(1), 222, 210-223.

Lechner, M., Liu, J., Masterson, L., and Fenton, T. R., *HPV-Associated Oropharyngeal Cancer: Epidemiology, Molecular Biology and Clinical Management*, **Nature Reviews. Clinical Oncology**, 19(5), 2022, 306–327.

Lee, J. E., Lee, S., Lee, H., Song, Y.-M., Lee, K., Han, M. J., Sung, J., and Ko, G., *Association of the Vaginal Microbiota with Human Papillomavirus Infection in a Korean Twin Cohort*, **PLoS ONE**, 8(5), 2013, 63-69.

Letian, T. and Tianyu, Z., *Cellular Receptor Binding and Entry of Human Papillomavirus*, **Virology Journal**, 7(1), 2010, 22-28.

Leung, E., Han, K., Zou, J., Zhao, Z., Zheng, Y., Wang, T. T., Rostami, A., Siu, L. L., Pugh, T. J., and Bratman, S. V., *HPV Sequencing Facilitates Ultrasensitive Detection of HPV Circulating Tumor DNA*, **Clinical Cancer Research**, 27(21), 2021, 5857–5868.

Li, R., Xiao, L., Gong, T., Liu, J., Li, Y., Zhou, X., Li, Y., and Zheng, X., *Role of Oral Microbiome in Oral Oncogenesis, Tumor Progression, and Metastasis*, **Molecular Oral Microbiology**, 38(1), 2023, 9–22.

Li, W., Liang, H., Lin, X., Hu, T., Wu, Z., He, W., Wang, M., Zhang, J., Jie, Z., Jin, X., Xu, X., Wang, J., Yang, H., Zhang, W., Kristiansen, K., Xiao, L., and Zou, Y., *A Catalog of Bacterial Reference Genomes from Cultivated Human Oral Bacteria*, **Biofilms and Microbiomes**, 9(1), 2023, 45-56.

Li, Z., Fu, R., Wen, X., Wang, Q., Huang, X., and Zhang, L., *The Significant Clinical Correlation of the Intratumor Oral Microbiome in Oral Squamous Cell Carcinoma Based on Tissue-Derived Sequencing*, **Frontiers in Physiology**, 13(1), 2023, 1089-1099.

Lim, Y. X. and D'Silva, N. J., *HPV-Associated Oropharyngeal Cancer: In Search of Surrogate Biomarkers for Early Lesions*, **Oncogene**, 43(8), 2024, 543–554.

Lim, Y. X., Mierzwa, M. L., Sartor, M. A., and D'Silva, N. J., *Clinical, Morphologic and Molecular Heterogeneity of HPV-Associated Oropharyngeal Cancer*, **Oncogene**, 42(40), 2023, 2939–2955.

Lin, H. and Peddada, S. D., *Analysis of Compositions of Microbiomes with Bias Correction*, **Nature Communications**, 11(1), 2020, 3514.

Lin, Y., Liang, X., Li, Z., Gong, T., Ren, B., Li, Y., and Peng, X., *Omics for Deciphering Oral Microecology*, **International Journal of Oral Science**, 16(1), 2024, 2-17.

Lipke, M. M., *An Armamentarium of Wart Treatments*, **Clinical Medicine and Research**, 4(4), 2006, 273–293.

Lu, Y., Xie, Z., Luo, G., Yan, H., Qian, H.-Z., Fu, L., Wang, B., Huang, R., Cao, F., Lin, H., You, R., Tan, L., Yu, T., Chen, M., Li, C., Liu, X., Lei, W., and Zou, H., *Global Burden of Oropharyngeal Cancer Attributable to Human Papillomavirus by Anatomical Subsite and Geographic Region*, **Cancer Epidemiology**, 78(1), 2022, 102-140.

Luo, Z.-Y., Chen, Q., Yang, H., Lin, M., Chen, C.-Y., Yang, C., and Yang, L.-Y., *The Prevalence and Genotype of Human Papillomavirus from Patients with Genital Warts in*

Eastern Guangdong Province, **Asian Pacific Journal of Cancer Prevention: APJCP**, 16(14), 2015, 5675–5679.

Mac, M. and Moody, C. A., *Epigenetic Regulation of the Human Papillomavirus Life Cycle*, **Pathogens**, 9(6), 2020, 48-53.

Maheshwari, Y., Handa, U., Aggarwal, P., and Goel, B., *Comparative Analysis of Conventional Cytology and Liquid-Based Cytology in the Detection of Carcinoma Cervix and Its Precursor Lesions*, **Journal of Cytology**, 40(3), 2023, 114–118.

Maier, T., *Oral Microbiome in Health and Disease: Maintaining a Healthy, Balanced Ecosystem and Reversing Dysbiosis*, **Microorganisms**, 11(6), 2023, 41-53.

Maity, T., *Homoeopathic Simillimum in Treatment of Verruca Plana (Flat Wart): A Case Report*, **Homœopathic Links**, 35(1), 2022, 191–197.

Makarewicz, J., Kaźmierczak-Siedlecka, K., Sobocki, B. K., Dobrucki, I. T., Kalinowski, L., and Stachowska, E., *Anti-Cancer Management of Head and Neck Cancers and Oral Microbiome—What Can We Clinically Obtain*, **Frontiers in Cellular and Infection Microbiology**, 14(1), 2024, 132-147.

Makhalanyane, T. P., Bezuidt, O. K. I., Pierneef, R. E., Mizrachi, E., Zeze, A., Fossou, R. K., Kouadjo, C. G., Duodu, S., Chikere, C. B., Babalola, O. O., Klein, A., Keyster, M., Du Plessis, M., Yorou, N. S., Hijri, M., Rossouw, T., Kamutando, C. N., Venter, S., Moleleki, L. N., and Murrell, C., *African Microbiomes Matter*, **Nature Reviews Microbiology**, 21(8), 2023, 479–481.

Malagón, T., Franco, E. L., Tejada, R., and Vaccarella, S., *Epidemiology of HPV-Associated Cancers Past, Present and Future: Towards Prevention and Elimination*, **Nature Reviews Clinical Oncology**, 21(7), 2024, 522–538.

Mann, A. E., O’Connell, L. M., Osagie, E., Akhigbe, P., Obuekwe, O., Omoigberale, A., Kelly, C., the DOMHaIN Study Team, Coker, M. O., and Richards, V. P., *Impact of HIV on the Oral Microbiome of Children Living in Sub-Saharan Africa, Determined by Using an rpoC Gene Fragment Metataxonomic Approach*, **Microbiology Spectrum**, 11(4), 2023, e00871-23.

De Martel, C., Georges, D., Bray, F., Ferlay, J., and Clifford, G. M., *Global Burden of Cancer Attributable to Infections in 2018: A Worldwide Incidence Analysis*, **The Lancet. Global Health**, 8(2), 2020, e180–e190.

Martin, M., *Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads*, **EMBnet.Journal**, 17(1), 2011, 10-17.

McKeon, M. G., Gallant, J.-N., Kim, Y. J., and Das, S. R., *It Takes Two to Tango: A Review of Oncogenic Virus and Host Microbiome Associated Inflammation in Head and Neck Cancer*, **Cancers**, 14(13), 2022, 31-40.

Min, Z., Yang, L., Hu, Y., and Huang, R., *Oral Microbiota Dysbiosis Accelerates the Development and Onset of Mucositis and Oral Ulcers*, **Frontiers in Microbiology**, 14(1), 2023, 106-110.

Mistry, H. B., Lebelo, R. L., Matshonyonge, F., Nchabeleng, M., Mathebula, M., Bogers, J.-P., Wood, N. H., Department of Periodontology and Oral Medicine, School of Oral Health Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa, HIV and Hepatitis Research Unit, National Health Laboratory service, Department of Virology, Sefako Makgatho Health Sciences University, Pretoria, South Africa, Department of Microbiology, Sefako Makgatho Health Sciences University, Pretoria, South Africa, Mecru Clinical Research Unit, Sefako Makgatho Health Sciences University, Pretoria, South Africa, and Faculty of Medicine and Health Sciences, Applied Molecular Biology Research Group (AMBIOR), Laboratory of Cell Biology and Histology, University of Antwerp, Belgium, *Oral and Oropharyngeal High-Risk HPV Prevalence, HIV Status, and Risk Behaviours in a Cohort of South African Men Who Have Sex with Men*, **AIMS Public Health**, 9(1), 2021, 129–141.

Morhason-Bello, I. O., Baisley, K., Pavon, M. A., Adewole, I. F., Bakare, R. A., De Sanjosé, S., Francis, S. C., and Watson-Jones, D., *Oral, Genital and Anal Human Papillomavirus Infections among Female Sex Workers in Ibadan, Nigeria*, **PLOS ONE**, 17(3), 2022, 265–269.

Morhason-Bello, I. O., Baisley, K., Pavon, M. A., Adewole, I. F., Bakare, R., Adebayo, S. A., De Sanjosé, S., Francis, S. C., and Watson-Jones, D., *Prevalence and Concordance of Penile, Anal, and Oral Human Papillomavirus Infections among Sexually Active Heterosexual Men in Ibadan, Nigeria*, **Cancer Causes & Control**, 36(1), 2025, 51–66.

Morhason-Bello, I. O., Baisley, K., Pavon, M. A., Adewole, I. F., Bakare, R., De Sanjosé, S., Francis, S. C., and Watson-Jones, D., *Prevalence and Genotype Specific Concordance of Oro-Genital and Anal Human Papillomavirus Infections among Sexually Active Nigerian Women*, **Infectious Agents and Cancer**, 16(1), 2021, 59–67.

Morshed, K., Polz-Gruska, D., Szymański, M., and Polz-Dacewicz, M., *Human Papillomavirus (HPV) - Structure, Epidemiology and Pathogenesis*, **The Polish Otolaryngology**, 68(5), 2014, 213–219.

Mougeot, J.-L. C., Beckman, M. F., Langdon, H. C., Lalla, R. V., Brennan, M. T., and Bahrani Mougeot, F. K., *Haemophilus Pittmaniae and Leptotrichia Spp. Constitute a Multi-Marker Signature in a Cohort of Human Papillomavirus-Positive Head and Neck Cancer Patients*, **Frontiers in Microbiology**, 12(1), 2021, 794–796.

Mremi, A., Mchome, B., Mlay, J., Schledermann, D., Blaakær, J., and Rasch, V., *Performance of HPV Testing, Pap Smear and VIA in Women Attending Cervical Cancer Screening in Kilimanjaro Region, Northern Tanzania: A Cross-Sectional Study Nested in a Cohort*, **BMJ Open**, 12(10), 2022, 64–71.

Münch, P. C., Franzosa, E. A., Stecher, B., McHardy, A. C., and Huttenhower, C., *Identification of Natural CRISPR Systems and Targets in the Human Microbiome*, **Cell Host & Microbe**, 29(1), 2021, 94–106.

Nagai, T., Shiba, T., Komatsu, K., Watanabe, T., Nemoto, T., Maekawa, S., Kobayashi, R., Matsumura, S., Ohsugi, Y., Katagiri, S., Takeuchi, Y., and Iwata, T., *Optimal 16S rRNA Gene Amplicon Sequencing Analysis for Oral Microbiota to Avoid the Potential Bias Introduced by Trimming Length, Primer, and Database*, **Microbiology Spectrum**, 12(12), 2024, 12–23.

Nishikawa, D., Suzuki, H., Beppu, S., Terada, H., Sawabe, M., and Hanai, N., *Near-Infrared Photoimmunotherapy for Oropharyngeal Cancer*, **Cancers**, 14(22), 2022, 56-62.

Ohihoin, A. G., Okwuraiwe, P. A., Musa, A. Z., Olorunfemi, G., Onwuamah, C. K., Ige, F., Amoo, O. S., Audu, R., Okogbo, F., Daniyan, B., Swende, T., Onyemelukwe, G. C., Daru, H., Usman, H., Shittu, O., Musa, J., Ezechi, O. C., and Ujah, I. A., *Prevalence and Predictors of High-Risk HPV in Nigeria*, **Advances in Infectious Diseases**, 12(04), 2022, 745–757.

Okoye, J. O., Ofodile, C. A., Adeleke, O. K., and Obioma, O., *Prevalence of High-Risk HPV Genotypes in Sub-Saharan Africa According to HIV Status: A 20-Year Systematic Review*, **Epidemiology and Health**, 43(1), 2021, 202-210.

Okoye, J. O., Okekpa, S. I., Chukwukelu, C. F., Onyekachi-Umeh, I. N., and Ngokere, A. A., *Racial Disparities in the Prevalence of Vaccine and Non-Vaccine HPV Types and Multiple HPV Infection between Asia and Africa: A Systematic Review*, **Asian Pacific Journal of Cancer Prevention**, 22(9), 2021, 2729–2741.

Okunade, K. S., *Human Papillomavirus and Cervical Cancer*, **Journal of Obstetrics and Gynaecology: The Journal of the Institute of Obstetrics and Gynaecology**, 40(5), 2020, 602–608.

Oliva, M., Schneeberger, P. H. H., Rey, V., Cho, M., Taylor, R., Hansen, A. R., Taylor, K., Hosni, A., Bayley, A., Hope, A. J., Bratman, S. V., Ringash, J., Singh, S., Weinreb, I., Perez-Ordoñez, B., Chepeha, D., Waldron, J., Xu, W., Guttman, D., Siu, L. L., Coburn, B., and Spreafico, A., *Transitions in Oral and Gut Microbiome of HPV+ Oropharyngeal Squamous Cell Carcinoma Following Definitive Chemoradiotherapy (ROMA LA-OPSCC Study)*, **British Journal of Cancer**, 124(9), 2021, 1543–1551.

Onu, E., Onu, E., Ejikeugwu, C., Azi, C., Ugwu, J., and Ogbu, O., *Seroprevalence and Molecular Investigation of Human Papillomavirus (HPV) Type-16 in HIV Positive Women in Abakaliki, Nigeria*, **International Journal of Virology**, 19(1), 2023, 1–5.

Onwuamah, C. K., Feng, N., Momoh, A. E., Uwandu, M., Ahmed, R. A., Idigbe, I., Vincent, G. D., Ogbu, C. A., Okonkwo, N., Sokei, J., Abimbola, B. S., Ojopagogo, T., Okoli, L. C., Adesina, M., Ezemelue, P. N., Sowunmi, O., Okwuzu, J., Labo-Popoola, O. H., Shaibu, J. O., Ohihoin, G. A., Nzeribe, E., David, A., Olaleye, O., Ofotokun, I., Dong, X., and Ezechi, O. C., *Prevalence and Risk Factors for High-Risk Human Papillomavirus Infection among Women from Three Southern Geopolitical Zones of Nigeria*, **Frontiers in Oncology**, 13(1), 2023, 125-130.

Onyango, S. O., Juma, J., De Paepe, K., and Van De Wiele, T., *Oral and Gut Microbial Carbohydrate-Active Enzymes Landscape in Health and Disease*, **Frontiers in Microbiology**, 12(1), 2021, 433-448.

Onywera, H., Williamson, A.-L., Mbulawa, Z. Z. A., Coetzee, D., and Meiring, T. L., *Factors Associated with the Composition and Diversity of the Cervical Microbiota of Reproductive-Age Black South African Women: A Retrospective Cross-Sectional Study*, **PeerJ**, 7(1), 2019, 74-88.

Osagie, E., Akhigbe, P., Idemudia, N., Obuekwe, O., Adebisi, R., Schlecht, N., Liu, J., Bromberg, Y., Eki-Udoko, F. E., Osazuwa-Peters, N., and Coker, M. O., *Human*

Papillomavirus, Human Immunodeficiency Virus, and Oral Microbiota Interplay in Nigerian Youth (HOMINY): A Prospective Cohort Study Protocol, **BMJ Open**, 15(2), 2025, 10-17.

Pan, Z., Wu, N., and Jin, C., *Intestinal Microbiota Dysbiosis Promotes Mucosal Barrier Damage and Immune Injury in HIV-Infected Patients*, **The Canadian Journal of Infectious Diseases & Medical Microbiology**, 2023(1), 2023, 80-96.

Peñaloza-Plascencia, M., Montoya-Fuentes, H., Flores-Martínez, S. E., Fierro-Velasco, F. J., Peñaloza-González, J. M., and Sánchez-Corona, J., *Molecular Identification of 7 Human Papillomavirus Types in Recurrent Respiratory Papillomatosis*, **Archives of Otolaryngology--Head and Neck Surgery**, 126(9), 2000, 1119–1123.

Peng, X., Cheng, L., You, Y., Tang, C., Ren, B., Li, Y., Xu, X., and Zhou, X., *Oral Microbiota in Human Systemic Diseases*, **International Journal of Oral Science**, 14(1), 2022, 14-15.

Perez Rosero, E., Heron, S., Jovel, J., O'Neil, C. R., Turvey, S. L., Parashar, P., and Elahi, S., *Differential Signature of the Microbiome and Neutrophils in the Oral Cavity of HIV-Infected Individuals*, **Frontiers in Immunology**, 12(1), 2021, 78-90.

Pisano, M., Giordano, F., Sangiovanni, G., Capuano, N., Acerra, A., and D'Ambrosio, F., *The Interaction between the Oral Microbiome and Systemic Diseases: A Narrative Review*, **Microbiology Research**, 14(4), 2023, 1862–1878.

de Planell-Mas, E., Martínez-Garriga, B., Zalacain, A. J., Vinuesa, T., and Viñas, M., *Human Papillomaviruses Genotyping in Plantar Warts*, **Journal of Medical Virology**, 89(5), 2017, 902–907.

Plotzker, R. E., Vaidya, A., Pokharel, U., and Stier, E. A., *Sexually Transmitted Human Papillomavirus: Update in Epidemiology, Prevention, and Management*, **Infectious Disease Clinics of North America**, 37(2), 2023, 289–310.

Posit team, *RStudio: Integrated Development Environment for R*, Boston, MA: Posit Software, PBC, **Advances in Infectious Diseases**, 14(3), 2024, 7-10.

Radaic, A., Ganther, S., Kamarajan, P., Grandis, J., Yom, S. S., and Kapila, Y. L., *Paradigm Shift in the Pathogenesis and Treatment of Oral Cancer and Other Cancers Focused on the Oralome and Antimicrobial-based Therapeutics*, **Periodontology 2000**, 87(1), 2021, 76–93.

Rahadiani, N., Habiburrahman, M., Stephanie, M., Handjari, D. R., and Krisnuhoni, E., *Estimated Projection of Oral Squamous Cell Carcinoma Annual Incidence from Twenty Years Registry Data: A Retrospective Cross-Sectional Study in Indonesia*, **PeerJ**, 11(1), 2023, 159-163.

Rai, A. K. and Mitchell, A. M., *Enterobacterial Common Antigen: Synthesis and Function of an Enigmatic Molecule*, **Molecular Biology**, 11(4), 2020, e01914-20.

Rajasekaran, J. J., Krishnamurthy, H. K., Bosco, J., Jayaraman, V., Krishna, K., Wang, T., and Bei, K., *Oral Microbiome: A Review of Its Impact on Oral and Systemic Health*, **Microorganisms**, 12(9), 2024, 17-27.

Reimers, L. L., Mehta, S. D., Massad, L. S., Burk, R. D., Xie, X., Ravel, J., Cohen, M. H., Palefsky, J. M., Weber, K. M., Xue, X., Anastos, K., Minkoff, H., Atrio, J., D'Souza, G., Ye, Q., Colie, C., Zolnik, C. P., Spear, G. T., and Strickler, H. D., *The Cervicovaginal Microbiota and Its Associations With Human Papillomavirus Detection in HIV-Infected and HIV-Uninfected Women*, **Journal of Infectious Diseases**, 214(9), 2016, 1361–1369.

Richards, S., *An Overview of Genital Warts*, **Nursing Standard (Royal College of Nursing (Great Britain): 1987)**, 28(24), 2014, 46–50.

Riddell, J., Brouwer, A. F., Walline, H. M., Campredon, L. P., Meza, R., Eisenberg, M. C., Andrus, E. C., Delinger, R. L., Yost, M. L., McCloskey, J. K., Thomas, T. B., Huang, S., Ferris, R. L., Shin, D. M., Fakhry, C., Ow, T., Li, D., Berlot, A., Carey, T. E., and Schlecht, N. F., *Oral Human Papillomavirus Prevalence, Persistence, and Risk-Factors in HIV-Positive and HIV-Negative Adults*, **Tumour Virus Research**, 13(1), 2022, 200–237.

Roman, B. R. and Aragonés, A., *Epidemiology and Incidence of HPV-Related Cancers of the Head and Neck*, **Journal of Surgical Oncology**, 124(6), 2021, 920–922.

Sabatini, M. E. and Chiocca, S., *Human Papillomavirus as a Driver of Head and Neck Cancers*, **British Journal of Cancer**, 122(3), 2020, 306–314.

Saliba, M., Ghossein, R., and Xu, B., *HPV-related Head and Neck Cancers: Pathology and Biology*, **Journal of Surgical Oncology**, 124(6), 2021, 923–930.

Santiago S, G., Angel, A., and Ignatio, B., *Papillomaviruses: Different Genes Have Different Histories*, **Trends in Microbiology**, 13(11), 2005, 514–521.

Sarkar, P., Malik, S., Laha, S., Das, S., Bunk, S., Ray, J. G., Chatterjee, R., and Saha, A., *Dysbiosis of Oral Microbiota During Oral Squamous Cell Carcinoma Development*, **Frontiers in Oncology**, 11(1), 2021, 614–628.

Sarkola, M. E., Grénman, S. E., Rintala, M. A. M., Syrjänen, K. J., and Syrjänen, S. M., *Human Papillomavirus in the Placenta and Umbilical Cord Blood*, **Acta Obstetrica et Gynecologica Scandinavica**, 87(11), 2008, 1181–1188.

Satanova, A., Kaidarova, D., Kukubassov, E., Bolatbekova, R., Kaldybekov, D., and Bertleuov, O., *The Global Prevalence of Human Papillomavirus Causing Cervical Cancer: A Literature Review*, **Oncologia Radiologia Kazakhstan**, 65(3), 2022, 42–46.

Schmitt, N. C., *HPV in Non-Oropharyngeal Head and Neck Cancer: Does It Matter*, **Annals of Translational Medicine**, 8(18), 2020, 1120–1120.

Sedghi, L., DiMassa, V., Harrington, A., Lynch, S. V., and Kapila, Y. L., *The Oral Microbiome: Role of Key Organisms and Complex Networks in Oral Health and Disease*, **Periodontology 2000**, 87(1), 2021, 107–131.

Seedat, R. Y., Combrinck, C. E., and Burt, F. J., *HPV Associated with Recurrent Respiratory Papillomatosis*, **Future Virology**, 8(5), 2023, 477–492.

Sheena, B. S., Hiebert, L., Han, H., Ippolito, H., Abbasi-Kangevari, M., Abbasi-Kangevari, Z., Abbastabar, H., Abdoli, A., Abubaker Ali, H., Adane, M. M., Adegboye, O. A., Adnani, Q. E. S., Advani, S. M., Afzal, M. S., Afzal, S., Aghaie Meybodi, M., Ahadinezhad, B.,

Ahinkorah, B. O., Ahmad, S., Ahmad, T., Ahmadi, S., Ahmed, H., Ahmed, M. B., Ahmed Rashid, T., Akalu, G. T., Akililu, A., Akram, T., Al Hamad, H., Alahdab, F., Alem, A. Z., Alem, D. T., Alhalaiqa, F. A. N., Alhassan, R. K., Ali, L., Ali, M. A., Alimohamadi, Y., Alipour, V., Alkhayyat, M., Almustanyir, S., Al-Raddadi, R. M., Altawalrah, H., Amini, S., Amu, H., Ancuceanu, R., Andrei, C. L., Andrei, T., Anoushiravani, A., Ansar, A., Anyasodor, A. E., Arabloo, J., Arab-Zozani, M., Argaw, A. M., Argaw, Z. G., Arshad, M., Artamonov, A. A., Ashraf, T., Atlaw, D., Ausloos, F., Ausloos, M., Azadnajafabad, S., Azangou-Khyavy, M., Azari Jafari, A., Azarian, G., Bagheri, S., Bahadory, S., Baig, A. A., Banach, M., Barati, N., Barrow, A., Batiha, A.-M. M., Bejarano Ramirez, D. F., Belgaumi, U. I., Berhie, A. Y., Bhagat, D. S., Bhardwaj, N., Bhardwaj, P., Bhattacharyya, K., Bhojaraja, V. S., Bijani, A., Biondi, A., Bodicha, B. B. A., Bojia, H. A., Bolor, A., Bosetti, C., Braithwaite, D., Briko, N. I., Butt, Z. A., Cámera, L. A., Chakinala, R. C., Chakraborty, P. A., Charan, J., Chen, S., Choi, J.-Y. J., Choudhari, S. G., Chowdhury, F. R., Chu, D.-T., Chung, S.-C., Cortesi, P. A., Cowie, B. C., Culbreth, G. T., Dadras, O., Dai, X., Dandona, L., Dandona, R., De La Hoz, F. P., Debela, S. A., Dedefo, M. G., Demeke, F. M., Demie, T. G. G., Demissie, G. D., Derbew Molla, M., Desta, A. A., Dhamnetiya, D., Dhimal, M. L., Dhimal, M., Didehdar, M., Doan, L. P., Dorostkar, F., Drake, T. M., Eghbalian, F., Ekholuenetale, M., El Sayed, I., El Sayed Zaki, M., Elhadi, M., Elmonem, M. A., Elsharkawy, A., Enany, S., Enyew, D. B., Erkhembayar, R., Eskandarieh, S., Esmaeilzadeh, F., Ezzikouri, S., Farrokhpour, H., Fetensa, G., Fischer, F., Foroutan, M., Gad, M. M., Gaidhane, A. M., Gaidhane, S., Galles, N. C., Gallus, S., Gebremeskel, T. G., Gebreyohannes, E. A., Ghadiri, K., Ghaffari, K., Ghafourifard, M., Ghamari, S.-H., Ghashghaee, A., Gholami, A., Gholizadeh, A., Gilani, A., Goel, A., Golechha, M., Goleij, P., Golinelli, D., Gorini, G., Goshu, Y. A., Griswold, M. G., Gubari, M. I. M., Gupta, B., Gupta, S., Gupta, V. B., Gupta, V. K., Haddadi, R., Halwani, R., Hamid, S. S., Hamidi, S., Hanif, A., Haque, S., Harapan, H., Hargono, A., Hariri, S., Hasaballah, A. I., Hasan, S. M. M., Hassanipour, S., Hassankhani, H., Hay, S. I., Hayat, K., Heidari, G., Herteliu, C., Heyi, D. Z., Hezam, K., Holla, R., Hosseini, M.-S., Hosseini, M., Hosseinzadeh, M., Hostiuc, M., Househ, M., Huang, J., Hussein, N. R., Iavicoli, I., Ibitoye, S. E., Ilesanmi, O. S., Ilic, I. M., Ilic, M. D., Irham, L. M., Islam, J. Y., Ismail, N. E., Jacobsen, K. H., Jadidi-Niaragh, F., Javadi Mamaghani, A., Jayaram, S., Jayawardena, R., Jebai, R., Jha, R. P., Joseph, N., Joukar, F., Kaambwa, B., Kabir, A., Kabir, Z., Kalhor, R., Kandel, H., Kanko, T. K. T., Kantar, R. S., Karaye, I. M., Kassa, B. G., Kemp Bohan, P. M., Keykhai, M., Khader, Y. S., Khajuria, H., Khan, G., Khan, I. A., Khan, J., Khan, M. A., Khanali, J., Khater, A. M., Khatib, M. N., Khodadost, M., Khoja, A. T., Khosravizadeh, O., Khubchandani, J., Kim, G. R., Kim, H., Kim, M. S., Kim, Y. J., Kocarnik, J. M., Kolahi, A.-A., Koteeswaran, R., Kumar, G. A., La Vecchia, C., Lal, D. K., Landires, I., Lasrado, S., Lazarus, J. V., Ledda, C., Lee, D. W., Lee, S., Lee, Y. Y., Levi, M., Li, J., Lim, S. S., Lobo, S. W., Lopukhov, P. D., Loureiro, J. A., MacLachlan, J. H., Magdy Abd El Razek, H., Magdy Abd El Razek, M., Majeed, A., Makki, A., Malekpour, M.-R., Malekzadeh, R., Malik, A. A., Mansour-Ghanaei, F., Mansournia, M. A., Martins-Melo, F. R., Matthews, P. C., Mendoza, W., Menezes, R. G., Meretoja, T. J., Mersha, A. G., Mestrovic, T., Miller, T. R., Minh, L. H. N., Mirica, A., Mirmoeeni, S., Mirrakhimov, E. M., Misra, S., Mithra, P., Moazen, B., Mohamadkhani, A., Mohammadi, M., Mohammed, S., Moka, N., Mokdad, A. H., Moludi, J., Momtazmanesh, S., Monasta, L., Moradi, G., Moradzadeh, M., Moradzadeh, R., Moraga, P., Mostafavi, E., Mubarik, S., Muniyandi, M., Murray, C. J. L., Naghavi, M., Naimzada, M. D., Narasimha Swamy, S., Natto, Z. S., Nayak, B. P., Nazari, J., Negroi, I., Negru, S. M., Nejadghaderi, S. A., Neupane Kandel, S., Nguyen, H. L. T., Ngwa, C. H., Niazi, R. K., Nnaji, C. A., Noubiap, J. J., Nowroozi, A., Nuñez-Samudio, V., Oancea, B., Ochir, C., Odukoya, O. O., Oh, I.-H., Olagunju, A. T., Olakunde, B. O., Omar Bali, A., Omer, E., Otstavnov, S. S., Oumer, B., Padubidri, J. R., Pana, A., Pandey, A., Park, E.-C., Pashazadeh Kan, F., Patel, U. K., Paudel,

U., Petcu, I.-R., Piracha, Z. Z., Pollok, R. C. G., Postma, M. J., Pourshams, A., Poustchi, H., Rabiee, M., Rabiee, N., Rafiei, A., Rafiei, S., Raghuram, P. M., Rahman, M., Rahmani, A. M., Rahmawaty, S., Rajesh, A., Ranasinghe, P., Rao, C. R., Rao, S. J., Rashidi, M., Rashidi, M.-M., Rawaf, D. L., Rawaf, S., Rawassizadeh, R., Rezaei, N., Rezapour, A., Rezazadeh-Khadem, S., Rodriguez, J. A. B., Rwegerera, G. M., Sabour, S., Saddik, B., Saeb, M. R., Saeed, U., Sahebkar, A., Saif-Ur-Rahman, K., Salah, S., Salimzadeh, H., Sampath, C., Samy, A. M., Sanabria, J., Sanmarchi, F., Santric-Milicevic, M. M., Sarveazad, A., Sathian, B., Sawhney, M., Seidu, A.-A., Sepanlou, S. G., Seylani, A., Shahabi, S., Shaikh, M. A., Shaker, E., Shakhmardanov, M. Z., Shannawaz, M., Shenoy, S. M., Shetty, J. K., Shetty, P. H., Shibuya, K., Shin, J. I., Shobeiri, P., Sibhat, M. M., Singh, A. D., Singh, J. A., Singh, S., Skryabin, V. Y., Skryabina, A. A., Sohrabpour, A. A., Song, S., Tabaeian, S. P., Tadesse, E. G., Taheri, M., Tampa, M., Tan, K.-K., Tavakoli, A., Tbakhi, A., Tefera, B. N., Tehrani-Banihashemi, A., Tesfaw, H. M., Thapar, R., Thavamani, A., Tohidast, S. A., Tollosa, D. N., Tosti, M. E., Tovani-Palone, M. R., Traini, E., Tran, M. T. N., Trihandini, I., Tusa, B. S., Ullah, I., Vacante, M., Valadan Tahbaz, S., Valdez, P. R., Varthya, S. B., Vo, B., Waheed, Y., Weldesenbet, A. B., Woldemariam, M., Xu, S., Yahyazadeh Jabbari, S. H., Yaseri, M., Yeshaw, Y., Yiğit, V., Yirdaw, B. W., Yonemoto, N., Yu, C., Yunusa, I., Zahir, M., Zaki, L., Zamani, M., Zamanian, M., Zastrozhin, M. S., Vos, T., Ward, J. W., and Dirac, M. A., *Global, Regional, and National Burden of Hepatitis B, 1990–2019: A Systematic Analysis for the Global Burden of Disease Study 2019*, **The Lancet Gastroenterology & Hepatology**, 7(9), 2022, 796–829.

Shiao, S. L., Kershaw, K. M., Limon, J. J., You, S., Yoon, J., Ko, E. Y., Guarnerio, J., Potdar, A. A., McGovern, D. P. B., Bose, S., Dar, T. B., Noe, P., Lee, J., Kubota, Y., Maymi, V. I., Davis, M. J., Henson, R. M., Choi, R. Y., Yang, W., Tang, J., Gargus, M., Prince, A. D., Zumsteg, Z. S., and Underhill, D. M., *Commensal Bacteria and Fungi Differentially Regulate Tumor Responses to Radiation Therapy*, **Cancer Cell**, 39(9), 2021, 1202–1213.

Shigeishi, H., *Association between Human Papillomavirus and Oral Cancer: A Literature Review*, **International Journal of Clinical Oncology**, 28(8), 2023, 982–989.

Silva, M. P. D., Silva, V. D. O., Pasetto, S., Ando-Suguimoto, E. S., Kawamoto, D., Mata, G. M. S. C., Murata, R. M., Mayer, M. P. A., and Chen, C., *Aggregatibacter Actinomycetemcomitans Outer Membrane Proteins 29 and 29 Parologue Induce Evasion of Immune Response*, **Frontiers in Oral Health**, 3(1), 2022, 835–842.

Singh, D., Vignat, J., Lorenzoni, V., Eslahi, M., Ginsburg, O., Lauby-Secretan, B., Arbyn, M., Basu, P., Bray, F., and Vaccarella, S., *Global Estimates of Incidence and Mortality of Cervical Cancer in 2020: A Baseline Analysis of the WHO Global Cervical Cancer Elimination Initiative*, **The Lancet Global Health**, 11(2), 2023, e197–e206.

Kjær, S. K., K, F., C, M., and T, I., *Long-Term Absolute Risk of Cervical Intraepithelial Neoplasia Grade 3 or Worse Following Human Papillomavirus Infection: Role of Persistence*, **Journal of the National Cancer Institute**, 102(19), 2010, 1478–88.

Smędra, A. and Berent, J., *The Influence of the Oral Microbiome on Oral Cancer: A Literature Review and a New Approach*, **Biomolecules**, 13(5), 2023, 81–95.

Sun, J., Tang, Q., Yu, S., Xie, M., Xie, Y., Chen, G., and Chen, L., *Role of the Oral Microbiota in Cancer Evolution and Progression*, **Cancer Medicine**, 9(17), 2020, 6306–6321.

Syrjänen, S., *Current Concepts on Human Papillomavirus Infections in Children*, **Acta Pathologica, Microbiologica et Immunologica Scandinavica**, 118(6–7), 2010, 89–94.

Syrjänen, S., *Oral Manifestations of Human Papillomavirus Infections*, **European Journal of Oral Sciences**, 126(1), 2018, 49–66.

Talapko, J., Erić, S., Meštrović, T., Stipetić, M. M., Juzbašić, M., Katalinić, D., Bekić, S., Muršić, D., Flam, J., Belić, D., Lešić, D., Fureš, R., Markanović, M., and Škrlec, I., *The Impact of Oral Microbiome Dysbiosis on the Aetiology, Pathogenesis, and Development of Oral Cancer*, **Cancers**, 16(17), 2024, 90–97.

The Galaxy Community, Abueg, L. A. L., Afgan, E., Allart, O., Awan, A. H., Bacon, W. A., Baker, D., Bassetti, M., Batut, B., Bernt, M., Blankenberg, D., Bombarely, A., Bretaudeau, A., Bromhead, C. J., Burke, M. L., Capon, P. K., Čech, M., Chavero-Díez, M., Chilton, J. M., Collins, T. J., Coppens, F., Coraor, N., Cuccuru, G., Cumbo, F., Davis, J., De Geest, P. F., De Koning, W., Demko, M., DeSanto, A., Begines, J. M. D., Doyle, M. A., Droesbeke, B., Erxleben-Eggenhofer, A., Föll, M. C., Formenti, G., Fouilloux, A., Gangazhe, R., Genthon, T., Goecks, J., Beltran, A. N. G., Goonasekera, N. A., Goué, N., Griffin, T. J., Grüning, B. A., Guerler, A., Gundersen, S., Gustafsson, O. J. R., Hall, C., Harrop, T. W., Hecht, H., Heidari, A., Heisner, T., Heyl, F., Hiltemann, S., Hotz, H.-R., Hyde, C. J., Jagtap, P. D., Jakiela, J., Johnson, J. E., Joshi, J., Jossé, M., Jum'ah, K., Kalaš, M., Kamieniecka, K., Kayikcioglu, T., Konkol, M., Kostykin, L., Kucher, N., Kumar, A., Kuntz, M., Lariviere, D., Lazarus, R., Bras, Y. L., Corguillé, G. L., Lee, J., Leo, S., Liborio, L., Libouban, R., Tabernero, D. L., Lopez-Delisle, L., Los, L. S., Mahmoud, A., Makunin, I., Marin, P., Mehta, S., Mok, W., Moreno, P. A., Morier-Genoud, F., Mosher, S., Müller, T., Nasr, E., Nekrutenko, A., Nelson, T. M., Oba, A. J., Ostrovsky, A., Polunina, P. V., Poterlowicz, K., Price, E. J., Price, G. R., Rasche, H., Raubenolt, B., Royaux, C., Sargent, L., Savage, M. T., Savchenko, V., Savchenko, D., Schatz, M. C., Seguinéau, P., Serrano-Solano, B., Soranzo, N., Srikakulam, S. K., Suderman, K., Syme, A. E., Tangaro, M. A., Tedds, J. A., Tekman, M., Cheng (Mike) Thang, W., Thanki, A. S., Uhl, M., Van Den Beek, M., Varshney, D., Vessio, J., Videm, P., Von Kuster, G., Watson, G. R., Whitaker-Allen, N., Winter, U., Wolstencroft, M., Zambelli, F., Zierep, P., and Zoabi, R., *The Galaxy Platform for Accessible, Reproducible, and Collaborative Data Analyses: 2024 Update*, **Nucleic Acids Research**, 52(1), 2024, 83–94.

Tuominen, H. and Rautava, J., *Oral Microbiota and Cancer Development*, **Pathobiology: Journal of Immunopathology, Molecular and Cellular Biology**, 88(2), 2021, 116–126.

Ugochukwu, I. C. I., Aneke, C. I., Idoko, I. S., Sani, N. A., Amoche, A. J., Mshiela, W. P., Ede, R. E., Ibrahim, N. D. G., Njoku, C. I. O., and Sackey, A. K. B., *Bovine Papilloma: Aetiology, Pathology, Immunology, Disease Status, Diagnosis, Control, Prevention and Treatment: A Review*, **Comparative Clinical Pathology**, 28(3), 2019, 737–745.

Upadhyay, R., Dhakal, A., Wheeler, C., Hoyd, R., Jagjit Singh, M., Karivedu, V., Bhateja, P., Bonomi, M., Valentin, S., Gamez, M. E., Konieczkowski, D. J., Baliga, S., Grecula, J. C., Blakaj, D. M., Gogineni, E., Mitchell, D. L., Denko, N. C., Spakowicz, D., and Jhawar, S. R., *Comparative Analysis of the Tumor Microbiome, Molecular Profiles, and Immune Cell Abundances by HPV Status in Mucosal Head and Neck Cancers and Their Impact on Survival*, **Cancer Biology & Therapy**, 25(1), 2024, 235–249.

Valls, J., Baena, A., Venegas, G., Celis, M., González, M., Sosa, C., Santin, J. L., Ortega, M., Soilán, A., Turcios, E., Figueroa, J., Rodríguez De La Peña, M., Figueredo, A., Beracochea,

A. V., Pérez, N., Martínez-Better, J., Lora, O., Jiménez, J. Y., Giménez, D., Fleider, L., Salgado, Y., Martínez, S., Bellido-Fuentes, Y., Flores, B., Tatti, S., Villagra, V., Cruz-Valdez, A., Terán, C., Sánchez, G. I., Rodríguez, G., Picconi, M. A., Ferrera, A., Mendoza, L., Calderón, A., Murillo, R., Wiesner, C., Broutet, N., Luciani, S., Pérez, C., Darragh, T. M., Jerónimo, J., Herrero, R., and Almonte, M., *Performance of Standardised Colposcopy to Detect Cervical Precancer and Cancer for Triage of Women Testing Positive for Human Papillomavirus: Results from the ESTAMPA Multicentric Screening Study*, **The Lancet Global Health**, 11(3), 2023, e350–e360.

Wang, S., Lu, Z., Wang, S., Liu, W., Gao, J., Tian, L., Wang, L., Zhang, X., Zhao, X., Wang, W., and Li, C., *The Inhibitory Effects and Mechanisms of Polymannuroguluronate Sulfate against Human Papillomavirus Infection in Vitro and in Vivo*, **Carbohydrate Polymers**, 241(1), 2020, 116-125.

Wang, S., Dang, L., Liu, S., Rezhake, R., Yan, H., Duan, X., Zhang, L., Zhang, L., Zhang, L., Su, M., Guo, F., Yan, C., Liu, M., Cao, X., Sun, M., Qiao, Y., and Zhao, F., *Cervical Cancer Screening via Visual Inspection With Acetic Acid and Lugol Iodine for Triage of HPV-Positive Women*, **JAMA Network Open**, 7(3), 2024, 244-252.

Wei, K., Ma, Y., Xu, J., Zheng, H., Xue, L., Chu, Y., Shi, Y., Sun, Z., and Sun, Q., *Potential Changes in Microorganisms and Metabolites Associated with Oral Cancer: A Preliminary Study*, **BMC Cancer**, 25(1), 2025, 61-71.

Wen, L., Mu, W., Lu, H., Wang, X., Fang, J., Jia, Y., Li, Q., Wang, D., Wen, S., Guo, J., Dai, W., Ren, X., Cui, J., Zeng, G., Gao, J., Wang, Z., and Cheng, B., *Porphyromonas Gingivalis Promotes Oral Squamous Cell Carcinoma Progression in an Immune Microenvironment*, **Journal of Dental Research**, 99(6), 2020, 666–675.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., and Yutani, H., *Welcome to the Tidyverse*, **Journal of Open Source Software**, 4(43), 2019, 76-86.

Willis, J. R. and Gabaldón, T., *The Human Oral Microbiome in Health and Disease: From Sequences to Ecosystems*, **Microorganisms**, 8(2), 2020, 30-38.

Witchey, D. J., Witchey, N. B., Roth-Kauffman, M. M., and Kauffman, M. K., *Plantar Warts: Epidemiology, Pathophysiology, and Clinical Management*, **The Journal of the American Osteopathic Association**, 118(2), 2018, 92-94.

Wood, N. H., Makua, K. S., Lebelo, R. L., Redzic, N., Benoy, I., Vanderveken, O. M., and Bogers, J., *Human Papillomavirus Prevalence in Oral and Oropharyngeal Rinse and Gargle Specimens of Dental Patients and of an HIV-Positive Cohort from Pretoria, South Africa*, **Advances in Virology**, 2020(1), 2020, 239-249.

Wu, K., Xu, B., Zhou, X., Guo, H., Du, G., Zhang, C., Chen, F., and Chen, X., *Microbiota and Metabolite Profiles of Saliva, Oral Swab and Cancer Tissue from Patients with Oral Squamous Cell Carcinoma (OSCC)*, **Infectious Agents and Cancer**, 20(1), 2025, 32-33.

Wu, L., Wang, W., Zhang, J., Wu, X., Chen, Y., Gu, X., Shao, H., Li, H., and Liu, W., *Detection of Five Types of HPV Genotypes Causing Anogenital Warts (Condyloma Acuminatum) Using PCR-Tm Analysis Technology*, **Frontiers in Microbiology**, 13, 2022, 857-860.

Xia, Q. and Pierson, S., *HPV Infection and Oral Microbiota: Interactions and Future Implications*, **International Journal of Molecular Sciences**, 26(4), 2025, 14-24.

Xu, Q., Wang, W., Li, Y., Cui, J., Zhu, M., Liu, Y., and Liu, Y., *The Oral-Gut Microbiota Axis: A Link in Cardiometabolic Diseases*, **Biofilms and Microbiomes**, 11(1), 2025, 11.

Yang, J., Guo, K., Zhang, A., Zhu, Y., Li, W., Yu, J., and Wang, P., *Survival Analysis of Age-Related Oral Squamous Cell Carcinoma: A Population Study Based on SEER*, **European Journal of Medical Research**, 28(1), 2023, 41-53.

Yoo, H., Won, S.-S., Choi, H.-C., Yoon, T.-J., Ye, S.-K., Park, T.-K., and Lee, K.-H., *Detection and Identification of Human Papillomavirus Types Isolated from Korean Patients with Flat Warts*, **Microbiology and Immunology**, 49(7), 2005, 633–638.

Yousefi, L., Leylabadlo, H. E., Poulak, T., Eslami, H., Taghizadeh, S., Ganbarov, K., Yousefi, M., Tanomand, A., Yousefi, B., and Kafil, H. S., *Oral Spirochetes: Pathogenic Mechanisms in Periodontal Disease*, **Microbial Pathogenesis**, 144, 2020, 104-123.

Zakrzewski, M., Gannon, O. M., Panizza, B. J., Saunders, N. A., and Antonsson, A., *Human Papillomavirus Infection and Tumor Microenvironment Are Associated with the Microbiota in Patients with Oropharyngeal Cancers—Pilot Study*, **Head & Neck**, 43(11), 2021, 3324–3330.

Zhang, W., Yin, Y., Jiang, Y., Yang, Y., Wang, W., Wang, X., Ge, Y., Liu, B., and Yao, L., *Relationship between Vaginal and Oral Microbiome in Patients of Human Papillomavirus (HPV) Infection and Cervical Cancer*, **Journal of Translational Medicine**, 22(1), 2024, 396.

Zhang, Y., D’Souza, G., Fakhry, C., Bigelow, E. O., Usyk, M., Burk, R. D., and Zhao, N., *Oral Human Papillomavirus Associated with Differences in Oral Microbiota Beta Diversity and Microbiota Abundance*, **The Journal of Infectious Diseases**, 226(6), 2022, 1098–1108.

Zheng, K., Egawa, N., Shiraz, A., Katakuse, M., Okamura, M., Griffin, H. M., and Doorbar, J., *The Reservoir of Persistent Human Papillomavirus Infection; Strategies for Elimination Using Anti-Viral Therapies*, **Viruses**, 14(2), 2022, 214.

Zhou, J., Hu, Z., Wang, L., Hu, Q., Chen, Z., Lin, T., Zhou, R., Cai, Y., Wu, Z., Zhang, Z., Yang, Y., Zhang, C., Li, G., Zeng, L., Su, K., Li, H., Su, Q., Zeng, G., Cheng, B., and Wu, T., *Tumor-Colonized Streptococcus Mutans Metabolically Reprograms Tumor Microenvironment and Promotes Oral Squamous Cell Carcinoma*, **Microbiome**, 12(1), 2024, 193.

Zhou, L., Li, Y., Wang, H., Qin, R., Han, Z., and Li, R., *Global Cervical Cancer Elimination: Quantifying the Status, Progress, and Gaps*, **BMC Medicine**, 23(1), 2025, 67.

Zilberman-Schapira, G., Zmora, N., Itav, S., Bashirdes, S., Elinav, H., and Elinav, E., *The Gut Microbiome in Human Immunodeficiency Virus Infection*, **BMC Medicine**, 14(1), 2016, 83.

Internet Sources

Chua, K. P., Ripp, F. *HiFi-16S-workflow: Nextflow pipeline to analyze PacBio HiFi full-length 16S data*. **Pacific Biosciences**. Accessed April, 2025, Available online: <https://github.com/PacificBiosciences/HiFi-16S-workflow>.

National Primary Health Care Development Agency. *Speech by the Executive Director/CEO of National Primary Health Care Development Agency (NPHCDA) at the National Flag-off of Human Papilloma Virus Vaccine Introduction (HPV-Vi) on the 24th of October 2023 – NPHCDA*, Accessed, June, 20, 2025, Available Online: <https://nphcda.ccurveconsult.com/speech-by-the-executive-director-ceo-of-national-primary-health-care-development-agency-nphcda-at-the-national-flag-off-of-human-papilloma-virus-vaccine-introduction-hpv-vi-on-the/>.

Appendices

Appendix I: Questionnaire

HOMINY Participant's Demographic Information and Medical History Form-Youth

Study ID:

Hospital Study (select one)		Group: Number:		HI		HEU		HUU
Visit number:		Indicate V1 – V4		Name of Interviewer/Study Staff Initials: _____				

A. General/ Demographic Information

Date of Birth: years / /

Age: months

Child's Gender: ☐ Male ☐ Female

Date of HIV Diagnosis (If this information is missing from the child's case note, (If child is in HI group): please ask the mother when her child was first diagnosed)

Day / Month / Year

Educational background

1. Have you ever been enrolled in school?	☐ Yes ☐ No
2 If yes to Q1, Are you currently enrolled in school?	☐ Yes ☐ No
3. If yes to Q1 or Q2, What is your current level of education?	PRIMARY _____ 1 JSS _____ 2 SSS _____ 3 CERTIFICATE/DIPLOMA _____ 4 UNIVERSITY _____ 5 Others _____ 6 Specify: _____
4. If no to Q1 or Q2, Why are you not in school?	1. Parents have no money 2. Parents not interested 3. I am not interested 4. I am too busy 5. Other, specify: _____

B. Labor and Delivery History

1.	Delivery method	1. Vaginal 2. C section 3. Assisted vaginal 4. Emergency C section 5. Unknown
----	-----------------	---

Baseline and Follow-up Dietary Questionnaire- Mother

Study: **H O M I N Y** / **2 0** Date of

ID Assignment:

ID: (select **H M 1** one only)

Hospital Number:

Name of Interviewer/Study Staff Initials: **HI** **HU**

A. Food frequency chart

Food items	Do you eat/drink this item? 0- No (skip to next item) 1- Yes	Frequency						
		Daily	4-5 times a week	2-3 times a week	Once a week	Less than once a week	Once a month	Rarely
a. Bread								
b. Rice								
c. Beans and soybean								
d. Eba/Amala/Fufu								
e. Maize/other corn meal								
f. Other Cereals and grain products (indomie, spaghetti)								
g. Nuts and seeds								
h. Meat and poultry/chicken								
i. Milk and related products (yoghurt, fura)								
j. Fish and products								
k. Eggs								

l. Starchy roots and tubers (Yam/yam pottage, cassava, potatoes)		☐	☐					
m. Fruits (fresh or canned)	☐	☐	☐	☐	☐	☐	☐	☐
n. Vegetables (fresh, canned or in soup)	☐		☐	☐	☐	☐	☐	☐
o. Cake/Buns/Puff-buff/Donut	☐	☐	☐	☐	☐	☐	☐	☐
p. Sweets/Candy/Chocolate	☐	☐	☐	☐	☐	☐	☐	☐
q. Biscuits/Cookies	☐	☐	☐	☐	☐	☐	☐	☐
r. Other snacks like chips, popcorn, pies and pastries	☐	☐	☐	☐	☐	☐	☐	☐
s. Ice-cream	☐	☐	☐	☐	☐	☐	☐	☐
t. Sugary beverages	☐	☐	☐	☐	☐	☐	☐	☐
u. Carbonated beverages (soda/coca-cola)	☐	☐	☐	☐	☐	☐	☐	☐
v. Caffeinated beverages	☐	☐	☐	☐	☐	☐	☐	☐
w. Alcoholic beverages	☐	☐	☐	☐	☐	☐	☐	☐
x. Other drinks like Kunu	☐	☐	☐		☐	☐	☐	☐
y. Other solids	☐	☐	☐		☐	☐		
Food items		Frequency						
	Do you eat/drink this item? 0-No (skip to next item) 1-Yes	Daily	4-5 times a week	2-3 times a week	Once a week	Less than once a week	Once a month	Rarely

FOR OFFICE USE ONLY

Data Tech:

Interviewer's Initials:

Appendix II: Informed consent document

Title of Study: Oral Microbiome Alterations Associated with HPV Infection in Edo State, Nigeria: A 16S rRNA Metagenomic Study

Principal Investigator: Kilsa Borgbara

Department: Biological Sciences

Institution: Lead City University

Contact Information: kilsa.borgbara@outlook.com, 08067771119

1. Introduction and Invitation

You are invited to participate in a research study conducted by Kilsa Borgbara and the research team at Lead City University. Before deciding, please read this form carefully to understand the study's purpose, procedures, potential risks and benefits, and your rights as a participant. Feel free to ask any questions.

2. Purpose of the Study

This research aims to understand the relationship between Human Papillomavirus (HPV), Human Immunodeficiency Virus (HIV), and the oral microbiome in young people in Nigeria. We seek to explore how these factors interact and potentially affect oral and overall health.

3. Procedures: What Will Happen If You Participate?

If you agree to participate, you will:

- **Provide a Saliva Sample:** Offer a small saliva sample into a sterile collection tube. This process is simple and painless.
- **Undergo an Oral Examination:** A trained researcher will visually examine your mouth, including gums, tongue, and cheeks. This brief examination is generally comfortable.
- **Complete a Questionnaire:** Answer questions about your general health, medical history (including any known HIV or HPV status, if comfortable sharing), lifestyle habits (e.g., smoking, diet), and oral hygiene practices. You may skip any questions you prefer not to answer.

4. Participation of Minors

If you are under 18 years old, your participation requires consent from your parent or legal guardian, in accordance with Nigerian research ethics guidelines. Additionally, if you are between 12 and 17 years old, your assent (agreement) is also required. This means that both you and your parent or guardian must agree to your participation. For children under 12, only parental or guardian consent is necessary.

5. Voluntary Participation and Withdrawal

Your participation is entirely voluntary. You may choose not to participate or withdraw at any time without penalty or loss of benefits. If you withdraw, any data collected up to that point may still be used in an anonymized form unless you request otherwise, where feasible.

6. Risks and Discomforts

Participation involves minimal risks:

- You might experience slight, temporary discomfort during the oral examination.
- Some questionnaire questions might be sensitive or personal. You have the right to skip any questions you do not wish to answer.

7. Potential Benefits

There may be no direct benefit to you. However, the information gathered will enhance understanding of health issues affecting young people in Nigeria, potentially leading to better prevention or treatment strategies in the future.

8. Confidentiality and Data Handling

We are committed to protecting your privacy:

- Your name or identifying information will not be linked to your samples or questionnaire answers in any reports or publications.
- A unique code will be assigned to your samples and data. The list linking your name to this code will be kept securely and separately from research data. Only the Principal Investigator and authorized research staff will have access to this list.
- All electronic data will be stored on password-protected computers, and paper records will be kept in locked cabinets in the researcher's office.
- Research data might be reviewed by authorized personnel from Lead City University Ethics Review Committee or regulatory agencies for monitoring purposes, but your identity will remain confidential.
- Anonymized data (data with all identifiers removed) may be shared with other researchers or deposited in scientific databases for future research, but it will not be possible to identify you from this data.

9. Compensation

You will not receive any payment for participating in this study.

10. Contact Information for Questions or Concerns

If you have any questions about the research study, please contact:

Principal Investigator: Kilsa Borgbara

Phone: 08067771119

Email: kilsa.borgbara@outlook.com

11. Consent Statement

I have read (or have had read to me) the information provided in this consent form. I have had the opportunity to ask questions, and all my questions have been answered to my satisfaction. I understand that my participation is voluntary and that I can withdraw at any time without penalty. I understand the procedures, risks, and benefits involved in this study. I understand how my confidentiality will be protected. I voluntarily agree to participate in this research study.

Participant's Printed Name
Date

Participant's Signature

Printed Name of Person
Date

Signature of Person

(Investigator or Designee) (Investigator or Designee)

Appendix III: Ethical Approval



UNIVERSITY OF BENIN TEACHING HOSPITAL

P.M.B. 1111 BENIN CITY NIGERIA

Telephone: 052-600418

Telex: 41120 NG

Website: ubth.org

CHAIRMAN, BOARD OF MANAGEMENT:

CHIEF ADEDOJA ADEWOLU, MFR

CHIEF MEDICAL DIRECTOR:

PROF. DARLINGTON E. OBASEKI

*MBBS (Benin), FMCPath
E-mail: darlobaseki@gmail.com*

DIRECTOR OF ADMINISTRATION:

M.O. JIMOH-KADIRI

B. Sc, (Hons) FJPM, Dip. Theo. AHAN

HEALTH RESEARCH ETHICS COMMITTEE APPROVAL

PROTOCOL NUMBER: ADM/E 22/A/VOL.VII/14831674

PROPOSAL TITLE: "HPV, HIV, AND ORAL MICROBIOTA INTERPLAY IN NIGERIAN YOUTH (HOMINY)"

SITE INVESTIGATOR(S): PROF OZOEMENE OBUEKWE, PROF AUGUSTINE OMOIGBERALE

DEPARTMENT/INSTITUTION: UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY, NIGERIA

DATE CONSIDERED: OCTOBER 7TH, 2022

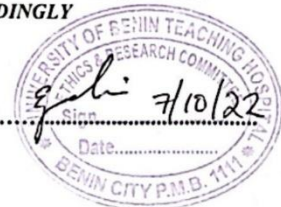
DECISION OF THE COMMITTEE: APPROVED

THIS APPROVAL DATES 7/10/2022 TO 7/10/2023. IF THERE IS DELAY IN STARTING THE RESEARCH, PLEASE INFORM THE HREC SO THAT THE DATES OF APPROVAL CAN BE ADJUSTED ACCORDINGLY

REMARK:

CHAIRMAN: PROF. (MRS) A.N. OFILI

SIGNATURE & DATE



SUPERVISOR (S):

DECLARATION BY INVESTIGATOR(S):

PROTOCOL NUMBER (please quote in all enquiries)

Note that no participant accrual or activity related to this research may be conducted outside of these dates. All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study. In multiyear research, endeavor to submit your annual re-port to the HREC early in order to obtain renewal of your approval and avoid disruption of your research. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit your research site without previous notification.

Signature & Date

[Signature] 11/10/22

Bio-data

A. Personal Information

Full Name: Kilsa Borgbara

Address: 25 Barika Street, Opp. UI Second gate, Ibadan, Nigeria

E-mail Address: kilsa.borgbara@outlook.com

Phone Number: +2348067771119

Nationality: Nigerian

Date of Birth: 3rd April 1992

Place of Origin: Gokana, Rivers State

Place of Birth: Kpor, Rivers State

Languages: English, Gokana

Name of Next of Kin: Barine Borgbara

Address of Next of Kinn: 161 Victoria, Port Harcourt, Nigeria

B. Educational Background

School Attended	Date	Qualifications
Sea Bird Nursery and Primary School	1998-2004	School Leaving Certificate
Emarid College	2003-2009	SSCE
University of Calabar	2012-2015	B.Sc. Genetics and Biotechnology
Lead City University, Ibadan	2023-(date)	M.Sc. Molecular Biology and Genomics

C. Work Experience

- a. Laboratory Manager - Inqaba Biotec West Africa Ltd [31 May 2021 –
Current]

- b. Area Sales Manager - Inqaba Biotec West Africa Ltd [1 Apr 2019 – 27 Feb 2021]
- c. Graduate Intern - International Institute of Tropical Agriculture [30 Jun 2018 – 30 Mar 2019]
- d. National Blood Transfusion Service - Donor Recruiter [31 May 2013 – 27 Mar 2014]
- e. University of Ibadan (Ibadan Journal of Agricultural Research) - Editorial assistant [1 Jun 2017 – 29 Jun 2018]
- f. Kues Systems Enterprise - Head, IT Unit [7 Feb 2016 – 29 Apr 2017]

D. Trainings and Certifications

- a. Sanger Sequencing Training - Inqaba Biotechnical Industries (Pty) Ltd [30 Jun 2021 – 30 Aug 2021]
- b. Next Generation Sequencing (PacBio) Training (2022) - Inqaba Biotechnical Industries (Pty) Ltd [1 Sep 2022 – 30 Sep 2022]
- c. Basic laboratory training on GMO analysis (2019) - International Centre for Genetic Engineering and Biotechnology [15 Sep 2019 – 19 Sep 2019]
- d. Basic first aid and CPR Basic first aid and CPR (2019) [24 Nov 2019 – 27 Nov 2019]
- e. Eukaryotic Genome Assembly: How to use Blobtoolkit For Quality Assessment Wellcome Connecting Science
- f. Business Etiquette and Communication for Impact Masterclass Eminence Corporate Solutions [13 Sep 2022 – 13 Sep 2022]
- g. 2023 Genome Sequencing Bioinformatics Course - WELLCOME Connecting Science [15 Sep 2023 – 10 Oct 2023]

- h. Laboratory Quality Management System and Preparation for International Accreditation Koukouvayia Consults and Inqaba Biotech West Africa [27 Nov 2023 – 29 Nov 2023]
- i. Introduction to Bioinformatics Pan African Bioinformatics Network for H3Africa [16 Apr 2024 – 15 Jul 2024]

E. PUBLICATIONS

- a. Comparative assessment of annual wormwood and local accessions of bitter leaf for antioxidant composition and activity Chukwurah, P., Brisibe, E., Ekwere, L., Ekerette, E. and Borgbara, K.
- b. What's in (y)our food? – Occurrence of GM-containing foods on the Nigerian market and compliance with national regulations Authors: Amedu, J., Adediji, A., Miracle, N., Anthony, A., Adeyemi, P., Ahmed, R., Atsumbe, S., Costly, M., Majekodunmi, A., Balogun, O., Akinpelu, O., Borgbara, K., Olufowobi, O., Tahir, H. J., Aroworamimo, L., & Asagbra, A. | Volume, Issue and Pages: Journal of Genetic Engineering and Biotechnology, 23(2), 100481.
- c. Unlocking African bioeconomy through biodiversity genomics and bioinformatics Authors: Hayah, I., Ezebuiro, V., Kagame, P., Kuja, J. O., Borgbara K et al.

University Compliance Certification

This is to certify that the thesis by Kilsa Borgbara with Matric no LCU/PG/005745 in the department of Biological Sciences (Molecular Biology and Genomics), Basic Medical and Applied Sciences, Lead City University, is in full compliance with the approved university format and style.

Signature

Date

Lead City University Ibadan DO NOT COPY

Plagiarism Report



Page 1 of 120 - Cover Page

Submission ID trn:oid::1:3360512122

Lcu Library

Borgbara_Kilsi_MSc LCU Library

- Quick Submit
- Quick Submit
- Lead City University

Document Details

Submission ID
trn:oid::1:3360512122

Submission Date
Oct 3, 2025, 4:46 PM GMT+1

Download Date
Oct 5, 2025, 11:24 PM GMT+1

File Name
Borgbara_Kilsi_MSc.docx

File Size
3.2 MB

105 Pages

23,863 Words

149,451 Characters



Page 1 of 120 - Cover Page

Submission ID trn:oid::1:3360512122

16% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Small Matches (less than 8 words)

Match Groups

-  **345** Not Cited or Quoted 16%
Matches with neither in-text citation nor quotation marks
-  **9** Missing Quotations 0%
Matches that are still very similar to source material
-  **0** Missing Citation 0%
Matches that have quotation marks, but no in-text citation
-  **0** Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

Top Sources

- 12%  Internet sources
- 12%  Publications
- 5%  Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.