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PENILE HUMAN PAPILLOMAVIRUS INFECTION AND ITS ASSOCIATED RISK FACTORS IN MEN WHO HAVE SEX WITH MEN IN KIGALI, RWANDA

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PENILE HUMAN PAPILLOMAVIRUS INFECTION AND ITS ASSOCIATED RISK FACTORS IN MEN WHO HAVE SEX WITH MEN IN KIGALI, RWANDA

By

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DECLARATION

I, Julie Raissa DUSABINEMA, hereby declare that this research project submitted to the University of Rwanda, for the degree of Master of Science in Biotechnology, is my own original work and has not been submitted before to any Institution by myself or any other person in fulfillment of the requirements for the award of any degree or any other qualification.

Julie Raissa DUSABINEMA

Signature

DEDICATION

To God, whose grace has sustained me with life, energy, and clarity throughout this academic path.

To my supervisors, your guidance and mentorship have been a constant compass. Thank you for your support and commitment to my growth as a researcher.

To my family, your patience, encouragement, and belief in me have been the foundation of this journey. I am grateful for every sacrifice you made.

To my friends, thank you for walking alongside me through every challenge and triumph, for your understanding, and for lifting my spirits when it mattered most.

This work is dedicated with deep appreciation to all of you.

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May God’s blessings be upon you all

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LIST OF ABREVIATIONS AND ACRONYMS

1. HPV – Human Papillomavirus
2. MSM – Men Who Have Sex with Men
3. HIV – Human Immunodeficiency Virus
4. ART – Antiretroviral Therapy
5. PCR – Polymerase Chain Reaction
6. AIDS – acquired immunodeficiency syndrome
7. SPSS – Statistical Package for the Social Sciences
8. RMRTH – Rwanda Military Referral and Teaching Hospital
9. DNA – Deoxyribonucleic Acid
10. CI – Confidence Interval
11. Z-score – Standard Score used in sample size calculation
12. S.E. – Standard Error
13. B – Regression Coefficient
14. Exp(B) – Exponentiated Coefficient (Odds Ratio) in Logistic Regression

ABSTRACT

Background: HPV infection poses a significant health risk, particularly for men who have sex with men (MSM). In Rwanda, limited data exist on penile HPV prevalence and genotypes. This study assessed high-risk HPV (hrHPV) prevalence, identified circulating genotypes, and examined related risk factors in this population

Methods: This cross-sectional study used 248 archived penile swab samples collected from Rwandan men who have sex with men (MSM) in Kigali. Molecular genotyping analysis used the AmpFire High-Risk HPV genotyping kit. Data analysis included descriptive statistics and logistic regression models to explore the association between hrHPV infection and participant age and HIV status.

Results: HrHPV was detected in 94 of participants (37.9%). Among the detected genotypes, HPV-16 had the highest frequency, 10 participants (10.6%), with HPV-52, 8 participants (8.5%), and HPV-39, 6 participants (6.4%) following. The prevalence of high-risk HPV was notably elevated in individuals having HIV. However, no significant association was observed between age and hrHPV status.

Conclusion: The high prevalence of hrHPV among Rwandan men who have sex with men (MSM) in Kigali, particularly those living with HIV, underscores the urgent need to extend HPV vaccination and screening services to this vulnerable group. Targeted public health interventions are essential to reduce the HPV-related disease burden in MSM populations.

Keywords: Human papillomavirus, high-risk HPV, MSM, HPV-16, genotype distribution, HIV co-infection, Kigali, Rwanda, molecular genotyping

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CHAPTER 1. INTRODUCTION

1.1. BACKGROUND

Human Papillomavirus (HPV) is the most widespread sexually transmitted infection (STI) globally, with men carrying a considerable share of the burden. A recent meta-analysis estimated that approximately 31% of men worldwide harbor genital HPV at any given time, and about 21% carry high-risk (hrHPV) types (Bruni et al., 2023). These oncogenic genotypes are strongly linked to cervical cancer in women, the fourth most frequently diagnosed cancer globally, as well as penile, anal, and oropharyngeal cancers in men (Aslanimehr et al., 2025). Despite its relevance to both sexes, the bulk of public health interventions and research has been directed toward women, particularly within the framework of cervical cancer prevention, leaving a substantial knowledge gap regarding HPV epidemiology in men, especially in high-risk groups such as men who have sex with men (MSM) (Borena et al., 2024).

Globally, men who have sex with men represent a key at-risk population because they experience both higher prevalence and persistence of HPV infection compared to heterosexual men. These factors substantially increase their risk of HPV-related cancers, including anal and penile cancers (Bruzzesi et al., 2024). Although their vulnerability is well-recognized, epidemiological data specific to men who have sex with men (MSM) remain limited, particularly in low- and middle-income countries. This scarcity of data constrains the design of targeted prevention programs, even though the group has consistently been identified as carrying a disproportionate share of HPV-related disease. HPV is transmitted primarily through direct skin-to-skin or mucosal contact during sexual activity. In men, infections are frequently asymptomatic, which allows the virus to remain undetected and contribute silently to ongoing transmission (Bruzzesi et al., 2024). Men who have sex with men (MSM) are especially susceptible to hrHPV acquisition and persistence, in part due to risk behaviors such as multiple sexual partnerships and receptive anal intercourse. Their risk is further compounded by HIV co-infection, which impairs immune clearance and facilitates viral persistence (Quinn et al., 2012). Globally, men who have sex with men are estimated to constitute between 2-5% of the adult male population, representing more than 25 million men (UNAID, 2022). In 2021, Rwanda had an estimated 18,100 men aged 18 and above who have sex with men (MSM), representing about 0.7% of adult men. Most are located in Kigali (around 7,842), with the rest distributed across the country's other provinces. These estimates are derived from a three-source capture-recapture study designed to support HIV surveillance and targeted public health programs (Tuyishime et al., 2023).

Table 1: Estimated Population Size of Men Who Have Sex with Men (MSM) in Rwanda by Province, 2021

Province	Estimated MSM Population (Age 18+)	Percentage of Total MSM Population
Kigali (Capital)	7842	43.3%
Western Province	2295	12.7%
Northern Province	2012	11.1%
Eastern Province	3012	16.6%
Southern Province	3039	16.8%
Total	18100	100%

In Sub-Saharan Africa, men who have sex with men are formally acknowledged as a key population within HIV and sexually transmitted infection (STI) programs, yet they continue to face legal, social, and structural barriers such as stigma, discrimination, and criminalization. These barriers hinder access to sexual and reproductive health services, thereby exacerbating their vulnerability to persistent HPV infection (Afolaranmi et al., 2025).

In Sub-Saharan Africa, studies consistently demonstrate elevated rates of hrHPV among men who have sex with men compared to the general male population. Research from South Africa, Kenya, and Uganda has reported hrHPV prevalence rates ranging from 45-67% in men who have sex with men, often with multiple concurrent genotype infections (Müller et al., 2016)(Oo et al., 2023). Such findings highlight the urgent need for targeted prevention efforts in the region. However, the epidemiology of HPV among men who have sex with men remains underexplored, leaving critical gaps in evidence to guide vaccination and screening policies.

In Rwanda, HPV prevention and control measures have largely centered on women, particularly through national vaccination campaigns for adolescent girls and cervical cancer screening initiatives. While these efforts have yielded significant progress in female populations, men and especially men who have sex with men (MSM) have been left outside the scope of HPV surveillance and vaccination programs (Murenzi et al., 2024). The absence of male-focused HPV initiatives may obscure the true burden of HPV-related disease in men and weaken the overall effectiveness of national cancer prevention strategies. Although men who have sex with men (MSM) are included in HIV prevention programs, their risk of hrHPV infection has not been systematically studied. This lack of local epidemiological data prevents the development of inclusive, evidence-based interventions that address HPV transmission and disease outcomes in this underserved group.

The biology of HPV infection in men further underscores the importance of surveillance and prevention. HPV enters the body through microabrasions in the epithelium and infects basal cells, where it may be cleared by immune responses or persist over time. Persistent infection can progress to intraepithelial neoplasia and, ultimately, invasive cancer (Foresta et al., 2011). Although penile cancer is relatively rare on a global scale, its incidence in parts of Africa is higher, ranging from 0.1 to 1.5 cases per 100,000 men (Ferlay et al., 2021). Penile cancers are strongly associated with hrHPV, particularly HPV-16 and HPV-18, which are also among the most common genotypes found in men who have sex with men. Other host and behavioral factors, including HIV status, circumcision, smoking, and sexual behavior, further influence the persistence and clinical consequences of infection (Bettampadi et al., 2021).

In Rwanda, men are neither routinely screened for HPV nor included in vaccination programs. Barriers such as low awareness, stigma surrounding sexual orientation, and limited access to men who have sex with men-sensitive services contribute to missed opportunities for prevention and care. Given the dual burden of HIV and HPV among men who have sex with men, there is an urgent need to generate local evidence that can inform policies and interventions tailored to this high-risk group.

This study, therefore, seeks to address this knowledge gap by investigating the prevalence of penile hrHPV, characterizing genotype distribution, and identifying associated risk factors among men who have sex with men in Rwanda. The findings will contribute crucial molecular and epidemiological evidence to guide the integration of men, particularly men who have sex with men, into national HPV prevention frameworks. In doing so, this research aims to support the development of inclusive, evidence-based public health programs that can reduce the long-term burden of HPV-related cancers in Rwanda

1.2. PROBLEM STATEMENT

Human Papillomavirus (HPV) infection poses a significant global health concern, with men who have sex with men (MSM) disproportionately affected due to their higher risk of persistent high-risk HPV (hrHPV) infections and associated cancers, including penile, anal, and oropharyngeal malignancies (Bruni et al., 2023). Despite major progress in Rwanda's cervical cancer prevention programs targeting women, men, and especially men who have sex with men, remain largely excluded from HPV surveillance and vaccination initiatives (ICO, 2016). At present, there is a critical lack of local data on penile hrHPV prevalence, genotype distribution, and risk factors in men who have sex with men, which limits the design of evidence-based and inclusive interventions.

HIV co-infection further heightens risk by reducing immune clearance of HPV, thereby increasing persistence and progression to malignancy (Murenzi et al., 2024). In addition, stigma, discrimination, and restricted access to men who have sex with men-sensitive healthcare services act as structural barriers to prevention and care. Unless these gaps are addressed, Rwanda's HPV prevention strategies may remain inadequate for controlling infection among key populations, perpetuating transmission and HPV-related disease burden.

This study, therefore, seeks to address this gap by investigating penile hrHPV prevalence, genotype distribution, and associated risk factors, including age and HIV status, among MSM in Kigali. The findings will provide much-needed evidence to guide equitable HPV vaccination, screening, and clinical management programs for this underserved population.

1.3. RESEARCH QUESTIONS

1. What is the prevalence of high-risk HPV infection in archived penile samples from men who have sex with men (MSM) in Kigali, Rwanda?
2. Which high-risk HPV genotypes are most frequently detected among penile samples from MSM in Kigali, Rwanda?
3. To what extent does age influence the likelihood of penile high-risk HPV infection among MSM in Kigali, Rwanda?
4. How does HIV status affect the distribution patterns of high-risk HPV genotypes in men who have sex with men populations in this region?

1.4. OBJECTIVES

1.4.1. General Objective:

To evaluate the prevalence, genotype distribution, and associated risk factors of penile high-risk human papillomavirus (hrHPV) infection among men who have sex with men (MSM) in Kigali, Rwanda.

1.4.2. Specific Objectives:

1. To evaluate the prevalence of high-risk HPV DNA in penile samples collected from MSM in Kigali, Rwanda.
2. To identify and characterize the specific high-risk HPV genotypes present in the penile samples from MSM in Kigali, Rwanda.
3. To assess the association between participant age and hrHPV infection status.
4. To determine the association between HIV status and hrHPV infection among MSM in Kigali, Rwanda.

CHAPTER 2. LITERATURE REVIEW

2.1. Epidemiology of Human Papillomavirus Infection in Men

Human Papillomavirus (HPV) is one of the greatest mutual transmitted viral infections through sexual contact. Although it has been recognized for its role in cervical cancer, it also significantly impacts men, particularly through genital and anal infections (Ospina-Henao et al., 2025). Over 200 HPV genotypes have been identified, with a subset categorized as high-risk HPV (hrHPV) due to their association with cancers (NIH MedlinePlus Magazine, 2020). Despite widespread recognition of HPV's role in cervical cancer, the impact of HPV infection in men remains understudied and underestimated, particularly in high-risk groups such as men who have sex with men (MSM).

HPV is often asymptomatic and transient in men, allowing the virus to persist often undetected and facilitate ongoing transmission (Giuliano et al., 2010). A 2023 WHO fact sheet reports that it is estimated that nearly one-third (31%) of men carry genital HPV infections, and approximately 21% carry hrHPV genotypes (Naidoo et al., 2024). In regions such as sub-Saharan Africa, HPV prevalence in men is also concerning. Findings from a 2020 regional analysis revealed that roughly one in three men 34.6% tested positive for the virus. Among men, men who have sex with men (MSM) are recognized globally as a population at higher risk for persistent hrHPV infection. Contributing factors include having multiple sexual partners, participating in receptive anal intercourse, and a higher likelihood of HIV infection, which can weaken immune responses and increase viral persistence. In the Rwandan context, men who have sex with men face particular challenges regarding HPV. A recent study documented high rates of persistent penile high-risk HPV among Rwandan men who have sex with men, signaling a significant public health concern (Murenzi et al., 2024).

In MSM, the burden is especially high, due in part to the increased likelihood of anal, penile, and oropharyngeal exposure. Studies from multiple regions have reported high hrHPV prevalence among men who have sex with men, particularly those who are HIV-positive (Sudenga et al., 2017). Globally, men who have sex with men comprise an estimated 2-5% of adult men, amounting to more than 25 million individuals, and represent a key population for targeted intervention (UNAID, 2022). However, social stigma, criminalization, and limited access to sexual health services in many African settings, including Rwanda, significantly restrict prevention and screening efforts for this group (Afolaranmi et al., 2025). Recent studies indicate that HIV co-infection significantly increases the persistence of HPV infections in men who have sex with men, thereby raising the risk of developing HPV-related malignancies

(Z. Zhang et al., 2024)(Biała et al., 2022). The immunosuppressive nature of HIV infection appears to hinder viral clearance, increasing the likelihood of persistent HPV infection and the development of related neoplasia (Verma et al., 2017).

The reported prevalence of penile HPV infection varies widely due to differences in sampling techniques, population characteristics, and regional epidemiology. A 2017 U.S survey showed that approximately 45.2 % of men aged 18-59 had detectable penile HPV DNA, with infection highest among those aged 18-24 (Deshmukh et al., 2017). Among MSM, recent data suggest that 61% and 73% carry genital HPV. Furthermore, evidence highlights that access to HPV vaccination and routine screening remains limited in many sub-Saharan African settings, creating significant gaps in preventive healthcare for high-risk male populations (Liu et al., 2023). Reported prevalence also differs across regions. Studies indicate that in sub-Saharan Africa, 35-45% of men may harbor HPV, compared with 40-50% in North America and 20-35% in Europe. Factors such as HIV status, circumcision, and age appear to influence these rates. These disparities necessitate localized epidemiological data to guide public health strategies, especially in underserved populations. Compiling prevalence data from multiple global studies in a comparative table could help illustrate the heightened burden among MSM relative to heterosexual men.

These rates are notably higher than in heterosexual men and carry significant implications for transmission risk, persistent infection, and related cancers in the anal and oropharyngeal regions, underscoring the urgent need for targeted HPV prevention, vaccination, and continuous epidemiological surveillance in this population (Ke et al., 2025).

2.2. Genotype Distribution in High-risk Populations

Globally, HPV-16 and HPV-18 are recognized as the most dominant high-risk HPV (hrHPV) genotypes due to their strong oncogenic potential. Together, they are responsible for the majority of HPV-related cancers, including cervical, anal, penile, and oropharyngeal cancers (Sun et al., 2025). However, the global HPV disease burden is not solely attributable to these two types. Other hrHPV genotypes, such as HPV-31, 33, 35, 39, 45, 52, and 58, also play a substantial role in cancer development and are increasingly recognized in epidemiological studies (Arbyn et al., 2021). These additional genotypes are of particular importance in high-risk populations, including men who have sex with men (MSM), where the prevalence and distribution of hrHPV infections often differ from those observed in the general population.

The distribution of hrHPV genotypes is influenced by several factors, including geographic location, sexual practices, and immunological status. (Seyoum et al., 2022) In high-income countries, HPV-16, 18, and 33 are reported as the most frequent genotypes among men who have sex with men. For instance, large-scale cohort studies in Europe and North America have consistently documented HPV-16 as the predominant type, followed by HPV-18 and 33, suggesting a relatively uniform distribution in these settings (Adeyanju et al., 2024). This pattern is also reflected in vaccine development, as the most widely used vaccines target these genotypes. By contrast, studies from low- and middle-income countries, particularly in Sub-Saharan Africa, reveal a broader diversity of hrHPV types. Research in these settings highlights HPV-52, HPV-58, and HPV-35 as being equally, if not more, significant than HPV-18 in men who have sex with men populations (Ouedraogo et al., 2023). A meta-analysis by (Tchouaket et al., 2023) reported similar genotype overlap between HIV-positive and general male populations in Sub-Saharan Africa, with types 16, 52, and 58. South Africa provides a striking example of the HR-HPV burden among men who have sex with men. A recent study revealed that over 60% of South African men who have sex with men were infected with hrHPV genotypes, with HPV-16 and HPV-18 being the most prevalent (Mistry et al., 2022). Some studies in sub-Saharan Africa have identified distinct genotype profiles compared to those in Western countries, suggesting that localized data are crucial for guiding prevention strategies.

From a public health standpoint, the circulation of diverse hrHPV types in men who have sex with men populations complicates prevention. Although current vaccines provide strong protection against the most carcinogenic strains (HPV-16 and HPV-18, as well as several others in the nonavalent vaccine), the prominence of additional types such as HPV-35 and HPV-39 in African men who have sex with men raises concerns about whether vaccine formulations developed in high-income countries fully address local risks (Naidoo et al., 2024). This makes regional genotype surveillance essential for guiding vaccination strategies. In summary, the distribution of hrHPV among men who have sex with men is heterogeneous and context-specific. While HPV-16 and 18 remain globally dominant, other types, including HPV-52, 58, 35, and 39, play a major role in Sub-Saharan Africa. Frequent co-infections, especially in HIV-positive men, further complicate prevention and highlight the importance of molecular epidemiology. Mapping these patterns is crucial for informing vaccine policy, tailoring screening strategies, and ultimately reducing HPV-associated cancers among men who have sex with men.

In men who have sex with men, multiple HPV infections are frequently reported, which may compound the risk of cancer transformation (Murenzi et al., 2020). Research comparing men who have sex with men and heterosexual men suggests that men who have sex with men (MSM) frequently carry multiple high-risk HPV types simultaneously, which may increase the likelihood of persistent infection. Within Africa, studies from Kenya, Uganda, and South Africa indicate that HPV-16, HPV-52, and HPV-66 are commonly observed among men who have sex with men, emphasizing the importance of collecting region-specific genotype data.

Table 2: Prevalence of High-Risk HPV in MSM vs Heterosexual Men in Sub-Saharan Africa.

Country	**High-Risk HPV Prevalence in MSM (%)**	**High-Risk HPV Prevalence in Heterosexual Men (%)**
Kenya	65%	35%
Uganda	62%	37%
South-Africa	67%	37%
Nigeria	58%	33%
Tanzania	57%	33%

2.3. BEHAVIORAL AND CLINICAL RISK FACTORS

The risk of HPV acquisition and persistence is influenced by behavioral and clinical factors. High-risk behaviors, including early sexual debut, multiple partners, and inconsistent condom use, are well-documented to increase HPV susceptibility (El-Zein et al., 2019). Receptive anal and intercourse further increase the risk in men who have sex with men.

Immunosuppression, particularly due to HIV infection, impairs the body's ability to clear HPV, leading to persistent infections. While antiretroviral therapy (ART) helps restore immune function, it does not eliminate HPV infection. Other contributing factors include smoking and the lack of circumcision, both of which have been associated with higher infection rates (Verma et al., 2017).

Some studies report inconsistent associations between condom use and HPV prevalence, reflecting the complex nature of behavioral risk factors. Evidence from Rwanda suggests that HIV-positive men who have sex with men exhibit higher rates of penile high-risk HPV than HIV-negative men who have sex with men, highlighting the combined impact of behavioral and immunological factors.

The dual burden of HPV and HIV significantly raises the risk of anal and penile cancers. Moreover, this situation is compounded by socioeconomic and structural barriers, such as limited access to sexual health services, economic constraints, low literacy, and stigma surrounding sexual orientation, which restrict men who have sex with men from utilizing preventive services and screening. These challenges are particularly pronounced in Rwanda and other African settings, where cultural norms and under-resourced health systems further hinder access to care (Z. Zhang et al., 2024).

Although behavioral and clinical factors increase the likelihood of acquiring HPV, disease progression ultimately depends on the biological dynamics of the virus within the male genital tract.

2.4. Pathophysiology and Disease Progression

Human papillomavirus (HPV) infects basal epithelial cells through microabrasions in the skin or mucosal surfaces. Once inside, the virus replicates within the squamous epithelium, hijacking the host cell machinery to facilitate its own replication cycle (Graham, 2017).

High-risk HPV types, such as HPV-16 and HPV-18, produce two major oncoproteins, E6 and E7, that play pivotal roles in carcinogenesis. The E6 oncoprotein binds to and promotes the degradation of the tumor suppressor protein p53, leading to the loss of cell cycle control and inhibition of apoptosis. Similarly, E7 binds to the retinoblastoma protein (pRb), freeing E2F transcription factors and driving the cell cycle forward, even in the presence of DNA damage (Y. Zhang et al., 2025).

The progression from initial infection to cancer involves several steps. Persistent infection with high-risk HPV types can lead to the integration of viral DNA into the host genome, disrupting the E2 gene and resulting in the overexpression of E6 and E7 oncoproteins. This integration is often accompanied by the accumulation of additional genetic mutations, contributing to genomic instability. Over time, these molecular alterations can lead to the development of precancerous lesions, such as penile intraepithelial neoplasia (PeIN), and, if left untreated, progression to invasive penile cancer (Kidd et al., 2017). The risk of progression to cancer is influenced by several factors, including the specific HPV genotype, the host's immune response, and the presence of co-infections. For instance, co-infection with HIV can impair immune surveillance, allowing for the persistence and progression of HPV-induced lesions (Saldaña-Rodríguez et al., 2023).

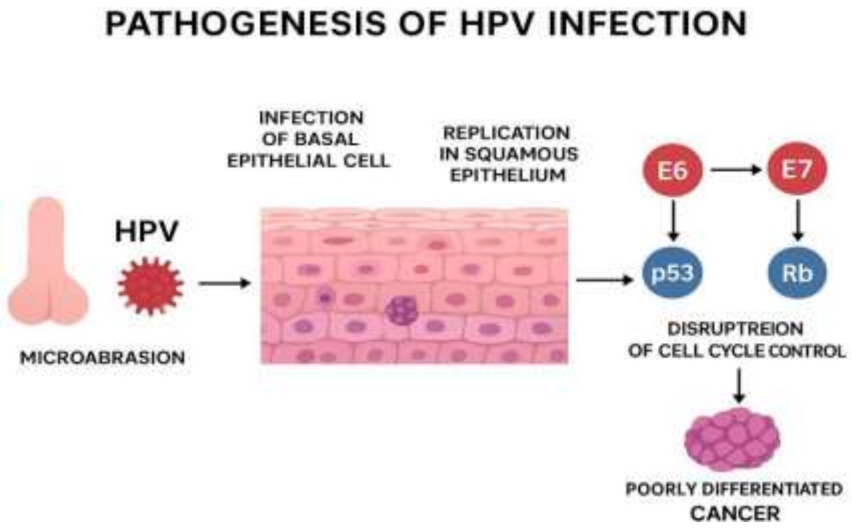

Figure 1: "Pathogenesis of hrHPV infection in men"

Understanding these molecular mechanisms is crucial for developing targeted prevention and therapeutic strategies for HPV-related cancers, including penile cancer.

2.5. Prevention and Control Measures

Prophylactic HPV vaccination remains the cornerstone of prevention against HPV-associated diseases. While it was initially directed toward adolescent girls and young women to reduce the burden of cervical cancer, there is growing recognition that HPV also poses a substantial health risk to men, particularly men who have sex with men (MSM). This has led to increasing global advocacy for the inclusion of boys and high-risk male groups, such as men who have sex with men, in national immunization programs (Rosado et al., 2023). Extending vaccination coverage to these populations is crucial, as it not only provides direct protection against penile, anal, and oropharyngeal cancers but also contributes to reducing overall HPV transmission within the wider community. Nevertheless, uptake among men who have sex with men remains limited across many settings due to social stigma, the absence of targeted vaccination strategies, inadequate health education, and the misconception that HPV is solely a women's health issue (DeSisto et al., 2025).

Screening for HPV-related disease in men is less established compared to cervical cancer screening programs in women. In men, potential approaches include molecular testing of penile or anal swabs and anal cytology, which can help detect high-risk HPV types or precancerous lesions. These strategies are especially important for HIV-positive men who have sex with men, who face a higher likelihood of persistent infections and progression to cancer (Ren et al., 2025). However, such screening services are rarely implemented as part of routine care in low- and middle-income countries, including Rwanda.

In Rwanda, the national HPV vaccination program has achieved strong coverage among adolescent girls aged 9-14 years, contributing to long-term cervical cancer prevention. Despite this success, men who have sex with men remain outside the current vaccination framework, leaving them vulnerable to HPV infection and related complications. Given the global guidance that recommends vaccinating men who have sex with men up to age 26, ideally before sexual debut, expanding Rwanda's program to include this group would address a significant gap in prevention. This is particularly relevant in light of emerging evidence on penile HPV infections among men who have sex with men in Rwanda, where the burden of high-risk genotypes has not been fully characterized. Incorporating men who have sex with men into vaccination strategies, alongside tailored health education and potential screening initiatives, could help reduce the prevalence of penile HPV, prevent HPV-associated cancers, and inform evidence-based policy updates in the country.

2.6. The Rwandan Context

In Rwanda, most HPV-related public health initiatives have focused on cervical cancer prevention in women. The national HPV vaccination program has substantially improved awareness and uptake among adolescent girls, yet male populations, particularly men who have sex with men (MSM), remain largely understudied and underserved within prevention strategies (Murenzi et al., 2024).

To date, there is limited published evidence on penile HPV infection and genotype distribution among MSM in Rwanda. This knowledge gap restricts the ability to fully assess the burden of infection and to design targeted interventions for this high-risk group.

Since the current vaccination strategy in Rwanda primarily targets females, understanding the genotype profile circulating among men is essential for evaluating the broader public health benefits of expanding vaccine coverage. Without local molecular surveillance data, developing tailored prevention and treatment approaches remains challenging. Behavioral factors, HIV status, and other clinical characteristics are likely to influence both the persistence and diversity of HPV genotypes, underscoring the need for locally relevant research (Murenzi et al., 2024).

The lack of epidemiological data on penile HPV prevalence and genotype distribution among men who have sex with men, therefore, represents a significant barrier to evidence-based intervention. This study seeks to address this gap by documenting infection patterns, genotype diversity, and demographic correlates in Rwandan men who have sex with men. Generating such evidence is critical to informing inclusive vaccination policies, strengthening diagnostic capacity, and shaping prevention strategies that account for the heightened risk of persistent HPV infections in HIV-positive men who have sex with men (MSM). Ultimately, expanding vaccination programs to include men who have sex with men, alongside improved molecular surveillance and the reduction of social barriers, represents an important step toward comprehensive HPV control in Rwanda.

CHAPTER 3. METHODOLOGY

3.1. Introduction

This chapter outlines the methods applied to achieve the objectives of the study on penile high-risk HPV infection and its associated risk factors among men who have sex with men in Kigali, Rwanda. It explains the study design, population, sampling, laboratory procedures, and statistical approaches used to generate and analyze the data. Ethical considerations are also highlighted to demonstrate compliance with research standards. The following sections present each component in detail, beginning with the study description.

3.2. Study Description

This study focused on Rwandan MSM, intending to assess the prevalence of penile hrHPV infection by identifying the circulating genotypes and exploring associated risk factors. Archived penile swab specimens collected from this population were analyzed to detect the presence of hrHPV and determine specific genotype profiles. The investigation also examined whether demographic variables such as age and HIV status were linked to hrHPV positivity. By incorporating detailed demographic data into the analysis, the study aimed to uncover meaningful patterns that could inform prevention strategies. The findings are expected to support national public health initiatives, particularly in strengthening Rwanda's targeted approach to HPV detection and vaccine coverage in MSM populations.

3.3. Study Design

This study adopted a laboratory-centered cross-sectional design. It relied on previously collected penile swab specimens and accompanying demographic and clinical data from a specific population of MSM in Rwanda.

3.4. Study Population

The study focused on adult males, aged 19 and above, who identify as men who have sex with men (MSM). These individuals had taken part in earlier research activities at the Rwanda Military Referral and Teaching Hospital, during which they contributed both penile swab samples and related clinical demographic information. Their existing records formed the basis for the analysis.

3.5. Sampling Technique

The study drew upon existing biological samples collected during an earlier research initiative. The Selection of samples was guided by specific inclusion criteria, which included the participants' age, documented HIV status, and the presence of sufficient volume and quality of stored penile swab material suitable for laboratory analysis.

3.6. Sample Size

To estimate the required number of study participants for estimating the incidence of hrHPV among the targeted people, Cochran’s formula was applied (Chanuan et al., 2021):

$$n=(Z^2\times p\times(1-p))/d^2$$

Where:

n is the acquired sample size

z represents the Z-score corresponding to a 95% confidence level (1.96)

p is the estimated prevalence of hrHPV (0.346, based on existing literature)

d is the acceptable margin of error (0.0592)

By substituting these values into the formula, the estimated sample size was 248 participants. This number was used to guide the inclusion of archived samples in the study. Using this sample size ensured reliable statistical power to estimate hrHPV prevalence and allowed for meaningful subgroup analysis based on age and HIV status.

$$n=\frac{(1.96)^2\times0.346\times(1-0.346)^2}{(0.0592)^2}$$

$$n= \sim248$$

3.7. Inclusion Criteria

The participants included in this study were individuals who self-identified as men who have sex with men. To meet inclusion requirements, they had to provide complete demographic information and contribute to penile swab specimens of sufficient quality and quantity for laboratory testing and examination.

3.8. Exclusion Criteria

Individuals were not eligible for inclusion if they declined to give informed consent, did not identify themselves as men who have sex with men, provided incomplete demographic details, or submitted penile swab specimens that were insufficient in quality or amount for dependable laboratory analysis.

3.9. Study Area

The practical component of the study involved key experimental methods that took place at the Einstein-Rwanda Research Laboratory, situated within the Rwanda Military Referral and Teaching Hospital in Kigali. This laboratory is nationally accredited and well-equipped for molecular diagnostics, housing tools such as the Atila PowerGene 9600 Plus Real-Time PCR system. It plays a central role in HPV research and infectious disease monitoring in Rwanda.

3.10. Laboratory Analysis

All samples used in this study had been previously collected and preserved at -80 °C until further processing. HPV genotyping was carried out using the AmpFire High-risk HPV genotyping kit, which detects and identifies high-risk HPV types through isothermal amplification.

The laboratory workflow consisted of several key steps:

1. Sample lysis: A 1ml aliquot of penile cell suspension underwent centrifugation, and the supernatant was discarded. The resulting pellet was treated with 100 µL of 40X lysis buffer and heated at 95°C for 20 minutes to ensure cell disruption and release of nucleic acids.
2. Master Mix Preparation: Four master mixes were prepared, each corresponding to a specific primer set (Mixes 1 through 4) targeting different HPV genotypes. Each primer mix was combined with a universal reaction mix according to the manufacturer’s protocol.
3. PCR Setup: For amplification, 20 µL of each master mix was dispensed into PCR strips, followed by the addition of 5 µL of the prepared lysate. Positive and negative controls were included in each run to validate results.
4. Amplification and Detection: Reactions were carried out on the Atila PowerGene 9600 Plus Real-Time PCR system at a constant temperature of 60 °C for one hour. Fluorescence signals were monitored in real time at one-minute intervals. Internal and negative controls were evaluated to confirm amplification quality and to ensure that no contamination was present.

3.11. Amplification and Results Interpretation

1. The positive control exhibited successful amplification with exponential curves, whereas the negative control showed no amplification, thus validating the experiment. Four tubes representing distinct HPV genotypes were examined for each specimen.
2. The fluorescent channel designations for each tube in the PCR analysis. In PM-1, FAM identifies HPV-31, HEX identifies HPV-51, ROX identifies HPV-39, and CY5 identifies HPV-16. PM-2 employs FAM for HPV-35, HEX for HPV-68, ROX for HPV-18, and CY5 for HPV-59. PM-3 utilizes FAM for HPV-33, HEX for Internal Control, ROX for HPV-66, and CY5 for HPV-45. In the end, PM-4 employs FAM for HPV-58, HEX for HPV-56, ROX for HPV-53, and CY5 for HPV-52.
3. The existence of exponential curves in the fluorescence channels signified the identification of particular high-risk HPV genotypes. In cases of multiplex infections, multiple curves were detected.

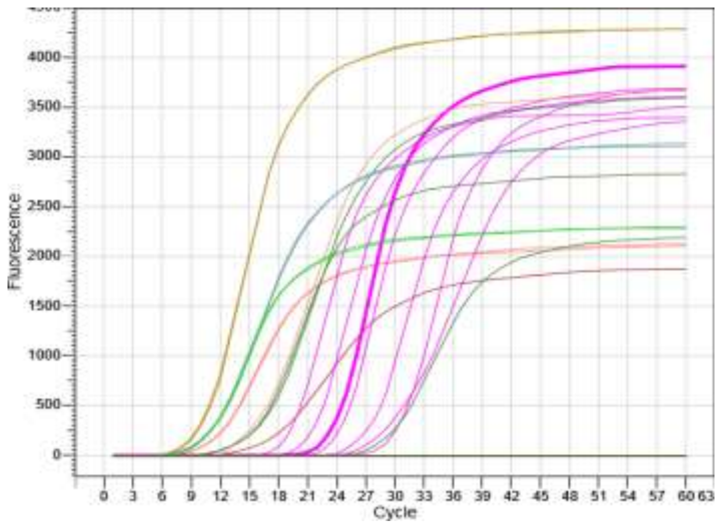

Figure 2: PCR CURVES

3.12. Demographic and Clinical Data Collection

Participant demographic details, such as age, were obtained from previously completed structured questionnaires. HIV status was extracted from documented clinical or laboratory test results available in the archived records.

3.13. Study Variables

3.13.1. Dependent variables

Penile samples were examined to determine whether high-risk HPV infection was present. For those samples that tested positive, the exact hrHPV genotypes were characterized.

3.13.2. Predictor variable

Age was analyzed both as a continuous variable and categorized into predefined age groups. HIV status was classified as either HIV-negative or HIV-positive based on confirmed laboratory results.

3.14. Data Analysis

To determine the overall prevalence, the number of individuals who carried one or more high-risk HPV genotypes was calculated based on laboratory genotyping results obtained using the AmpFire HPV detection assay. Descriptive statistics were then applied to summarize the distribution of specific hrHPV genotypes among the positive cases. The frequency and percentage of each genotype were tabulated to identify the most commonly circulating types.

Age was analyzed both as a continuous variable and within defined age categories to evaluate its association with hrHPV infection. This relationship was first explored using bivariate analysis with Chi-square tests, and subsequently assessed through multivariable logistic regression to account for possible confounding factors. The association between HIV status and hrHPV infection was also studied using Chi-square tests, comparing infection rates between HIV-positive and HIV-negative individuals.

All statistical analyses were conducted in SPSS to ensure rigorous and standardized data handling.

3.15. Ethical Consideration

This study received ethical clearance from the Rwanda National Ethics Committee under approval number 100/RNEC/2020. The research involved the analysis of archived penile swab specimens and previously documented demographic and clinical information, all obtained during an earlier study. At the point of original collection, participants provide written consent that explicitly allows the future use of their de-identified data in related scientific investigations. To ensure strict discretion, data access was limited exclusively to authorized members of the research team, and all personal identifiers were removed before analysis.

CHAPTER 4. RESULTS

4.1. Prevalence of hrHPV Infection Among Men who have sex with Men in Kigali

Among the 248 individuals analyzed, HrHPV was detected in 94 of 248 participants (37.9%), whereas 62.1% showed no evidence of infection. This proportion indicates that nearly four out of every ten participants were infected with hrHPV, underscoring its considerable burden in the study population. These results highlight the importance of strengthening HPV screening and prevention initiatives, particularly within the men who have sex with men community in Kigali, Rwanda.

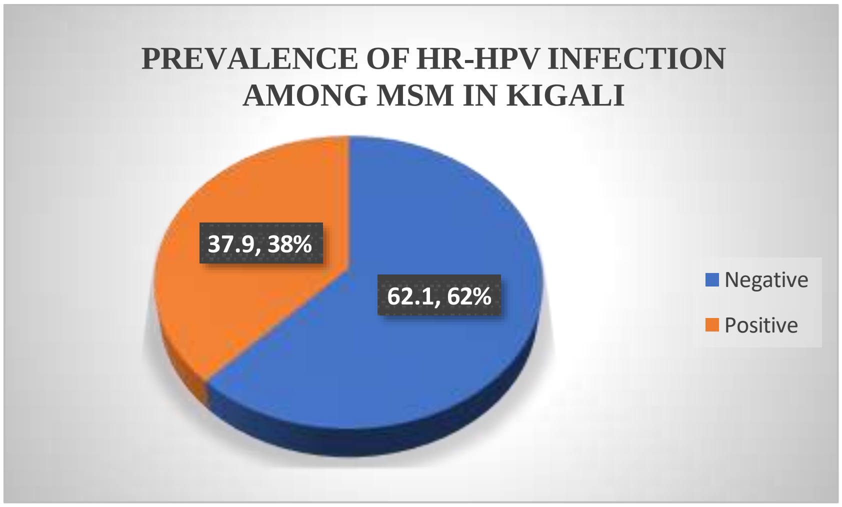

Figure 3: Prevalence of hrHPV Infection among MSM in Kigali.

4.2. Genotypic Profile of hrHPV Genotypes among Men who have sex with Men in Kigali

HPV-16 was the most frequently detected genotype, identified in 10 participants (10.6%), followed by HPV-52 in 8 participants (8.5%) and HPV-39 in 6 participants (6.4%). Other genotypes detected included HPV-56 in 5 participants (5.3%), HPV-59 in 5 participants (5.3%), HPV-35 in 4 participants (4.3%), HPV-45 in 4 participants (4.3%), HPV-66 in 2 participants (2.1%), HPV-31 in 2 participants (2.1%), and HPV-18, HPV-33, HPV-53, and HPV-58 each in 1 participant (1.1%). Multiple HPV infections were also identified, although each specific combination occurred in only one participant (1.1%). Notably, HPV-16 appeared both as a single infection and in combination with other types, confirming its predominant role in this population.

Table 3: HPV Genotype Distribution among MSM in Kigali.

HPV Genotype	**Frequency (N)**	**Percent (%)**
Single infections		
HPV-16	10	10.6
HPV-52	8	8.5
HPV-39	6	6.4
HPV-56	5	5.3
HPV-59	5	5.3
HPV-35	4	4.3
HPV-45	4	4.3
HPV-66	2	2.1
HPV-31	2	2.1
HPV-18	1	1.1
HPV-33	1	1.1
HPV-53	1	1.1
HPV-58	1	1.1
Multiple infections		
HPV-18, 52	1	1.1
HPV-18, 51	1	1.1
HPV-39, 53	1	1.1
HPV-31, 45, 53	1	1.1
HPV-31, 53, 66	1	1.1
HPV-31, 59	1	1.1
HPV-39, 45	1	1.1
HPV-39, 56	1	1.1
HPV-39, 56, 66	1	1.1
HPV-39, 66, 68	1	1.1
HPV-39, 58	1	1.1
HPV-45, 52, 59, 68	1	1.1
HPV-45, 53, 59	1	1.1
HPV-51, 66	1	1.1
HPV-52, 53	1	1.1
HPV-53, 66	1	1.1
HPV-53, 68	1	1.1
HPV-56, 58	1	1.1
HPV-58, 59	1	1.1
HPV-16, 66	1	1.1
HPV-16, 18, 35, 45, 51, 53, 58, 59	1	1.1
HPV-16, 18, 51	1	1.1
HPV-16, 18, 51, 59	1	1.1
HPV-16, 18, 52, 53	1	1.1
HPV-16, 18, 66	1	1.1
HPV-16, 31, 51	1	1.1
HPV-16, 35	1	1.1

HPV-16, 39, 51	1	1.1
Total	**94**	**100**

4.3. Association of hrHPV Infection with Age in Men who have sex with Men in Kigali

The logistic regression model assessing the association between age and hrHPV infection revealed that age group was not a statistically significant predictor. Specifically, the age group variable yielded a p-value of 0.261, which exceeds the conventional cutoff for statistical significance set at 0.05. This proposes that the likelihood of testing positive for hrHPV does not differ significantly across age categories.

Despite the lack of statistical significance, the odds ratio 1.324 indicates a trend toward increased risk of HPV infection in older age groups. When age was analyzed as a continuous variable, the regression coefficient was 0.044, suggesting a modest increase in the odds of hrHPV positivity with each additional year of age. However, this association also failed to reach statistical significance, with a p-value of 0.074. Although the corresponding odds ratio of 1.045 points to a slight upward trend, it should be interpreted cautiously given the non-significant result.

Table 4: Association of hrHPV Infection with Age

Variable	**B (Coefficient)**	**p-value**	**Odds Ratio**
Age Group	0.281	0.261	1.324
AGE	0.044	0.074	1.045
Constant	-0.856	0.014	0.425
Constant (Age)	-1.69	0.013	0.184

4.4. Association Between hrHPV Infection and HIV Status

A Pearson Chi-Square test was performed to assess the association between the variables, yielding a value of 6.409 with 1 degree of freedom and a p-value of 0.011, indicating a statistically significant association at the conventional 0.05 level. Since some expected cell counts may have been small, Fisher's Exact Test was also calculated, producing a p-value of 0.014, which similarly confirms a significant association. The analysis included 248 valid cases.

Table 5: Association Between hrHPV Infection and HIV Status in MSM in Kigali.

Test	**Value**	**Degrees of Freedom (df)**	**p-value**
Pearson Chi-Square	**6.409**	**1**	**0.011**
Fisher's Exact Test			**0.014**
N of Valid Cases	**248**		

CHAPTER 5: DISCUSSION

5. 1. Prevalence of High-Risk HPV Infection Among MSM in Kigali

This study revealed that 37.9% of penile samples from men who have sex with men (MSM) in Kigali tested positive for high-risk HPV (hrHPV). Such a proportion indicates a considerable burden of hrHPV within this community. The observed prevalence aligns with regional findings, where rates range from 47% in South Africa (Müller et al., 2016). Similarly, research conducted in Kenya reported a prevalence of 51.3 %, with HPV-16, HPV-52, and HPV-35 as the most frequently detected genotypes (Oo et al., 2023). These results highlight the urgent need to strengthen HPV prevention and screening efforts tailored specifically to MSM in Rwanda, a group often excluded from national HPV programs.

5.2. Identification and Distribution of hrHPV Genotypes among MSM in Kigali

Among hrHPV-positive participants, HPV-16 was the most frequent genotype (10.6%), followed by HPV-52 and HPV-39. These findings are consistent with studies in other African contexts, where HPV-16 consistently dominates, although HPV-39 and HPV-52 also appear commonly. Multiple concurrent infections were detected, but each combination was rare. The diversity of circulating genotypes may be influenced by behavioral patterns, limited vaccine coverage, and the structure of sexual networks within the MSM community in Rwanda. For example, the elevated detection of HPV-39 in our study might be attributed to limited vaccine coverage or differences in sexual network structures within local MSM communities. The occurrence of multiple concurrent hrHPV infections in some participants further supports findings from other African studies. In Tanzania, MSM frequently harbored multiple high-risk genotypes, often with HPV-16 co-infected alongside HPV-39 or HPV-52 (Nyitray et al., 2022). Comparable observations have been reported in Nigeria, where co-infections involving HPV-16 and genotypes such as HPV-35, HPV-45, and HPV-56 were documented among MSM (de Zambotti, 2016). The presence of multiple genotypes is clinically important, as it may elevate the likelihood of persistent infection and accelerate progression to precancerous or malignant lesions, particularly in immunocompromised individuals.

5.3. Association Between Age and hrHPV Infection Among MSM in Kigali

No statistically significant association was found between age and hrHPV infection, although a slight trend suggested higher prevalence in older participants. This absence of significance may be explained by the relatively small sample size and the predominance of younger individuals in the study cohort. While age did not appear to be a major predictor in this group, future research with larger and more age-diverse samples, ideally using longitudinal designs, is needed to clarify the role of age in HPV acquisition and persistence.

5.4. Association Between hrHPV Infection and HIV Status among MSM in Kigali

A significant relationship was identified between HIV status and hrHPV infection. Men who have sex with men living with HIV were more likely to test positive for hrHPV, underscoring the effect of immunosuppression on HPV persistence. This association echoes previous studies in the region. For instance, research conducted in Uganda showed significantly higher HPV prevalence among HIV-positive MSM compared to those who were HIV-negative (Nakigozi et al., 2024). Immunosuppression linked to HIV infection is thought to hinder the clearance of HPV, allowing infections to persist and increasing the risk of cancers such as penile and anal cancers (Verma et al., 2017).

5.5. Implications

The findings of this study reveal a significant gap in Rwanda’s HPV prevention efforts. Despite the high burden of hrHPV among MSM, current programs remain largely female-focused, overlooking the needs of this high-risk group. The absence of targeted prevention strategies contributes to continued vulnerability, particularly among HIV-positive MSM, who face greater challenges in clearing infections. These results underscore the need for more inclusive approaches to HPV control in Rwanda.

6. LIMITATIONS

This study has several limitations that should be considered when interpreting its findings. First, the sample size was relatively small and demographically limited, with a majority of participants falling within the 19-29 age bracket. This concentration of younger individuals may reduce the applicability to a broader cohort of the results to wider MSM people in Rwanda and restrict the ability to detect age-specific patterns in HPV infection and genotype prevalence. The limited representation of older adults may have masked potential associations between increasing age and persistent HPV infection, a phenomenon known to vary across the lifespan.

Second, the cross-sectional nature of the study restricts the ability to conclude causality or the timeline of infection. Because participants were only observed at a single point in time, it is not possible to determine whether the detected hrHPV infections were recently acquired or long-standing. Longitudinal research that follows individuals over time would be more appropriate for examining persistence, clearance, and risk progression associated with different HPV genotypes.

Another methodological limitation concerns the use of the AmpFire HPV Genotyping Kit, which is restricted to detecting a panel of 15 well-established hrHPV types. While this panel includes the most common oncogenic strains, it does not account for less frequent or newly emerging genotypes. Consequently, the true diversity of circulating hrHPV types may have been underestimated, potentially missing novel or region-specific variants with public health significance.

Selection bias may also have influenced the results. Since the study relied on archived samples and partially on self-reported demographic or clinical data, there is a risk that the sample does not fully reflect the wider MSM community. Participants who agreed to provide specimens or who were retained in previous datasets may differ in important ways from those who were not included, potentially skewing the findings.

Lastly, the study was geographically constrained to a single area, which may not capture regional variability in HPV epidemiology across Rwanda. Factors such as healthcare access, sexual behavior, and vaccination uptake can differ by location and influence genotype distribution and prevalence. Broader geographic sampling in future studies would be necessary to build a more representative national profile of HPV among MSM.

7. CONCLUSION

The findings offer essential localized evidence on both the prevalence and molecular diversity of penile hrHPV among men who have sex with men in Rwanda, addressing a previously underexplored area in national HPV research. The findings confirm a substantial burden of hrHPV within this population and reinforce the urgent need for targeted prevention strategies. HPV-16 emerged as the most prevalent genotype, in line with its well-established role in anogenital carcinogenesis.

A particularly noteworthy observation is the elevated prevalence of hrHPV among individuals living with HIV, highlighting the intersection of these two infections and the necessity for integrated public health responses. To reduce the long-term health consequences associated with HPV, national vaccination and screening initiatives must be adapted to include MSM, especially those who are immunocompromised.

Finally, this study underscores the importance of further investigations using broader and more demographically varied cohorts. Future research should aim to clarify the role of age and other socio-demographic variables in shaping HPV infection risk, which will contribute to the design of inclusive and evidence-based intervention strategies in Rwanda.

8. RECOMMENDATIONS

1. The Ministry of Health (MoH) and Rwanda Biomedical Center (RBC) should broaden HPV vaccination to include men who have sex with men. Given the 37.9% prevalence of high-risk HPV observed in this study, especially the predominance of HPV-16, a genotype covered by existing vaccines, there is a clear need to expand Rwanda's HPV immunization efforts to include MSM. Prioritizing individuals in the young age group, which showed the highest representation in the sample, may help curb transmission and reduce the incidence of HPV-related cancers. Targeted immunization of this high-risk demographic could substantially lower the burden of disease in MSM populations.
2. Healthcare providers and sexual health clinics should implement routine screening for high-risk HPV (hrHPV) among men who have sex with men. The elevated rate of hrHPV infections among HIV-positive men who have sex with men, as identified in this study, supports the need for regular HPV screening in this subgroup. Integrating penile HPV testing into routine sexual health services for men who have sex with men, particularly those living with HIV, would allow for earlier identification and clinical management of high-risk infections. The use of

non-invasive sample collection methods, such as those employed in this research, can increase the accessibility and acceptability of screening programs, potentially improving uptake.

3. Public health authorities, NGOs, and community-based organizations (CBOs) should expand education and awareness campaigns tailored to MSM. There is a pressing need for focused public health campaigns that inform MSM about the connection between HPV and HIV and encourage proactive health-seeking behaviors. Educational initiatives should address common misconceptions, reduce stigma, and promote awareness of the importance of vaccination and regular screening. Increasing knowledge within the MSM community will empower individuals to make informed decisions and foster safer sexual practices.
4. MoH, hospital administrators, and training institutions should improve access to men who have sex with men-sensitive healthcare services. Barriers to care remain a major challenge for men who have sex with men, particularly in accessing HPV-related services. It is essential to train healthcare professionals in providing culturally competent and non-discriminatory care to this population. Establishing dedicated health centers or drop-in clinics tailored for MSM can create safer, more inclusive environments that encourage service utilization. Such changes are critical to increasing vaccination rates and early detection of HPV-related conditions.
5. Research institutions, universities, and funding agencies should support larger and longitudinal studies on HPV among men who have sex with men. To gain a more complete picture of HPV infection dynamics among men who have sex with men in Rwanda, future research should involve larger, more diverse samples and adopt longitudinal study designs. Including participants from different age ranges, geographic regions, and socioeconomic backgrounds would allow researchers to explore how various factors influence infection risk, persistence, and response to public health interventions. Longitudinal follow-up would also help identify patterns over time and provide evidence for more effective, targeted strategies.

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