Centro de Investigación Científica de Yucatán, A.C.

Posgrado en Ciencias Biológicas

Association between penile skin microbiota and subclinical human papillomavirus infection

Tesis que presenta

DIEGO ALBERTO GARZA GONÁLEZ

En opción al título de

DOCTOR EN CIENCIAS

(Ciencias Biológicas: BIOTECNOLOGÍA)

Mérida, Yucatán, México

2025

CENTRO DE INVESTIGACIÓN CIENTÍFICA DE YUCATÁN, A. C.
POSGRADO EN CIENCIAS BIOLÓGICAS

RECONOCIMIENTO

Por medio de la presente, hago constar que el trabajo de tesis de Diego Alberto Garza González, titulado **Association between penile skin microbiota and subclinical human papillomavirus infection**, fue realizado en la Unidad de Biotecnología, en la línea de investigación Biotecnología de microorganismos, en el laboratorio de metagenómica del Centro de Investigación Científica de Yucatán, A.C. bajo la dirección de la Dra. Ingrid Aileen O'Connor Sánchez, dentro de la opción de Biotecnología, perteneciente al Programa de Posgrado en Ciencias Biológicas de este Centro y en codirección con la Dra. María del Refugio González Losa del Laboratorio de Virología del Centro de Investigaciones Regionales "Dr. Hideyo Noguchi" de la Universidad Autónoma de Yucatán.

Atentamente

Dr. José Luis Hernández Stefanoni
Director de Docencia

Mérida, Yucatán, México, a 2 de septiembre de 2025

Mérida, Yucatán, México, a 18 de Agosto de 2025

DECLARACIÓN DE PROPIEDAD

Declaro que la información contenida en la sección de Materiales y Métodos, los Resultados y Discusión de este documento proviene de las actividades de investigación realizadas durante el período que se me asignó para desarrollar mi trabajo de tesis, en las Unidades y Laboratorios del Centro de Investigación Científica de Yucatán, A.C., y que a razón de lo anterior y en contraprestación de los servicios educativos o de apoyo que me fueron brindados, dicha información, en términos de la Ley Federal del Derecho de Autor y la Ley de la Propiedad Industrial, le pertenece patrimonialmente a dicho Centro de Investigación. Por otra parte, en virtud de lo ya manifestado, reconozco que de igual manera los productos intelectuales o desarrollos tecnológicos que deriven o pudieran derivar de lo correspondiente a dicha información, le pertenecen patrimonialmente al Centro de Investigación Científica de Yucatán, A.C., y en el mismo tenor, reconozco que si derivaren de este trabajo productos intelectuales o desarrollos tecnológicos, en lo especial, estos se regirán en todo caso por lo dispuesto por la Ley Federal del Derecho de Autor y la Ley de la Propiedad Industrial, en el tenor de lo expuesto en la presente Declaración.

Firma: _____

Nombre: Diego Alberto Garza González

Este trabajo se llevó a cabo en la Unidad de Biotecnología del Centro de Investigación Científica de Yucatán, A.C., y forma parte del proyecto finciado por el SECIHTI en la convocatoria Ciencia de Frontera 2019 titulado *Estudio de la composición de la microbiota genital en hombres y su relación con la infección por el virus de papiloma humano: generando conocimiento de frontera con impacto en la salud* (CF-2019 – 87172) bajo la dirección de la Dra. Ingrid Aileen O'Connor Sánchez y Dra. María del Refugio González Losa.

ACKNOWLEDGMENTS:

To my thesis advisors, Dr. Ingrid Aileen O'Connor Sánchez and Dr. María del Refugio González Losa, for giving me the opportunity to collaborate on this highly fruitful project.

To my thesis committee members, Dr. Laura Conde Ferraez, Dr. Elsa Beatriz Góngora Castillo, and Dr. Ian Carroll, for their invaluable contributions to improving this thesis work and my professional development.

To the Centro de Investigación Científica de Yucatán (CICY), for providing the infrastructure and resources necessary for my graduate studies in a supportive environment.

To the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI), for awarding me Doctoral Scholarship No. 800614, without which my graduate studies would not have been possible. I also acknowledge the funding from the project "Study of the Genital Microbiota Composition in Men and Its Relationship with Human Papillomavirus Infection: Generating Cutting-Edge Knowledge with Health Impact" (No. 87172), which made this thesis and its accompanying academic outputs feasible.

To my lab colleagues, Perla Contreras, Alejandra Sallas, Jorge Tzec, and Olivia Zapata, for their encouragement.

To M en C. Jesús G. Gómez Carballo for his extensive support in the virology lab; to Q.B.B Mirbella Cáceres Farfán for her assistance in the metagenomics lab; and to Ing. Luis Puc Canul for his help with managing and setting up pipelines on CICY's supercomputing equipment.

ACADEMIC PRODUCTS:

Oral presentation titled "Differences in the penile skin microbiota of HPV-positive Yucatecan men", delivered as part of the Symposium VI: Bioinformatics and Omics at the XI Congress of Biotechnology and Bioengineering of the South-Southeast Region, held in San Francisco de Campeche, Campeche, Mexico, on November 10, 2022.

Oral presentation at the 1st Congress of Young Scientists of the Mexican Southeast , awarded by the Sociedad Científica Juvenil (Youth Scientific Society), Mérida Chapter. Mérida, Yucatán, Mexico, May 24, 2024.

Science Communication: M.Sc. Diego Garza González participated in outreach activities for the Centro de Investigación Científica de Yucatán (CICY), appearing on the podcast Cienciasional (La Kimbomba radio-CICY) to discuss "What does metagenomics study? From bees to Humanpapiloma viruses." May 30, 2024.

Poster Presentation: "Association between subclinical Human Papillomavirus infections and penile skin microbiota" at the XII Congress of Biotechnology and Bioengineering of the Southeast and III Symposium on Comprehensive Utilization of Fish, Seafood, and Algal Biomass, organized by the Mexican Society of Biotechnology and Bioengineering, South-Southeast Delegation. Mérida, Yucatán, Mexico. October 31, 2024.

Poster Presentation: Diego Alberto Garza-Gonzalez, Jesus Gomez-Carballo, Stephany Burgos-Vela, Laura Conde-Ferráez, Ian Carroll, Aileen O'Connor-Sanchez, and Maria del Refugio Gonzalez-Losa presented "Association of the Penile Microbiota and Human Papillomavirus Subclinical Infections: A Cross-Sectional Study Among Mexican Men" at the 2024 STI Prevention Conference. Atlanta, GA, USA. September 16–19, 2024.

Research article: Garza-González DA, Gómez-Carballo JG, Burgos-Vela SD, Conde-Ferraéz L, Carroll IM, O'Connor-Sánchez A, González-Losa MR. Penile microbiota dysbiosis associates with subclinical HPV infection in HIV-negative men. **International Journal of STD & AIDS**. In press. Accepted 2025 Aug 08.

INDEX

FIGURES INDEX

TABLES INDEX

RESUMEN

La infección por el virus del papiloma humano (VPH) es una infección de transmisión sexual frecuente y con potencial oncogénico. Si bien su asociación con la microbiota vaginal ha sido ampliamente documentada, se sabe poco sobre su relación con la microbiota de la piel del pene (MPP). Este estudio exploró si existe disbiosis de la MPP en hombres no circuncidados con infección subclínica por VPH. Acudieron un total de 103 hombres de entre 18 y 45 años de Mérida, México, de los cuales 70 fueron negativos (68%) y 33 positivos (32%) para VPH. Se excluyeron aquellos con VIH, uso reciente de antibióticos o lesiones genitales. Las muestras se obtuvieron mediante hisopado del pene y se analizó la MPP a través de secuenciación de amplicones de la región v4 gen del gen 16S rRNA. También se empleó metagenómica tipo shotgun en un subconjunto de muestras para mayor resolución taxonómica. Los hombres VPH-positivos mostraron una mayor riqueza microbiana ($p = 0.003$), especialmente quienes portaban genotipos de alto riesgo. El análisis identificó cinco tipos de estado comunitario (CST), y el CST-5, dominado por bacterias del género *Finegoldia*, fue el asociado con VPH (OR = 3.11; IC95%: 1.22–8.22). Este grupo también presentó un aumento en la abundancia de géneros anaerobios oportunistas como *Peptoniphilus* y *Anaerococcus*, junto con una disminución de comensales como *Corynebacterium* y *Staphylococcus*. La metagenómica por shotgun confirmó la presencia de especies como *Finegoldia magna* y *Peptoniphilus genitalis*, así como cepas de *Staphylococcus* no detectadas por 16S. Estos resultados sugieren que la infección por VPH se asocia con una disbiosis del PSM caracterizada por un predominio de anaerobios oportunistas y pérdida de bacterias comensales, lo que podría afectar la inmunidad local. Aunque el diseño transversal impide establecer causalidad, los hallazgos abren nuevas líneas de investigación sobre el papel del microbioma en la patogénesis del VPH. Se recomienda continuar con estudios longitudinales dirigidos a las especies aquí señaladas para establecer causalidad.

ABSTRACT

Human papillomavirus (HPV) infection is a common sexually transmitted infection with oncogenic potential. While its association with the vaginal microbiota has been widely documented in women, little is known about its relationship with the penile skin microbiota (PSM) in men. This study explored whether there is PSM dysbiosis in uncircumcised men with subclinical HPV infection. A total of 103 men aged 18 to 45 years in Mérida, Mexico, participated in the study, of whom 70 were HPV-negative and 33 were HPV-positive. Those with HIV, recent antibiotic use, or genital lesions were excluded. Samples were obtained by swabbing the penis, and PSM was analyzed by sequencing amplicons from the v4 region of the 16S rRNA gene. Shotgun metagenomics was also applied to a subset of samples to enhance taxonomic resolution. HPV-positive men showed greater microbial richness ($p = 0.003$), especially those carrying high-risk genotypes. The analysis identified five community state types (CSTs), and CST-5, dominated by bacteria of the genus *Finegoldia*, was most strongly associated with HPV (OR = 3.11; 95% CI: 1.22–8.22). This group also showed an increase in the abundance of opportunistic anaerobic genera such as *Peptoniphilus* and *Anaerococcus*, along with a decrease in commensals such as *Corynebacterium* and *Staphylococcus*. Shotgun metagenomics confirmed the presence of species such as *Finegoldia magna* and *Peptoniphilus genitalis*, as well as *Staphylococcus* strains not detected by 16S. These results suggest that HPV infection is associated with PSM dysbiosis characterized by a predominance of opportunistic anaerobes and loss of commensal bacteria, which could affect local immunity and favor viral persistence. Although the cross-sectional design prevents establishing causality, the findings open new lines of research on the role of the microbiome in HPV pathogenesis. Longitudinal studies targeting the species identified here are recommended to establish causality

INTRODUCTION

The human microbiome plays a critical role in health and disease, with microbial communities influencing host immunity, barrier integrity, and infection susceptibility. While research on the gut and vaginal microbiomes has expanded rapidly in the last decade, other anatomical sites remain less understood. One such site is the penile skin, whose microbiota is shaped by local tissue structure, hygiene practices, and host immune factors, yet remains relatively understudied (Grice & Segre, 2011; Gonçalves et al., 2022).

HPV is the most common sexually transmitted infection worldwide (Weinstock et al., 2004). In men, HPV infections are often transient, but persistent infection can lead to genital warts and several cancers, including penile and anal malignancies (Gu et al., 2020). While the relationship between vaginal microbiota composition and HPV persistence has been well-documented (Gillet et al., 2011; Mitra et al., 2020), much less is known about how microbial communities on the penile skin might influence HPV acquisition, persistence, or clearance. This gap is notable, particularly given men's role in HPV transmission and the limited understanding of microbial factors that may modulate viral infections at this site (Giuliano et al., 2008).

The penile skin provides a unique microenvironment, with anatomical and histological variation across the penis. In uncircumcised men, the glans supports an anaerobic niche that harbors specific bacterial taxa such as *Finegoldia* and *Anaerococcus* (Humphrey, 2014; Price et al., 2010). Notably, circumcision has been shown to reduce HPV prevalence and alter microbial composition, suggesting a possible link between the penile microbiota and HPV infection risk (Castellsagué et al., 2002; Hernandez et al., 2008; Price et al., 2010).

This thesis investigates the composition and structure of the penile skin microbiota in uncircumcised men with subclinical HPV infection. Using high-resolution microbiome profiling methods, including 16S rRNA gene sequencing and shotgun metagenomics, this work aims to characterize the microbial communities present and explore associations with HPV status. The findings add to the limited knowledge of microbial communities on the penile skin and their potential relationship with HPV.

CHAPTER I

BACKGROUND

1.1 The human skin as an ecosystem

Covering over 1.8 m², the skin hosts diverse microhabitats, primarily categorized as dry, moist, or sebaceous (Grice & Segre, 2011). The skin is a harsh environment, constantly eroded by hygiene, sexual activity, and scratching. Additionally, it is in a perpetual state of shedding and renewal with limited nutrients (e.g. sweat, sebum, and skin cells debris).

Human skin consists of distinct layers: epithelium, epidermis, and dermis. Functionally, physical and chemical barriers inhibit microbial growth. The skin's physical barrier is the stratum corneum, the outermost layer of the epidermis, composed of keratinocytes known as squames. Squames are flattened, enucleated dead cells replaced roughly every two weeks (Fuchs & Raghavan, 2002). This layer sheds continuously, making microbial attachment difficult.

The chemical barrier is primarily formed by sweat gland secretions. While its primary role is evaporative thermoregulation, sweat also inhibits microbial growth through salt, electrolytes, and acidification (Grice & Segre, 2011). Sebaceous glands ~~also~~ secrete sebum, a lipid-rich substance. While sebum primarily coats follicles and lubricates skin, its anoxic nature supports facultative anaerobes that hydrolyze triglycerides and release free fatty acids onto the skin (Marples et al., 1971). These fatty acids help maintain the skin's acidic pH (approximately 5.6). Some commensals metabolize these fatty acids and release antimicrobial compounds, forming an additional microbial barrier against foreign microbes (Bomar et al., 2016). This resident microbial community is the skin microbiota.

1.1.1 The penile shaft might be different ecosystems compared to other skin sites

From a histological standpoint, the glans and penile shaft are different. The penile shaft is covered by thin, glandular skin containing sweat glands (Dinotta et al., 2013), whereas the glans is a mucosal surface lined by partially keratinized squamous epithelium without adnexal structures (Humphrey, 2014). In uncircumcised men, the prepuce creates a protected interspace, further differentiating the glans microenvironment.

The most relevant aspect may be smegma production at the base of the glans (corona) by sebaceous glands. Smegma is an oily mix of shed skin cells, oils, and moisture (Fahmy, 2020). Smegma can accumulate and trigger inflammation, likely by promoting bacterial growth. Circumcision does not prevent smegma production but may reduce its buildup by easing hygiene. The glans and shaft differ in ecosystem conditions (e.g., sweat vs. sebaceous glands, exposed vs. covered), potentially sustaining distinct microbiota.

Circumcision lowers the anaerobic bacterial load in the glans (Price et al., 2010). Among the few studies characterizing penile skin microbiota (~10), none distinguish between shaft and glans communities. Most protocols pool samples from both sites into a single collection (Gonçalves et al., 2022). This methodological shortcut may mask anatomical differences in microbiota composition across studies.

1.1.2 Dysbiosis: when the skin ecosystem equilibrium is compromised

Environmental equilibrium can be disrupted by antibiotics, skin trauma, or pathogen colonization, leading to dysbiosis. Dysbiosis refers to a harmful shift in the resident microbial community, typically involving loss of commensals, rise of pathobionts, or reduced diversity (Petersen & Round, 2014). Ecologically, dysbiosis represents a disruption in microbial equilibrium that overwhelms the system's resistance and resilience, often due to environmental or host-related stressors, resulting in persistent shifts toward a new stable state (potentially harmful) (Levy et al., 2017).

Skin disorders can emerge when the commensal microbiota becomes dysbiotic. For example, psoriatic lesions show an imbalance between *Corynebacterium* and *Cutibacterium* (Quan et al., 2020). This finding is counterintuitive, since *Corynebacterium* is a common skin symbiont; however, certain species can drive inflammation in response to host physiology (Ridaura et al., 2018). Another notable feature of the skin microbiota in clinical settings is that certain species or strains can be harmful, making accurate identification essential.

1.2 The penile skin microbiota (PSM)

1.2.1 The skin microbiota is a conserved community

The skin microbiota includes four dominant phyla: Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Among these, the most prevalent skin genera are *Corynebacterium* and *Cutibacterium* (both in Actinobacteria, with the latter formerly known as *Propionibacterium*), and *Staphylococcus* (Firmicutes) (Grice et al., 2009).

Despite the skin's harsh environment, its microbiota is well adapted, with each genus utilizing nutrients from sebum or sweat. *Staphylococcus* is a facultative aerobe that thrives in moist areas. *Cutibacterium* is anaerobic but aerotolerant, preferring sebum-rich sites. Meanwhile, *Corynebacterium* species are generally aerobic or facultatively anaerobic and inhabit both sebum-rich and moist areas (Byrd et al., 2018; Swaney et al., 2023). These niche preferences shape the spatial organization of skin microbiota. As a result, the skin microbiota is conserved rather than acquired, due to its adaptation to the skin's unique environment (Oh et al., 2016).

The skin microbiota not only resides on the skin but also performs key functions, including producing antimicrobial compounds and metabolites that modulate immune signaling (Bomar et al., 2016; Ridaura et al., 2018).

1.2.2 The penile skin microbiota appears to be more diverse than that of other regions of the skin

Penile skin microbiota composition has been examined in a limited number of studies (reviewed in Gonçalves et al., 2022). To date, no studies have specifically characterized the normal penile skin microbiota or its ecosystem, as most available data come from comparisons across clinical conditions (Carda-Diéguez et al., 2019; Ghemrawi et al., 2021; Kigozi et al., 2020; C. M. Liu et al., 2015, 2017; Mehta et al., 2022, 2020; Nelson et al., 2012; Onywera, Williamson, Cozzuto, et al., 2020; Onywera, Williamson, Ponomarenko, et al., 2020; Price et al., 2010; Prodger et al., 2021; Watchorn et al., 2021; Zozaya et al., 2016).

The penile microbiota remains poorly defined, partly due to limited studies addressing its anatomical niches, such as moist or sebaceous regions. Notable features include smegma

production or accumulation at the glans and moisture along the shaft; sexual practices (e.g., vaginal, anal, oral) may also enrich the microbiota. These conditions may support a distinct microbial community compared to other skin sites (Grice & Segre, 2011; Harris-Tryon & Grice, 2022). The most common families include Corynebacteriaceae, Prevotellaceae, Clostridiales Family XI, Porphyromonadaceae, Staphylococcaceae, Bifidobacteriaceae, and Lactobacillaceae (Nelson et al., 2012; Onywera, Williamson, Ponomarenko, et al., 2020; Price et al., 2010). Frequently reported genera include *Staphylococcus, Corynebacterium, Finegoldia, Anaerococcus, Prevotella, Lactobacillus, Peptoniphilus*, and *Dialister*, with less frequent detection of *Porphyromonas*, *Mobiluncus*, and *Peptostreptococcus*. No studies to date have characterized penile bacterial communities at the species or strain level.

1.2.3 Anaerobic opportunistic pathogens

Several Gram-positive anaerobic cocci (GPAC), once considered benign commensals of the skin and mucosa, have emerged as significant opportunistic pathogens, particularly in dysbiotic conditions. Key genera include *Anaerococcus*, *Finegoldia*, *Peptoniphilus*, *Parvimonas*, and *Peptostreptococcus* (Murphy & Frick, 2013). These bacteria are strict anaerobes, incapable of growth in oxygenated environments, yet often thrive in polymicrobial biofilms where oxygen is depleted by aerobic colonizers such as *Staphylococcus* or *Streptococcus spp*. (Murphy & Frick, 2013).

Anerococcus spp., including *A. vaginalis* and *A. lactolyticus*, are frequently isolated from chronic wounds and biofilms (Dowd et al., 2008; A. Han et al., 2011). These bacteria are weakly saccharolytic, indole-negative, and produce butyrate as a major metabolic end product (Ezaki et al., 2001). *Peptoniphilus spp*., especially *P. harei* and *P. ivorii*, dominate in pressure ulcers, and some species like *P. indolicus* have been recovered from eye discharges and vaginal infections (Dowd et al., 2008; Murdoch et al., 1988). They do not ferment carbohydrates and rely on peptones and amino acids (Murphy & Frick, 2013).

Finegoldia magna, considered the most pathogenic GPAC, is frequently found in pure cultures from abscesses and bone or joint infections (Brazier et al., 2008). While strictly anaerobic, it shows some aerotolerance (Murdoch, 1998). Although not typically facultative, some GPAC demonstrate enhanced survival in oxygen-limited conditions and contribute significantly to chronic

skin infections by forming polymicrobial communities that impair healing (A. Han et al., 2011; Murphy & Frick, 2013).

1.3 Human Papillomavirus (HPV)

1.3.1 General characteristics

HPV is a non-enveloped, double-stranded DNA virus with a genome of approximately 8 kb. Its circular genome encodes eight proteins: E1–E7, involved in replication, transcription, and cellular transformation; and L1 and L2 capsid structural proteins. It also contains a noncoding regulatory region known as the long control region (Brianti et al., 2017).

Human papillomaviruses belong to the family *Papillomaviridae*, formally recognized by the International Committee on Taxonomy of Viruses (ICTV). Within this hierarchy, they are placed in distinct taxonomic ranks that include family, genus, species and types, following standardized rules for viral classification (Bernard et al., 2010). Their classification is based on sequence variation of the L1 gene (Arroyo Mühr et al., 2021; Burk et al., 2013), which provides a conserved yet discriminative region suitable for phylogenetic analysis.

ICTV guidelines use percentage identity thresholds to separate taxonomic levels (Table 1.1) Papillomaviruses within the same species share 71–89% L1 nucleotide identity, while members of the same genus share more than 60% but less than 71% identity. Viruses below 60% identity belong to different genera. These cutoffs, combined with phylogenetic tree topology and biological features, ensure precise and reproducible placement of each papillomavirus within the official taxonomic hierarchy. As of November 2025, the International HPV Reference Center had recognized 229 HPV genotypes, while GenBank listed approximately 800 unique putative types (Arroyo Mühr et al., 2021). HPV genotypes are commonly classified as high-risk (HPV-HR) or low-risk (HPV-LR) in scientific literature.

Table 1.1. Taxonomy and homology percentage of HPV

Taxonomic Level	L1 Gene Identity Percentage
Genus	Members of the same genus share more than 60% L1 identity
Species	Members of the same species share 60% to 70% L1 identity
Type	Distinct types within the same species must share 71% to 89% L1 identity
Variant	For a variant of a known type to exist, the L1 identity must differ by less than 2%

Human papillomaviruses (HPVs) can also be grouped according to their clinical relevance into high-risk (HR) and low-risk (LR) types (de Sanjosé et al., 2018). HPVs are also broadly classified as mucosal, infecting the anogenital tract and upper aerodigestive mucosa, or cutaneous, affecting keratinized skin and causing warts on the hands, feet, or other skin surfaces (Gheit, 2019). This functional classification reflects their typical disease associations or tissue tropism rather than strict taxonomic placement. HR types, such as HPV16 and HPV18, are strongly linked to the development of malignant lesions, including cervical, anogenital, and oropharyngeal cancers. They can integrate into the host genome and disrupt cell cycle regulation through the expression of oncogenes (Münger et al., 2004). In contrast, LR types, such as HPV6 and HPV11, are typically associated with benign lesions like genital warts (Egawa & Doorbar, 2017). A more comprehensive list of types is presented in Table 1.2.

Table 1.2 Classification of Human papillomaviruses types by oncogenic risk and tissue tropism

Category	HPV Types
High risk (confirmed carcinogens)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Possible high risk	26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85, 97
Low risk mucosal	6, 11, 40, 42, 43, 44, 54, 61, 72, 81, 89
Low risk cutaneous	1, 2, 3, 4, 7, 10, 27, 28, 29, 41, 57, 63

1.3.2 HPV and its clinical manifestations

HPV is transmitted through direct skin-to-skin or skin-to-mucosa contact. Infection begins when the virus enters the basal epithelial layer, typically through microlesions in the upper strata. Most men remain asymptomatic (Giuliano et al., 2010), and 75% clear the infection within a year (Giuliano et al., 2008). However, some men develop clinical disease, typically presenting as genital warts or, less commonly, cancer.

References to genital warts appear in classical medical literature, beginning with Hippocrates (460–370 BC), who described anal warts as condylomas (Littré, 1861). Genital warts are benign lesions caused mainly by HPV genotypes 6 and 11. However, their high transmission and recurrence rates can significantly impact quality of life, particularly in individuals with compromised immunity, such as those with HIV, where clearance is impaired (Abu El-Hamd et al., 2019). Although benign, genital warts often pose a psychological burden due to their visible and occasionally disfiguring appearance (Jeynes et al., 2009).

Genital warts also pose a burden for sexual partners due to their high transmissibility. For example, among heterosexual couples, 68% of male partners of women with genital warts also had warts (Krebs & Helmkamp, 1991). Similarly, up to 64% of sexual partners develop warts (Oriel, 1971). Men with a history of genital warts or asymptomatic infection have an increased risk of acquiring new HPV infections, suggesting that warts may act as reservoirs for self-inoculation (Brenda Y. Hernandez et al., 2011).

HPV infections are linked to anogenital cancers, including cervical, vulvar, vaginal, penile, and anal cancer. In men, HPV has been detected in 50% of penile cancer lesions (W. Gu et al., 2020). Penile cancer is more commonly diagnosed in men over 60 (Ingles et al., 2015), while genital warts are more prevalent among younger men aged 18–30 (Anic et al., 2011). Men with subclinical infection represent the largest group and have the greatest impact on infection dynamics. Therefore, this thesis focuses exclusively on subclinical infections.

1.4 Microbiota and HPV

1.4.1 Microbiota and viral infections

Microbial symbionts, viruses, and human cells inevitably interact within their shared environment, though not always directly.

Direct interactions between viruses and microbiota can either enhance or inhibit viral infectivity (Robinson & Pfeiffer, 2014). For example, the mouse mammary tumor virus (MMTV) relies on commensal microbiota for effective transmission, as germ-free mice cannot be infected through the natural route (Kane et al., 2011). Similarly, bacterial flagellin increases viral attachment to human epithelial cells (Benedikz et al., 2019).

Viruses can also benefit indirectly when the microbiota enters a dysbiotic state. Such dysbiosis may initiate or sustain disease by disrupting microbiota-driven immune regulation. In this disrupted environment, beneficial commensals decline while pathobionts emerge, contributing to disease.

Studies on HPV and vaginal microbiota have shown that shifts from a Lactobacillus-dominant community, along with increased species richness, were associated with HPV acquisition (Gillet et al., 2011). *Lactobacillus*-dominant communities maintain low vaginal pH by producing lactic acid. When dysbiosis disrupts this balance, pH increases, creating a more permissive environment for pathogens (Łaniewski et al., 2018).

To date, only one study has examined the relationship between microbiota and HPV in men (Onywera, Williamson, Cozzuto, et al., 2020). However, the cohort included men over 60 and some individuals co-infected with HIV. As a result, no study has exclusively investigated the penile skin microbiota in HPV-positive, but HIV-negative men.

Onywera et al. (2020) reported an increased abundance of anaerobes (*Prevotella*, *Peptoniphilus*, and *Dialister*) alongside reduced *Corynebacterium*, indicating a dysbiotic state. They also applied community state type (CST) analysis, finding that CST-1, dominated by *Corynebacterium*, had fewer HPV-positive samples, though HPV was detected across all CSTs. Overall, evidence linking penile skin microbiota to HPV remains limited.

IMPORTANCE OF THE PRESENT STUDY

HPV is the most common sexually transmitted virus, posing a significant health burden for both men and women (Weinstock et al., 2004). Although men face a lower risk of HPV-related cancers, they contribute to its transmission while asymptomatic.

In women, HPV infections are linked to vaginal microbiota dysbiosis; however, it remains unclear whether subclinical HPV infections in men are associated with shifts in the penile skin microbiota (PSM). This question is relevant because sexual partners may be exposed not only to the virus but also to an altered microbiota, with unknown consequences. To date, only one study has compared the PSM of subclinical HPV-positive and HPV-negative men, reporting increased abundance of *Prevotella*, *Dialister*, and *Peptoniphilus* in HPV-positive samples. However, the results are inconclusive due to inclusion of men co-infected with HIV and HPV. Additionally, the study focused exclusively on men who have sex with women (MSW), excluding men who have sex with men (MSM), a group at higher risk for HPV-related conditions (Meites et al., 2022). This study aims to determine whether PSM of the shaft is associated with HPV infection, providing a basis for future causal inference models and in vivo experimentation.

This thesis will contribute to the field by: (1) generating HPV infection data from Yucatecan men, including both MSW and MSM; (2) identifying taxonomic groups associated with subclinical HPV infections; and (3) expanding current knowledge of microbiota profiles linked to HPV infection.

GENERAL OBJECTIVE AND SPECIFIC OBJECTIVES

To characterize potential patterns of dysbiosis in the penile skin microbiota associated with subclinical HPV infections in uncircumcised men.

SPECIFIC OBJECTIVES

- To compare microbial diversity (alpha and beta diversity) between HPV-positive and HPV-negative uncircumcised men.
- To characterize the bacterial composition of the penile skin microbiota in uncircumcised men with subclinical HPV infection
- To identify differentially abundant bacterial taxa (genera/ASVs) associated with subclinical HPV infection.
- To explore the taxonomic profiles of pooled penile skin samples using shotgun metagenomics in order to identify key taxa at the lowest possible taxonomic level

STUDY DESIGN

1.1. Study rationale

We hypothesized that subclinical HPV infection is associated with dysbiosis of the PSM, characterized by an increased abundance of anaerobic opportunistic bacteria and a reduction in beneficial commensals. The analysis began at the community level to identify differences in the diversity metrics between HPV-negative and HPV-positive penile skin microbiota. Alpha diversity metrics were assessed by HPV status, providing an overview of species richness (*S*) and diversity (*H'*) using the Shannon index. Greater richness reflects a higher number of species, while increased diversity with constant richness indicates greater evenness (i.e., more balanced species proportions). Subsequently, to characterize the bacterial composition of the penile skin microbiota a Community State Type (CST) analysis was performed to cluster samples based on similarity; highlighting potential microbiota-HPV associations. Next, a taxa-specific analysis was conducted to identify taxonomic groups of interest. These groups are selected based on differential abundance analysis.

Once the 16S survey is completed, we expect to identify candidate genera or ASVs associated with HPV subclinical infection. However, 16S-based profiling is inherently limited in its taxonomic resolution, often failing to discriminate at the species or strain level. This limitation is critical, as clinical relevance and mechanistic hypotheses frequently depend on fine-scale taxonomic identification. Therefore, the final part of this thesis will focus on exploring the possibility of refining these taxonomic classifications through shotgun metagenomics. By employing this approach, we can analyze longer genomic fragments and obtain richer genetic information, which could lead to more accurate taxonomic classification. This higher-resolution data will allow us to identify species or strain-level features potentially linked to HPV infection, providing a foundation for future hypothesis-driven studies and targeted microbiome manipulation.

1.2. Participants and sample collection

The project was approved by the ethics committee of the “Centro de Investigaciones Regionales Dr. Hideyo Noguchi” of the “Universidad Autónoma de Yucatán” (identification number: CEI- 01-2020).

To address the study objectives, we employed a multi-dataset strategy based on a cohort of 103 participants, including 70 HPV-negative and 33 HPV-positive individuals. Among the HPV-positive group, 33 tested positive in the penile shaft and 26 in the glans. To avoid confounding factors, these samples were analyzed separately, with one chapter dedicated to the glans and another to the penile shaft.

A recruitment campaign under the slogan in spanish "*Por ti, por los que amas: Detección VPH en Hombres*" (For you, for those you love: HPV Screening in Men) was conducted via social networks (Facebook™) to invite sexually active men aged 18-45 living in the city of Merida, Mexico (Figure 1.1). Exclusion criteria were: presence of genital warts, HIV positivity, antibiotic use within the 4 weeks prior to sample collection, and the presence of genital lesions. Enrolled participants were instructed to abstain from sexual intercourse and refrain from genital hygiene for 24 hours prior to sample collection.

Trained project staff obtained two samples by swabbing the penile glans or the penile shaft of each subject with a flocked dacron swab moistened with saline solution. One sample was preserved in 50% ethanol, while the other sample was preserved in DNA/RNA shield solution (Zymo Research).

Figure 1.1 Cover photo from the Facebook page "Por ti, por los que amas: Detección VPH en Hombres"

1.3. Detection of HPV DNA and genotyping

DNA from samples preserved in ethanol was extracted for HPV identification using a commercial kit (DNeasy Blood & Tissue, Qiagen). DNA quantification and purity were determined using a Nanodrop 2000 (Thermo) spectrophotometer. DNA quality was assessed by amplifying the human beta-globin gene (Saiki et al., 1986). The presence of HPV was determined using classical PCR with two pairs of universal primers targeting the genes L1, MY09/MY11 and L1C1 (Husnjak et al., 2000). Negative controls included PCR reactions without DNA, while the positive control comprised the HPV-16 genome cloned in the pBR322 vector (kindly donated by E.M. De Villiers).

HPV genotyping was performed using a proprietary real-time PCR assay (Biomédicos de Mérida S.A. de C.V., a certified diagnostic laboratory under ISO 15189:2012). The assay detects high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and low-risk genotypes (6, 11, 13, 40, 42, 43, 44, 54, 61, 70, 72, 81, 84, 89, and 91). Men who had at least one high risk genotype were considered as HR-HPV positive, and only those who had only low risk genotypes were considered low risk.

1.4. 16S rRNA amplicon sequence analyses

Sequence analyses were performed using R 4.2.2 (Team, 2013). Raw pair-end sequences were processed in DADA2 1.18 (Callahan et al., 2016) for denoising, merging, and chimera removal. Reads were trimmed (truncLen = c(230, 130)) to maintain Phred scores >20. ASV inference used pooled reads dada(pool = TRUE) for sensitive variant detection. Sequences were merged (≥50 bp overlap), and chimeras were removed removeBimeraDenovo(method="pooled", minFoldParentOverAbundance = 8).

Classification was performed using the implementation of the RDP naive Bayesian classifier in DADA2 to search ASVs against the Silva database 138.1 (Quast et al., 2013), assignTaxonomy(minBoot= 50). Samples were rarefied to the depth of the sample with the lowest number of reads. Finally, to capture general patterns of the resident microbiota and to remove species that might be unique to an individual, ASV's were filtered out if they were present in less than 10% of the samples.

Alpha diversity metrics (Observed species, Chao1 and Shannon diversity) were calculated using the *vegan* package (Oksanen et al., 2007). A Non-metric multidimensional scaling (NMDS) based on Jensen-Shannon divergence distance with permutational multivariate analyses of variance (PERMANOVAs) was constructed using *vegan*. The Jensen-Shannon divergence was selected over Bray-Curtis as a beta diversity metric because it measures compositional differences by considering subtle shifts across the entire microbial community, including less abundant taxa. This sensitivity is particularly valuable when detecting early or subclinical dysbiosis associated with HPV infection, where ecological changes may be subtle yet meaningful.

1.5. Statistical analyses

The statistical analyses were performed using R 4.2.2 and the *rstatix* package (Kassambara, 2019). Comparison of the HPV and negative characteristics were conducted using Fisher's exact test. Linear regression models were constructed using the *lm* function from the base R package to assess the relationships between diversity metrics and predictor variables, including HPV status, HPV risk based on genotype, and sexual preference.

For each diversity metric (observed species, Chao1, and Shannon diversity), separate models were fitted as follows: `lm(div_metric ~ hpv_status + sex_pref)` this model evaluated the association between HPV status (positive or negative) and microbial richness, controlling for sexual preference. `lm(div_metric ~ hpv_risk + sex_pref)` this model evaluated the association between HPV risk (high-risk, low-risk, or negative) and microbial richness, controlling for sexual preference.

For community composition comparisons, the Adonis nonparametric test was used. The distance matrix was used to calculate the effect size (R^2 value) that showed the extent of variation explained by the metadata category.

CHAPTER II

Alpha and beta diversity of the penile glans microbiota in relation to HPV infection

2. INTRODUCTION

The glans is a mucosal surface that differs histologically and immunologically from the keratinized shaft skin. It is lined by partially keratinized squamous epithelium and lacks adnexal glands (Humphrey, 2014; Dinotta et al., 2013). In uncircumcised men, the preputial space creates a moist, low-oxygen niche conducive to anaerobic colonization (Price et al., 2010; Fahmy, 2020). Smegma, composed of exfoliated cells and lipids, may accumulate and promote inflammation when not properly cleared in uncircumcised men (Fahmy, 2020).

A clinically relevant remark is that circumcision reduces HPV risk (B. Y. Hernandez et al., 2008); this environmental shift is accompanied with a decreased load of anaerobic bacteria. Despite its clinical relevance, the microbial ecology of the glans remains poorly characterized, partly because sampling often combines swabs from the shaft and glans, potentially masking region-specific microbial signatures (Gonçalves et al., 2022).

Previous studies in women have shown that a shift away from protective *Lactobacillus*-dominated communities with increased microbial richness correlates to persistent HPV infection, suggesting that viral persistence can be influenced by local microbiota (Gillet et al., 2011; Mitra et al., 2020). However, evidence for analogous interactions at the male genital mucosa remains scarce. Notably, studies examining penile microbiota in relation to HPV infection often include confounding factors such as circumcision status, HIV coinfection, or advanced age (Onywera et al., 2020; Liu et al., 2017). As such, whether subclinical HPV infection is associated with measurable microbial alterations in the glans of HIV-negative, uncircumcised men remains an open question.

This chapter explores that gap by analyzing the glans microbiota at the community level, using ecological metrics to assess diversity and composition. Alpha diversity metrics (such as observed richness and the Shannon index) capture within-sample variability, while beta diversity compares differences across individuals. Dysbiosis may manifest as increased richness due to colonization

by opportunistic taxa, or as altered community structure marked by the dominance of specific groups (Petersen and Round, 2014; Levy et al., 2017). By applying these metrics, we aim to determine whether subclinical HPV infection is associated with ecological disruption in the glans microbiota.

3. METHODS

Samples were processed as described in the study design section. In this chapter, samples were rarefied to 8,812 reads, the depth of the sample with the lowest number of reads.

4. RESULTS

4.1. General characteristics of the study population

A total of 96 men were included in the study. Their basic characteristics are shown in Table 2.1. The median age was 29 years. HPV was detected in 26 (27%) men, with 10 high-risk, 5 low-risk, and 11 untyped infections (Table 2.2).

Sexual preference was the only factor significantly linked to HPV status (Fisher's test, $p > 0.05$). Men who have sex with women (MSW) were 4.31times (95% CI: 1.34-15.25) more likely to be HPV positive than men who have sex with men (MSM). Consequently, subsequent multiple linear regression models were adjusted to control for the effect of sexual preference.

Table 2.1 General characteristics of the male population studied.

General characteristic	*# HPV-negative (%)*	*# HPV-positive (%)*	*p.value*
Sampled men	70 (72.9%)	26 (27.1%)	
Age			0.93
20-24	13 (13.5%)	5 (5.2%)	
25-29	27 (28.1%)	8 (8.3%)	
30-49	30 (31.2%)	13 (13.5%)	
Sex of sexual partners			**0.01**
Men who have sex with men (MSM)	28 (29.2%)	4 (4.2%)	
Men who have sex with women (MSW)	42 (43.8%)	22 (22.9%)	
Number of sexual partners*			0.14
0-4	9 (9.4%)	1 (1.0%)	
5-9	18 (18.8%)	7 (7.3%)	
10-19	22 (22.9%)	7 (7.3%)	
<20	20 (20.8%)	11 (11.5%)	
Tobacco use			0.36
Non-smoker	47 (46%)	21 (21%)	
Smoker	23 (22%)	5 (5%)	

*Sum of previous and current sexual partners. P values are calculated using Fisher's exact test

Table 2.2 Low and High risk genotypes

Code	Genotypes	HPV-risk
VM 3	40, 84	Low risk
VM 9	58	High risk
VM 10	52, 59, 66, 89	High risk
VM 30	31, 52	High risk
VM 50	6, 53, 91	Low risk
VM 61	91	Low risk
VM 62	43, 51, 56, 84, 91	High risk
VM 65	51, 61, 84	High risk
VM 68	45, 51, 73, 91	High risk
VM 100	66	Low risk
VM 113	45, 84	High risk
VM 130	33	High risk
VM 148	16, 66, 89, 91	High risk
VM 162	13, 43, 45, 53, 66, 84, 91	High risk
VM 186	NA	—
VM 50	NA	—
VM 59	NA	—

VM 115	NA	—
VM 150	NA	—
VM 155	NA	—
VM 41	NA	—
VM 120	NA	—
VM 190	NA	—
VM 88	NA	—

NA=No amplify any genotype

Genotypes considered as High-risk 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59

4.2. Alpha diversity

We used multiple linear regression to assess the association between HPV infection and microbial diversity, adjusting for sexual preference (MSM vs MSW). Compared to HPV-negative participants, HPV-positive men showed no significant associations with any alpha diversity indices (Table 2.3), though Shannon diversity trended higher (coefficient = 0.28, p = 0.07).

To examine differences by HPV genotype, we stratified a second set of models by risk group, using HPV-negative men as the reference (Table 2.4). Men with high-risk HPV (HR-HPV) had significantly higher diversity than HPV-negative men in three of four metrics: observed species (coefficient = 10.04, p = 0.02), Shannon diversity (0.48, p = 0.04), and Pielou's evenness (0.10, p = 0.03). Low-risk HPV (LR-HPV) was not associated with any diversity measures, and sexual preference remained non-significant across all models.

Despite significant associations for HR-HPV, the overall models did not reach statistical significance (Table 2.5), suggesting HPV status and sexual preference explained little variance.

Table 2.3 Multiple linear regression analysis for the relationship between HPV status, sexual preference, and microbial diversity.

Diversity metric	Variable	Coefficient	Standard error	p-value
Species Observed	HPV status (Positive)	6.16	3.40	0.96
	Sexual preference (MSW)	-0.14	3.60	0.08
Species richness (Chao1)	HPV status (Positive)	4.68	4.06	0.25
	Sexual preference (MSW)	-1.54	4.29	0.72
Shannon	HPV status (Positive)	0.28	0.16	0.07
	Sexual preference (MSW)	-0.03	0.16	0.81
Evenness	HPV status (Positive)	0.06	0.03	0.06
	Sexual preference (MSW)	-0.008	0.03	0.81

Results from multiple linear regression models assessing the relationship between HPV status, sexual preference, and microbial diversity (Observed species, species richness (Chao1), Shannon, and Pielou evenness) adjusting for sexual preference (MSM). Coefficients, standard errors and p.values for each model are shown, significant values are in bold. MSW: Men who have sex with women.

Table 2.4 Multiple linear regression analysis for the relationship between HPV genotypes and microbial diversity.

Diversity metric	Variable	Coefficient	Standard error	p-value
Species Observed	LR-HPV	-1.32	5.40	0.80
	HR-HPV	10.04	4.45	**0.02**
	Sexual preference (MSW)	-3.69	3.74	0.32
Species richness (Chao1)	LR-HPV	-2.67	6.64	0.69
	HR-HPV	8.28	5.47	0.13
	Sexual preference (MSW)	-5.51	6.60	0.23
Shannon	LR-HPV	-0.01	0.27	0.96
	HR-HPV	0.48	0.23	**0.04**
	Sexual preference (MSW)	-0.08	-0.44	0.65
Evenness	LR-HPV	0.001	0.05	0.98
	HR-HPV	0.10	0.04	**0.03**
	Sexual preference (MSW)	-0.01	0.04	0.80

Results from multiple linear regression models assessing the relationship between HPV genotypes (LR and HR vs. Negative) and microbial diversity (Observed species, species richness (Chao1), Shannon, and Pielou evenness), adjusting for sexual preference (MSM). Coefficients, standard errors and p.values for each model are shown, significant values are in bold. Negative HPV status was used as the reference group. MSW: Men who have sex with woman.

Table 2.5 Models summary for multiple linear regression analysis evaluating the relationship between HPV genotypes and microbial diversity.

	Residual Std. Error	R^2 (Adjusted)	F-statistic (DF)	Model p-value
Observed species	11.19	0.054	1.86 (3, 42)	0.15
Chao1 richness	13.76	0.003	1.06 (3, 42)	0.37
Shannon	0.578	0.040	1.63 (3, 42)	0.19
Pielou's evenness	0.122	0.049	1.78 (3, 42)	0.16

Note: None of the models reached statistical significance (all $p > 0.15$), indicating that HPV risk group and sexual preference explained only a small portion of the variability in microbial diversity.

4.3. Beta diversity

NMDS ordination revealed overlapping in microbial community composition between HPV-positive and HPV-negative individuals (Figure 2.1). The PERMANOVA analysis revealed no effect of infection status on microbial composition (adonis2: $R^2 = 0.03513$, $p = 0.09$). The PERMANOVA was not significant when evaluating according to genotype risk (adonis2 $p = 0.385$).

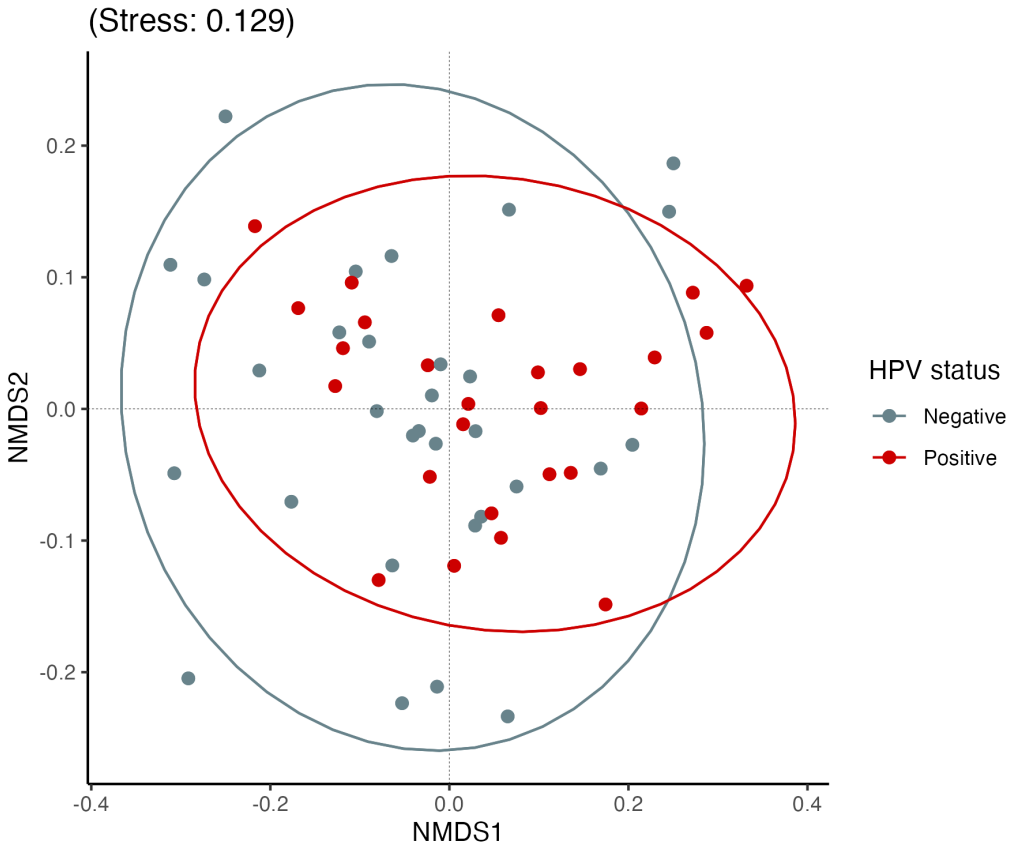

Figure 2.1 Beta diversity of the penile glans on a NMDS computed by Jensen-Shannon distance of the glans microbiota. Ellipses represent 95% confidence intervals. HPV-positive samples are closer together compared to HPV-negative samples (adonis2 $R^2 = 0.03513$, $p = 0.09$).

5. DISCUSSION

This cross-sectional study is the first to examine the relationship between subclinical HPV infection and the microbial ecology of the penile glans in uncircumcised, HIV-negative men. The analysis aimed to identify signs of dysbiosis in the glans microbiota associated with HPV infection. Exploratory analyses showed no differences in alpha diversity metrics between HPV-positive and HPV-negative men. None of the global regression models reached statistical significance (F-test $p > 0.05$), and intra-group variability was substantial. These results do not provide conclusive evidence of diversity differences linked to HPV infection and highlight the need for studies with larger populations to clarify potential microbial shifts associated with specific HPV genotypes.

In microbial ecology, dysbiosis can appear as changes in alpha diversity, indicating colonization by opportunistic taxa or reduced diversity associated with community restructuring (Petersen and Round, 2014; Levy et al., 2017). Metrics such as observed richness and the Shannon index quantify within-sample diversity. Studies of the vaginal microbiota have demonstrated that persistent HPV infection is associated with both increased richness and a loss of protective *Lactobacillus* dominance (Gillet et al., 2011; Mitra et al., 2020), indicating that viral establishment is driven by changes in the microbiota. This chapter applies the same framework to the penile glans.

Beta diversity analyses showed no significant compositional differences between HPV-positive and HPV-negative individuals (PERMANOVA $R^2 = 0.035$, $p = 0.09$), nor when stratified by genotype risk ($p = 0.385$). The strong overlap in NMDS ordination supported the compositional stability of the glans microbiota despite HPV infection, within the limits of a cross-sectional design. This anatomical resilience is biologically plausible. The glans, a partially keratinized mucosal surface without adnexal glands (Humphrey, 2014; Dinotta et al., 2013), resides in a semi-anaerobic niche enclosed by the preputial space in uncircumcised men (Price et al., 2010). These features may confer resistance to environmental perturbations, unlike the more exposed, glandular, sweat-rich shaft.

A major challenge in this study was the anatomical discordance in HPV detection, with many participants testing positive at one site but negative at the other. This discordance complicates ecological interpretation, as HPV stage (acquisition or clearance) likely affects microbial dynamics

in ways not captured by cross-sectional data (Giuliano et al., 2008). Methodologically, it introduces ambiguity in modeling. Treating glans and shaft samples as independent overlooks within-subject correlations, while paired designs assume synchronicity that may not reflect biological reality. These limitations highlight the need for in-depth studies using targeted sampling and host immune profiling to clarify the spatiotemporal dynamics of site-specific HPV infections.

Although the entire penis may harbor HPV, inadequate sampling can yield false negatives, which has critical implications for clinical decision-making. Avoiding urethral sampling reduces invasiveness and improves patient comfort, addressing a key barrier to diagnostic participation (Wang et al 2021). However, the observed pattern of site-specific positivity warrants serious and rigorous evaluation to ensure accurate interpretation and effective clinical management. However, this goes beyond the scope of this work.

This chapter provides foundational insights into glans-specific microbial ecology. It supports future research on how anatomical differences may shape HPV-microbiota interactions. Advanced approaches, such as meta-transcriptomics or spatial profiling, may reveal subtle functional shifts in the glans not captured by 16S rRNA sequencing (Gonçalves et al., 2022).

In summary, HPV infection was not associated with differences in microbial diversity on the glans, and overall community structure remained stable. This resilience may reflect the glans unique microenvironment or the transient nature of HPV infections in men.

CHAPTER III

Alpha and beta diversity of the penile shaft microbiota in relation to HPV infection

1. INTRODUCTION

The penile shaft forms a distinct cutaneous habitat, lined with keratinized stratified squamous epithelium and abundant in eccrine and sebaceous glands (Grice & Segre, 2011; Humphrey, 2014). These adnexal structures release sweat and sebum, creating lipid-rich micro-niches subject to fluctuations in moisture and oxygen due to air exposure, friction, and hygiene practices. This dynamic environment supports diverse skin commensals, including *Staphylococcus* and *Corynebacterium*, along with facultative and obligate anaerobes (Byrd et al., 2018).

Chapter II reported trends in dysbiosis signals in the glans microbiota associated with subclinical HPV infection. Alpha diversity shifts appeared only in high-risk genotype carriers, and beta diversity analyses showed no clear separation between infected and uninfected individuals. A possible explanation is that the glans semi-anaerobic niche, enclosed by the prepuce, resists transient perturbations from HPV. In contrast, the shaft's alternating occluded–exposed state and by far more extensive in surface may increase susceptibility to ecological shifts linked to viral either infection or behavioral factors.

Several participants showed discordant HPV detection between the glans and shaft, indicating independent infection status across sites. More individuals tested positive on the shaft than on the glans, increasing the effective sample size and enhancing statistical power for shaft-based analyses. Previous studies often pooled glans and shaft samples, obscuring region-specific associations (Gonçalves et al., 2022). By focusing on the shaft, this chapter aims to increase statistical power, as the shaft represents a less colonized environment with fewer available nutrients compared to the smegma-rich glans. A more limited microbial community may exhibit stronger responses to HPV infection. Additionally, analyzing only the shaft reduces the risk of a dilution effect. If glans and shaft communities differ, combining them could obscure HPV-associated signals. This site-specific approach enhances sensitivity to detect microbial differences linked to infection.

This chapter characterizes the penile shaft microbiota in uncircumcised, HIV-negative men with and without subclinical HPV infection. Alpha diversity will be quantified using observed ASV

richness, Chao1, and the Shannon index to assess within-sample complexity. Beta diversity will be evaluated through NMDS ordination based on Jensen–Shannon divergence and PERMANOVA to test for compositional shifts by infection status and genotype risk. This targeted analysis will reveal whether the shaft microbiota displays dysbiotic patterns.

2. METHODS

2.1. Sample processing and diversity analyses for the penile shaft

Samples were processed as described in the study design section. This chapter included subjects who were negative in both anatomical sites and positive only in the shaft of the penis. All remaining samples were rarefied to 12,223 sequences per sample, the minimum observed depth.

3. RESULTS

3.1. General characteristics of the study population

A total of 103 men were included in this chapter. Their basic characteristics are shown in Table 3.1. In this data set HPV was detected in 33 (32.03%) men, with 15 high-risk, 10 low-risk, and 8 untyped infections (Table 3.2).

Sexual preference was the only factor significantly linked to HPV status (Fisher's test, $p = 0.013$). Men who have sex with women (MSW) were 3.69 times (95% CI: 1.21-13.73) more likely to be HPV positive than men who have sex with men (MSM). Consequently, subsequent multiple linear regression models were adjusted to control for the effect of sexual preference.

Table 3.1 General characteristics of the male population studied.

General characteristic	*# HPV-negative (%)*	*# HPV-positive (%)*	*p.value*
Sampled men	70 (68%)	33 (32%)	
Age			0.88
20-24	13 (13%)	6 (6%)	
25-29	27 (26%)	11 (11%)	
30-49	30 (29%)	16 (16%)	
Sex of sexual partners			0.01
Men who have sex with men (MSM)	28 (27%)	5 (5%)	
Men who have sex with women (MSW)	42 (41%)	28 (27%)	
Number of sexual partners*			0.57
0-4	9 (9%)	1 (1%)	
5-9	18 (17%)	10 (10%)	
10-19	22 (21%)	11 (11%)	
<20	20 (19%)	11 (11%)	
Unanswered	1 (1%)	0 (0%)	
Tobacco use			0.65
Non-smoker	47 (46%)	24 (23%)	
Smoker	23 (22%)	9 (8%)	

*Sum of previous and current sexual partners. P values are calculated using Fisher's exact test

Table 3.2 General characteristics of the male population studied

Code	Genotypes	HPV-risk
VM 002	61, 42, 89	Low risk
VM 003	84, 40	Low risk
VM 009	58	High risk
VM 0010	66, 52, 59, 89	High risk
VM 0021	91	Low risk
VM 0028	66	Low risk
VM 0030	31, 52	High risk
VM 0039	56, 84, 53, 39	High risk
VM 0050	6 ,53 ,91	Low risk
VM 0061	91	Low risk
VM 0062	56, 51, 84, 43, 91	High risk
VM 0064	45, 40 ,91	High risk
VM 0065	51, 61, 84	High risk
VM 0068	51, 73, 45, 91	High risk
VM 0075	51, 84, 13	High risk
VM 0100	66	Low risk
VM 0105	44	Low risk
VM 0113	84, 45	High risk

VM 0130	33	High risk
VM 0148	66, 16, 89, 91	High risk
VM 0158	51, 59	High risk
VM 0162	66, 84, 45, 43, 53, 13, 91	High risk
VM 0163	66, 59, 43	High risk
VM 0191	61	Low risk
VM 0196	66, 84, 33	High risk

Genotypes considered as High-risk 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59

3.2. Alpha diversity

Multiple linear regression analyses revealed that HPV-positive men exhibited significantly higher observed and predicted richness (S.obs: +9.34 ASVs, p = 0.003; Chao1: +8.33 ASVs, p = 0.02) compared to HPV-negative individuals (Table 2.3). However, no significant differences were observed in Shannon diversity (p = 0.23) or Pielou's evenness (p = 0.68) between the two groups.

When stratified by HPV genotype present (Table 2.4), men with high-risk (HR) genotypes showed significantly higher observed richness (S.obs: +11.79 ASVs, p = 0.003; Chao1: +10.06 ASVs, p = 0.03) compared to HPV-negative individuals, while no significant differences were observed for low-risk (LR) genotypes (S.obs: p = 0.16; Chao1: p = 0.26).

Table 3.3 Multiple linear regression analysis for the relationship between HPV status, sexual preference, and microbial diversity.

Diversity metric	Variable	Coefficient	Standard error	p-value
Species Observed	HPV status (Positive)	9.34	3.08	**0.003**
	Sexual preference (MSM)	-0.7	2.87	0.806
Species richness (Chao1)	HPV status (Positive)	8.33	3.75	**0.029**
	Sexual preference (MSM)	-1.05	3.49	0.765
Shannon	HPV status (Positive)	0.16	0.14	0.234
	Sexual preference (MSM)	0.06	0.13	0.655
Evenness	HPV status (Positive)	0.01	0.03	0.681
	Sexual preference (MSM)	0.02	0.03	0.577

Results from multiple linear regression models assessing the relationship between HPV status, sexual preference, and microbial diversity (Observed species, species richness (Chao1), Shannon, and Pielou evenness) adjusting for sexual preference (MSM). Coefficients, standard errors and p.values for each model are shown, significant values are in bold. MSM: Men who have sex with men.

Table 3.4 Multiple linear regression analysis for the relationship between HPV genotypes and microbial diversity.

Diversity metric	Variable	Coefficient	Standard error	p-value
Species Observed	LR-HPV	6.07	4.35	0.166
	HR-HPV	11.79	3.84	**0.003**
	Sexual preference (MSM)	-1.39	2.94	0.638
Species richness (Chao1)	LR-HPV	6.03	5.32	0.261
	HR-HPV	10.06	4.7	**0.035**
	Sexual preference (MSM)	-1.53	3.59	0.672
Shannon	LR-HPV	0.04	0.19	0.822
	HR-HPV	0.25	0.17	0.142
	Sexual preference (MSM)	0.03	0.13	0.806
Evenness	LR-HPV	-0.02	0.04	0.736
	HR-HPV	0.03	0.04	0.39
	Sexual preference (MSM)	0.01	0.03	0.726

Results from multiple linear regression models assessing the relationship between HPV genotypes (LR and HR vs. Negative) and microbial diversity (Observed species, species richness (Chao1), Shannon, and Pielou evenness), adjusting for sexual preference (MSM). Coefficients, standard errors and p.values for each model are shown, significant values are in bold. Negative HPV status was used as the reference group. MSM: Men who have sex with men.

3.3. Beta diversity

NMDS ordination revealed overlapping in microbial community composition between HPV-positive and HPV-negative individuals (Figure 3.1). However, the PERMANOVA analysis revealed a modest but statistically significant effect of infection status on microbial composition (adonis2: R^2 = 0.0298, p = 0.0130). The PERMANOVA was not significant when evaluating according to genotype risk (adonis2 p = 0.1289).

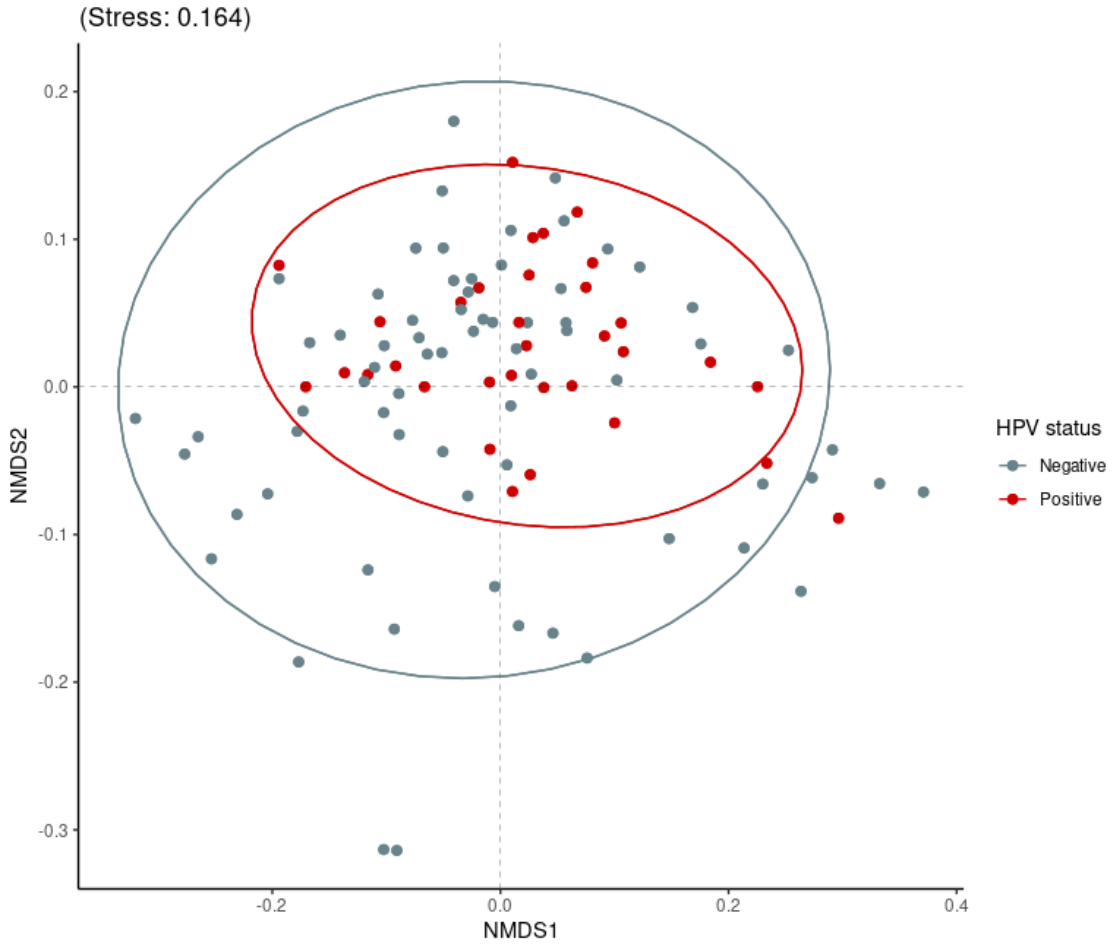

Figure 3.1 Beta diversity of the penile glans on a NMDS computed by Jensen-Shannon distance of the penile skin microbiota. Ellipses represent 95% confidence intervals. HPV-positive samples are closer together compared to HPV-negative samples (adonis2 R^2 = 0.03513, p = 0.09).

4. DISCUSSION

A total of 103 men were included in this cross-sectional analysis of penile shaft microbiota to identify microbial differences between HPV-positive and HPV-negative individuals. Alpha and beta diversity analyses revealed significant differences in species richness and modest but consistent divergence in community composition associated HR-HPV infection.

The analysis of shaft microbiota in this chapter followed the same bioinformatic pipeline as described previously, with two methodological improvements. First, a larger number of samples increased statistical power. Second, rarefaction was performed at 12,223 reads per sample, approximately 4,000 more than in the glans dataset. This higher sequencing depth improved the resolution for detecting low-abundance ASVs and reduced stochastic variability in diversity estimates (Schloss 2024). These refinements increased the robustness and resolution of our findings for the penile shaft.

Here, as observed in the glans, sexual preference was the only host variable significantly associated with HPV status in the penile shaft. Men who have sex with women (MSW) were more likely to be HPV-positive than men who have sex with men (MSM), and this association remained robust after adjustment in multivariable models. This consistent pattern across anatomical sites suggests that behavioral exposure plays a key role in HPV acquisition, especially in asymptomatic cases. While MSM face elevated risk for HPV-related oncogenic outcomes compared to MSW, (such as markedly higher incidence of anal cancer) (van Aar et al., 2013; Farahmand, Monavari, and Tavakoli, 2021; Daling et al., 1982) global data indicate that overall HPV prevalence is similar between MSM and MSW (Kusters et al., 2023). The higher prevalence among MSW in this study may reflect their role as asymptomatic carriers who contribute to transmission without clinical symptoms.

Regarding alpha diversity, men with high-risk HPV (HR-HPV) showed significantly greater species richness, both in observed and predicted ASV's, compared to HPV-negative individuals. Shannon diversity and Pielou's evenness did not reach statistical significance, though both displayed a non-significant upward trend. This pattern suggests that 1) HR-HPV infection may be linked to the presence of additional low-abundance taxa without altering the relative distribution of dominant species, 2) there is a more permissive environment that allows the increased richness.

The finding that only species richness increased significantly in HR-HPV carriers indicate early or subclinical dysbiosis. Unlike severe imbalances characterized by shifts in dominance, mild dysbiosis may involve the accumulation of rare taxa without disrupting overall structure (Shan, Lee, and Chang 2022). Similar “richness-only” dysbiosis has been reported in vaginal microbiota during transitional states between community types, where richness increases occur without changes in evenness or dominance (Zeng et al. 2022). In the penile shaft, new ASVs in HR-HPV individuals could reflect microenvironmental changes (yet not described in the natural history of infection) or reduced ecological stability. This does not imply pathogenic shifts but may indicate a transient imbalance (Shan, Lee, and Chang 2022).

Beta diversity analysis based on Jensen–Shannon divergence (JSD) revealed modest but statistically significant differences in community composition between HPV-positive and HPV-negative individuals ($R^2 = 0.0298$, $p = 0.0130$). JSD was chosen over Bray–Curtis dissimilarity because it accounts for full taxonomic probability distributions, is less sensitive to zeros, and avoids overweighting dominant taxa (Gajer et al., 2012). Given the high richness and sparsity of penile skin microbiota, JSD is well suited to detect subtle shifts driven by minority ASVs. Although small, the observed effect size supports partial community-level changes associated with HPV status.

Interpreting NMDS ordinations based on JSD requires anatomical context. In the vagina, infection status often drives distinct clustering due to dominance shifts in taxa like *Lactobacillus* (Gajer et al., 2012). Penile skin communities, by contrast, proved to be more diverse and are compositionally diffuse. This results in broader overlap across ordination space. Nonetheless, samples from HPV-positive individuals tended to cluster more closely with one another than with controls, suggesting weak but consistent structuring within the infected group. This pattern is consistent with ecological drift or compositional tuning, rather than abrupt restructuring.

In conclusion, these findings indicate that the penile shaft microbiota shows measurable but limited shifts in the presence of HR-HPV infection. These findings highlight the importance of adequate sample size for detecting ecological signals and that microbial shifts can occur even in subclinical infections. Increased richness, modest compositional divergence, and persistent behavioral associations across sites support the value of further research on microbial correlates

of subclinical HPV carriage in male genital skin. The inclusion of participants with site-specific HPV discordance may introduce dilution effects in global comparisons, reinforcing the value of site-specific and stratified analyses.

CHAPTER IV

Community state types of the penile skin microbiota and HPV status

1. INTRODUCTION

Community State Types (CSTs) are discrete categories used to describe recurring patterns of microbial community composition, typically defined by taxonomic dominance and co-abundance structure. Originally developed for vaginal microbiota research (Ravel et al., 2011; Gajer et al., 2012), CSTs provide a reproducible, ecologically meaningful framework for classifying microbial states based on unsupervised clustering of 16S rRNA gene profiles.

The first step in identifying CSTs involves computing a pairwise distance matrix that quantifies dissimilarity between microbiome samples. This process transforms taxonomic abundance tables into a structure suitable for clustering. Several distance metrics are commonly used, each capturing different ecological dimensions (Anderson et al. 2011). Bray–Curtis dissimilarity measures differences in taxon abundance and emphasizes dominant species but ignores shared absences. Jensen–Shannon divergence (JSD), a smoothed and symmetric form of Kullback–Leibler divergence, compares entire taxonomic distributions and is robust to sparsity and extreme values. UniFrac distances (weighted or unweighted) incorporate phylogenetic information and detect shifts among evolutionarily distinct taxa (Chen et al. 2021). The choice of distance metric influences CST resolution and interpretability and should reflect the ecological characteristics of the sampled environment or data set properties.

After computing the distance matrix, hierarchical clustering is typically applied to define CSTs (Namkung 2020). Ward's linkage is commonly used due to its ability to generate compact clusters by minimizing within-cluster variance. Complete linkage emphasizes maximum inter-sample distance and forms tight, conservative clusters, while single linkage uses minimum distance and is sensitive to outliers, often producing chain-like groupings. Average linkage (UPGMA) offers a balanced approach by averaging all pairwise distances. The clustering algorithm affects cluster shape, separation, and interpretability in visualizations such as heatmaps and ordination plots.

Validating the quality of clustering results is essential to ensure that the identified CSTs represent biologically meaningful and statistically robust groupings. Internal validation indices offer a principled approach to assess cluster compactness, separation, and consistency based solely on

intrinsic data structure (Handl, Knowles, and Kell 2005). They evaluate how well the data form natural groupings without relying on predefined class labels such as disease status or clinical categories (Handl, Knowles, and Kell 2005; Brock et al. 2008). Connectivity measures the extent to which neighboring samples are assigned to the same cluster. Low values indicate preservation of local structure, which is especially important in ecological datasets shaped by gradual gradients. The Dunn Index quantifies the ratio of the smallest inter-cluster distance to the largest intra-cluster diameter. High Dunn values suggest well-separated and cohesive clusters. Silhouette Width offers an intuitive evaluation at the sample level by comparing average dissimilarity within a cluster to that with the nearest neighboring cluster. Values range from −1 to 1, with higher scores indicating better-defined cluster membership. These indices can be computed across different numbers of clusters to identify the most stable and interpretable solution. The clValid R package (Brock et al. 2008) supports simultaneous comparison of clustering algorithms and distance metrics using these indices, offering a comprehensive framework for selecting optimal CST configurations in microbiome studies.

In women, CSTs have clarified links between microbial composition and mucosal health (Brooks et al. 2017). CSTs dominated by *Lactobacillus crispatus* or *L. gasseri* are associated with low vaginal pH and reduced risk of sexually transmitted infections, including HPV and HIV (Carrillo-Ng et al. 2021). In contrast, CSTs rich in strict anaerobes such as *Gardnerella*, *Prevotella*, and *Atopobium* are linked to bacterial vaginosis, inflammation, and persistent HPV infection (Gajer et al., 2012; Brotman et al., 2014). The CST model also enables longitudinal tracking of microbial shifts across menstrual cycles, antibiotic use, and disease progression. Despite its success in vaginal microbiome studies, the CST framework has not been systematically applied to the penile microbiota. Given the penile role in sexual transmission networks, identifying stable and recurring CSTs in this environment could clarify microbial contributions to HPV acquisition and persistence.

This chapter adopts a CST-based approach following the methodology of Gajer et al. (2012) to classify penile shaft microbiota into community state types (CSTs). The objetive is to explore the ecological structure of the penile shaft and examine potential associations between CST membership and HPV infection status. Glans samples were excluded from this CST analysis for both methodological and biological reasons. First, many individuals showed discordant HPV results between anatomical sites, testing positive in either the glans or the shaft but not both. This

introduces uncertainty in defining the infection status of the sampled community and complicates CST–HPV interpretation. Secondly, prior analyses (Chapter II) revealed no clear HPV-associated differences in glans diversity or composition, indicating weaker ecological signal at that site. Restricting the analysis to shaft samples improves ecological resolution and supports more reliable inferences about HPV-related community shifts.

2. METHODS

To characterize community structure patterns among penile shaft samples, we used hierarchical clustering to define community state types (CSTs) based on microbial composition. All analyses were conducted in R 4.2.2. Amplicon sequence variants (ASVs) were inferred using DADA2 (Callahan et al., 2016) with pooled error modeling and classified against the SILVA 138.1 database (Quast et al., 2013). Taxa present in fewer than 10% of samples were excluded to focus on prevalent community members. Samples were rarefied to a uniform sequencing depth of 12,223 reads.

Relative abundance tables were generated after normalizing counts per sample. For visualization, the top 10 ASVs ranked by mean relative abundance across samples were retained. Heatmaps were created using the *ComplexHeatmap* package (Gu 2022), and taxonomic labels were manually curated by combining genus and family identifiers for incompletely resolved taxa.

This chapter adopts a CST-based approach following the methodology of Gajer et al. (2012). Microbial profiles were clustered using Jensen–Shannon divergence (JSD) as the dissimilarity. Hierarchical clustering was performed with Ward's linkage method (ward.D2). The optimal number of clusters was determined using a Gaussian mixture model fitted with the *Mclust* package (Scrucca et al. 2016). Five CSTs were selected based on model fit. Cluster robustness was assessed using resampling-based stability analysis with 1000 bootstrap iterations (Hennig and Imports 2015) using *fpc* package. Cluster stability was quantified using the Jaccard similarity index. Clusters with average Jaccard values >0.85 were considered highly stable. Each sample was assigned to a CST based on dendrogram structure. Cluster validity was confirmed visually using heatmaps and metadata annotations.

Associations between CSTs and HPV status were tested using Fisher's exact test. CST-5 was further compared against all other CSTs in a binary analysis. Taxa enriched in CST-5 were identified using Wilcoxon rank-sum tests and adjusted for multiple comparisons using the Benjamini–Hochberg method.

3. RESULTS

To estimate the optimal number of clusters, we applied Gaussian finite mixture modeling via the *Mclust* algorithm. This unsupervised approach suggested a nine-component solution as optimal. The clustering table revealed the following sample distribution across components: 4, 15, 10, 8, 22, 14, 7, 20, and 3 samples per cluster, respectively. Despite the statistical suggestion of nine components, we chose a biologically interpretable five-cluster solution for downstream analysis. This decision was guided by prior CST literature in vaginal microbiota (Gajer et al., 2012; Ravel et al., 2011), and the need for sufficient statistical power within each group.

To evaluate cluster stability, we used bootstrap resampling (B = 1000) and computed Jaccard similarity indices for each cluster (Table 4.1). Three of five CSTs surpassed the commonly accepted threshold for stability (Avg. Jaccard > 0.75), and one cluster (CST-5) was highly stable (Avg. Jaccard = 0.92, instability = 0.003). Cluster instability ranged from 0.01 to 0.21 across the five groups. These results indicate that the selected CST solution offers a reproducible and coherent representation of microbial community types in the penile shaft.

Table 4.1 Cluster stability metrics for the five penile CSTs

CST Cluster	Average Jaccard Index	Instability
CST-1	0.748	0.154
CST-2	0.739	0.224
CST-3	0.844	0.062
CST-4	0.726	0.137
CST-5	0.927	0.003

Jaccard similarity and instability scores obtained from 1000 bootstrap iterations using Ward's method and Jensen-Shannon divergence.

The bacterial community was characterized and grouped into five distinct Community State Types (CSTs) (Figure 4.1 Table 4.2). CST-1 was dominated by *Staphylococcus* (n = 11), CST-2

by *Corynebacterium* (n = 20), CST-3 by *Fenollaria* and *Prevotella* (n = 14), CST-4 by *Mannheimia* (n = 12), and CST-5 by *Finegoldia* (n = 46).

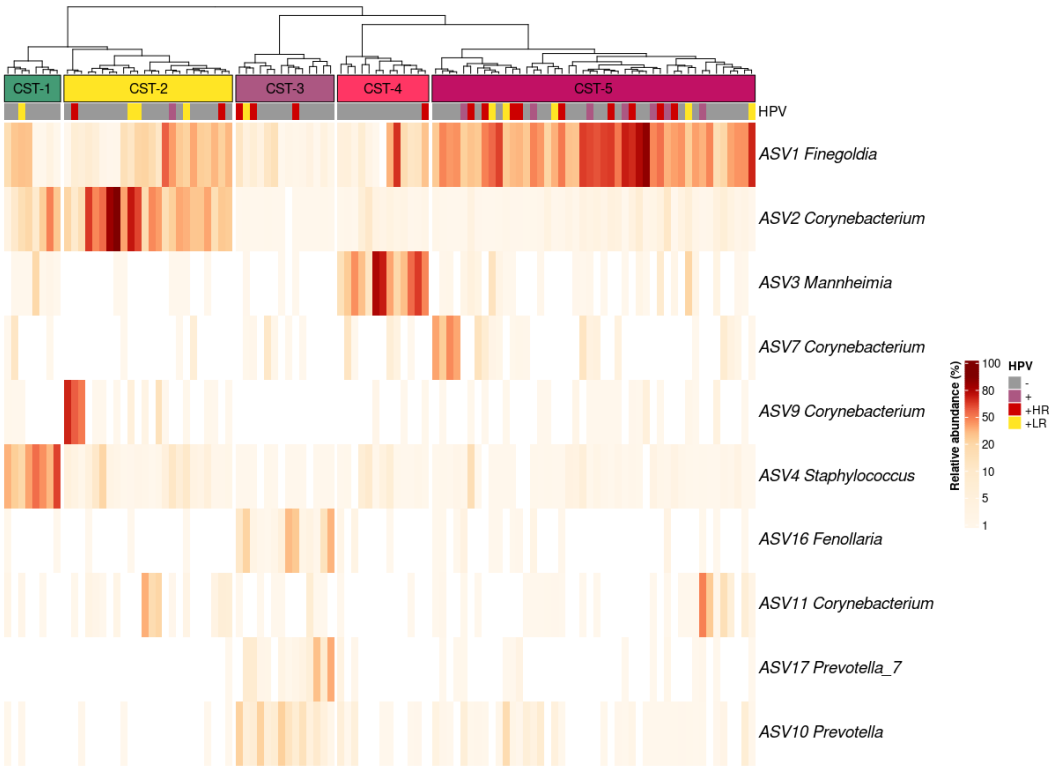

Figure 4.1 Community State Types of the penile skin microbiota. Heatmap representing the relative abundance of the most abundant ASV's in the penile skin microbiota of subclinical HPV-positive and HPV-negative samples. The dendrogram was constructed using the Ward's linkage clustering based on the Jensen-Shannon Divergence distance.

Table 4.2 Community State Types and their microbial diversity metrics in the penile skin microbiota of HPV-infected men.

CST	Dominant Taxa (relative abundance)	Number of samples	# HPV-positive (%)
CST-5	*Finegoldia* (45%)	46	21 (84%)
CST-2	*Corynebacterium* (41%)	20	5 (33.3%)
CST-3	*Fenollaria* (11%)	14	4 (40%)
CST-4	*Mannheimia* (41%)	12	1 (9.1%)
CST-1	*Staphylococcus* (31%)	11	2 (22.2%)

Summary of the five identified Community State Types (CSTs) in the penile skin microbiota of HPV-infected individuals. The table shows the dominant taxa for each CST, the number of individuals belonging to each CST, the prevalence of each CST, and the mean alpha diversity metrics (S.chao1, S.obs, evenness, and Shannon index) for each CST.

All CSTs included at least one HPV-positive sample (Table 4.2). CST-5 had the highest proportion of HPV-positive individuals (84%), while CST-1 had the lowest (22%). Although Fisher's exact test showed no overall association between CSTs and HPV status ($p = 0.085$), CST-5 exhibited a trend towards encompassing most HPV-positive samples.

Comparing CST-5 to all other CSTs combined (CST-1 to CST-4) revealed a significant association (Fisher's exact test: $p = 0.01$; OR = 3.11, CI95%: 1.22 - 8.22), indicating a three-fold increased likelihood of HPV positivity in CST-5.

CST-5 was dominated by *Finegoldia* (45.13% relative abundance). To investigate other taxonomic drivers of this CST, we compared the mean abundance of taxa between CST-5 and the other CSTs combined (Table 4.3). This revealed significant differences in several taxa. ASVs belonging to the genera *Peptoniphilus*, *Negativicoccus*, *Anaerococcus*, and *Prevotella_7* showed higher abundance in CST-5, whereas beneficial commensals such as *Corynebacterium* and *Staphylococcus* were significantly less represented.

Table 4.3 Relative abundance of ASV's in men grouped in CST5 versus CST1 to CST-4

ASV	Genus	Frequency in CST5	Frequency in CST1 to CST4	Mean relative abundance in CST-5 (%)	Mean relative abundance in CST-1 to 4 (%)	p.adj
ASV1	*Finegoldia*	46 (45%)	54 (52%)	45.13	15.31	< .001
ASV145	*Finegoldia*	31 (30%)	17 (17%)	0.2	0.08	0.004
ASV17	*Prevotella_7*	13 (13%)	14 (14%)	0.37	8.02	0.003
ASV171	*Peptoniphilus*	12 (12%)	10 (10%)	0.05	0.37	0.033
ASV18	*Campylobacter*	27 (26%)	25 (24%)	0.73	3.97	0.029
ASV2	*Corynebacterium*	46 (45%)	56 (54%)	2.03	19.49	0.018
ASV24	*Negativicoccus*	36 (35%)	34 (33%)	1.43	0.6	0.039
ASV30	*Porphyromonas*	8 (8%)	13 (13%)	0.15	3.96	0.018
ASV4	*Staphylococcus*	43 (42%)	53 (51%)	1.15	8.01	0.023
ASV40	*Anaerococcus*	34 (33%)	26 (25%)	1.02	0.14	< .001
ASV51	*Staphylococcus*	17 (17%)	29 (28%)	0.05	1	< .001
ASV53	*Corynebacterium*	33 (32%)	36 (35%)	0.09	0.4	0.003
ASV63	*Peptoniphilus*	15 (15%)	15 (15%)	0.67	0.94	0.047
ASV73	*Finegoldia*	33 (32%)	15 (15%)	0.24	0.02	0.044
ASV97	*Staphylococcus*	16 (16%)	28 (27%)	0.46	0.28	0.039

Comparison of relative abundance of microbial taxa between CST-5 and other skin bacterial community (SBC) groups using the Wilcoxon rank-sum test. P-values were adjusted using the Bonferroni method.

4. DISCUSSION

In this chapter we applied community state type (CST) modeling to the penile skin microbiota, adapting a framework originally developed for the vaginal microbiome (Ravel et al., 2011; Gajer et al., 2012). CSTs offer an ecologically intuitive classification of microbial configurations, allowing comparison of distinct community structures potentially linked to host phenotypes such as HPV status. We identified CST-5 as associated with HPV infection. This CST displayed a community profile marked by reduced levels of beneficial commensals and enrichment of opportunistic anaerobes, consistent with features of dysbiosis.

Although CST modeling has focused mainly on the vaginal microbiome, where *Lactobacillus*-dominated states are considered protective, penile CSTs showed markedly different compositions. Five CSTs were identified this number was selected based on internal validation metrics, including connectivity and Jaccard stability from bootstrapped clustering. These indices confirmed that the five-cluster solution was compact, well-separated, and statistically robust (Handl et al., 2005; Brock et al., 2008). CST-5 showed particularly high stability (Jaccard > 0.85), supporting its biological relevance. Increasing the number of clusters led to over-partitioning, while fewer clusters masked dominant patterns.

The main CSTs were defined by taxa such as *Finegoldia*, *Corynebacterium*, *Fenollaria*, *Mannheimia* and *Staphylococcus*. However, unlike the strong stratification observed in female CSTs, our CST groupings showed no clear dominance patterns and were more compositionally diffuse. In all clusters, no single genus exceeded 50% relative abundance, indicating that community composition was spread across multiple taxa. For instance, CST-3 which was nominally dominated by *Fenollaria*, showed only 11% relative abundance of this genus. A larger sample size and additional studies in uncircumcised populations are needed to reinforce the observations reported here and advance understanding of CSTs in the male genital microbiota.

These findings are consistent with previous reports describing the penile microbiome as a diverse, skin-like environment colonized by anaerobic and facultative anaerobic bacteria (Onywera, Williamson, Cozzuto, et al., 2020). They also partially align with studies linking penile anaerobic dysbiosis to increased susceptibility to HIV infection (C. M. Liu et al., 2017). While immune markers were not assessed in this study, the CST-5 group showed elevated abundances of

genera such as *Prevotella* and *Peptoniphilus*, previously described as pro-inflammatory (C. M. Liu et al., 2017). Additionally, we did not find associations between CSTs and HPV genotype risk, contrasting with reports in women (Brotman et al., 2014). These discrepancies likely reflect anatomical differences between penile and vaginal mucosa.

For downstream analysis, CST-5 was compared against all other CSTs grouped together. This binary comparison was justified by CST-5's distinct composition, dominated by *Finegoldia*, *Peptoniphilus*, and *Anaerococcus*, anaerobic taxa associated with vaginal dysbiosis and mucosal inflammation (Brotman et al., 2014; Murphy and Frick, 2013). *Finegoldia* and *Peptoniphilus* produce proteolytic enzymes and volatile fatty acids that may affect epithelial barrier integrity and immune tone (Murphy and Frick, 2013). Their consistent presence in CST-5 supports their role as ecological indicators of potential community disruption. In contrast, other CSTs were either more heterogeneous or enriched in *Corynebacterium* or *Staphylococcus*, genera common in healthy cutaneous communities (Byrd et al., 2018). CST-5 may represent a disrupted or inflammatory state, although its ecological and clinical roles in male genital microbiota remain unclear. No statistically significant association was observed between CST membership and HPV genotype risk. CSTs represent broad community states that may resist subtle or transient shifts, especially in the absence of surface damage. Still, CST-5 was overrepresented among HPV-positive individuals, showing that anaerobe-rich communities are associated with infection.

The study presented in this chapter has some limitations. First, although the overall sample size was adequate for penile shaft analyses, some CSTs included relatively few samples. A larger sample size could help clarify the typical composition and membership of each CST. Second, the cross-sectional design limits causal inference regarding microbial dynamics and HPV infection. Third, the use of 16S rRNA amplicon sequencing constrains taxonomic resolution. While certain genera and ASVs were identified, species or strain-level identities remain uncertain, which limits clinical interpretability.

In conclusion, this CST-based analysis introduces a structured framework for describing penile microbiota and investigating its links to HPV. Although no strong associations with genotype risk were detected, the identification of distinct anaerobe-rich CSTs offers a foundation for future longitudinal and mechanistic studies. CSTs represent a scalable tool for uncovering ecologically

meaningful patterns in male genital microbiomes, extending their utility beyond the vaginal context.

CHAPTER V

Shotgun sequencing: Addressing some limitations of amplicon sequencing for fine taxonomic classification.

1. INTRODUCTION

The skin hosts a narrow microbiota that plays a role in maintaining health and influencing disease states. Previous studies based on 16S rRNA gene amplicon sequencing have characterized differences in microbial diversity between healthy individuals and those with skin infections, revealing imbalances (dysbiosis) associated with pathology (Harris-Tryon & Grice, 2022). While these amplicon-based approaches provide valuable ecological insights, they face inherent limitations when precise taxonomic identification is required for clinical applications (Johnson et al., 2019).

Standard 16S rRNA analyses usually target short hypervariable regions (V3–V4 or V4, spanning 300–600 bp) of the full-length 16S gene (~1,500 bp) (Janda & Abbott, 2007). This partial coverage, combined with incomplete databases (Abellan-Schneyder et al., 2021) and high sequence similarity among related anaerobic species (Ghebremedhin et al., 2008), often limits reliable classification beyond the genus level. This resolution constraint proved particularly limiting for our HPV-associated penile skin microbiota study (Chapter II), where we identified *Finegoldia*, *Anaerococcus*, and *Negativicoccus* as consistently elevated in infections, while *Peptoniphilus* and *Staphylococcus* exhibited strain-specific variation patterns (Some ASV's were high and other were in low abundance) and highlighting the need for strain-level resolution beyond what 16S sequencing can offer.

In Chapter II, the penile skin microbiota of HPV-infected and healthy individuals was compared in more than 100 samples by 16S sequencing. While this approach was essential for an initial screening due to its cost-effectiveness at large scale, it left critical questions unanswered regarding the specific species or strains involved. This limitation is clinically significant, as different strains within a species can vary dramatically in their virulence factors and antibiotic resistance profiles.

To address these limitations while maintaining methodological and economic feasibility, we implemented a two-phase analytical strategy. The initial 16S screening identified candidate opportunistic anaerobic bacteria associated with infection, while this subsequent study employs shotgun metagenomics on selected samples to achieve species and potentially strain-level resolution (Tickle et al., 2013). Shotgun metagenomics provides several key advantages: it sequences all microbial DNA present, enables analysis of multiple genomic regions, and offers compatibility with more comprehensive reference databases (Quince et al., 2017).

The primary objective of this part of the study was to characterize the taxonomy of opportunistic anaerobic bacteria previously flagged in our 16S analysis. By focusing on these specific targets through shotgun metagenomics, we aimed to reach their species-level identification. The enhanced taxonomic resolution provided by shotgun metagenomics could reveal previously unrecognized species or strains among penile skin opportunistic anaerobes, potentially improving diagnostic accuracy and guiding more targeted *in vitro* analyses.

2. METHODS

2.1. Sample selection

We selected three HPV-positive samples based on the relative abundance of the target genera *Peptoniphilus*, *Finegoldia*, *Anaerococcus Staphylococcus* and *Negativicoccus.* Additionally, three HPV-negative samples were randomly selected as controls.

2.2. Metagenomic DNA extraction and sequencing

Metagenomic DNA was extracted from each DNA/RNA shield-preserved sample using a ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research) following the manufacturer's protocol. DNA quality was assessed using a NanoDrop spectrophotometer (ThermoScientific). Then all the DNA was pooled in a single tube for sequencing. Samples were sent for whole genome sequencing through the NovaSeqXPlus, 150PE (Novogene, CA, USA).

2.3. Metagenomic sequence analysis

The raw FASTQ files obtained from shotgun metagenomic sequencing were first subjected to quality control using Trimmomatic v0.39 (Bolger et al., 2014). This process included the removal of Illumina adapters, quality trimming of reads using a sliding window approach (4 bases, minimum quality score of 20), and discarding reads shorter than 50 bp. To remove human DNA contamination, the reads were then aligned against the GRCh38 reference genome using Bowtie2 v2.4.5 (Langmead & Salzberg, 2012) in sensitive mode, retaining only unaligned reads for downstream analysis.

Metagenomic assembly was performed using MEGAHIT v1.2.9 (Li et al., 2015). Taxonomic classification was conducted using Kraken2 v2.1.2 with the Standard Plus PF database (Wood et al., 2019). Kraken2 is a high-resolution taxonomic classifier that uses a k-mer–based approach, offering advantages over 16S rRNA surveys. It analyzes short DNA fragments (default 35 bp) across the genome for species- and strain-level resolution. A curated reference database excludes non-informative regions, keeping only k-mers unique to taxa or with key variations. Kraken2 splits input sequences into overlapping k-mers and matches them to this database. K-mers matching multiple taxa are discarded. A sequence is classified if over 50% of its k-mers uniquely match one taxon. For example, if 83 of 166 k-mers in a 200 bp contig match Finegoldia magna, it is classified accordingly.

3. RESULTS

Shotgun metagenomic analysis of pooled penile skin samples yielded 139,113 contigs (172.17 Mbp) in the HPV-negative group and 182,365 contigs (177.73 Mbp) in the HPV-positive group, with N50 values of 4,013 bp and 1,512 bp, respectively. Taxonomic classification succeeded for 85.15% of HPV-negative and 84.72% of HPV-positive sequences, leaving 14.85–15.28% unclassified. Despite more fragmented assemblies in the HPV-positive pool (average contig length: 974 bp vs. 1,237 bp), both groups exhibited comparable performance in assembly and classification.

To complement the metagenomic analysis, we characterized contigs classified by Kraken2 and annotated them for gene content. Table 5.1 summarizes assembly metrics for selected taxa of

interest, including contig counts, length ranges, total k-mers, and gene numbers. *Finegoldia magna*, *Peptoniphilus genitalis*, *and Staphylococcus epidermidis* yielded the highest numbers of contigs (586, 322, and 407, respectively) and the largest total gene counts, indicating high representation in the sample. Among *Anaerococcus spp*, *A. prevotii* DSM 20548 was prominent, with 191 contigs and over 400 genes, while *A. nagyae* and *A. vaginalis* appeared with fewer contigs and lower gene content.

Notably, single long contigs, such as the 1,313 bp contig for *Staphylococcus warneri* and the 1,112 bp contig for *S. hominis subsp. Hominis*, may reflect partial genome recovery or low abundance. Abundant taxa yielded extensive functional profiles, with over 700 predicted genes in *F. magna* and more than 450 in *S. epidermidis*. In contrast, low-abundance species typically contained fewer than 10 annotated genes.

Although the pooled design precluded statistical comparisons, distinct trends in relative abundance were observed (Table 5.2). *Finegoldia* was highly enriched in HPV-positive samples (12.51% vs. 1.93%), composed exclusively of the species *F. magna*, including strain ATCC 53516 (4.41% vs. 0.72%) and ATCC 29328 (0.03% vs. 0.01%).

Table 5.1. Summary of assembled contigs and gene annotations by taxon.

Taxon	#contigs	Min. length (bp)	Max. length (bp)	Total k-mers	Gene count
Anaerococcus	7	318	1,985	211	7
Anaerococcus mediterraneensis	9	340	3,555	405	10
Anaerococcus nagyae	2	1,552	2,016	114	7
Anaerococcus obesiensis	9	432	10,611	814	22
Anaerococcus prevotii DSM 20548	191	311	16,496	40,078	404
Anaerococcus sp. Marseille-Q7828	3	348	4,435	83	4
Anaerococcus vaginalis	5	470	1,504	16	5
Finegoldia magna	586	301	20,531	38,729	773
Finegoldia magna ATCC 53516	55	269	8,451	1,277	72
Peptoniphilus	8	340	5,757	295	11
Peptoniphilus genitalis	322	302	30,597	12,388	432
Peptoniphilus harei	112	312	18,457	5,663	162
Staphylococcus	6	351	2,886	917	6

Staphylococcus epidermidis	407	315	4,051	51,745	455
Staphylococcus haemolyticus	185	301	10,722	19,392	202
Staphylococcus hominis	288	301	3,489	30,858	316
Staphylococcus hominis subsp. *hominis*	1	1,112	1,112	15	1
Staphylococcus hominis subsp. *novobiosepticus*	1	711	711	2	1
Staphylococcus warneri	1	1,313	1,313	5	1

Summary of contig-level genomic features for 19 bacterial taxa identified in penile skin metagenomes. For each taxon, the table reports the number of assembled contigs, minimum and maximum contig lengths, total k-mers, and number of genes that informed that taxonomy.

Table 5.2. Species and strain-level classification of penile skin opportunistic anaerobes in HPV-positive pools by shotgun metagenomics

Taxa	HPV-negative relative abundance	HPV-positive relative abundance
Peptoniphilus	3.09	7.78
Peptoniphilus genitalis	1.50	5.89
Peptoniphilus harei	1.43	1.71
Peptoniphilus equinus	0.08	0.07
Anaerococcus	2.11	3.41
Anaerococcus prevotii	1.09	1.45
Anaerococcus prevotii DSM 20548	1.09	1.45
Anaerococcus vaginalis	0.27	0.55
Anaerococcus obesiensis	0.27	0.59
Anaerococcus mediterraneensis	0.24	0.40
Anaerococcus nagyae	0.04	0.09
unclassified Anaerococcus	0.04	0.08
Anaerococcus sp. Marseille-Q7828	0.04	0.08

Anaerococcus degeneri	0.01	<0.01
Finegoldia	1.93	12.51
Finegoldia magna	1.93	12.51
Finegoldia magna ATCC 53516	0.72	4.41
Finegoldia magna ATCC 29328	0.01	0.03
Staphylococcus	0.04	4.13
Staphylococcus hominis	0.01	1.30
Staphylococcus hominis subsp. hominis	<0.01	0.07
Staphylococcus aureus	<0.01	0.03
Staphylococcus haemolyticus	<0.01	1.05
Staphylococcus capitis	<0.01	0.01
Staphylococcus epidermidis	<0.01	1.60
Staphylococcus hominis subsp. novobiosepticus	NA	0.02
Staphylococcus haemolyticus JCSC1435	NA	0.01

Peptoniphilus also showed higher abundance in HPV-positive samples (7.78% vs. 3.09%), primarily *P. genitalis* (5.89% vs. 1.5%) and *P. harei* (1.71% vs. 1.43%) were found. *P. equinus* was detected at similar low levels in both groups.

Anaerococcus was moderately more abundant in HPV-positive samples (3.41% vs. 2.11%), with diverse species such as *A. prevotii* (1.45% vs. 1.09%), *A. vaginalis* (0.55% vs. 0.27%), *A. obesiensis*, *A. mediterraneensis*, and *A. nagyae* present in both groups. Unclassified *Anaerococcus* and rare species such as *A. degeneri* and *A. sp. Marseille-Q7828* were also detected at levels <0.1%.

Importantly, shotgun sequencing revealed *Staphylococcus* species that were undetected by 16S-based methods. Total *Staphylococcus* abundance was notably higher in HPV-positive samples (4.13% vs. 0.04%), including S. hominis (1.3%), S. epidermidis (1.6%), and S. haemolyticus (1.05%). Subspecies-level resolution showed S. hominis subsp. hominis (0.07%) and *S. hominis subsp. novobiosepticus* (0.02%) only in HPV-positive samples. Additional species such as S. aureus (0.03%) and *S. capitis* (0.01%) were also exclusive to this group. The strain *S. haemolyticus* JCSC1435 was detected at 0.01% in HPV-positive samples.

These findings confirm and expand upon previous 16S observations, highlighting consistent enrichment of opportunistic anaerobic species in HPV-positive penile skin while providing higher taxonomic resolution and revealing additional species associated with HPV infection.

4. DISCUSSION

The final part of this study employed shotgun metagenomics to resolve the taxonomic composition of penile skin microbiota in HPV infection, building upon previous 16S rRNA data. While assembly metrics revealed slightly more fragmented contigs in HPV-positive samples, N50 values indicate adequate assembly for taxonomic profiling. Additionally, classification rates were comparable between HPV-positive and negative pools (~85%), validating the robustness of our taxonomic profiles. The analysis confirmed the prominence of opportunistic anaerobic genera, *Finegoldia*, *Peptoniphilus*, and *Anaerococcus*, previously identified by 16S sequencing, while providing species and strain-level resolution. *Finegoldia magna* emerged as the dominant opportunistic anaerobe, and *Peptoniphilus genitalis* was identified as a previously unrecognized, potentially HPV-associated species.

The near-exclusive detection of *F. magna* (particularly strain ATCC 53516) in HPV-positive samples suggests this opportunistic anaerobe may thrive much better in the HPV-infected microenvironment. This aligns with emerging evidence that *F. magna* can modulate inflammation (van der Krieken et al., 2023), potentially exacerbating HPV persistence.

Ecologically, the presence of anaerobic-dominated CSTs on penile skin highlights questions about dysbiosis and microbial resilience. Unlike the vaginal microbiome, where dysbiosis often involves loss of *Lactobacillus* dominance, penile communities are diverse even in presumed

healthy states. In this context, dysbiosis may reflect enrichment of pro-inflammatory or metabolically active taxa rather than a simple loss of protective species. *Finegoldia* and *Peptoniphilus*, for example, are known to stimulate cytokine production and may alter local immune responses (Murphy & Frick, 2013; van der Krieken et al., 2023).

Within the *Peptoniphilus* genus, *P. genitalis* was only recently described ((Abou Chacra et al., 2024), but several related species have already been linked to genital dysbiosis in women (Diop et al., 2019) and may contribute synergistically to HPV pathogenesis. *Anaerococcus* species showed high diversity, highlighting the need for species-level classification in microbiome studies. The strain-specific patterns observed for *Staphylococcus* (e.g., *S. epidermidis* exclusively in HPV+ samples) further demonstrate that shotgun metagenomics can reveal microbial signatures that are not so evident using only 16S approaches.

Our findings should be interpreted considering the pooled sample design, which precluded statistical analysis of abundances and individual-level correlations. The 15% unclassified sequences suggest gaps in reference databases, particularly for opportunistic anaerobic skin microbiota. Future studies should combine deeper sequencing with species cultivation for validation to isolate clinically relevant strains.

By bridging the resolution gap between 16S screening and clinical microbiology, this final part of the study provides the first species and strain-level characterization of opportunistic anaerobic bacteria in HPV-associated penile skin microbiota. The identification of *F. magna* and *P. genitalis* as candidate cofactors, along with strain-specific *Staphylococcus* patterns, offers testable hypotheses for future mechanistic studies.

CHAPTER VI

Overall discussion, conclusions and perspectives

DISCUSSION

This thesis study included a total of 103 uncircumcised, HIV-negative men. The design was cross-sectional and exploratory, aimed at identifying associations between subclinical infections and the penile skin microbiota.

The main motivation for studying subclinical (asymptomatic) infections lies in their effectiveness as transmission routes, since men can unknowingly act as carriers, contributing to transmission to their sexual partners. Men who do not engage in receptive intercourse have lower risk compared to those who do, particularly men who have sex with men (Choi et al., 2021; Dunne et al., 2006). In women, the oncogenic risk is well established due to the histological characteristics of receptive tissues such as the cervix and rectum (Łaniewski et al., 2018, 2020; Parkin, 2006). In contrast, the penis is highly keratinized, and the skin serves as a strong barrier that limits viral access to the dermis, where HPV must reach to complete its infection cycle (de Sanjosé et al., 2018). This makes penile infection a distinct model from mucosal tissues. However, the glans represents a semi-mucosal surface within the same anatomical structure, introducing intra-organ variation. Moreover, circumcision lowers the risk of acquiring HPV, adding further complexity (Price et al., 2010). Studying subclinical infections in the context of the microbiota avoids confounding from clinical manifestations such as cancer lesions or genital warts, which likely alter the skin ecosystem. For example, a wart may create physical niches **(**like ridges and cavities**)** that may harbor distinct microbial communities. Here, we focused on the pre-lesion environment to examine whether early shifts in the microbiota could serve as indicators of HPV infection before overt symptoms appear.

While the vaginal microbiota has been extensively studied due to its easily characterizable community, including the development of CST methodology, the penile microbiota has received comparatively limited attention. The introduction of community state types (CSTs) enabled classification of microbial profiles dominated by *Lactobacillus* or anaerobes, facilitating research into dysbiosis and sexually transmitted infection risk (Ravel et al., 2011). This approach later

extended to studies of female CSTs in relation to male partners, gradually bringing focus to the male genital microbiota (C. M. Liu et al., 2015). However, the penile microbiome has often been viewed from a reductionist perspective. As an external environment resembling skin rather than internal mucosa, it was assumed to host a limited number of symbionts, similar to other cutaneous surfaces. This led to the delayed development of a theoretical framework addressing the ecology of the penile microbiota.

Although significant changes such as circumcision are now known to affect microbial composition (Price et al., 2010), important knowledge gaps remain. For instance, it is unclear whether microbial differences exist across anatomical regions such as the glans, shaft, scrotum, and urethral meatus. Similarly, behavioral and lifestyle factors, such as partner type (MSM vs. MSW), hair removal methods (laser, wax, razor), piercings, tattoos, and underwear type, may influence microbial communities but have yet to be systematically studied to draw conclusions.

The most notable aspect of studying sexually transmitted diseases (STDs) is that these infections are shared between sexual partners, creating a dynamic that sustains their circulation over time. Although the causative agents of STDs have been well documented, other contributing factors have only recently begun to receive attention. One such factor is the human microbiome, which has been linked to a wide range of conditions, from metabolic to psychiatric disorders (Q. Liu et al., 2025; Ruiz-Malagón et al., 2025).

In the context of STDs, it is well established that certain infections facilitate the acquisition or progression of others (Galvin & Cohen, 2004). HIV, for example, compromises immune function and increases susceptibility to additional infections. This raises the broader question of whether an altered microbiota in one partner could influence the microbial community of another, even in the absence of a recognized infection. The potential for microbial exchange due to intimacy and its downstream consequences has important public health implications. If co-occurrence patterns can be identified between specific microbiota profiles and infection outcomes, these synergies should be addressed to prevent compounding effects.

In the case of HPV, its interaction with HIV has been frequently discussed (Dreyer, 2018; Konopnicki et al., 2013), often in a unidirectional framework where HIV is the primary infection and HPV a secondary concern. Medical attention tends to prioritize HIV, while HPV-related issues

such as genital warts or cancer are treated as consequences. This is evident among individuals who have achieved HIV viral suppression but continue to experience HPV-related complications (Dreyer, 2018; R. N. Werner et al., 2017). For this reason, any cofactor that contributes to HPV persistence or pathogenicity becomes relevant. Given the potential for the microbiota to facilitate viral transmission or modulate host response (C. H. Kim, 2018; Shi et al., 2017), identifying microbial patterns associated with HPV could clarify the role of microbial co-factors. Such insights may inform preventive strategies and interventions, particularly if certain microbiota states can favor the establishment of viral infection or its clearance.

Here, a cross-sectional experimental design was selected based on budgetary constraints, participant behavior, and theoretical considerations. From a financial perspective, cross-sectional studies allow for participant recruitment and sampling at a single time point, minimizing costs associated with follow-up, staffing, and logistics. This approach enables faster data collection and more efficient resource use. In contrast, longitudinal studies require repeated visits and sustained investment over time. Participant behavior also influenced the choice of design. Longitudinal studies face higher dropout rates due to extended follow-up periods (Lajous et al., 2005), which can compromise internal validity and reduce the effective sample size. Theoretically, given the limited number of existing studies exploring these associations, a cross-sectional approach offers a necessary first step. Establishing reproducible observations is essential before conducting longitudinal research aimed at tracking clearly defined microbial or clinical patterns.

Demonstrating causality between a microorganism and its interaction with the immune system requires controlled experimental conditions. For example, studies have isolated specific strains (such as *Finegoldia magna*, *Corynebacterium accolens* or *Staphylococcus epidermidis*) performed genomic characterization, and evaluated their immunological effects using cellular models (keratinocytes, PBMCs) and animal systems (H. J. Kim et al., 2019; Ridaura et al., 2018; van der Krieken et al., 2023). These experiments included live or inactivated bacteria, cytokine measurements, and functional assessment of immune responses such as IFN-λ production or AMP expression. Without this multifaceted approach, causal links cannot be established, and indirect or confounding effects cannot be ruled out. Proving causality requires microbial isolation, molecular characterization, and controlled application in cell or animal models. Immune markers such as cytokines or AMPs must be quantified. Only with well-defined systems and appropriate

controls can specific and direct immune interactions be demonstrated. This approach requires a completely different experimental setup and the prior identification of well-established taxa of interest. Therefore, the first step is to detect patterns and, if possible, narrow the list of candidate taxa suitable for further causal investigation.

Another important aspect is the benefit of analyzing the shaft and glans separately. These anatomical sites differ in epithelial structure, moisture, exposure, and immune environment, leading to distinct microbial communities. Separate analysis improves resolution and avoids compositional dilution caused by pooling samples with discordant microbiota or HPV status. In our dataset, many individuals were HPV-positive at one site but not the other, reinforcing the need for independent analysis to preserve biological signal and prevent confounding. This approach strengthens ecological interpretation and increases statistical power by reducing within-subject heterogeneity.

Importantly to mention is the non-invasive swab sampling that here was employed as it offers a practical, low-discomfort method for collecting penile microbiota, enhancing participant comfort and study compliance. Unlike urethral or biopsy-based methods, swabbing minimizes invasiveness, reducing anxiety and refusal rates (Qi et al., 2014). This approach facilitates broader participation, especially in asymptomatic individuals, and supports large-scale, community-based microbiome research. Moreover, self-collected samples can also be applied, which has been broadly use in vaginal microbiome research (Berggrund et al., 2020; Brotman et al., 2014).

Moving into the methodological aspects related to the 16S rRNA amplicon sequencing strategy, several key points must be considered. The first is primer selection. The 16S rRNA gene spans approximately 1,500 base pairs and contains conserved and hypervariable regions, labeled V1 through V9 (Větrovský & Baldrian, 2013). Among the most commonly used primer sets are those targeting the V4 and V3–V4 regions. These differ mainly in the amplicon size: around 300 bp for V4 and ~460–500 bp for V3–V4. In theory, longer amplicons offer greater taxonomic resolution. However, this advantage is often counterbalanced by practical limitations. If DNA is degraded or fragmented (due to suboptimal preservation, heat exposure, or inefficient extraction) read quality declines (Videnska et al., 2019). In such cases, the overlap between forward and reverse reads

may be insufficient, leading to high sequence loss during read merging, sometimes exceeding 50%. This is particularly relevant for paired-end sequencing, where the reverse read contributes additional nucleotide information beyond the overlap region. Because the reverse read often exhibits lower quality than the forward read, this extra information can be minimal or unusable. Consequently, only forward reads remain usable, and in the case of V3–V4, these alone lack sufficient resolution. For this reason, when suboptimal sample quality is anticipated, targeting the shorter V4 region is preferred. It yields a more consistent amplicon with near-complete overlap, increasing the proportion of usable sequences.

The assumption that an additional 200 bp always improve taxonomic resolution is debatable (Wang et al., 2007; J. J. Werner et al., 2012). While longer fragments can capture more informative sites, many bacterial species differ by only one or two nucleotides across the full-length gene. Even the complete 16S gene (~1,500 bp) struggles to distinguish phylogenetically close species, such as those within the diverse genera *Staphylococcus* and *Corynebacterium* commonly found on skin (Ghebremedhin et al., 2008). Moreover, the reference database used for taxonomic assignment heavily influences resolution (J. J. Werner et al., 2012). If a species 16S gene is not included in the database, a closely related taxon will be assigned instead.

Recognizing these technical constraints allows researchers to prioritize reliability and reproducibility. A shorter, high-quality amplicon is preferable, even if taxonomic resolution is limited to the genus level. Further refinement can be achieved through complementary approaches such as bacterial isolation, multilocus sequence analysis, shotgun metagenomics, or whole-genome sequencing.

From a computational perspective, using pool=TRUE in DADA2 (Callahan et al., 2016) causes all samples to be analyzed simultaneously, considering the total abundance of each sequence across the dataset. This improves the ability to distinguish sequencing errors from true biological variants, as rare sequences gain statistical support when observed repeatedly across different samples, strengthening their inference. Applying pool=TRUE increases sensitivity for detecting low-abundance variants that might be missed when processing samples individually. This is particularly advantageous in studies with many samples and high microbial diversity. Although it

requires greater computational resources, it allows for more accurate reconstruction of the microbial community at single-nucleotide resolution.

Although some authors recommended against normalization, we applied rarefaction in this study (Willis, 2019). Rarefaction is a normalization method that randomly subsamples each sample to the same number of sequences (Schloss, 2024), allowing comparisons under equivalent sequencing depth. This controls for bias introduced by unequal sampling effort, which can distort richness, Shannon diversity, or beta-diversity metrics. Without normalization, samples with higher sequencing depth may appear artificially more diverse, leading to false group differences. Rarefaction prevents such distortions and ensures comparability across samples.

Finally, it is important to note a limitation in Chapter V, where pooled samples were used to generate composite profiles. This approach aimed to maximize taxonomic resolution by enriching the sample content. However, pooling compromises interpretability by eliminating sample individuality, making relative abundance an average that cannot support statistical comparisons. As a result, all findings from Chapter V should be interpreted with caution.

One of the main findings of this study was the observation of significant differences in microbial diversity between HPV-negative and HPV-positive individuals. These differences were statistically significant for richness-based diversity metrics, both observed and estimated. An increase in richness is a common hallmark of microbial dysbiosis (Petersen & Round, 2014), and as such, this pattern may reflect a disrupted penile skin microbiota in HPV-infected men. Notably, these differences were only significant in the penile shaft and not in the glans. This may be explained by (a) the lower number of glans samples analyzed, which reduces statistical power, and (b) the naturally higher abundance of anaerobic taxa in the glans due to its anatomical and physiological characteristics. In this context, the relative increase of anaerobic taxa in HPV-positive individuals may have been more easily detected on the penile shaft, where anaerobes are typically less dominant, while remaining masked in the already anaerobe-enriched glans microbiota (Price et al., 2010).To this end, circumcised men are expected to have a glans microbiota more similar to that of the penile shaft.

A common hallmark of dysbiosis—or any ecological disturbance—is a shift in species proportions, where dominant taxa decline and opportunistic species increase (Carding et al., 2015). This is

typically reflected in metrics of community evenness. In the vaginal microbiota, for instance, dysbiosis is frequently characterized by the depletion of *Lactobacillus*-dominated communities (Y. Han et al., 2021). The *Lactobacillus* community play a central role in maintaining low pH through lactic acid production, which competitively suppresses opportunistic anaerobes. Their reduction allows anaerobes to proliferate, creating a permissive environment for HPV acquisition and progression. In contrast, the Shannon diversity index, which integrates both richness and evenness into a single abstract metric, is often used as a general-purpose indicator of community diversity (Daly et al., 2018). While useful for broad comparisons, its versatility can be a limitation when detecting subtle but biologically meaningful shifts. In our case, although significant differences in richness were observed, Shannon diversity remained unchanged. This suggests that community-wide structure is largely conserved as other skin environments (Oh et al., 2016), and that HPV-associated shifts may involve low-abundance, yet biologically relevant taxa rather than dramatic restructuring of the overall microbiota.

At this point, considering the penile microbiota as an ecological community, two main hypotheses can be proposed to explain the observed increase in microbial richness associated with HPV infection. First, a higher richness typically implies a more permissive environment that allows the coexistence of a broader range of microbial taxa (Petersen & Round, 2014). HPV is known for its ability to evade immune detection (de Sanjosé et al., 2018), which allows it to persist subclinical for extended periods—often up to two years—before being cleared. Our cross-sectional design limits the ability to determine the temporal sequence of infection and microbiome shifts, and immune parameters were not directly measured to assess local immune activity.

The more plausible hypothesis, supported by previous literature, is that a pre-existing dysbiotic state characterized by the enrichment of opportunistic anaerobes, predisposes individuals to HPV infection. This is consistent with findings from a Ugandan cohort (C. M. Liu et al., 2017), in which men with higher relative abundances of anaerobes were at increased risk of HIV acquisition. While that study involved HIV rather than HPV, the ecological and immunological mechanisms are not mutually exclusive. Dysbiosis may facilitate viral acquisition by compromising mucosal immunity, disrupting epithelial barriers, or generating a pro-inflammatory microenvironment (Levy et al., 2017; Petersen & Round, 2014). Furthermore, several anaerobic taxa have been shown to modulate host immunity or enhance viral infectivity either directly or indirectly (Lam et al., 2018;

Lima-Junior et al., 2021; Robinson & Pfeiffer, 2014). These microbial shifts may therefore not only reflect a permissive niche but actively contribute to viral pathogenesis. Future longitudinal and mechanistic studies are needed to disentangle the directionality and causality of these associations.

Non-metric multidimensional scaling revealed partial separation between HPV-positive and HPV-negative samples. HPV-negative individuals clustered tightly, indicating a conserved microbiota in that group. HPV-positive individuals displayed greater dispersion, reflecting interindividual microbiota variability. These patterns suggested that HPV infection induced a microbial shift rather than community replacement. This observation aligned with unchanged Shannon diversity, which integrated richness and evenness. Penile skin hosted communities shaped by endogenous and environmental influences, including personal hygiene habits (Oh et al., 2016). These findings emphasized anatomical context and ecological complexity when interpreting infection-related community differences.

The CST framework, initially developed in vaginal microbiota research (Gajer et al., 2012; Ravel et al., 2011), offers a robust ecological classification system based on taxonomic dominance and co-abundance structure. Its adaptation to penile skin microbiota analysis is justified for three main reasons. First, CSTs allow summarizing complex, high-dimensional microbiome data into ecologically interpretable community types, facilitating comparisons across individuals and populations. Second, although the penile skin lacks the strong Lactobacillus dominance typical of healthy vaginal communities, both niches exhibit recurrent compositional states influenced by host anatomy, physiology, and behavior, making CST-based clustering relevant for detecting dysbiosis patterns. Third, CSTs integrate subtle compositional differences that may be overlooked by diversity metrics alone, enabling the identification of high-risk microbial states associated with HPV infection. Moreover, grouping individuals into CSTs can amplify biological signal: samples within the same CST already share structural and ecological similarities, so any association with a clinical or behavioral trait is tested against a background of reduced heterogeneity. This approach can therefore increase statistical power and biological interpretability when investigating protective or pathogenic microbiota states.

Unlike NMDS ordination of beta diversity, which provides a continuous representation of inter-sample dissimilarities (Anderson et al., 2011), CST analysis discretizes the microbiota into distinct compositional states (Gajer et al., 2012). NMDS is exploratory, showing gradients of variation without imposing categorical boundaries, and is useful for visualizing community overlap or separation. In contrast, CST clustering forces a partitioning of samples into recurrent patterns, emphasizing dominant taxa and co-abundance relationships that may correspond to biologically meaningful states. While NMDS can reveal broad ecological trends, CSTs facilitate hypothesis testing on state-specific associations with clinical or environmental variables, making them complementary but conceptually distinct analytical tools.

In microbiome clustering, Bray–Curtis dissimilarity and Euclidean distance are among the most widely used metrics due to their straightforward interpretation and compatibility with abundance data (Alberdi & Gilbert, 2019). However, Ravel et al. (2011) recommended Jensen–Shannon divergence (JSD) for CST analysis, as it compares full taxonomic probability distributions, is less sensitive to zero inflation, and captures patterns in both dominant and rare taxa. This property makes JSD particularly suitable for high-dimensional, compositional datasets such as the penile skin microbiota. Regarding linkage algorithms, single and complete linkage can produce elongated or uneven clusters, particularly in heterogeneous communities (Handl et al., 2005). Ward.D2 linkage, by contrast, minimizes within-cluster variance at each step, yielding more compact and ecologically coherent groups. In this study, hierarchical clustering with JSD + Ward.D2 produced the most stable and interpretable solution.

Here, CST-5 emerged as the microbial community state most strongly associated with HPV infection. This CST was characterized by the dominance of Gram-positive anaerobic cocci (GPAC) such as *Finegoldia*, *Peptoniphilus*, and *Anaerococcus*, genera previously linked to dysbiotic conditions in both skin and mucosal niches (Dowd et al., 2008; Murphy & Frick, 2013). These taxa thrive in low-oxygen microenvironments and are often detected in polymicrobial biofilms, where they may contribute to inflammation and impaired epithelial defense (A. Han et al., 2011). The enrichment of GPAC in CST-5 mirrors findings from vaginal microbiota studies, where anaerobe-dominated CSTs have been associated with HPV persistence and higher viral loads (Gillet et al., 2011; Mitra et al., 2020). Conversely, CST-5 displayed a marked reduction in commensal genera such as *Corynebacterium* and *Staphylococcus*, which are key contributors to

skin barrier integrity and immune modulation (Bomar et al., 2016; Byrd et al., 2018). This loss of protective taxa may facilitate viral persistence by reducing colonization resistance and altering local immune signaling.

The functional profile of CST-5 suggests a dysbiotic, inflammation-prone microenvironment. The dominance of GPAC genera indicates a community adapted to oxygen-limited niches, capable of producing metabolic by-products like short-chain fatty acids (e.g., butyrate) and proteolytic enzymes that can disrupt epithelial integrity (Ezaki et al., 2001; Murphy & Frick, 2013). These conditions may favor localized inflammation, increase epithelial permeability, and modulate host immune responses toward a less effective antiviral state (Levy et al., 2017; Petersen & Round, 2014). Concurrently, the reduction in abundance of beneficial commensals like *Corynebacterium* and *Staphylococcus,* which are known for their role in producing antimicrobial peptides and regulating skin pH (Bomar et al., 2016; Byrd et al., 2018), may reduce colonization resistance, allowing opportunistic taxa to proliferate. This may explain the increased richness observed in our HPV-positive samples. Similar functional shifts have been documented in vaginal CSTs dominated by anaerobes, which are associated with persistent HPV and higher risk of neoplastic progression (Mitra et al., 2020). Thus, CST-5 may represent a pro-inflammatory, immune-modulating state conducive to viral persistence.

In summary, CST-5 may be defined as a high-risk microbial signature, whereas CSTs enriched in commensals such as *Corynebacterium* and *Staphylococcus* likely represent protective states. However, this remains as a hypothesis. Validation in larger, longitudinal cohorts is warranted to support and confirm these statements.

The shotgun metagenomics (Chapter V) expanded upon 16S rRNA results by delivering species and strain-level resolution of the penile skin microbiota in HPV infection. High-quality assemblies were obtained for key taxa, with some contigs exceeding 30 kb, enabling confident classification of opportunistic anaerobes. Classification rates were consistent across HPV-positive and HPV-negative pools (~85%), validating both sequencing and analysis pipelines. The high abundance of *F. magna* (strain ATCC 53516) in HPV-positive samples suggests preference to the infected microenvironment, reinforcing its potential role in modulating local inflammation and influencing viral persistence. *Peptoniphilus genitalis* was newly detected in this anatomical context, extending

prior links to genital dysbiosis in women (Abou Chacra et al., 2024), as most studies only report at genus level (Gonçalves et al., 2022; C. M. Liu et al., 2017).

The ecological implications differ from vaginal dysbiosis models, where loss of *Lactobacillus* dominance is typical. In penile skin, even presumed healthy states are diverse; dysbiosis may instead manifest as enrichment of pro-inflammatory or metabolically active taxa. *F. magna* and *P. genitalis* are known to stimulate cytokine production (Zeeuwen et al., 2017), potentially altering epithelial immunity, while *Anaerococcus* spp. remain functionally understudied at the species level. The 15% of sequences that remained unclassified highlight gaps in current reference databases, particularly for skin-associated anaerobes. These results establish a foundation for mechanistic studies linking strain-resolved microbial profiles to HPV infection.

CONCLUSIONS

This study demonstrates a statistically significant association between penile skin microbiota dysbiosis and subclinical HPV infection in uncircumcised, HIV-negative men. HPV-positive individuals exhibited a distinct microbial profile, marked by increased richness of opportunistic anaerobes such as *Finegoldia* and *Peptoniphilus*, accompanied by a reduction in beneficial commensals *Corynebacterium* and *Staphylococcus*. Dysbiosis in penile skin microbiota is not defined by loss of dominant taxa (as in women), but by enrichment of opportunists. CSTs and diversity indices reveal complementary facets of microbiota–HPV interactions.

Given the public health relevance of persistent HPV in male populations, both as asymptomatic carriers and as individuals at risk of anogenital neoplasia, these results underscore the need for longitudinal and mechanistic studies to determine whether shifts in microbial community structure precede infection or are induced by it.

It is important to highlight that the increase in opportunistic anaerobic bacteria observed in our cohort has also been reported in circumcised male populations from Uganda and South Africa. This consistent finding, despite methodological and population differences, suggests a robust biological pattern associated with HPV infection. Future longitudinal studies integrating microbiome, cytokine, and viral load data will be essential to confirm whether CST-5 actively drives immune suppression or is simply a biomarker of an HPV-permissive state.

PERSPECTIVES

The results of this research not only reveal an association between penile microbiota dysbiosis and HPV infection, but also point toward new scientific and clinical frontiers. As this work is now concluding, several questions emerge.

A critical next step will be the design of longitudinal studies to capture the temporal dynamics of this relationship. Does microbial dysbiosis precede and facilitate viral infection, or is it instead a consequence of HPV colonization? This unresolved temporal sequence could be clarified through close monitoring of newly infected male cohorts, combining microbial profiling with local immune markers. On the mechanistic side, the consistent presence of *Finegoldia magna* raises compelling questions about its potential role in modulating host immunity. In vitro studies could explore whether this anaerobic bacterium, known for its ability to evade immune defenses, interferes with

type I interferon production, a key element of the antiviral response. Diversity patterns could serve as a potential early biomarker of HPV infection, and understanding these patterns will be essential to clarify causal relationships between dysbiosis and HPV infection.

These insights open opportunities for developing microbiota-based diagnostics or risk stratification tools. Penile microbiome modulation, for instance through topical probiotics or selective antibiotics, represents a possible intervention strategy. While vaginal probiotics have shown translational success, applications to the penile microbiome remain unexplored.

REFERENCES

Abellan-Schneyder, Isabel, Monica S. Matchado, Sandra Reitmeier, Alina Sommer, Zeno Sewald, Jan Baumbach, Markus List, and Klaus Neuhaus. 2021. “Primer, Pipelines, Parameters: Issues in 16S rRNA Gene Sequencing.” mSphere 6 (1). https://doi.org/10.1128/mSphere.01202-20.

Abou Chacra, Linda, Marion Bonnet, Mégane Heredia, Gabriel Haddad, Nicholas Armstrong, Stéphane Alibar, Florence Bretelle, and Florence Fenollar. 2024. “Peptoniphilus Genitalis Sp. Nov. and Mobiluncus Massiliensis Sp. Nov.: Novel Bacteria Isolated from the Vaginal Microbiome.” Current Microbiology 81 (4): 97.

Abu El-Hamd, Mohammed, Hanan Abdel Rady Assaf, Ahmed Sedky, and Shrouk Haggag Mohammad. 2019. “Possible Role of Interleukin 21 and Interleukin 33 in Patients with Genital Warts.” Dermatologic Therapy 32 (5): e13063.

Amador-Molina, Alfredo, José Fernando Hernández-Valencia, Edmundo Lamoyi, Adriana Contreras-Paredes, and Marcela Lizano. 2013. “Role of Innate Immunity against Human Papillomavirus (HPV) Infections and Effect of Adjuvants in Promoting Specific Immune Response.” Viruses 5 (11): 2624–42.

Andrade Pessoa Morales, Julia, Camila Marconi, Mariam El-Zein, Jacques Ravel, Gabriel Victor da Silva Pinto, Rosana Silveira, Moises Diogode Lima, et al. 2022. “Vaginal Microbiome Components as Correlates of Cervical Human Papillomavirus Infection.” The Journal of Infectious Diseases 226 (6): 1084–97.

Anic, Gabriella M., Ji-Hyun Lee, Heather Stockwell, Dana E. Rollison, Yougui Wu, Mary R. Papenfuss, Luisa L. Villa, et al. 2011. “Incidence and Human Papillomavirus (HPV) Type Distribution of Genital Warts in a Multinational Cohort of Men: The HPV in Men Study.” The Journal of Infectious Diseases 204 (12): 1886–92.

Arroyo Mühr, Laila Sara, Carina Eklund, and Joakim Dillner. 2021. “Misclassifications in Human Papillomavirus Databases.” Virology 558 (June):57–66.

Benedikz, Elizabeth K., Dalan Bailey, Charlotte N. L. Cook, Daniel Gonçalves-Carneiro, Michelle M. C. Buckner, Jessica M. A. Blair, Timothy J. Wells, et al. 2019. “Bacterial Flagellin Promotes Viral Entry via an NF-kB and Toll Like Receptor 5 Dependent Pathway.” Scientific Reports 9 (1): 7903.

Bolger, Anthony M., Marc Lohse, and Bjoern Usadel. 2014. “Trimmomatic: A Flexible Trimmer for Illumina Sequence Data.” Bioinformatics (Oxford, England) 30 (15): 2114–20.

Bomar, Lindsey, Silvio D. Brugger, Brian H. Yost, Sean S. Davies, and Katherine P. Lemon. 2016. “Corynebacterium Accolens Releases Antipneumococcal Free Fatty Acids from Human Nostril and Skin Surface Triacylglycerols.” mBio 7 (1): e01725–15.

Brazier, J., D. Chmelar, L. Dubreuil, G. Feierl, M. Hedberg, S. Kalenic, E. Könönen, et al. 2008. “European Surveillance Study on Antimicrobial Susceptibility of Gram-Positive Anaerobic Cocci.” International Journal of Antimicrobial Agents 31 (4): 316–20.

Brianti, Pina, Eduardo De Flammineis, and Santo Raffaele Mercuri. 2017. “Review of HPV-Related Diseases and Cancers.” The New Microbiologica 40 (2): 80–85.

Burk, Robert D., Ariana Harari, and Zigui Chen. 2013. “Human Papillomavirus Genome Variants.” Virology 445 (1-2): 232–43.

Byrd, Allyson L., Yasmine Belkaid, and Julia A. Segre. 2018. “The Human Skin Microbiome.” Nature Reviews. Microbiology 16 (3): 143–55.

Carda-Diéguez, Miguel, Nívia Cárdenas, Marina Aparicio, David Beltrán, Juan M. Rodríguez, and Alex Mira. 2019. “Variations in Vaginal, Penile, and Oral Microbiota After Sexual Intercourse: A Case Report.” Frontiers of Medicine 6 (August):178.

Carrillo-Ng, Hugo, Lorena Becerra-Goicochea, Yordi Tarazona-Castro, Luis Pinillos-Vilca, Luis J. Del Valle, Miguel Angel Aguilar-Luis, Carmen Tinco-Valdez, et al. 2021. “Variations in Cervico-Vaginal Microbiota among HPV-Positive and HPV-Negative Asymptomatic Women in Peru.” BMC Research Notes 14 (1): 4.

Castellsagué, Xavier, F. Xavier Bosch, Nubia Muñoz, Chris J. L. M. Meijer, Keerti V. Shah, Silvia de Sanjose, José Eluf-Neto, et al. 2002. “Male Circumcision, Penile Human Papillomavirus Infection, and Cervical Cancer in Female Partners.” The New England Journal of Medicine 346 (15): 1105–12.

Choi, Yoojin, Mona Loutfy, Robert S. Remis, Juan Liu, Anuradha Rebbapragada, Sanja Huibner, Jason Brunetta, et al. 2021. “HPV Genotyping and Risk Factors for Anal High-Risk HPV Infection in Men Who Have Sex with Men from Toronto, Canada.” Scientific Reports 11 (1): 4779.

Dinotta, F., M. R. Nasca, and G. Micali. 2013. “Anatomy of Male Genitalia.” Atlas of Male Genital Disorders. https://doi.org/10.1007/978-88-470-2787-9.pdf#page=11.

Diop, Khoudia, Awa Diop, Caroline Michelle, Magali Richez, Jaishriram Rathored, Florence Bretelle, Pierre-Edouard Fournier, and Florence Fenollar. 2019. “Description of Three New Peptoniphilus Species Cultured in the Vaginal Fluid of a Woman Diagnosed with Bacterial Vaginosis: Peptoniphilus Pacaensis Sp. Nov., Peptoniphilus Raoultii Sp. Nov., and Peptoniphilus Vaginalis Sp. Nov.” MicrobiologyOpen 8 (3): e00661.

Dowd, Scot E., Yan Sun, Patrick R. Secor, Daniel D. Rhoads, Benjamin M. Wolcott, Garth A. James, and Randall D. Wolcott. 2008. “Survey of Bacterial Diversity in Chronic Wounds Using Pyrosequencing, DGGE, and Full Ribosome Shotgun Sequencing.” BMC Microbiology 8 (March):43.

Dunne, Eileen F., Carrie M. Nielson, Katherine M. Stone, Lauri E. Markowitz, and Anna R. Giuliano. 2006. “Prevalence of HPV Infection among Men: A Systematic Review of the Literature.” The Journal of Infectious Diseases 194 (8): 1044–57.

Ezaki, T., Y. Kawamura, N. Li, Z. Y. Li, L. Zhao, and S. Shu. 2001. “Proposal of the Genera Anaerococcus Gen. Nov., Peptoniphilus Gen. Nov. and Gallicola Gen. Nov. for Members of the Genus Peptostreptococcus.” International Journal of Systematic and Evolutionary Microbiology 51 (Pt 4): 1521–28.

Fahmy, Mohamed A. Baky. 2020. “Smegma.” In Normal and Abnormal Prepuce, edited by Mohamed A. Baky Fahmy, 153–61. Cham: Springer International Publishing.

Fuchs, Elaine, and Srikala Raghavan. 2002. “Getting under the Skin of Epidermal Morphogenesis.” Nature Reviews. Genetics 3 (3): 199–209.

Ghebremedhin, B., F. Layer, W. König, and B. König. 2008. “Genetic Classification and Distinguishing of Staphylococcus Species Based on Different Partial Gap, 16S rRNA, hsp60, rpoB, sodA, and Tuf Gene Sequences.” Journal of Clinical Microbiology 46 (3): 1019–25.

Ghemrawi, Mirna, Andrea Ramírez Torres, George Duncan, Rita Colwell, Manoj Dadlani, and Bruce McCord. 2021. “The Genital Microbiome and Its Potential for Detecting Sexual Assault.” Forensic Science International. Genetics 51 (March):102432.

Gillet, Evy, Joris Fa Meys, Hans Verstraelen, Carolyne Bosire, Philippe De Sutter, Marleen Temmerman, and Davy Vanden Broeck. 2011. “Bacterial Vaginosis Is Associated with Uterine Cervical Human Papillomavirus Infection: A Meta-Analysis.” BMC Infectious Diseases 11 (1): 10.

Giuliano, Anna R., Gabriella Anic, and Alan G. Nyitray. 2010. “Epidemiology and Pathology of HPV Disease in Males.” Gynecologic Oncology 117 (2 Suppl): S15–19.

Giuliano, Anna R., Ji-Hyun Lee, William Fulp, Luisa L. Villa, Eduardo Lazcano, Mary R. Papenfuss, Martha Abrahamsen, et al. 2011. “Incidence and Clearance of Genital Human Papillomavirus Infection in Men (HIM): A Cohort Study.” The Lancet 377 (9769): 932–40.

Giuliano, Anna R., Beibei Lu, Carrie M. Nielson, Roberto Flores, Mary R. Papenfuss, Ji-Hyun Lee, Martha Abrahamsen, and Robin B. Harris. 2008. “Age-Specific Prevalence, Incidence, and Duration of Human Papillomavirus Infections in a Cohort of 290 US Men.” The Journal of Infectious Diseases 198 (6): 827–35.

Gonçalves, Micael F. M., Ângela Rita Fernandes, Acácio Gonçalves Rodrigues, and Carmen Lisboa. 2022. “Microbiome in Male Genital Mucosa (Prepuce, Glans, and Coronal Sulcus): A Systematic Review.” Microorganisms 10 (12): 2312.

Grice, Elizabeth A., Heidi H. Kong, Sean Conlan, Clayton B. Deming, Joie Davis, Alice C. Young, NISC Comparative Sequencing Program, et al. 2009. “Topographical and Temporal Diversity of the Human Skin Microbiome.” Science 324 (5931): 1190–92.

Grice, Elizabeth A., and Julia A. Segre. 2011. “The Skin Microbiome.” Nature Reviews. Microbiology 9 (4): 244–53.

Gu, Weijie, Peipei Zhang, Guiming Zhang, Jiaquan Zhou, Xuefei Ding, Qifeng Wang, Beihe Wang, et al. 2020. “Importance of HPV in Chinese Penile Cancer: A Contemporary Multicenter Study.” Frontiers in Oncology 10 (September):1521.

Han, Anne, Jonathan M. Zenilman, Johan H. Melendez, Mark E. Shirtliff, Alessandra Agostinho, Garth James, Philip S. Stewart, et al. 2011. “The Importance of a Multifaceted Approach to Characterizing the Microbial Flora of Chronic Wounds.” Wound Repair and Regeneration 19 (5): 532–41.

Harris-Tryon, Tamia A., and Elizabeth A. Grice. 2022. “Microbiota and Maintenance of Skin Barrier Function.” Science 376 (6596): 940–45.

Hernandez, Brenda Y., Yurii B. Shvetsov, Marc T. Goodman, Lynne R. Wilkens, Pamela J. Thompson, Xuemei Zhu, James Tom, and Lily Ning. 2011. “Genital and Extra-Genital Warts Increase the Risk of Asymptomatic Genital Human Papillomavirus Infection in Men.” Sexually Transmitted Infections 87 (5): 391–95.

Hernandez, B. Y., L. R. Wilkens, X. Zhu, K. McDuffie, P. Thompson, Y. B. Shvetsov, L. Ning, and M. T. Goodman. 2008. “Circumcision and Human Papillomavirus Infection in Men: A Site-Specific Comparison.” The Journal of Infectious Diseases 197 (6): 787–94.

Humphrey, Peter A. 2014. “Male Urethra and External Genitalia Anatomy.” In Advanced Male Urethral and Genital Reconstructive Surgery, edited by Steven B. Brandes and Allen F. Morey, 17–23. New York, NY: Springer New York.

Husnjak, K., M. Grce, L. Magdić, and K. Pavelić. 2000. “Comparison of Five Different Polymerase Chain Reaction Methods for Detection of Human Papillomavirus in Cervical Cell Specimens.” Journal of Virological Methods 88 (2): 125–34.

Ingles, Donna J., Hui-Yi Lin, William J. Fulp, Staci L. Sudenga, Beibei Lu, Matthew B. Schabath, Mary R. Papenfuss, et al. 2015. “An Analysis of HPV Infection Incidence and Clearance by Genotype and Age in Men: The HPV Infection in Men (HIM) Study.” Papillomavirus Research (Amsterdam, Netherlands) 1 (December):126–35.

Janda, J. Michael, and Sharon L. Abbott. 2007. “16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls.” Journal of Clinical Microbiology 45 (9): 2761–64.

Jeynes, C., M. C. Chung, and R. Challenor. 2009. “‘Shame on You’--the Psychosocial Impact of Genital Warts.” International Journal of STD & AIDS 20 (8): 557–60.

Johnson, Jethro S., Daniel J. Spakowicz, Bo-Young Hong, Lauren M. Petersen, Patrick Demkowicz, Lei Chen, Shana R. Leopold, et al. 2019. “Evaluation of 16S rRNA Gene Sequencing for Species and Strain-Level Microbiome Analysis.” Nature Communications 10 (1): 5029.

Kane, Melissa, Laure K. Case, Karyl Kopaskie, Alena Kozlova, Cameron MacDearmid, Alexander V. Chervonsky, and Tatyana V. Golovkina. 2011. “Successful Transmission of a Retrovirus Depends on the Commensal Microbiota.” Science 334 (6053): 245–49.

Kigozi, Godfrey, Cindy M. Liu, Daniel Park, Zoe R. Packman, Ronald H. Gray, Rupert Kaul, Aaron A. R. Tobian, et al. 2020. “Foreskin Surface Area Is Not Associated with Sub-Preputial Microbiome Composition or Penile Cytokines.” PloS One 15 (6): e0234256.

Kim, Hyun Jik, Ara Jo, Yung Jin Jeon, Sujin An, Kang-Mu Lee, Sang Sun Yoon, and Jae Young Choi. 2019. “Nasal Commensal Staphylococcus Epidermidis Enhances Interferon-λ-Dependent Immunity against Influenza Virus.” Microbiome 7 (1): 80.

Krebs, H. B., and B. F. Helmkamp. 1991. “Treatment Failure of Genital Condylomata Acuminata in Women: Role of the Male Sexual Partner.” American Journal of Obstetrics and Gynecology 165 (2): 337–39; discussion 339–40.

Krieken, Danique A. van der, Gijs Rikken, Thomas H. A. Ederveen, Patrick A. M. Jansen, Diana Rodijk-Olthuis, Luca D. Meesters, Ivonne M. J. J. van Vlijmen-Willems, et al. 2023. “Gram-Positive Anaerobic Cocci Guard Skin Homeostasis by Regulating Host-Defense Mechanisms.” iScience 26 (4): 106483.

Langmead, Ben, and Steven L. Salzberg. 2012. “Fast Gapped-Read Alignment with Bowtie 2.” Nature Methods 9 (4): 357–59.

Łaniewski, Paweł, Dominique Barnes, Alison Goulder, Haiyan Cui, Denise J. Roe, Dana M. Chase, and Melissa M. Herbst-Kralovetz. 2018. “Linking Cervicovaginal Immune Signatures, HPV and Microbiota Composition in Cervical Carcinogenesis in Non-Hispanic and Hispanic Women.” Scientific Reports 8 (1). https://doi.org/10.1038/s41598-018-25879-7.

Levy, Maayan, Aleksandra A. Kolodziejczyk, Christoph A. Thaiss, and Eran Elinav. 2017. “Dysbiosis and the Immune System.” Nature Reviews. Immunology 17 (4): 219–32.

Li, Dinghua, Chi-Man Liu, Ruibang Luo, Kunihiko Sadakane, and Tak-Wah Lam. 2015. “MEGAHIT: An Ultra-Fast Single-Node Solution for Large and Complex Metagenomics Assembly via Succinct de Bruijn Graph.” Bioinformatics (Oxford, England) 31 (10): 1674–76.

Li, Hui, Zhen Xu, Chaoqi Liu, Jinfang Deng, Ziqin Li, Yongqin Zhou, and Zhiying Li. 2025. “Probiotics: A New Approach for the Prevention and Treatment of Cervical Cancer.” Probiotics and Antimicrobial Proteins, February. https://doi.org/10.1007/s12602-025-10479-5.

Lin, Tian-Lan, Chao Cheng, Wei-Ting Zeng, Fang Duan, Yin-Hui Pei, Xiu-Ping Liu, Fu Shang, and Kai-Li Wu. 2021. “Anti-Viral Activity of Staphylococcus Aureus Lysates against Herpes Simplex Virus Type-I Infection: An in Vitro and in Vivo Study.” International Journal of Ophthalmology 14 (10): 1463–72.

Littré, Émile Maximilien Paul. 1861. Oeuvres complètes d’Hippocrates. Baillière.

Liu, Cindy M., Bruce A. Hungate, Aaron A. R. Tobian, Jacques Ravel, Jessica L. Prodger, David Serwadda, Godfrey Kigozi, et al. 2015. “Penile Microbiota and Female Partner Bacterial Vaginosis in Rakai, Uganda.” mBio 6 (3): e00589.

Liu, Cindy M., Jessica L. Prodger, Aaron A. R. Tobian, Alison G. Abraham, Godfrey Kigozi, Bruce A. Hungate, Maliha Aziz, et al. 2017. “Penile Anaerobic Dysbiosis as a Risk Factor for HIV Infection.” mBio 8 (4). https://doi.org/10.1128/mBio.00996-17.

Marples, R. R., D. T. Downing, and A. M. Kligman. 1971. “Control of Free Fatty Acids in Human Surface Lipids by Corynebacterium Acnes.” The Journal of Investigative Dermatology 56 (2): 127–31.

Mehta, Supriya D., Debarghya Nandi, Walter Agingu, Stefan J. Green, Fredrick O. Otieno, Dulal K. Bhaumik, and Robert C. Bailey. 2022. “Longitudinal Changes in the Composition of the Penile Microbiome Are Associated With Circumcision Status, HIV and HSV-2 Status, Sexual Practices, and Female Partner Microbiome Composition.” Frontiers in Cellular and Infection Microbiology 12 (July):916437.

Mehta, Supriya D., Dan Zhao, Stefan J. Green, Walter Agingu, Fredrick Otieno, Runa Bhaumik, Dulal Bhaumik, and Robert C. Bailey. 2020. “The Microbiome Composition of a Man’s Penis Predicts Incident Bacterial Vaginosis in His Female Sex Partner With High Accuracy.” Frontiers in Cellular and Infection Microbiology 10 (August):433.

Mei, Ling, Tao Wang, Yueyue Chen, Dongmei Wei, Yueting Zhang, Tao Cui, Jian Meng, et al. 2022. “Dysbiosis of Vaginal Microbiota Associated with Persistent High-Risk Human Papilloma Virus Infection.” Journal of Translational Medicine 20 (1): 12.

Meites, Elissa, Timothy J. Wilkin, and Lauri E. Markowitz. 2022. “Review of Human Papillomavirus (HPV) Burden and HPV Vaccination for Gay, Bisexual, and Other Men Who Have Sex with Men and Transgender Women in the United States.” Human Vaccines & Immunotherapeutics 18 (1): 2016007.

Mitra, Anita, David A. MacIntyre, George Ntritsos, Ann Smith, Konstantinos K. Tsilidis, Julian R. Marchesi, Phillip R. Bennett, Anna-Barbara Moscicki, and Maria Kyrgiou. 2020. “The Vaginal Microbiota Associates with the Regression of Untreated Cervical Intraepithelial Neoplasia 2 Lesions.” Nature Communications 11 (1): 1999.

Murdoch, D. A. 1998. “Gram-Positive Anaerobic Cocci.” Clinical Microbiology Reviews 11 (1): 81–120.

Murdoch, D. A., I. J. Mitchelmore, and Soad Tabaqchali. 1988. “Peptostreptococcus Micros in Polymicrobial Abscesses.” Lancet 331 (8585): 594.

Murphy, Elizabeth Carmel, and Inga-Maria Frick. 2013. “Gram-Positive Anaerobic Cocci--Commensals and Opportunistic Pathogens.” FEMS Microbiology Reviews 37 (4): 520–53.

Nelson, David E., Qunfeng Dong, Barbara Van der Pol, Evelyn Toh, Baochang Fan, Barry P. Katz, Deming Mi, et al. 2012. “Bacterial Communities of the Coronal Sulcus and Distal Urethra of Adolescent Males.” PloS One 7 (5): e36298.

Nielson, Carrie M., Roberto Flores, Robin B. Harris, Martha Abrahamsen, Mary R. Papenfuss, Eileen F. Dunne, Lauri E. Markowitz, and Anna R. Giuliano. 2007. “Human Papillomavirus Prevalence and Type Distribution in Male Anogenital Sites and Semen.” Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology 16 (6): 1107–14.

Oh, Julia, Allyson L. Byrd, Morgan Park, NISC Comparative Sequencing Program, Heidi H. Kong, and Julia A. Segre. 2016. “Temporal Stability of the Human Skin Microbiome.” Cell 165 (4): 854–66.

Onywera, Harris, Anna-Lise Williamson, Luca Cozzuto, Sarah Bonnin, Zizipho Z. A. Mbulawa, David Coetzee, Julia Ponomarenko, and Tracy L. Meiring. 2020. “The Penile Microbiota of Black South African Men: Relationship with Human Papillomavirus and HIV Infection.” BMC Microbiology 20 (1): 78.

Onywera, Harris, Anna-Lise Williamson, Julia Ponomarenko, and Tracy L. Meiring. 2020. “The Penile Microbiota in Uncircumcised and Circumcised Men: Relationships With HIV and Human Papillomavirus Infections and Cervicovaginal Microbiota.” Frontiers of Medicine 7 (July):383.

Oriel, J. D. 1971. “Natural History of Genital Warts.” The British Journal of Venereal Diseases 47 (1): 1–13.

Parkin, Donald Maxwell. 2006. “The Global Health Burden of Infection-Associated Cancers in the Year 2002.” International Journal of Cancer. Journal International Du Cancer 118 (12): 3030–44.

Petersen, Charisse, and June L. Round. 2014. “Defining Dysbiosis and Its Influence on Host Immunity and Disease.” Cellular Microbiology 16 (7): 1024–33.

Plummer, Martyn, Catherine de Martel, Jerome Vignat, Jacques Ferlay, Freddie Bray, and Silvia Franceschi. 2016. “Global Burden of Cancers Attributable to Infections in 2012: A Synthetic Analysis.” The Lancet. Global Health 4 (9): e609–16.

Price, Lance B., Cindy M. Liu, Kristine E. Johnson, Maliha Aziz, Matthew K. Lau, Jolene Bowers, Jacques Ravel, et al. 2010. “The Effects of Circumcision on the Penis Microbiome.” PloS One 5 (1): e8422.

Prodger, Jessica L., Alison G. Abraham, Aaron Ar Tobian, Daniel E. Park, Maliha Aziz, Kelsey Roach, Ronald H. Gray, et al. 2021. “Penile Bacteria Associated with HIV Seroconversion, Inflammation, and Immune Cells.” JCI Insight 6 (8). https://doi.org/10.1172/jci.insight.147363.

Quan, Cheng, Xiao-Ying Chen, Xia Li, Feng Xue, Li-Hong Chen, Na Liu, Bo Wang, et al. 2020. "Psoriatic Lesions Are Characterized by Higher Bacterial Load and Imbalance between Cutibacterium and Corynebacterium." Journal of the American Academy of Dermatology 82 (4): 955–61.

Quince, Christopher, Alan W. Walker, Jared T. Simpson, Nicholas J. Loman, and Nicola Segata. 2017. "Shotgun Metagenomics, from Sampling to Analysis." Nature Biotechnology 35 (9): 833–44.

Ridaura, Vanessa K., Nicolas Bouladoux, Jan Claesen, Y. Erin Chen, Allyson L. Byrd, Michael G. Constantinides, Eric D. Merrill, Samira Tamoutounour, Michael A. Fischbach, and Yasmine Belkaid. 2018. "Contextual Control of Skin Immunity and Inflammation by Corynebacterium." The Journal of Experimental Medicine 215 (3): 785–99.

Robinson, Christopher M., and Julie K. Pfeiffer. 2014. "Viruses and the Microbiota." Annual Review of Virology 1:55–69.

Saiki, R. K., T. L. Bugawan, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1986. "Analysis of Enzymatically Amplified Beta-Globin and HLA-DQ Alpha DNA with Allele-Specific Oligonucleotide Probes." Nature 324 (6093): 163–66.

Swaney, Mary Hannah, Amanda Nelsen, Shelby Sandstrom, and Lindsay R. Kalan. 2023. "Sweat and Sebum Preferences of the Human Skin Microbiota." Microbiology Spectrum 11 (1): e0418022.

Tickle, Timothy L., Nicola Segata, Levi Waldron, Uri Weingart, and Curtis Huttenhower. 2013. "Two-Stage Microbial Community Experimental Design." The ISME Journal 7 (12): 2330–39.

Watchorn, Richard E., Ellen H. A. van den Munckhof, Koen D. Quint, Joseph Eliahoo, Maurits N. C. de Koning, Wim G. V. Quint, and Christopher B. Bunker. 2021. "Balanopreputial Sac and Urine Microbiota in Patients with Male Genital Lichen Sclerosus." International Journal of Dermatology 60 (2): 201–7.

Weinstock, Hillard, Stuart Berman, and Willard Cates Jr. 2004. “Sexually Transmitted Diseases among American Youth: Incidence and Prevalence Estimates, 2000.” Perspectives on Sexual and Reproductive Health 36 (1): 6–10.

Wood, Derrick E., Jennifer Lu, and Ben Langmead. 2019. “Improved Metagenomic Analysis with Kraken 2.” bioRxiv. bioRxiv. https://doi.org/10.1101/762302.

Zeng, Mi, Xin Li, Xiaoyang Jiao, Xiaochun Cai, Fen Yao, Shaomin Xu, Xiaoshan Huang, Qiaoxin Zhang, and Jianqiang Chen. 2022. “Roles of Vaginal Flora in Human Papillomavirus Infection, Virus Persistence and Clearance.” Frontiers in Cellular and Infection Microbiology 12:1036869.

Zozaya, Marcela, Michael J. Ferris, Julia D. Siren, Rebecca Lillis, Leann Myers, M. Jacques Nsuami, A. Murat Eren, Jonathan Brown, Christopher M. Taylor, and David H. Martin. 2016. “Bacterial Communities in Penile Skin, Male Urethra, and Vaginas of Heterosexual Couples with and without Bacterial Vaginosis.” Microbiome 4 (April):16.

Abellan-Schneyder, I., Matchado, M. S., Reitmeier, S., Sommer, A., Sewald, Z., Baumbach, J., List, M., & Neuhaus, K. (2021). Primer, pipelines, parameters: Issues in 16S rRNA gene sequencing. *MSphere*, *6*(1). https://doi.org/10.1128/mSphere.01202-20

Abou Chacra, L., Bonnet, M., Heredia, M., Haddad, G., Armstrong, N., Alibar, S., Bretelle, F., & Fenollar, F. (2024). Peptoniphilus genitalis sp. nov. and Mobiluncus massiliensis sp. nov.: Novel Bacteria Isolated from the Vaginal Microbiome. *Current Microbiology*, *81*(4), 97. https://doi.org/10.1007/s00284-023-03584-7

Abu El-Hamd, M., Assaf, H. A. R., Sedky, A., & Mohammad, S. H. (2019). Possible role of interleukin 21 and interleukin 33 in patients with genital warts. *Dermatologic Therapy*, *32*(5), e13063. https://doi.org/10.1111/dth.13063

Alberdi, A., & Gilbert, M. T. P. (2019). A guide to the application of Hill numbers to DNA-based diversity analyses. *Molecular Ecology Resources*, *19*(4), 804–817. https://doi.org/10.1111/1755-0998.13014

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., Sanders, N. J., Cornell, H. V., Comita, L. S., Davies, K. F., Harrison, S. P., Kraft, N. J. B., Stegen, J. C., & Swenson, N. G. (2011). Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. *Ecology Letters*, *14*(1), 19–28. https://doi.org/10.1111/j.1461-0248.2010.01552.x

Anic, G. M., Lee, J.-H., Stockwell, H., Rollison, D. E., Wu, Y., Papenfuss, M. R., Villa, L. L., Lazcano-Ponce, E., Gage, C., Silva, R. J. C., Baggio, M. L., Quiterio, M., Salmerón, J., Abrahamsen, M., & Giuliano, A. R. (2011). Incidence and human papillomavirus (HPV) type distribution of genital warts in a multinational cohort of men: the HPV in men study. *The Journal of Infectious Diseases*, *204*(12), 1886–1892. https://doi.org/10.1093/infdis/jir652

Arroyo Mühr, L. S., Eklund, C., & Dillner, J. (2021). Misclassifications in human papillomavirus databases. *Virology*, *558*, 57–66. https://doi.org/10.1016/j.virol.2021.03.002

Benedikz, E. K., Bailey, D., Cook, C. N. L., Gonçalves-Carneiro, D., Buckner, M. M. C., Blair, J. M. A., Wells, T. J., Fletcher, N. F., Goodall, M., Flores-Langarica, A., Kingsley, R. A., Madsen, J., Teeling, J., Johnston, S. L., MacLennan, C. A., Balfe, P., Henderson, I. R., Piddock, L. J. V., Cunningham, A. F., & McKeating, J. A. (2019). Bacterial flagellin promotes viral entry via an NF-kB and Toll Like Receptor 5 dependent pathway. *Scientific Reports*, *9*(1), 7903. https://doi.org/10.1038/s41598-019-44263-7

Berggrund, M., Gustavsson, I., Aarnio, R., Lindberg, J. H., Sanner, K., Wikström, I., Enroth, S., Bunikis, I., Olovsson, M., & Gyllensten, U. (2020). Temporal changes in the vaginal microbiota in self-samples and its association with persistent HPV16 infection and CIN2. *Virology Journal*, *17*(1), 147. https://doi.org/10.1186/s12985-020-01420-z

Bernard, H.-U., Burk, R. D., Chen, Z., van Doorslaer, K., zur Hausen, H., & de Villiers, E.-M. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*, *401*(1), 70–79. https://doi.org/10.1016/j.virol.2010.02.002

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics (Oxford, England)*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bomar, L., Brugger, S. D., Yost, B. H., Davies, S. S., & Lemon, K. P. (2016). Corynebacterium accolens releases antipneumococcal free fatty acids from human nostril and skin surface triacylglycerols. *MBio*, *7*(1), e01725-15. https://doi.org/10.1128/mBio.01725-15

Brazier, J., Chmelar, D., Dubreuil, L., Feierl, G., Hedberg, M., Kalenic, S., Könönen, E., Lundgren, B., Malamou-Ladas, H., Nagy, E., Sullivan, A., Nord, C. E., & ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria. (2008). European surveillance study on antimicrobial susceptibility of Gram-positive anaerobic cocci. *International Journal of Antimicrobial Agents*, *31*(4), 316–320. https://doi.org/10.1016/j.ijantimicag.2007.11.006

Brianti, P., De Flammineis, E., & Mercuri, S. R. (2017). Review of HPV-related diseases and cancers. *The New Microbiologica*, *40*(2), 80–85. https://www.ncbi.nlm.nih.gov/pubmed/28368072

Brock, G., Pihur, V., Datta, S., & Datta, S. (2008). clValid: AnRPackage for Cluster Validation. *Journal of Statistical Software*, *25*(4). https://doi.org/10.18637/jss.v025.i04

Brooks, J. P., Buck, G. A., Chen, G., Diao, L., Edwards, D. J., Fettweis, J. M., Huzurbazar, S., Rakitin, A., Satten, G. A., Smirnova, E., Waks, Z., Wright, M. L., Yanover, C., & Zhou, Y.-H. (2017). Changes in vaginal community state types reflect major shifts in the microbiome. *Microbial Ecology in Health and Disease*, *28*(1), 1303265. https://doi.org/10.1080/16512235.2017.1303265

Brotman, R. M., Shardell, M. D., Gajer, P., Tracy, J. K., Zenilman, J. M., Ravel, J., & Gravitt, P. E. (2014). Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. *The Journal of Infectious Diseases*, *210*(11), 1723–1733. https://doi.org/10.1093/infdis/jiu330

Burk, R. D., Harari, A., & Chen, Z. (2013). Human papillomavirus genome variants. *Virology*, *445*(1–2), 232–243. https://doi.org/10.1016/j.virol.2013.07.018

Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews. Microbiology*, *16*(3), 143–155. https://doi.org/10.1038/nrmicro.2017.157

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Carda-Diéguez, M., Cárdenas, N., Aparicio, M., Beltrán, D., Rodríguez, J. M., & Mira, A. (2019). Variations in Vaginal, Penile, and Oral Microbiota After Sexual Intercourse: A Case Report. *Frontiers of Medicine*, *6*, 178. https://doi.org/10.3389/fmed.2019.00178

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health and Disease*, *26*, 26191. https://doi.org/10.3402/mehd.v26.26191

Carrillo-Ng, H., Becerra-Goicochea, L., Tarazona-Castro, Y., Pinillos-Vilca, L., Del Valle, L. J., Aguilar-Luis, M. A., Tinco-Valdez, C., Silva-Caso, W., Martins-Luna, J., Peña-Tuesta, I., Aquino-Ortega, R., & Del Valle-Mendoza, J. (2021). Variations in cervico-vaginal microbiota among HPV-positive and HPV-negative asymptomatic women in Peru. *BMC Research Notes*, *14*(1), 4. https://doi.org/10.1186/s13104-020-05422-6

Chen, B., He, X., Pan, B., Zou, X., & You, N. (2021). Comparison of beta diversity measures in clustering the high-dimensional microbial data. *PloS One*, *16*(2), e0246893. https://doi.org/10.1371/journal.pone.0246893

Choi, Y., Loutfy, M., Remis, R. S., Liu, J., Rebbapragada, A., Huibner, S., Brunetta, J., Smith, G., Reko, T., Halpenny, R., Kaul, R., & Grennan, T. (2021). HPV genotyping and risk factors for anal high-risk HPV infection in men who have sex with men from Toronto, Canada. *Scientific Reports*, *11*(1), 4779. https://doi.org/10.1038/s41598-021-84079-y

Daly, A., Baetens, J., & De Baets, B. (2018). Ecological Diversity: Measuring the Unmeasurable. In *Mathematics* (Vol. 6, Issue 7, p. 119). https://doi.org/10.3390/math6070119

de Sanjosé, S., Brotons, M., & Pavón, M. A. (2018). The natural history of human papillomavirus infection. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, *47*, 2–13. https://doi.org/10.1016/j.bpobgyn.2017.08.015

Dinotta, F., Nasca, M. R., & Micali, G. (2013). Anatomy of male genitalia. *Atlas of Male Genital Disorders*. https://doi.org/10.1007/978-88-470-2787-9.pdf#page=11

Diop, K., Diop, A., Michelle, C., Richez, M., Rathored, J., Bretelle, F., Fournier, P.-E., & Fenollar, F. (2019). Description of three new Peptoniphilus species cultured in the vaginal fluid of a woman diagnosed with bacterial vaginosis: Peptoniphilus pacaensis sp. nov., Peptoniphilus raoultii sp. nov., and Peptoniphilus vaginalis sp. nov. *MicrobiologyOpen*, *8*(3), e00661. https://doi.org/10.1002/mbo3.661

Dowd, S. E., Sun, Y., Secor, P. R., Rhoads, D. D., Wolcott, B. M., James, G. A., & Wolcott, R. D. (2008). Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiology*, *8*, 43. https://doi.org/10.1186/1471-2180-8-43

Dreyer, G. (2018). Clinical implications of the interaction between HPV and HIV infections. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, *47*, 95–106. https://doi.org/10.1016/j.bpobgyn.2017.08.011

Dunne, E. F., Nielson, C. M., Stone, K. M., Markowitz, L. E., & Giuliano, A. R. (2006). Prevalence of HPV infection among men: A systematic review of the literature. *The Journal of Infectious Diseases*, *194*(8), 1044–1057. https://doi.org/10.1086/507432

Egawa, N., & Doorbar, J. (2017). The low-risk papillomaviruses. *Virus Research*, *231*, 119–127. https://doi.org/10.1016/j.virusres.2016.12.017

Ezaki, T., Kawamura, Y., Li, N., Li, Z. Y., Zhao, L., & Shu, S. (2001). Proposal of the genera Anaerococcus gen. nov., Peptoniphilus gen. nov. and Gallicola gen. nov. for members of the genus Peptostreptococcus. *International Journal of Systematic and Evolutionary Microbiology*, *51*(Pt 4), 1521–1528. https://doi.org/10.1099/00207713-51-4-1521

Fahmy, M. A. B. (2020). Smegma. In M. A. B. Fahmy (Ed.), *Normal and Abnormal Prepuce* (pp. 153–161). Springer International Publishing. https://doi.org/10.1007/978-3-030-37621-5_17

Fuchs, E., & Raghavan, S. (2002). Getting under the skin of epidermal morphogenesis. *Nature Reviews. Genetics*, *3*(3), 199–209. https://doi.org/10.1038/nrg758

Gajer, P., Brotman, R. M., Bai, G., Sakamoto, J., Schütte, U. M. E., Zhong, X., Koenig, S. S. K., Fu, L., Ma, Z. S., Zhou, X., Abdo, Z., Forney, L. J., & Ravel, J. (2012). Temporal dynamics of the human vaginal microbiota. *Science Translational Medicine*, *4*(132), 132ra52. https://doi.org/10.1126/scitranslmed.3003605

Galvin, S. R., & Cohen, M. S. (2004). The role of sexually transmitted diseases in HIV transmission. *Nature Reviews. Microbiology*, *2*(1), 33–42. https://doi.org/10.1038/nrmicro794

Ghebremedhin, B., Layer, F., König, W., & König, B. (2008). Genetic classification and distinguishing of Staphylococcus species based on different partial gap, 16S rRNA,

hsp60, rpoB, sodA, and tuf gene sequences. *Journal of Clinical Microbiology*, *46*(3), 1019–1025. https://doi.org/10.1128/JCM.02058-07

Gheit, T. (2019). Mucosal and cutaneous human Papillomavirus infections and cancer biology. *Frontiers in Oncology*, *9*, 355. https://doi.org/10.3389/fonc.2019.00355

Ghemrawi, M., Torres, A. R., Duncan, G., Colwell, R., Dadlani, M., & McCord, B. (2021). The genital microbiome and its potential for detecting sexual assault. *Forensic Science International. Genetics*, *51*, 102432. https://doi.org/10.1016/j.fsigen.2020.102432

Gillet, E., Meys, J. F., Verstraelen, H., Bosire, C., De Sutter, P., Temmerman, M., & Broeck, D. V. (2011). Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC Infectious Diseases*, *11*(1), 10. https://doi.org/10.1186/1471-2334-11-10

Giuliano, A. R., Anic, G., & Nyitray, A. G. (2010). Epidemiology and pathology of HPV disease in males. *Gynecologic Oncology*, *117*(2 Suppl), S15-9. https://doi.org/10.1016/j.ygyno.2010.01.026

Giuliano, A. R., Lu, B., Nielson, C. M., Flores, R., Papenfuss, M. R., Lee, J.-H., Abrahamsen, M., & Harris, R. B. (2008). Age-specific prevalence, incidence, and duration of human papillomavirus infections in a cohort of 290 US men. *The Journal of Infectious Diseases*, *198*(6), 827–835. https://doi.org/10.1086/591095

Gonçalves, M. F. M., Fernandes, Â. R., Rodrigues, A. G., & Lisboa, C. (2022). Microbiome in Male Genital Mucosa (Prepuce, Glans, and Coronal Sulcus): A Systematic Review. *Microorganisms*, *10*(12), 2312. https://doi.org/10.3390/microorganisms10122312

Grice, E. A., Kong, H. H., Conlan, S., Deming, C. B., Davis, J., Young, A. C., NISC Comparative Sequencing Program, Bouffard, G. G., Blakesley, R. W., Murray, P. R., Green, E. D., Turner, M. L., & Segre, J. A. (2009). Topographical and temporal diversity of the human skin microbiome. *Science*, *324*(5931), 1190–1192. https://doi.org/10.1126/science.1171700

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews. Microbiology*, *9*(4), 244–253. https://doi.org/10.1038/nrmicro2537

Gu, W., Zhang, P., Zhang, G., Zhou, J., Ding, X., Wang, Q., Wang, B., Wei, Y., Jin, S., Ye, D., & Zhu, Y. (2020). Importance of HPV in Chinese Penile Cancer: A Contemporary Multicenter Study. *Frontiers in Oncology*, *10*, 1521. https://doi.org/10.3389/fonc.2020.01521

Gu, Z. (2022). Complex heatmap visualization. *IMeta*, *1*(3), e43. https://doi.org/10.1002/imt2.43

Han, A., Zenilman, J. M., Melendez, J. H., Shirtliff, M. E., Agostinho, A., James, G., Stewart, P. S., Mongodin, E. F., Rao, D., Rickard, A. H., & Lazarus, G. S. (2011). The importance of a multifaceted approach to characterizing the microbial flora of chronic wounds. *Wound Repair and Regeneration*, *19*(5), 532–541. https://doi.org/10.1111/j.1524-475X.2011.00720.x

Han, Y., Liu, Z., & Chen, T. (2021). Role of vaginal Microbiota dysbiosis in gynecological diseases and the potential interventions. *Frontiers in Microbiology*, *12*, 643422. https://doi.org/10.3389/fmicb.2021.643422

Handl, J., Knowles, J., & Kell, D. B. (2005). Computational cluster validation in post-genomic data analysis. *Bioinformatics* , *21*(15), 3201–3212. https://doi.org/10.1093/bioinformatics/bti517

Harris-Tryon, T. A., & Grice, E. A. (2022). Microbiota and maintenance of skin barrier function. *Science*, *376*(6596), 940–945. https://doi.org/10.1126/science.abo0693

Hennig, C., & Imports, M. (2015). Package 'fpc.' *Flexible Procedures for Clustering*. ftp://ftp.netbsd.hu/pub/CRAN/web/packages/fpc/fpc.pdf

Hernandez, B. Y., Wilkens, L. R., Zhu, X., McDuffie, K., Thompson, P., Shvetsov, Y. B., Ning, L., & Goodman, M. T. (2008). Circumcision and human papillomavirus infection in men: a site-specific comparison. *The Journal of Infectious Diseases*, *197*(6), 787–794. https://doi.org/10.1086/528379

Hernandez, Brenda Y., Shvetsov, Y. B., Goodman, M. T., Wilkens, L. R., Thompson, P. J., Zhu, X., Tom, J., & Ning, L. (2011). Genital and extra-genital warts increase the risk of asymptomatic genital human papillomavirus infection in men. *Sexually Transmitted Infections*, *87*(5), 391–395. https://doi.org/10.1136/sti.2010.048876

Humphrey, P. A. (2014). Male Urethra and External Genitalia Anatomy. In S. B. Brandes & A. F. Morey (Eds.), *Advanced Male Urethral and Genital Reconstructive Surgery* (pp. 17–23). Springer New York. https://doi.org/10.1007/978-1-4614-7708-2_2

Husnjak, K., Grce, M., Magdić, L., & Pavelić, K. (2000). Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical

cell specimens. *Journal of Virological Methods*, *88*(2), 125–134. https://doi.org/10.1016/s0166-0934(00)00194-4

Ingles, D. J., Lin, H.-Y., Fulp, W. J., Sudenga, S. L., Lu, B., Schabath, M. B., Papenfuss, M. R., Abrahamsen, M. E., Salmeron, J., Villa, L. L., Ponce, E. L., & Giuliano, A. R. (2015). An analysis of HPV infection incidence and clearance by genotype and age in men: The HPV Infection in Men (HIM) Study. *Papillomavirus Research (Amsterdam, Netherlands)*, *1*, 126–135. https://doi.org/10.1016/j.pvr.2015.09.001

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, *45*(9), 2761–2764. https://doi.org/10.1128/JCM.01228-07

Jeynes, C., Chung, M. C., & Challenor, R. (2009). 'Shame on you'--the psychosocial impact of genital warts. *International Journal of STD & AIDS*, *20*(8), 557–560. https://doi.org/10.1258/ijsa.2008.008412

Johnson, J. S., Spakowicz, D. J., Hong, B.-Y., Petersen, L. M., Demkowicz, P., Chen, L., Leopold, S. R., Hanson, B. M., Agresta, H. O., Gerstein, M., Sodergren, E., & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, *10*(1), 5029. https://doi.org/10.1038/s41467-019-13036-1

Kane, M., Case, L. K., Kopaskie, K., Kozlova, A., MacDearmid, C., Chervonsky, A. V., & Golovkina, T. V. (2011). Successful transmission of a retrovirus depends on the

commensal microbiota. *Science*, *334*(6053), 245–249. https://doi.org/10.1126/science.1210718

Kassambara, A. (2019). rstatix: Pipe-friendly framework for basic statistical tests. *CRAN: Contributed Packages*. https://cir.nii.ac.jp/crid/1360864422589421952

Kigozi, G., Liu, C. M., Park, D., Packman, Z. R., Gray, R. H., Kaul, R., Tobian, A. A. R., Abraham, A. G., Ssekasanvu, J., Kagaayi, J., & Prodger, J. L. (2020). Foreskin surface area is not associated with sub-preputial microbiome composition or penile cytokines. *PloS One*, *15*(6), e0234256. https://doi.org/10.1371/journal.pone.0234256

Kim, C. H. (2018). Immune regulation by microbiome metabolites. *Immunology*, *154*(2), 220–229. https://doi.org/10.1111/imm.12930

Kim, H. J., Jo, A., Jeon, Y. J., An, S., Lee, K.-M., Yoon, S. S., & Choi, J. Y. (2019). Nasal commensal Staphylococcus epidermidis enhances interferon-λ-dependent immunity against influenza virus. *Microbiome*, *7*(1), 80. https://doi.org/10.1186/s40168-019-0691-9

Konopnicki, D., De Wit, S., & Clumeck, N. (2013). HPV and HIV coinfection: A complex interaction resulting in epidemiological, clinical and therapeutic implications. *Future Virology*, *8*(9), 903–915. https://doi.org/10.2217/fvl.13.69

Krebs, H. B., & Helmkamp, B. F. (1991). Treatment failure of genital condylomata acuminata in women: role of the male sexual partner. *American Journal of Obstetrics and Gynecology*, *165*(2), 337–339; discussion 339-40. https://doi.org/10.1016/0002-9378(91)90087-8

Lajous, M., Mueller, N., Cruz-Valdéz, A., Aguilar, L. V., Franceschi, S., Hernández-Avila, M., & Lazcano-Ponce, E. (2005). Determinants of prevalence, acquisition, and persistence of human papillomavirus in healthy Mexican military men. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *14*(7), 1710–1716. https://doi.org/10.1158/1055-9965.EPI-04-0926

Lam, K. C., Vyshenska, D., Hu, J., Rodrigues, R. R., Nilsen, A., Zielke, R. A., Brown, N. S., Aarnes, E.-K., Sikora, A. E., Shulzhenko, N., Lyng, H., & Morgun, A. (2018). Transkingdom network reveals bacterial players associated with cervical cancer gene expression program. *PeerJ*, *6*(e5590), e5590. https://doi.org/10.7717/peerj.5590

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Łaniewski, P., Barnes, D., Goulder, A., Cui, H., Roe, D. J., Chase, D. M., & Herbst-Kralovetz, M. M. (2018). Linking cervicovaginal immune signatures, HPV and microbiota composition in cervical carcinogenesis in non-Hispanic and Hispanic women. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-25879-7

Łaniewski, P., Ilhan, Z. E., & Herbst-Kralovetz, M. M. (2020). The microbiome and gynaecological cancer development, prevention and therapy. *Nature Reviews. Urology*, *17*(4), 232–250. https://doi.org/10.1038/s41585-020-0286-z

Levy, M., Kolodziejczyk, A. A., Thaiss, C. A., & Elinav, E. (2017). Dysbiosis and the immune system. *Nature Reviews. Immunology*, *17*(4), 219–232. https://doi.org/10.1038/nri.2017.7

Li, D., Liu, C.-M., Luo, R., Sadakane, K., & Lam, T.-W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics (Oxford, England)*, *31*(10), 1674–1676. https://doi.org/10.1093/bioinformatics/btv033

Lima-Junior, D. S., Krishnamurthy, S. R., Bouladoux, N., Collins, N., Han, S.-J., Chen, E. Y., Constantinides, M. G., Link, V. M., Lim, A. I., Enamorado, M., Cataisson, C., Gil, L., Rao, I., Farley, T. K., Koroleva, G., Attig, J., Yuspa, S. H., Fischbach, M. A., Kassiotis, G., & Belkaid, Y. (2021). Endogenous retroviruses promote homeostatic and inflammatory responses to the microbiota. *Cell*, *184*(14), 3794-3811.e19. https://doi.org/10.1016/j.cell.2021.05.020

Littré, É. M. P. (1861). *Oeuvres complètes d'Hippocrates*. Baillière. https://play.google.com/store/books/details?id=LY9MAAAAcAAJ

Liu, C. M., Hungate, B. A., Tobian, A. A. R., Ravel, J., Prodger, J. L., Serwadda, D., Kigozi, G., Galiwango, R. M., Nalugoda, F., Keim, P., Wawer, M. J., Price, L. B., & Gray, R. H. (2015). Penile Microbiota and Female Partner Bacterial Vaginosis in Rakai, Uganda. *MBio*, *6*(3), e00589. https://doi.org/10.1128/mBio.00589-15

Liu, C. M., Prodger, J. L., Tobian, A. A. R., Abraham, A. G., Kigozi, G., Hungate, B. A., Aziz, M., Nalugoda, F., Sariya, S., Serwadda, D., Kaul, R., Gray, R. H., & Price, L. B. (2017).

Penile Anaerobic Dysbiosis as a Risk Factor for HIV Infection. *MBio*, *8*(4). https://doi.org/10.1128/mBio.00996-17

Liu, Q., Fang, W., Zheng, P., Xie, S., Jiang, X., Luo, W., Han, L., Zhao, L., Lu, L., Zhai, L., Yu, D. J., Yang, W., Lin, C., Fang, X., & Bian, Z. (2025). Multi-kingdom microbiota analysis reveals bacteria-viral interplay in IBS with depression and anxiety. *Npj Biofilms and Microbiomes*, *11*(1), 129. https://doi.org/10.1038/s41522-025-00760-4

Marples, R. R., Downing, D. T., & Kligman, A. M. (1971). Control of free fatty acids in human surface lipids by Corynebacterium acnes. *The Journal of Investigative Dermatology*, *56*(2), 127–131. https://doi.org/10.1111/1523-1747.ep12260695

Mehta, S. D., Nandi, D., Agingu, W., Green, S. J., Otieno, F. O., Bhaumik, D. K., & Bailey, R. C. (2022). Longitudinal Changes in the Composition of the Penile Microbiome Are Associated With Circumcision Status, HIV and HSV-2 Status, Sexual Practices, and Female Partner Microbiome Composition. *Frontiers in Cellular and Infection Microbiology*, *12*, 916437. https://doi.org/10.3389/fcimb.2022.916437

Mehta, S. D., Zhao, D., Green, S. J., Agingu, W., Otieno, F., Bhaumik, R., Bhaumik, D., & Bailey, R. C. (2020). The Microbiome Composition of a Man's Penis Predicts Incident Bacterial Vaginosis in His Female Sex Partner With High Accuracy. *Frontiers in Cellular and Infection Microbiology*, *10*, 433. https://doi.org/10.3389/fcimb.2020.00433

Meites, E., Wilkin, T. J., & Markowitz, L. E. (2022). Review of human papillomavirus (HPV) burden and HPV vaccination for gay, bisexual, and other men who have sex with men

and transgender women in the United States. *Human Vaccines & Immunotherapeutics*, *18*(1), 2016007. https://doi.org/10.1080/21645515.2021.2016007

Mitra, A., MacIntyre, D. A., Ntritsos, G., Smith, A., Tsilidis, K. K., Marchesi, J. R., Bennett, P. R., Moscicki, A.-B., & Kyrgiou, M. (2020). The vaginal microbiota associates with the regression of untreated cervical intraepithelial neoplasia 2 lesions. *Nature Communications*, *11*(1), 1999. https://doi.org/10.1038/s41467-020-15856-y

Münger, K., Baldwin, A., Edwards, K. M., Hayakawa, H., Nguyen, C. L., Owens, M., Grace, M., & Huh, K. (2004). Mechanisms of human papillomavirus-induced oncogenesis. *Journal of Virology*, *78*(21), 11451–11460. https://doi.org/10.1128/JVI.78.21.11451-11460.2004

Murdoch, D. A. (1998). Gram-positive anaerobic cocci. *Clinical Microbiology Reviews*, *11*(1), 81–120. https://doi.org/10.1128/cmr.11.1.81

Murdoch, D. A., Mitchelmore, I. J., & Tabaqchali, S. (1988). Peptostreptococcus micros in polymicrobial abscesses. *Lancet*, *331*(8585), 594. https://doi.org/10.1016/s0140-6736(88)91393-1

Murphy, E. C., & Frick, I.-M. (2013). Gram-positive anaerobic cocci--commensals and opportunistic pathogens. *FEMS Microbiology Reviews*, *37*(4), 520–553. https://academic.oup.com/femsre/article-abstract/37/4/520/2399123

Namkung, J. (2020). Machine learning methods for microbiome studies. *Journal of Microbiology* , *58*(3), 206–216. https://doi.org/10.1007/s12275-020-0066-8

Nelson, D. E., Dong, Q., Van der Pol, B., Toh, E., Fan, B., Katz, B. P., Mi, D., Rong, R., Weinstock, G. M., Sodergren, E., & Fortenberry, J. D. (2012). Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PloS One*, *7*(5), e36298. https://doi.org/10.1371/journal.pone.0036298

Oh, J., Byrd, A. L., Park, M., NISC Comparative Sequencing Program, Kong, H. H., & Segre, J. A. (2016). Temporal Stability of the Human Skin Microbiome. *Cell*, *165*(4), 854–866. https://doi.org/10.1016/j.cell.2016.04.008

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. (2007). The vegan package. *Community Ecology Package*, *10*(631–637), 719. https://scholar.google.com/citations?view_op=view_citation&hl=en&citation_for_view=2WBRFVIAAAAJ:QUX0mv85b1cC

Onywera, H., Williamson, A.-L., Cozzuto, L., Bonnin, S., Mbulawa, Z. Z. A., Coetzee, D., Ponomarenko, J., & Meiring, T. L. (2020). The penile microbiota of Black South African men: relationship with human papillomavirus and HIV infection. *BMC Microbiology*, *20*(1), 78. https://doi.org/10.1186/s12866-020-01759-x

Onywera, H., Williamson, A.-L., Ponomarenko, J., & Meiring, T. L. (2020). The Penile Microbiota in Uncircumcised and Circumcised Men: Relationships With HIV and Human Papillomavirus Infections and Cervicovaginal Microbiota. *Frontiers of Medicine*, *7*, 383. https://doi.org/10.3389/fmed.2020.00383

Oriel, J. D. (1971). Natural history of genital warts. *The British Journal of Venereal Diseases*, *47*(1), 1–13. https://doi.org/10.1136/sti.47.1.1

Parkin, D. M. (2006). The global health burden of infection-associated cancers in the year 2002. *International Journal of Cancer. Journal International Du Cancer*, *118*(12), 3030–3044. https://doi.org/10.1002/ijc.21731

Petersen, C., & Round, J. L. (2014). Defining dysbiosis and its influence on host immunity and disease. *Cellular Microbiology*, *16*(7), 1024–1033. https://doi.org/10.1111/cmi.12308

Price, L. B., Liu, C. M., Johnson, K. E., Aziz, M., Lau, M. K., Bowers, J., Ravel, J., Keim, P. S., Serwadda, D., Wawer, M. J., & Gray, R. H. (2010). The effects of circumcision on the penis microbiome. *PloS One*, *5*(1), e8422. https://doi.org/10.1371/journal.pone.0008422

Prodger, J. L., Abraham, A. G., Tobian, A. A., Park, D. E., Aziz, M., Roach, K., Gray, R. H., Buchanan, L., Kigozi, G., Galiwango, R. M., Ssekasanvu, J., Nnamutete, J., Kagaayi, J., Kaul, R., & Liu, C. M. (2021). Penile bacteria associated with HIV seroconversion, inflammation, and immune cells. *JCI Insight*, *6*(8). https://doi.org/10.1172/jci.insight.147363

Qi, S.-Z., Wang, S.-M., Shi, J.-F., Wang, Q.-Q., Chen, X.-S., Sun, L.-J., Liu, A., Zhang, N., Jiang, N., Siva, P., Xu, X.-L., & Qiao, Y.-L. (2014). Human papillomavirus-related psychosocial impact of patients with genital warts in China: a hospital-based cross-sectional study. *BMC Public Health*, *14*(1), 739. https://doi.org/10.1186/1471-2458-14-739

Quan, C., Chen, X.-Y., Li, X., Xue, F., Chen, L.-H., Liu, N., Wang, B., Wang, L.-Q., Wang, X.-P., Yang, H., & Zheng, J. (2020). Psoriatic lesions are characterized by higher bacterial load and imbalance between Cutibacterium and Corynebacterium. *Journal of the American Academy of Dermatology*, *82*(4), 955–961. https://doi.org/10.1016/j.jaad.2019.06.024

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, *41*(Database issue), D590-6. https://doi.org/10.1093/nar/gks1219

Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, *35*(9), 833–844. https://doi.org/10.1038/nbt.3935

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S. K., McCulle, S. L., Karlebach, S., Gorle, R., Russell, J., Tacket, C. O., Brotman, R. M., Davis, C. C., Ault, K., Peralta, L., & Forney, L. J. (2011). Vaginal microbiome of reproductive-age women. *Proceedings of the National Academy of Sciences of the United States of America*, *108 Suppl 1*, 4680–4687. https://doi.org/10.1073/pnas.1002611107

Ridaura, V. K., Bouladoux, N., Claesen, J., Chen, Y. E., Byrd, A. L., Constantinides, M. G., Merrill, E. D., Tamoutounour, S., Fischbach, M. A., & Belkaid, Y. (2018). Contextual control of skin immunity and inflammation by Corynebacterium. *The Journal of Experimental Medicine*, *215*(3), 785–799. https://doi.org/10.1084/jem.20171079

Robinson, C. M., & Pfeiffer, J. K. (2014). Viruses and the Microbiota. *Annual Review of Virology*, *1*, 55–69. https://doi.org/10.1146/annurev-virology-031413-085550

Ruiz-Malagón, A. J., Rodríguez-Sojo, M. J., Redondo, E., Rodríguez-Cabezas, M. E., Gálvez, J., & Rodríguez-Nogales, A. (2025). Systematic review: The gut microbiota as a link between colorectal cancer and obesity. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, *26*(4), e13872. https://doi.org/10.1111/obr.13872

Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B., & Erlich, H. A. (1986). Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. *Nature*, *324*(6093), 163–166. https://doi.org/10.1038/324163a0

Schloss, P. D. (2024). Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses. *MSphere*, *9*(2), e0035423. https://doi.org/10.1128/msphere.00354-23

Scrucca, L., Fop, M., Murphy, T. B., & Raftery, A. E. (2016). Mclust 5: Clustering, classification and density estimation using Gaussian finite mixture models. *The R Journal*, *8*(1), 289–317. https://doi.org/10.32614/rj-2016-021

Shan, Y., Lee, M., & Chang, E. B. (2022). The Gut Microbiome and Inflammatory Bowel Diseases. *Annual Review of Medicine*, *73*, 455–468. https://doi.org/10.1146/annurev-med-042320-021020

Shi, N., Li, N., Duan, X., & Niu, H. (2017). Interaction between the gut microbiome and mucosal immune system. *Military Medical Research*, *4*(1). https://doi.org/10.1186/s40779-017-0122-9

Swaney, M. H., Nelsen, A., Sandstrom, S., & Kalan, L. R. (2023). Sweat and sebum preferences of the human skin Microbiota. *Microbiology Spectrum*, *11*(1), e0418022. https://doi.org/10.1128/spectrum.04180-22

Tickle, T. L., Segata, N., Waldron, L., Weingart, U., & Huttenhower, C. (2013). Two-stage microbial community experimental design. *The ISME Journal*, *7*(12), 2330–2339. https://doi.org/10.1038/ismej.2013.139

van der Krieken, D. A., Rikken, G., Ederveen, T. H. A., Jansen, P. A. M., Rodijk-Olthuis, D., Meesters, L. D., van Vlijmen-Willems, I. M. J. J., van Cranenbroek, B., van der Molen, R. G., Schalkwijk, J., van den Bogaard, E. H., & Zeeuwen, P. L. J. M. (2023). Gram-positive anaerobic cocci guard skin homeostasis by regulating host-defense mechanisms. *IScience*, *26*(4), 106483. https://doi.org/10.1016/j.isci.2023.106483

Větrovský, T., & Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PloS One*, *8*(2), e57923. https://doi.org/10.1371/journal.pone.0057923

Videnska, P., Smerkova, K., Zwinsova, B., Popovici, V., Micenkova, L., Sedlar, K., & Budinska, E. (2019). Stool sampling and DNA isolation kits affect DNA quality and bacterial composition following 16S rRNA gene sequencing using MiSeq Illumina platform. *Scientific Reports*, *9*(1), 13837. https://doi.org/10.1038/s41598-019-49520-3

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Watchorn, R. E., van den Munckhof, E. H. A., Quint, K. D., Eliahoo, J., de Koning, M. N. C., Quint, W. G. V., & Bunker, C. B. (2021). Balanopreputial sac and urine microbiota in patients with male genital lichen sclerosus. *International Journal of Dermatology*, *60*(2), 201–207. https://doi.org/10.1111/ijd.15252

Weinstock, H., Berman, S., & Cates, W., Jr. (2004). Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. *Perspectives on Sexual and Reproductive Health*, *36*(1), 6–10. https://doi.org/10.1363/psrh.36.6.04

Werner, J. J., Koren, O., Hugenholtz, P., DeSantis, T. Z., Walters, W. A., Caporaso, J. G., Angenent, L. T., Knight, R., & Ley, R. E. (2012). Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *The ISME Journal*, *6*(1), 94–103. https://doi.org/10.1038/ismej.2011.82

Werner, R. N., Westfechtel, L., Dressler, C., & Nast, A. (2017). Anogenital warts and other HPV-associated anogenital lesions in the HIV-positive patient: a systematic review and meta-analysis of the efficacy and safety of interventions assessed in controlled clinical trials. *Sexually Transmitted Infections*, *93*(8), 543–550. https://doi.org/10.1136/sextrans-2016-053035

Willis, A. D. (2019). Rarefaction, Alpha Diversity, and Statistics. *Frontiers in Microbiology*, *10*, 2407. https://doi.org/10.3389/fmicb.2019.02407

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. In *bioRxiv*. bioRxiv. https://doi.org/10.1101/762302

Zeeuwen, P. L. J. M., Ederveen, T. H. A., van der Krieken, D. A., Niehues, H., Boekhorst, J., Kezic, S., Hanssen, D. A. T., Otero, M. E., van Vlijmen-Willems, I. M. J. J., Rodijk-Olthuis, D., Falcone, D., van den Bogaard, E. H. J., Kamsteeg, M., de Koning, H. D., Zeeuwen-Franssen, M. E. J., van Steensel, M. A. M., Kleerebezem, M., Timmerman, H. M., van Hijum, S. A. F. T., & Schalkwijk, J. (2017). Gram-positive anaerobe cocci are underrepresented in the microbiome of filaggrin-deficient human skin. *The Journal of Allergy and Clinical Immunology*, *139*(4), 1368–1371. https://doi.org/10.1016/j.jaci.2016.09.017

Zeng, M., Li, X., Jiao, X., Cai, X., Yao, F., Xu, S., Huang, X., Zhang, Q., & Chen, J. (2022). Roles of vaginal flora in human papillomavirus infection, virus persistence and clearance. *Frontiers in Cellular and Infection Microbiology*, *12*, 1036869. https://doi.org/10.3389/fcimb.2022.1036869

Zozaya, M., Ferris, M. J., Siren, J. D., Lillis, R., Myers, L., Nsuami, M. J., Eren, A. M., Brown, J., Taylor, C. M., & Martin, D. H. (2016). Bacterial communities in penile skin, male urethra, and vaginas of heterosexual couples with and without bacterial vaginosis. *Microbiome*, *4*, 16. https://doi.org/10.1186/s40168-016-0161-6