IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF HUMAN PAPILLOMAVIRUS INFECTION AMONG WOMEN LIVING WITH AND WITHOUT HIV IN SELECTED HEALTH FACILITIES IN LIMPOPO PROVINCE, SOUTH AFRICA

By

Rixongile Rhenny Rikhotso (11604205)

A thesis submitted in fulfillment of the requirements for the award of the degree of Doctor of Philosophy (PhD) in Microbiology

To the Department of Biochemistry and Microbiology,
Faculty of Science, Engineering and Agriculture
University of Venda
Private bag X5050
Thohoyandou
0950
South Africa

Supervisor: Prof Pascal Obong Bessong
University of Venda, South Africa

Co-supervisor: Prof Emma McKim Mitchell
University of Virginia, United States of America

2025

© University of Venda

DECLARATION

I, **Rikhotso Rixongile Rhenny**, hereby declare that this thesis for the award of Doctor of Philosophy in Microbiology at the University of Venda, hereby submitted by me, has not been submitted for a degree at this or any other University, and that it is my work in design and execution and that all the reference materials contained therein have been duly acknowledged.

Student: Rikhotso Rixongile Rhenny (11604205)



Signature:……. **Date:** 08 July 2025

© University of Venda

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude to all the study participants and clinicians whose contributions were invaluable to the collection of samples. Their support and dedication played a crucial role in the success of this work.

This PhD program has been a life-challenging experience, comprising both my triumphant moments and lowest points during which I often contemplated giving up. However, amidst it all, I am eternally thankful to Almighty God for continuously safeguarding and ensuring my sanity, blessing me with excellent health, and granting me wisdom. His divine protection throughout this journey is beyond words. When I think of where I come from, I cannot help, but wholeheartedly shower Him with praise and profound gratitude. Glory to Almighty God!!

To my amazing and dear brother, Rodney Rikhotso, as I type these words, tears run down my cheeks. The unwavering support you have provided me throughout my academic journey will forever hold a special place in my heart. I express my deepest appreciation to you. Thank you so much for always believing in my dreams and never doubting my abilities. Your PRAYERS, attentive ears, and unending support have been my hiding place, a place of safety during times of need. We did it again “nwana mhani na papa” (child of my father and my mother). I love you so much.

To my supervisor, Professor Pascal Obong Bessong, I express my sincere gratitude for the opportunity and guidance you have provided me since I enrolled for this PhD program until its completion. This journey was not without its challenges, tears were shed but you never gave up on me. Your words of encouragement, valuable support and wise scientific input sustained me throughout. I will forever carry with me all the valuable life lessons, skills and experiences gained under your supervision and guidance. I am who I am today because of you. Thank you, Professor, “Ndza khensa” (I thank you).

To my co-supervisor Professor Emma McKim Mitchell, your kindness and sweetness knows no bounds. I extend my most heartfelt gratitude to you Prof, for your unwavering patience throughout this journey. You consistently made time for me despite your extremely busy schedule. Your invaluable scientific contributions played a huge role in the success of this work. Thank you sincerely for all you have done for me Prof.

To my incredible family, you have been nothing less, but the epitome of excellence from day one until the completion of my doctoral program. I am profoundly grateful for the unending

© University of Venda

support you have shown, both emotionally and financially. I extend special appreciation to my aunties (Vusiwana Rikhotso, Rirhandzu Rikhotso, Tinyiko Rikhotso, Grace Rikhotso (deceased), Dalcy Ndzeru, Elsinah Ndzeru and Mayda Ndzeru), my sisters (Dineo Rikhotso and Muswa Rikhotso), my brother (Mandla Rikhotso), and my uncles (Kenneth Ndzeru and Gara Ndzeru). You are all amazing.

To my remarkable grandmother Phophi Ndzeru, I am filled with immense joy that I managed to complete my program while you are still with us, allowing me to express my appreciation while you can witness it. Thank you so much for all the life lessons and unconditional love you have shown me from day one. You are truly amazing, and I am profoundly grateful. “Ndia livhuwa, Aa” (I am grateful to you).

This one is very difficult. In loving memory of my grandmother, Mphephu Fokisa Rikhotso, who passed away two days before my birthday **(23 April 2023)** and, more sadly, before I handed in my thesis and graduated. I express my gratitude for the everlasting support and love she bestowed upon me. Her unwavering belief in my dreams will forever be cherished. Continue resting in peace my Anne.

To the members of the HIV/AIDS and Global Health Research Program at the University of Venda, I wish to express my profound and heartfelt gratitude to each one of you for being part of my academic journey. I hold dear all the scientific input you have provided, which has played an important role in the success of my work.

I would also like to extend my deepest appreciation to my amazing friends, Tinyiko Rikhotso, Gudani Managa, and Vutomi Hlungwane, for consistently carrying my right hand throughout both my shiny and rainy days. Your unwavering support has been a true definition of sisterhood.

To Risala Rikhotso, my wonderful daughter who holds a very special place in my heart. You came into my life at the appropriate time. Your contagious laughter, smile and our unending video calls strengthened me daily to see this work through to its completion. Thank you! As you grow older, there will come a time when you read and understand this message. PLEASE, when I am no more, engrave this thesis into your heart and take it to another level. I love you immensely.

© University of Venda

DEDICATION

*This thesis is dedicated to three special people in the entire universe, my guardian angels in heaven: My mother (**Veronica Latani Rikhotso)**, my father (**Robert Gezani Rikhotso)** and my princess (**sister**), whom I never had an opportunity to meet nor hold. Thank you all for always watching over me. Until we meet again, for now, may your beautiful souls continue resting in perfect peace.*

I LOVE YOU ALL….

© University of Venda

ABSTRACT

Background: Co-infection of human papillomavirus (HPV) and human immunodeficiency virus (HIV) has been well established. Both viruses are sexually transmitted with paramount public health implications due to their interaction with cervical cancer (CC). Globally, the estimated prevalence of HPV is 11.7%, with high-risk (hr-) HPV 16 and HPV 18 attributing for most cases of CC. Due to the high genetic diversity of HPV, it is important to study the virus in different geographic locations, people, and in persons living with HIV, to inform the selection of genes for vaccine improvements and potential vaccine trial sites. Consequently, it is important to describe the prevalence, genotype distribution and genomes of HPV in Limpopo Province, where there is limited data. The general objective of this study was to therefore to identify and characterize HPV DNA among women living with and without HIV in selected health facilities in Limpopo Province, South Africa. The specific objectives were: (1) To provide a narrative literature review on the prevalence and distribution of selected cervical HPV genotypes among women living with and without HIV in South Africa; (2) To determine the prevalence of cervical HPV infection among women living with and without HIV in selected health facilities in Limpopo Province, South Africa; (3) To describe HPV genotypes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa; and (4) To generate and characterize HPV full genomes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.

Methods: A systematic review of the available literature was conducted in the quest to understand and determine the prevalence and distribution of cervical HPV among women living with HIV (WLWH) and those without HIV in South Africa, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) workflow. Records were retrieved from PubMed and Web of Science databases.

An analytic cross-sectional study on HPV prevalence and genotype distribution among 450 women who were living with and without HIV from selected health facilities in Limpopo Province was done. In this regard, total DNA was purified from cervical specimens and amplified using a double-nested polymerase chain reaction (PCR) strategy, targeting the L1 gene. A sample that shows an amplification product of 450 bp in the first nested PCR or a product of 150 bp in the second nested reaction was taken as positive for the presence of HPV DNA. Statistical analysis, using models in R-statistical package, was done to determine comparative inferences of the presence of HPV DNA and associated risk factors. HPV

© University of Venda

genotypes were determined through deep sequencing of the 450 bp PCR products on an Illumina MiniSeq platform. The generated sequences were quality assured and analyzed for viral genotypes.

Rolling circle amplification (RCA) was used for enriching DNA in samples that tested positive for HPV DNA to generate and characterize near-full length HPV genomes. The enriched DNA was purified with AMPure XP beads and quantified with Qubit, followed by DNA library preparation, and then sequenced on an Illumina MiniSeq platform following the manufacturer's protocols.

Results

Prevalence and distribution of selected cervical HPV genotypes in South Africa

A total of sixty-nine (69) articles met the inclusion criteria for determining the prevalence and distribution of cervical HPV genotypes among South African women who were either living with HIV or not. These articles were on studies done in 5 of the 9 South African Provinces. The studies were conducted between 1989-2021. Sequencing was the least genotyping tool used in these studies, while most studies utilized PCR-based hybridization commercial kits. Compared to other investigated genotypes, HPV 16, HPV 18, and HPV 35 were predominant in the study population regardless of the HIV status.

Prevalence of HPV DNA and genotype distribution in selected health facilities in Limpopo Province, South Africa

The HPV DNA was detected in cervical specimens from 147 of 450 women (32.7%), significantly higher at 52.21% ($p = 0.00$) among WLWH compared to women living without HIV. Forty-eight samples were of acceptable quality and analyzed for viral diversity. The study detected 41 HPV genotypes, all of which belong to the *Alphapapillomavirus* genus of the *Papillomaviridae* family. In general, HPV 45 (16.7%) was the predominant genotype. However, HPV 81 (18.8%) and HPV 56 (25.0%) were the most common genotypes among women living with and without HIV, respectively. Multiple infections and high-risk HPV genotypes were more common among WLWH.

Characterization of whole genomes of cervical human papillomavirus

Six full genomes of HPV genotypes 34 (n=1), 35 (n=1), 42 (n=1), 45 (n=2), and 90 (n=1) are reported herein. The study further identified variant sub-lineages, including the A1 sub-lineages of hr-HPV 35 and hr-HPV 45. The genome sequences are available at the National Center for Biotechnology Information (NCBI) in the Sequence Read Archive (SRA) database.

© University of Venda

Interpretations and conclusions

Prevalence and distribution of selected cervical HPV genotypes in South Africa

The narrative review of the literature indicated that there are limited studies on cervical HPV prevalence and genotype distribution in South Africa. Therefore, further research is required to identify HPV genotypes, including the use of sensitive approaches such as next generation sequencing. This will enhance our understanding of HPV geo-diversity patterns in various provinces across South Africa, and subsequently inform decisions on screening strategies, and selection of geographically relevant genes for vaccine improvement and development. The high prevalence of hr-HPV 16, HPV 18 and HPV 35 regardless of HIV status in the country is a public health concern, considering their significant contribution to the burden of CC. Consequently, routine HPV screening and prevention measures such as HPV vaccination for all individuals remain critical. Because current vaccines exclude HPV 35 as a target, there is a need to consider this genotype in future vaccine development efforts.

Prevalence of HPV DNA and genotype distribution in selected health facilities in Limpopo Province, South Africa

The study reported a relatively high prevalence of HPV infection among the study participants, which signified the burden of HPV in the province. The prevalence was significantly higher among WLWH, including more HPV genotypes and hr-HPV genotypes as compared to women living without HIV, potentially due to immunosuppression. Considering the high rate of HPV-HIV co-infection observed in this study and other studies in South Africa, prioritizing women living with HIV for cervical HPV screening and HPV vaccination is recommended. Strengthening integrated HPV and HIV screening programs and ensuring regular monitoring of cervical health among WLWH remains a public health imperative. Moreover, the study also showed that women living with and without HIV may have different profiling in terms of HPV infections which is important in intervention strategies, example development of targeted HPV screening strategies. The current study also fills a significant gap of limited data by providing baseline information on the prevalence and genotype distribution of cervical HPV infection among women living with and without HIV in the study area. The study findings further encourage national-level surveillance with the inclusion of expanded regional representation.

Characterization of whole genomes of cervical HPV

Near full-length genomes of six HPV genotypes were successfully characterized in the study population. These sequences could serve as a valuable resource for further research and vaccine development studies. The identified variant sub-lineages may underscore the need to strengthen HPV vaccine coverage, routine cervical screening, and genomic monitoring in

© University of Venda

underrepresented areas such as Limpopo Province. Moreover, this data supports the development of risk stratification models aimed at identifying women at high-risk for persistent infection and progression to CC, thereby enabling early, tailored interventions to reduce the HPV related disease burden.

Keywords: Human papillomavirus; Genotypes; Genomes; Human immunodeficiency virus; Limpopo Province

© University of Venda

SCIENTIFIC COMMUNICATIONS FROM THIS WORK

Published article

Rixongile R. Rikhotso, Emma M. Mitchell, Daniel T. Wilson, Aubrey Doede, Nontokozo D. Matume, Pascal O. Bessong. Prevalence and distribution of selected cervical human papillomavirus genotypes in HIV infected and HIV uninfected women in South Africa, 1989-2021: A narrative review. *Southern African Journal of Infectious Diseases*. 2022 Jun;*37*(1). pp.1-10.

Submitted article

Rixongile R. Rikhotso, Emma M. Mitchell, Pascal O. Bessong. Prevalence and genotype distribution of cervical human papillomavirus among women living with and without HIV in selected health facilities in Limpopo Province, South Africa. *Southern African Journal of Infectious Diseases.*

Conference presentations

Rixongile Rhenny Rikhotso, Emma Mitchell, Pascal Bessong. Prevalence and distribution of HPV genotypes covered by Gardasil-9 vaccine among women residing in the Limpopo Province, South Africa (**Oral presentation).** Faculty of Science, Engineering and Agriculture's Research Celebration, University of Venda, Research Conference Centre, Thohoyandou Limpopo Province, South Africa. 27-28 June 2022.

Rixongile Rhenny Rikhotso, Samuel Mark Eger, Emma McKim Mitchell, Pascal Obong Bessong. Next Generation Sequencing Analysis of HPV L1 gene to determine HPV types in HIV infected and HIV non-infected in northern South Africa **(Poster Presentation).** Minority Health International Research Training (MHIRT) Summer Research Symposium. Corner Building, University of Virginia, Charlottesville, United States of America. 2 August 2019.

Rixongile Rikhotso, Emma Mitchell, Pascal Bessong. Prevalence of human papillomavirus DNA and risk factors among HIV infected and HIV non-infected women residing in Vhembe and Capricorn districts, Limpopo Province, South Africa **(Poster presentation).** Emerging Issues in Oncogenic Virus Research. San Pietro, Bevagna (Manduria), Italy. 7-12 June 2022.

Rixongile Rhenny Rikhotso, Emma McKim Mitchell, Pascal Obong Bessong. Prevalence and genetic characterization of cervical Alpha-papillomaviruses in a cohort of HIV infected and

© University of Venda

HIV uninfected women in Limpopo Province, South Africa (**Poster presentation**). The South African Society of Biochemistry and Molecular Biology (SASBMB). Protea Hotel, Ranch Resort, Polokwane, South Africa. 7-10 July 2024.

© University of Venda

University of Venda
Creating Future Leaders

TABLE OF CONTENTS

DECLARATION i
ACKNOWLEDGEMENTS ii
DEDICATION iv
ABSTRACT v
SCIENTIFIC COMMUNICATIONS FROM THIS WORK ix
TABLE OF CONTENTS xi
LIST OF FIGURES xv
LIST OF ABBREVIATIONS AND ACRONYMS xviii
STRUCTURE OF THE THESIS xx
CHAPTER ONE 1
1.1 GENERAL INTRODUCTION 2
1.2 PROBLEM STATEMENT AND RATIONALE 3
1.3 STUDY HYPOTHESIS 5
1.4 RESEARCH QUESTIONS 5
1.5 MAIN OBJECTIVE 5
1.5.1 Specific objectives 5
1.6 REFERENCES 7
CHAPTER TWO 14
ABSTRACT 15
2.1 INTRODUCTION AND STUDY RATIONALE 16
2.2 RESEARCH QUESTIONS 17
2.3 MAIN OBJECTIVE 18
2.3.1 Specific objectives 18
2.4 MATERIALS AND METHODS 18
2.4.1 Eligibility criteria 18
2.4.2 Information source 18
2.4.3 Search strategy 18
2.4.4 Selection process and study risk of bias assessment 19
2.4.5 Data collection process 20
2.4.6 Data items 20
2.4.7 Synthesis method 21
2.4.8 Analysis for human papillomavirus genotypes prevalence and distribution in South Africa 29

© University of Venda

2.4.9 Statistical analysis 30
2.5 RESULTS 30
2.5.1 Characteristics of analyzed studies 30
2.5.2 Sample type, collection mode, and human papillomavirus genotyping approaches 31
2.5.3 High-risk human papillomavirus genotype distribution and human immunodeficiency virus status 31
2.6 DISCUSSION 35
2.7 REFERENCES 40
CHAPTER THREE 56
ABSTRACT 57
3.1 INTRODUCTION AND STUDY RATIONALE 58
3.2 LITERATURE REVIEW 59
3.2.1 HPV and cervical cancer 59
3.2.2 Classification of human papillomavirus 59
3.2.3 HPV virion, genome structure and organization 61
3.2.4 Viral proteins and their functions 62
3.2.5 Epidemiology of HPV and cervical cancer 62
3.2.5.1 General population 62
3.2.5.2 Normal cervical cytology 63
3.2.5.3 Cutaneous warts 63
3.2.5.4 Ano-genital warts 63
3.2.5.5 Cervical cancer (CC) 64
3.2.6 Risk factors for HPV infection 64
3.2.7 Routes of transmission 65
3.2.7.1 Horizontal transmission 65
3.2.7.2 Vertical transmission 66
3.2.8 Vaccines for HPV 66
3.2.8.1 South Africa and HPV vaccination 66
3.2.8.2 The HPV vaccination rollout programs in South Africa 67
3.2.9 The HPV detection methods 68
3.2.9.1 Nucleic acid-hybridization assays 69
3.2.9.2 Signal-amplification assays 69
3.2.9.3 Nucleic acid-amplification assays 69
3.2.9.3.1 Polymerase chain reaction (PCR) 69
3.2.9.3.2 The linear array® (LA) genotyping method 70
3.2.9.3.3 Real-time PCR and Abbott real-time PCR 70

© University of Venda

3.2.9.3.4 INNO-Lipa 70
3.2.9.4 HPV sequencing 71
3.2.10 Summary of HPV research over the years 71
3.3 HYPOTHESIS 72
3.4 RESEARCH QUESTIONS 72
3.5 MAIN OBJECTIVE 72
3.6 SPECIFIC OBJECTIVES 72
3.7 MATERIALS AND METHODS 73
3.7.1 Study design and sample size 73
3.7.2 Study area, study population and participant recruitment 73
3.7.3 Inclusion and exclusion criteria 74
3.7.4 Data collection 74
3.7.5 Specimen collection and processing 74
3.7.6 DNA extraction 74
3.7.7 Detection of HPV DNA by double nested PCR 75
3.7.8 Next generation sequencing with the Illumina Miniseq platform for HPV genotyping 76
3.7.9 Sequences control check 76
3.7.10 Data analysis 77
3.7.10.1 Statistical analysis 77
3.7.10.2 HPV Genotyping 77
3.8 RESULTS 79
3.8.1 Characteristics of the study population 79
3.8.2 Prevalence of HPV DNA 81
3.8.3 Distribution of HPV genotypes 82
3.8.3.1 Prevalence and distribution of known carcinogenic HPV genotypes 83
3.8.3.2 Prevalence and distribution of HPV genotypes incorporated in the Gardasil-9 vaccine 85
3.8.4 Risk factors associated with HPV infection 86
3.9 DISCUSSION 88
3.10 REFERENCES 92
CHAPTER FOUR 112
ABSTRACT 113
4.1 INTRODUCTION AND STUDY RATIONALE 114
4.2 LITERATURE REVIEW 114
4.2.1 Genome sequencing of HPV 114
4.2.2 HPV lineages and sub-lineages 115

© University of Venda

4.3 RESEARCH QUESTION....115
4.4 MAIN OBJECTIVE....115
4.4.1 Specific objectives....116
4.5 MATERIALS AND METHODS....116
4.5.1 Study specimens....116
4.5.2 Specimen collection, DNA enrichment and sequencing....116
4.6 DATA ANALYSIS....116
4.6.1 Genome characterization....116
4.6.2 HPV lineages or sub-lineages determination....117
4.7 RESULTS....117
4.7.1 Generated and characterized HPV genomes....117
4.7.1.1 Characterized HPV 35 genome and associated lineages/sub-lineages....118
4.7.1.2 Characterized HPV 45 genome and associated lineages/sub-lineages....119
4.7.1.3 Characterized HPV 34 genome and associated lineages/sub-lineages....121
4.7.1.4 Characterized HPV 42 and 90 genomes and associated lineages/sub-lineages....123
4.8 PHYLOGENETIC ANALYSIS OF GENERATED HPV FULL GENOMES....124
4.9 DISCUSSION....126
4.10 REFERENCES....130
CHAPTER FIVE....138

© University of Venda

LIST OF FIGURES

Chapter 1

Figure 1.1: Illustration of ano-genital cancers caused by human papillomaviruses ranging from cervical to anal cancers 2

Chapter 2

Figure 2.1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA). 21

Chapter 3

Figure 3.1: Classification of human papillomavirus highlighting the 5 individual genera belonging to papillomaviridae family 61

Figure 3. 2: Illustration of HPV genotype 16 circular genome organization 62

Figure 3.3: The HPV genotypes and their estimated percentage of CC and genital warts cases covered by Cervarix and Gardasil-4 67

Figure 3.4: Illustration of the HPV classification test utilized to HPV detection 68

Figure 3.5: Illustration of human papillomavirus research over time 72

Figure 3.6: Map indicating Vhembe and Capricorn districts 74

Figure 3.7: Illustration of the study methodology, from sample collection through sequence analysis.....78

Figure 3.8: Representation of the gel UV visualization of the nested PCR amplicons on 2.5% agarose gel.....81

Figure 3.9: Representation of the gel UV visualization of the double-nested PCR amplicons on 2.5% agarose gel. 81

Figure 3.10: The distribution of 41 HPV genotypes detected in a study population.....82

Figure 3.11: The frequency distribution of HPV genotypes by HIV status.....83

Figure 3.12: The distribution of carcinogenic HPV genotypes (x-axis) and their frequencies (y-axis) in a study population 84

Figure 3.13: The carcinogenic HPV genotype distribution among the HIV negative (blue) and HIV positive (brown) group.....84

Figure 3.14: The occurrence of HPV genotypes covered by the Gardasil-9 HPV vaccine among women in a study population. 85

Figure 3.15: The distribution of HPV genotypes incorporated in Gardasil-9 vaccine among women living with and without HIV in the study population.....86

© University of Venda

Chapter 4

Figure 4.1: A representative of read distribution and coverage in study sequence. 118
Figure 4.2: A circular representation of HPV 35 genome (7,880 bp), sample ORHC-103...118
Figure 4.3: A rooted phylogenetic tree between study consensus sequence genome and reference sequences. 119
Figure 4.4: A circular representation of HPV 45 genome (7,859 bp), sample ORHC-031...120
Figure 4.5: A circular representation of HPV 45 genome (7,858 bp), sample ORHC-005...120
Figure 4.6: A rooted phylogenetic tree showing a relationship between HPV 45 study sequences and downloaded reference sequences.. 121
Figure 4.7: A circular representation of HPV 34 genome (7,747 bp), sample OSHC-183…122
Figure 4.8: A phylogenetic tree illustrating a relationship between HPV 34 study sequence and downloaded reference sequences. 123
Figure 4.9: A circular representation of HPV 42 genome (7,894 bp), sample ODF-035.....124
Figure 4.10: A circular representation of HPV 90 genome (8,063 bp), sample ODF-007…124
Figure 4.11: Phylogenetic analysis of HPV genomes of genotypes 34, 45, 42, 45 and 90 and HPV reference sequences.....125

© University of Venda

LIST OF TABLES

Chapter 2

Table 2.1: Characteristics of the 69 articles analyzed to achieve objective 1 and 2 23
Table 2.2: Human papillomavirus studies used to achieve objectives 3 and 4 29
Table 2.3: Estimated prevalence of 14 known hr-HPV genotypes across 46 studies regardless of HIV status in South Africa 32
Table 2.4: Estimated prevalence of known 14 hr-HPV and 2 lr-HPV genotypes (incorporated in Gardasil 9) with HIV status in South Africa 33

Chapter 3

Table 3.1: Functions of HPV proteins. 62
Table 3.2: Oligonucleotide specifications and target sizes 75
Table 3.3: Cycling conditions used during the first round of PCR 75
Table 3.4: Socio-demographic and clinical characteristics of the study population 80
Table 3.5: Risk factors associated with HPV infection 87

© University of Venda

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-retroviral Therapy
ASC	American Cancer Society
ASCCP	American Society for Colposcopy and Cervical Pathology
Bp	Base Pairs
CC	Cervical Cancer
CI	Confidence interval
CIN	Cervical Intraepithelial Neoplasia
DNA	Deoxyribonucleic Acid
DNTP	Deoxynucleoside Triphosphate
E1-2,4-7	Early Genes
EBV	Epstein-Bar virus
EIA	Enzyme Immune Assay
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HR	High-risk
IARC	International Agency For Research on Cancer
ICC	Invasive Cervical Cancer
Kb	Kilobase
L1-2	Late Genes
LA	Linear Array
LCR	Long Control Region
LMICs	Low to Middle Income Countries
LR	Low-risk
Min	Minute (s)
$MgCl_2$	Magnesium Chloride
NCR	Non-coding Region
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
OR	Odd ratios
ORF	Open Reading Frame
PAVE	Papillomavirus Episteme

© University of Venda

PCR	Polymerase Chain Reaction
PRB	Probable
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSB	Possible
QC	Quality Control
RCA	Rolling Circle Amplification
RFLP	Restriction Fragment Length Polymorphism
RPM	Revolution Per Minute
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for Social Sciences
SRA	Sequence Read Archive
STIs	Sexually Transmitted Infections
URR	Upstream Regulatory Region
USA	United States of America
WLWH	Women Living With HIV
VLPs	Virus-like Particles

© University of Venda

STRUCTURE OF THE THESIS

ETHICAL CONSIDERATIONS

This study received ethical approval from the Human and Clinical Trials Research Committee of the University of Venda (SMNS/20/MBY/12/1003). Permission to access public healthcare facilities was obtained from the Limpopo Provincial Department of Health (Ref 4/2/2). Informed consent was obtained from each participant prior to enrolment into the study and specimen collection. The privacy and confidentiality of each participant was protected throughout the study.

This thesis is structured as follows:

Chapter 1 is a general introduction which lays a brief foundation on HPV infection and cervical cancer (CC). It highlights the primary prevention tools, such as vaccines, available for the control of HPV infection and associated disease. The study problem statement, rationale, hypothesis, research questions and objectives are stated in this chapter.

Chapter 2 describes a narrative review of the literature on the prevalence and distribution of selected cervical HPV genotypes among women living with and without HIV in South Africa between 1989-2021. This chapter has its own abstract, introduction and study rationale, research questions, objectives, materials and methods, results, a discussion and conclusion sections. The following research questions are investigated in Chapter 2:

1. How are cervical HPV studies distributed across provinces in South Africa?
2. What detection methods are used for identifying HPV genotypes in South Africa?
3. What is the prevalence and distribution of high-risk HPV (hr-HPV) genotypes among women in a general population, and among those with normal and abnormal cervical cytology?
4. What is the prevalence and distribution of hr-HPV or lr-HPV genotypes by HIV status?

Chapter 3 deals with an analytical cross-sectional study on the prevalence of cervical HPV DNA and HPV genotype distribution among women living with and without HIV in selected health facilities in Limpopo Province, South Africa. This chapter has its own abstract, introduction and study rationale, literature review, hypothesis, research questions, objectives, materials and methods, results, a discussion and conclusion sections. The following research questions are investigated in Chapter 3:

© University of Venda

1. What is the prevalence of cervical HPV infection among women living with and without HIV in selected health facilities in Limpopo Province, South Africa?
2. What are the HPV genotypes infecting women in the study population?

Chapter 4 describes complete or near-complete HPV genomes obtained from the study population. Genomes were generated by next generation sequencing. This chapter has its own abstract, introduction and study rationale, literature review, research question, objectives, materials and methods, results, a discussion and conclusion sections. The main question investigated was, what are the characteristics of HPV genomes in selected health facilities in Limpopo Province, South Africa.

Chapter 5 presents a synthesis of the whole study, and proffered recommendations.

© University of Venda

CHAPTER ONE

INTRODUCTION AND RATIONALE OF THE STUDY

© University of Venda

1.1 GENERAL INTRODUCTION

Since the discovery of human papillomaviruses (HPVs) in the 1950s, these diverse group of viruses have been identified worldwide including South Africa (García et al., 2016; Beyazit et al., 2018; Mbulawa et al., 2018; Kuguyo et al., 2021; Zhou et al., 2024). Human papillomaviruses are a form of vastly heterogeneous double-stranded deoxyribonucleic acid (DNA) viruses belonging to the *papillomaviridae* family (Latsuzbaia et al., 2020). They are mostly transmitted sexually and are a major cause of cervical cancer (CC) (Einstein et al., 2009), among other cancers (Figure 1.1).

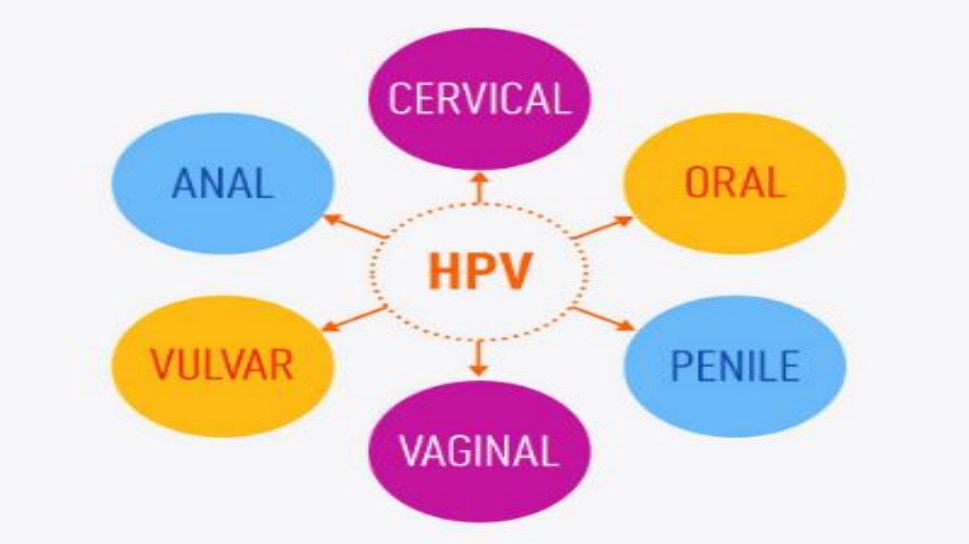


Figure 1.1: *Illustration of ano-genital cancers caused by human papillomaviruses ranging from cervical to anal cancers (Source: Kirkel, 2020).*

Worldwide, it has been estimated that 25.7 million people are living with HIV, and about 80% were reported in Southern and Eastern Africa in 2019 (Avert, 2019; WHO, 2019). Notably, the highest rates of HIV prevalence by global comparisons have been reported in South Africa, with about 8.23 million people living with the virus (Statistics South Africa, 2021). The prevalence of HIV infection among women aged between 15-49 years is about 19% (WHO, 2019; UNAIDS, 2019). Some studies have shown the existence of an association between specific cancers and HIV infection (Stelzle et al., 2021). It is in this context that CC, among other cancers, has been defined as a form of cancer associated with HIV (Chirenje et al., 2005; Moodley et al., 2006; Mbulaiteye et al., 2011; WHO, 2016). South Africa ranked fourth in the world with the highest number of CC among women living with HIV (WLWH) (Stelzle et al., 2021). It is estimated that there were about 10 702 new CC cases in 2020 among South African women, which resulted in approximately 5 870 deaths (Bruni et al., 2021). Women living with HIV have been observed to have an increased burden of multiple HPV infections, pre-cancerous lesions, and persistent HPV infections when compared to women living without HIV

© University of Venda

(Liu et al., 2018; Marembo et al., 2019). Moreover, WLWH are more susceptible to an increased HPV prevalence (Azis et al., 2023). Therefore, the growing incidence of HIV among South African women in particular, makes HPV-HIV co-infection a public health concern. Moreover, the literature highlights that about 80% of individuals who are sexually active in the general population will be infected with HPV in their lifetime (Centers for Disease Control and Prevention, 2004; Magalhães et al., 2021; Tao et al., 2022), further raising the need for continuous HPV detection for early interventions.

There is currently no cure for HPV infection beyond the current prevention measures offered by the available HPV vaccines. The Food and Drug Administration (FDA) in the United States of America (USA) has approved three therapeutic vaccines. These vaccines offer protection against selected low-risk (lr-) (HPV 6 and HPV 11) and high-risk (hr-) (HPV 16, HPV 18, HPV 31, HPV 33, HPV 45, HPV 52 and HPV 58) genotypes. However, not all countries are benefiting from these vaccines due to a number of factors, including poverty-induced constraints.

Two of the three HPV vaccines (Cervarix and Gardasil-4) are currently available in South Africa (World Health Organization, 2014). Cervarix is primarily available through a public school-based program as part of the nationwide vaccination programme (Expanded programme on immunization) launched by South Africa Department of Health, targeting Grade 4 girls, aged 9 years or older (Botha and Richter, 2015; Mbulawa et al., 2018). The shortcomings of the South African HPV school-based vaccination programme include: non-coverage for girls not in school or girls in non-public schools; and non-inclusion for boys who may also need the vaccine. Moreover, low HPV vaccine coverage in some sub-districts found within Mpumalanga and KwaZulu-Natal provinces of South Africa is one of the challenges facing this programme (Amponsah-Dacosta et al., 2022). Furthermore, regardless of the success of the high update of the vaccine in some provinces, the private sector (private schools) has been shown to have a limited uptake (Richter, 2015). A significant lack of knowledge and awareness about HPV and CC and the high cost of the vaccine contribute, in general, to the limited uptake of HPV vaccine (Richter, 2015).

1.2 PROBLEM STATEMENT AND RATIONALE

In South Africa, CC ranks second, the leading cause of cancer-associated mortality among South African women (Olorunfemi et al., 2018). Women who are living with HIV have the highest burden of CC (Dhokotera et al., 2022). In the year 2018, 63% of women who were diagnosed with CC were living with HIV, with 53% of CC cases attributable to HIV in the

© University of Venda

country (Stelzle et al., 2021), a call for concern considering the growing rates of HIV in South Africa and necessitates for more studies on HPV-HIV co-infection to promote early intervention on the affected individuals and improve future screening and vaccination tactics.

HPV infection is prevalent globally. However, viral prevalence and genotype distribution can differ by population and region (Liu et al., 2023). Globally, the estimated prevalence of HPV is 11.7%, with Latin America and Eastern Europe reporting a high prevalence of 16.1% and 21.4%, respectively. However, the highest prevalence has been reported in Sub-Saharan Africa (24.0%) (Bruni et al., 2010). The high burden of HIV in these regions may contribute to the high HPV prevalence. A South African study reported an HPV prevalence of 96.6% among women with an abnormal cytology (Taku et al., 2021). However, HPV prevalence has been shown to vary across South Africa (Richter et al., 2013; Mbatha et al., 2017; Taku et al., 2020; Mbulawa et al., 2021). Other studies show a higher prevalence of HPV among HIV seropositive individuals compared to HIV seronegative individuals (Tartaglia et al., 2017); meanwhile others report no difference between the two populations (Mbulawa et al., 2021).

Human papillomavirus genotypes 16 and 18 were reported to be more prevalent in Europe, while HPV 52 and HPV 58 were more common in Asia (Clifford et al., 2005; de Sanjosé et al., 2007; Vinodhini et al., 2012). Other reports highlight that HPV 16 and HPV 18 account for most cases of CC (Fani et al., 2020). However, other reports are showing that after HPV 16, HPV 58 is the second most prevalent cause of CC among women living with HIV (Mayaud et al., 2003). The differences in sexual behaviour, HPV vaccination rates, and cultural and socioeconomic factors could play a role in these differences (Burk et al., 1996; Kim et al., 2012; Llanos et al., 2018; Pauli et al., 2022). In Africa, HPV 35, HPV 45, and HPV 58 are prevalent in women diagnosed with squamous cell carcinoma (Clifford et al., 2003a) and high-grade squamous intraepithelial lesion (Clifford et al., 2003b). A study conducted by Taku and colleagues in the Eastern-Cape province of South Africa showed that HPV 35, HPV 45 and HPV 58 were the top three detected genotypes regardless of the HIV (Taku et al., 2021). Furthermore, the same study highlighted that CIN II women with multiple infections were more prone to be infected with HPV 35, HPV 45, and HPV 58 (HIV seropositive) and HPV 16, HPV 35 and HPV 58 (HIV seronegative). A study by Mbulawa. (2021) found that HPV 59, HPV 61, and HPV 62, were the predominant genotypes in the general population. Due to the high genetic diversity of HPV, it is important to study the virus in different geographic locations, people, and in persons living with HIV, to inform the selection of genes for vaccine improvements and potential vaccine trial sites. Screening for HPV infection is further significant to detect early infections, particularly of hr-HPV genotypes, to inform monitoring and management. There is

© University of Venda

a growing use of HPV polymerase chain reaction (PCR) hybridization-based assays in South Africa for the detection of HPV genotypes. These assays target a number of known HPV genotypes, potentially missing other genotypes of importance. Meiring et al. (2012) highlighted that the diagnostic molecular tools used for detecting HPV DNA should be able to identify and genotype HPV in the specimen. In this regard, next-generation sequencing (NGS) could be an alternative strategy for the universal identification and characterization of HPV DNA (Arroyo et al., 2013; Kocjan et al., 2013; Mühr et al., 2015; Murahwa et al., 2018; Galati et al., 2020; Poljak et al., 2020). Next-generation sequencing can also achieve the sequencing of whole genomes. Furthermore, this approach is known for detecting novel HPV genotypes and variants (Mühr et al., 2015; Siqueira et al., 2016; Latsuzbaia et al., 2018), as well as known HPV genotypes existing as a minority population. However, NGS is not readily available for HPV genomics in resource limited settings such as Limpopo Province, South Africa.

1.3 STUDY HYPOTHESIS

- There is a high variety of cervical HPV genotypes among WLWH in selected health facilities in Limpopo Province, South Africa.

1.4 RESEARCH QUESTIONS

1. What is the prevalence and distribution of selected cervical HPV genotypes among women living with and without HIV in South Africa?
2. What is the prevalence of cervical HPV infection among women in selected health facilities in Limpopo Province, South Africa?
3. What are the HPV genotypes infecting women in the study population?
4. What are the characteristics of HPV genomes from the study population?

1.5 MAIN OBJECTIVE

- The main objective of this study was to identify and characterize HPV DNA among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.

1.5.1 Specific objectives

The specific objectives were:

1. To provide a narrative literature review on the prevalence and distribution of selected cervical HPV genotypes among women living with and without HIV in South Africa.
2. To determine the prevalence of cervical HPV infection among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.

© University of Venda

3. To describe HPV genotypes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.
4. To generate and characterize HPV full genomes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.

© University of Venda

1.6 REFERENCES

1. Amponsah-Dacosta, E., Blose, N., Nkwinika, V.V. and Chepkurui, V., 2022. Human papillomavirus vaccination in South Africa: programmatic challenges and opportunities for integration with other adolescent health services? *Frontiers in Public Health*, *10*, p.799984.

2. Arroyo, L.S., Smelov, V., Bzhalava, D., Eklund, C., Hultin, E. and Dillner, J., 2013. Next generation sequencing for human papillomavirus genotyping. *Journal of Clinical Virology*, *58*(2), pp. 437-442.

3. Avert. HIV and AIDS in East and Southern Africa Regional Overview. 2019. Available online: https://wwwavertorg/printpdf/node/393 (accessed on 23 February 2023).

4. Aziz, H., Sattar, A.A., Mahmood, H., Fatima, S., Khurshid, M. and Faheem, M., 2023. Prevalence of HPV types in HIV-positive and negative females with normal cervical cytology or dysplasia. *Journal of Clinical Laboratory Analysis*, *37*(4), p.e24851.

5. Beyazit, F., Sılan, F., Gencer, M., Aydin, B., Paksoy, B., Unsal, M.A. and Ozdemir, O., 2018. The prevelance of human papillomavirus (HPV) genotypes detected by PCR in women with normal and abnormal cervico-vaginal cytology. *Ginekologia polska*, *89*(2), pp.62-67.

6. Botha, M.H. and Richter, K.L., 2015. Cervical cancer prevention in South Africa: HPV vaccination and screening both essential to achieve and maintain a reduction in incidence. *South African medical journal*, *105*(1), pp.33-34.

7. Bruni, L., Albero, G., Serrano, B., Mena, M.J., Collado, J.J., Gómez, D., Muñoz, J., Bosch, F.X. and de Sanjosé, S., 2021. *Human Papillomavirus and Related Diseases in South Africa: Summary Report 22 October 2021.* ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). [Accessed 10 December 2022].

8. Bruni, L., Diaz, M., Castellsagué, M., Ferrer, E., Bosch, F.X. and de Sanjosé, S., 2010. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *Journal of Infectious Diseases*, *202*(12), pp.1789-1799.

9. Burk, R.D., Ho, G.Y., Beardsley, L., Lempa, M., Peters, M. and Bierman, R., 1996. Sexual behavior and partner characteristics are the predominant risk factors for genital human

© University of Venda

papillomavirus infection in young women. *Journal of Infectious Diseases*, *174*(4), pp.679-689.

10. Centers for Disease Control and Prevention, 2004. Genital HPV infection—CDC fact sheet. *Centers for Disease Control and Prevention*.

11. Chirenje, Z.M., 2005. HIV and cancer of the cervix. *Best practice & research Clinical obstetrics & gynaecology*, *19*(2), pp.269-276.

12. Clifford, G.M., Gallus, S., Herrero, R., Munoz, N., Snijders, P.J., Vaccarella, S., Anh, P.T., Ferreccio, C., Hieu, N.T., Matos, E. and Molano, M., 2005. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *The Lancet*, *366*(9490), pp.991-998.

13. Clifford, G.M., Smith, J.S., Aguado, T. and Franceschi, S., 2003. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *British journal of cancer*, *89*(1), pp.101-105.

14. Clifford, G.M., Smith, J.S., Plummer, M., Munoz, N. and Franceschi, S., 2003. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *British journal of cancer*, *88*(1), pp.63-73.

15. De Sanjosé, S., Diaz, M., Castellsagué, X., Clifford, G., Bruni, L., Muñoz, N. and Bosch, F.X., 2007. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *The Lancet infectious diseases*, *7*(7), pp.453-459.

16. Dhokotera, T., Asangbeh, S., Bohlius, J., Singh, E., Egger, M., Rohner, E., Ncayiyana, J., Clifford, G.M., Olago, V. and Sengayi-Muchengeti, M., 2022. Cervical cancer in women living in South Africa: a record linkage study of the National Health Laboratory Service and the National Cancer Registry. *ecancermedicalscience*, 16, p.1348.

17. Einstein, M.H., Schiller, J.T., Viscidi, R.P., Strickler, H.D., Coursaget, P., Tan, T., Halsey, N. and Jenkins, D., 2009. Clinician's guide to human papillomavirus immunology: Knowns and unknowns. *The Lancet Infectious Diseases*, *9*(6), pp. 347-356.

18. Fani, M., Mahmoodi, P., Emadzadeh, M., Avan, A., Karimi, E., Ferns, G.A., Rezayi, M. and Amiri, I.S., 2020. Correlation of human papillomavirus 16 and 18 with cervical cancer

© University of Venda

and their diagnosis methods in Iranian women: A systematic review and meta-analysis. *Current problems in cancer*, *44*(1), p.100493.

19. Galati, L., Brancaccio, R.N., Robitaille, A., Cuenin, C., Luzi, F., Fiorucci, G., Chiantore, M.V., Marascio, N., Matera, G., Liberto, M.C. and Donà, M.G., 2020. Detection of human papillomaviruses in paired healthy skin and actinic keratosis by next generation sequencing. *Papillomavirus Research*, *9*, p. 100196.

20. García Muentes, G.D., García Rodríguez, L.K., Burgos Galarraga, R.I., Almeida Carpio, F. and Ruiz Cabezas, J.C., 2016. Genotypes distribution of human papillomavirus in cervical samples of Ecuadorian women. *Revista Brasileira de Epidemiologia*, *19*, pp.160-166.

21. Kim, K.D., Kim, J.J., Kim, S.M., No, J.H. and Kim, Y.B., 2012. Prevalence and determinants of high-risk human papillomavirus infection in women with high socioeconomic status in Seoul, Republic of Korea. *Asian Pacific Journal of Cancer Prevention*, *13*(1), pp.269-273.

22. Kirkel, J. 2020. published in January 28 2022. January is Cervical Cancer awareness month; young

23. Kocjan, B.J., Steyer, A., Sagadin, M., Hošnjak, L. and Poljak, M., 2013. Novel human papillomavirus type 174 from a cutaneous squamous cell carcinoma. *Genome Announcements*, *1*(4), pp. e00445-13.

24. Kuguyo, O., Dube Mandishora, R.S., Thomford, N.E., Makunike-Mutasa, R., Nhachi, C.F., Matimba, A. and Dandara, C., 2021. High-risk HPV genotypes in Zimbabwean women with cervical cancer: Comparative analyses between HIV-negative and HIV-positive women. *PLoS One*, *16*(9), p.e0257324.

25. Latsuzbaia, A., Arbyn, M., Dutta, S., Fischer, M., Gheit, T., Tapp, J., Tommasino, M., Weyers, S. and Mossong, J., 2018. Complete genome sequence of a novel human gamma papillomavirus isolated from a cervical swab in Luxembourg. *Genome Announcements*, *6*(11), pp. e00114-18.

26. Latsuzbaia, A., Wienecke-Baldacchino, A., Tapp, J., Arbyn, M., Karabegović, I., Chen, Z., Fischer, M., Mühlschlegel, F., Weyers, S., Pesch, P. and Mossong, J., 2020. Characterization and diversity of 243 complete human papillomavirus genomes in cervical swabs using next generation sequencing. *Viruses*, *12*(12), p. 1437.

© University of Venda

27. Liu, J., Li, H., Yang, F., Ren, Y., Xia, T., Zhao, Z., Cao, X., Wang, Z., Yin, M. and Lu, S., 2018. Epidemiology and clinical profile of cutaneous warts in Chinese college students: A cross-sectional and follow-up study. *Scientific Reports*, *8*(1), pp. 1-8.

28. Liu, H., Zeng, C., Jiang, M., Dai, Y., Xu, M., Zhou, F., Wang, Y., Pulliero, A., Sobierajski, T., Nesser, W. and Matsuura, M., 2023. Study on the prevalence and subtypes of human papillomavirus infection among women in the Xuhui District, Shanghai City, China. *Translational Cancer Research*, *12*(10), p.2923.

29. Llanos, A.A., Tsui, J., Rotter, D., Toler, L. and Stroup, A.M., 2018. Factors associated with high-risk human papillomavirus test utilization and infection: a population-based study of uninsured and underinsured women. *BMC Women's Health*, *18*, pp.1-14.

30. Magalhães, G.M., Vieira, É.C., Garcia, L.C., Carvalho-Leite, D., de Lourdes Ribeiro, M., Guedes, A.C.M. and Araújo, M.G., 2021. Update on human papilloma virus-part I: epidemiology, pathogenesis, and clinical spectrum. *Anais Brasileiros de Dermatologia*, *96*, pp.1-16.

31. Marembo, T., Mandishora, R.D. and Borok, M., 2019. Use of multiplex polymerase chain reaction for detection of high-risk human papillomavirus genotypes in women attending routine cervical cancer screening in Harare. *Intervirology*, *62*(2), pp.90-95.

32. Mayaud, P., Weiss, H.A., Lacey, C.J., Gill, D.K. and Mabey, D.C., 2003. Genital human papillomavirus genotypes in northwestern Tanzania. *Journal of Clinical Microbiology*, *41*(9), pp.4451-4453.

33. Mbatha, J.N., Taylor, M., Kleppa, E., Lillebo, K., Galapaththi-Arachchige, H.N., Singh, D., Kjetland, E.F., Baay, M.F. and Mkhize-Kwitshana, Z.L., 2017. High-risk human papillomavirus types in HIV-infected and HIV-uninfected young women in KwaZulu-Natal, South Africa: implications for vaccination. *Infectious Diseases*, *49*(8), pp.601-608.

34. Mbulaiteye, S.M., Bhatia, K., Adebamowo, C. and Sasco, A.J., 2011. HIV and cancer in Africa: mutual collaboration between HIV and cancer programs may provide timely research and public health data. *Infectious agents and cancer*, *6*, pp.1-12.

35. Mbulawa, Z., Kufa-Chakezha, T., Williamson, A.L. and Kularatne, R., 2018. Sentinel surveillance of human papillomavirus genotypes among young women attending public healthcare facilities in South Africa, 2017. *Public health surveillance*, p.91.

© University of Venda

36. Mbulawa, Z.Z., Somdyala, N.I., Mabunda, S.A. and Williamson, A.L., 2021. High human papillomavirus prevalence among females attending high school in the Eastern Cape Province of South Africa. *PLoS One*, *16*(6), p.e0253074.

37. Meiring, T.L., Salimo, A.T., Coetzee, B., Maree, H.J., Moodley, J., Hitzeroth, I.I., Freeborough, M.J., Rybicki, E.P. and Williamson, A.L., 2012. Next-generation sequencing of cervical DNA detects human papillomavirus types not detected by commercial kits. *Virology Journal*, *9*(1), pp. 1-10.

38. Moodley, J.R., Hoffman, M., Carrara, H., Allan, B.R., Cooper, D.D., Rosenberg, L., Denny, L.E., Shapiro, S. and Williamson, A.L., 2006. HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study. *BMC cancer*, *6*, pp.1-6.

39. Mühr, L.S.A., Bzhalava, D., Lagheden, C., Eklund, C., Johansson, H., Forslund, O., Dillner, J. and Hultin, E., 2015. Does human papillomavirus-negative condylomata exist? *Virology*, *485*, pp. 283-288.

40. Murahwa, A.T., Meiring, T.L., Mbulawa, Z.Z. and Williamson, A.L., 2018. Complete genome sequences of four novel human Gamma papillomavirus types, HPV-219, HPV-220, HPV-221, and HPV-222, isolated from penile skin swabs from South African men. *Genome Announcements*, *6*(25), pp. e00584-18.

41. Olorunfemi, G., Ndlovu, N., Masukume, G., Chikandiwa, A., Pisa, P.T. and Singh, E., 2018. Temporal trends in the epidemiology of cervical cancer in South Africa (1994–2012). *International journal of cancer*, *143*(9), pp.2238-2249.

42. Tartaglia, E., Falasca, K., Vecchiet, J., Sabusco, G.P., Picciano, G., Di Marco, R. and Ucciferri, C., 2017. Prevalence of HPV infection among HIV-positive and HIV-negative women in Central/Eastern Italy: Strategies of prevention. *Oncology Letters*, *14*(6), pp.7629-7635.

43. Pauli, S., Kops, N.L., Bessel, M., Lina Villa, L., Moreno Alves Souza, F., Mendes Pereira, G.F., Neves Hugo, F., POP-Brazil Study Group Comerlato Juliana 1 Bandeira Isabel 1 Fernandes Bruna 1 Fetzner Tiago 1 Soto Milena Mantelli Dall 1 Baptista Thais 1 Mello Barbara Pereira 2 Mota Giana 2 and Wendland, E., 2022. Sexual practices and HPV infection in unvaccinated young adults. *Scientific reports*, *12*(1), p.12385.

© University of Venda

44. Poljak, M., Valenčak, A.O., Domjanič, G.G., Xu, L. and Arbyn, M., 2020. Commercially available molecular tests for human papillomaviruses: A global overview. *Clinical Microbiology and Infection*, *26*(9), pp. 1144-1150.

45. Richter, K., 2015. Implementation of HPV vaccination in South Africa. *Pretoria: Public Health Association of South Africa*.

46. Richter, K., Becker, P., Horton, A. and Dreyer, G., 2013. Age-specific prevalence of cervical human papillomavirus infection and cytological abnormalities in women in Gauteng Province, South Africa. *South African medical journal*, *103*(5), pp.313-317.

47. Siqueira, J.D., Alves, B.M., Prellwitz, I.M., Furtado, C., Meyrelles, Â.R., Machado, E.S., Seuánez, H.N., Soares, M.A. and Soares, E.A., 2016. Identification of novel human papillomavirus lineages and sublineages in HIV/HPV-coinfected pregnant women by next-generation sequencing. *Virology*, *493*, pp. 202-208.

48. Statistics South Africa. Mid-year population estimates 2021 [homepage on the Internet]. [cited 2023 January 15] Available from: www.statssa.gov.za.

49. Stelzle, D., Tanaka, L.F., Lee, K.K., Khalil, A.I., Baussano, I., Shah, A.S., McAllister, D.A., Gottlieb, S.L., Klug, S.J., Winkler, A.S. and Bray, F., 2021. Estimates of the global burden of cervical cancer associated with HIV. *The lancet global health*, *9*(2), pp.e161-e169.

50. Strickler, H.D., Burk, R.D., Fazzari, M., Anastos, K., Minkoff, H., Massad, L.S., Hall, C., Bacon, M., Levine, A.M., Watts, D.H. and Silverberg, M.J., 2005. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus–positive women. *Journal of the National Cancer Institute*, *97*(8), pp.577-586.

51. Taku, O., Businge, C.B., Mdaka, M.L., Phohlo, K., Basera, W., Garcia-Jardon, M., Meiring, T.L., Gyllensten, U., Williamson, A.L. and Mbulawa, Z.Z., 2020. Human papillomavirus prevalence and risk factors among HIV-negative and HIV-positive women residing in rural Eastern Cape, South Africa. *International Journal of Infectious Diseases*, *95*, pp. 176-182.

52. Taku, O., Mbulawa, Z.Z., Phohlo, K., Garcia-Jardon, M., Businge, C.B. and Williamson, A.L., 2021. Distribution of human papillomavirus (HPV) genotypes in HIV-negative and HIV-positive women with cervical intraepithelial lesions in the Eastern Cape Province, South Africa. *Viruses*, *13*(2), p.280.

© University of Venda

53. Tao, Y., Shao, H., Zhang, T., Pu, J. and Tang, C., 2022. Factors Influencing Men's Attitudes toward HPV Vaccination in Males Included in the Chinese National Immunization Program. *Vaccines*, *10*(7), p.1054.

54. Tartaglia, E., Falasca, K., Vecchiet, J., Sabusco, G.P., Picciano, G., Di Marco, R. and Ucciferri, C., 2017. Prevalence of HPV infection among HIV-positive and HIV-negative women in Central/Eastern Italy: Strategies of prevention. *Oncology Letters*, *14*(6), pp.7629-7635.

55. UNAIDS. Aidsinfo. 2019. Available online: http://aidsinfounaidsorg/ (accessed on 23 February 2023).

56. Vinodhini, K., Shanmughapriya, S., Das, B.C. and Natarajaseenivasan, K., 2012. Prevalence and risk factors of HPV infection among women from various provinces of the world. *Archives of gynecology and obstetrics*, *285*, pp.771-777.

57. WHO. HIV/AIDS: Data and Statistics. 2019. Available online: https://wwwwhoint/hiv/data/en/ (accessed on 23 February 2023).

58. World Health Organization, 2014. Human papillomavirus vaccines: WHO position paper, October 2014. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire*, *89*(43), pp.465-491.

59. World Health Organization, 2016. *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach*. World Health Organization

60. Zhou, Y.X., Ma, X.H., Wang, T.T., Qu, X.L. and Zhang, X.Q., 2024. Analysis of age-specified and genotype distribution of HPV multiple infections in the Chinese population. *Scientific Reports*, *14*(1), p.2678.

© University of Venda

CHAPTER TWO

PREVALENCE AND DISTRIBUTION OF SELECTED CERVICAL HUMAN PAPILLOMAVIRUS TYPES IN HIV INFECTED AND HIV UNINFECTED WOMEN IN SOUTH AFRICA, 1989-2021: A NARRATIVE REVIEW

© University of Venda

ABSTRACT

Background and objective: Human papillomavirus (HPV) infection, a causative agent of cervical cancer, is of great concern, more so in populations with high HIV prevalence, such as South Africa. This review aimed at examining the prevalence and distribution of selected cervical human papillomavirus genotypes among women living with and without HIV in South Africa.

Methods: Keywords were used to conduct searches in PubMed and Web of Science databases. Data was assessed by two authors independently in order to facilitate data integrity. The study inclusion criteria comprised records on cervical HPV, HPV genotyping and HPV genotype distribution among women in South Africa. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software.

Results: Sixty-nine reviewed articles met the inclusion criteria for analysis. Data on cervical HPV prevalence and genotype distribution was available only for five of the nine South African provinces. Only 4/69 studies used sequencing as an approach to identify HPV genotypes. In a general population, HPV 16 was the most frequent (8.80%), followed by HPV 35 (4.86%), HPV 18 (4.14%), HPV 58 and HPV 52 with the frequency of 3.65% and 3.62%, respectively. Furthermore, HPV 70 (0.74%) was found to be the least frequent genotype of HPV. There was a higher prevalence of HPV genotypes 16, 18, and 35 in individuals living with HIV or not. In addition, HPV 6, HPV 11 and HPV 70 were the least frequent genotypes from these populations. Lastly, HPV 16 was the predominant genotype among women with normal (2.03%) and abnormal cervical cytology (6.60%).

Conclusion: The high prevalence of hr-HPV 16, HPV 18 and HPV 35 irrespective of HIV status in the country is a public health concern, considering their significant contribution to the burden of CC. Thus, prevention measures such as HPV vaccination and routine screening for all individuals remain essential. Expansion of HPV genotyping using advanced technologies such as NGS will enhance knowledge concerning patterns of HPV genotype distribution in various provinces of South Africa. Such knowledge will further help in decision making for improvement of the current diagnostics, as well as future vaccine development and assessment. Because available vaccines exclude HPV 35 targets, there is a need to consider this genotype in future vaccine development approaches.

Keywords: Human papillomavirus, Genotypes; Prevalence; HIV; South Africa

© University of Venda

2.1 INTRODUCTION AND STUDY RATIONALE

Human papillomavirus (HPV) and human immunodeficiency virus (HIV) are leading sexually transmitted infections (STIs) (Lissouba et al., 2013) that cause a significant burden of disease in low- and middle-income countries (Moodley et al., 2006; De Sanjosé et al., 2012). Cervical cancer (CC) is underlined as an HIV-associated malignancy and rated the second most frequent cancer among South African women of all age groups (Bruni et al., 2015) after breast cancer. About 70% of CC cases are caused by persistent infection of high-risk human papillomavirus (hr-HPV)16 and hr-HPV 18 (Reid et al., 1982). In 2020, there were 10 702 new reported cases of CC among South African women, irrespective of HIV status, 5 870 of whom died because of the disease (Bruni et al., 2021). Globally, South Africa has the highest HIV infection rates, with an estimated 8.23 million people living with HIV in 2021 (Statistics South Africa, 2022). Women were the most affected group in this regard. However, the introduction and free access to anti-retroviral therapy (ART) has increased the life span of individuals living with HIV and acquired immunodeficiency syndrome (AIDS) (Hogg et al., 2008), leading to an increasing number of people ageing with HIV infection. Women living with HIV (WLWH) subsequently experience life expectancy approaching that of persons living without HIV, although, they still have an increased incidence of developing CC at a later stage (Loke et al., 2017), probably due to their compromised immune system (Palefsky, 2009).

According to the International HPV Reference Center, there are 228 HPV genotypes known to date (Karolinska Institutet, 2021), of which about 40 have been reported to infect the ano-genital region (De Villiers et al., 2004). Based upon the potential interconnection between various HPV genotypes and CC, HPV was thereafter stratified into two main groups, namely: high-risk (hr-) and low-risk (lr-) genotypes primarily known to be cancerous (Leszczyszyn et al., 2014) and non-cancerous (Bhatia et al., 2013), respectively.

There is currently no cure for HPV infection. However, prevention is possible through vaccination. To date, three HPV vaccines have been developed, which offers protection against some of the hr genotypes. The global uptake of these vaccines varies geographically (Loke et al., 2017). Cervarix is a bivalent vaccine that covers HPV 16 and HPV 18, while Gardasil is a quadrivalent vaccine offering protection against HPV 6, HPV 11, HPV 16 and HPV 18 (WHO, 2014). Furthermore, Gardasil-9 is a nonavalent vaccine and comprises the four HPV genotypes covered by the quadrivalent vaccine, with the addition of genotypes HPV 31, HPV 33, HPV 45, HPV 52, and HPV 58 (Luxembourg and Moeller, 2017). However, in South Africa, only Cervarix and Gardasil-4 are currently available (WHO, 2014).

© University of Venda

In collaboration with the Department of Basic Education, the Department of Health launched a South African HPV vaccination campaign in 2014 (Botha and Richter, 2015). The campaign specifically targets Grade 4 public-school children aged 9-years and above and involved the delivery of two doses of vaccine 6 (six) months apart (Delany-Moretlwe et al., 2018; Ngcobo et al., 2019). In 2014, it was reported that more than 350 000 school girls received the vaccine in more than 16 000 public schools (Delany-Moretlwe et al., 2018). Within the same year of the programme's launch, 86.6% coverage was attained (Delany-Moretlwe et al., 2018). These campaigns and programmes play a role in the reduction of HPV infections and effectively control and prevent CC development in the later stage. However, issues of cost and unavailability of vaccines limit the progress of these programmes as well as the limitation of the available HPV vaccines to cover all the prevalent HPV or hr-HPV genotypes.

Immunocompromised individuals, especially seropositive women, are at high-risk of HPV infections (Mbulawa et al. 2012) and associated pathologies (Kelly et al., 2017; Taku et al., 2021). Studies have shown a high prevalence of hr-HPV genotypes in HIV seropositive women than seronegative women (Mbulawa et al., 2014; Clifford et al., 2016). Other studies indicate multiple hr-HPV infections in HIV seropositive women (McDonald et al., 2014; Mbulawa et al., 2018). However, other studies indicate a high prevalence of HPV infections in women regardless of their HIV status (McDonald et al., 2014; Mbulawa et al., 2021). There are uncertainties on the prevalence and distribution of HPV genotypes regarding HIV status. Therefore, more studies are needed to understand which hr-HPV genotypes are associated with HIV and how these hr-HPV genotypes contribute to the development of CC in this population, especially in South Africa where there is a high prevalence of HIV infection.

This study was set up to determine the prevalence and distribution of selected cervical human papillomavirus genotypes in HIV infected and HIV uninfected women in South Africa.

2.2 RESEARCH QUESTIONS

1. How are cervical HPV studies distributed across provinces in South Africa?
2. What detection methods are used for identifying HPV genotypes in South Africa?
3. What is the prevalence and distribution of hr-HPV genotypes among women in a general population, and among those with normal and abnormal cervical cytology?
4. What is the prevalence and distribution of hr-HPV or lr-HPV by HIV status?

© University of Venda

2.3 MAIN OBJECTIVE

To determine the prevalence and distribution of selected cervical human papillomavirus genotypes among women living with and without HIV in South Africa.

2.3.1 Specific objectives

1. To assess the distribution of cervical HPV studies across provinces in South Africa.
2. To identify detection methods used for HPV genotypes in South Africa.
3. To determine the prevalence and distribution of hr-HPV genotypes among women in general, and among those with a normal and abnormal cervical cytology
4. To investigate the prevalence and distribution of hr-HPV and or lr-HPV genotypes by HIV status.

2.4 MATERIALS AND METHODS

2.4.1 Eligibility criteria

Research articles focusing on cervical HPV infection in South Africa were sought. Records on cervical HPV, HPV genotyping and genotype distribution published among South African women formed part of the study's inclusion criteria. Five major exclusion criteria were designated: (1) not geographically related (South Africa must have been at least one of the countries included); (2) review articles, clinical trials and preface; (3) poster presentations, letters to the editor and correspondence; (4) not HPV or cervical (HPV or CC) related and (5) HPV in men. Full-text articles published in English were included as well. It is worth noting that, comparative studies conducted between South Africa and other countries were included in the final analysis. However, in such studies, only South African data relevant to our review was extracted.

2.4.2 Information source

Published reports on the prevalence and cervical HPV genotypes among South African women living with and without HIV were carefully searched and compiled. In addition, articles that did not differentiate HPV prevalence and genotypes by HIV status were also included to respond to the objectives which were independent of HIV status. All the reports were identified through PubMed and Web of Science databases last consulted on 01 July 2021.

2.4.3 Search strategy

All the records were sourced by using an advanced search option for both databases. In addition, all fields were also searched. The following search strategies were generated per database:

© University of Venda

PubMed: ((((HPV) OR ((Human Papillomavirus))) OR ((Human Papilloma virus))) OR (Papillomaviridae)) AND ((South Africa*))

("HPV"[All Fields] OR ("alphapapillomavirus"[MeSH Terms] OR "alphapapillomavirus"[All Fields] OR ("human"[All Fields] AND "papillomavirus"[All Fields]) OR "human papillomavirus"[All Fields]) OR ("papillomaviridae"[MeSH Terms] OR "papillomaviridae"[All Fields] OR ("human"[All Fields] AND "papilloma"[All Fields] AND "virus"[All Fields]) OR "human papilloma virus"[All Fields]) OR ("papillomaviridae"[MeSH Terms] OR "papillomaviridae"[All Fields])) AND ("South"[All Fields] AND "africa*"[All Fields])

TRANSLATIONS

Human Papillomavirus

"alphapapillomavirus"[MeSH Terms] OR "alphapapillomavirus"[All Fields] OR ("human"[All Fields] AND "papillomavirus"[All Fields]) OR "human papillomavirus" [All Fields]

Papillomaviridae

"papillomaviridae"[MeSH Terms] OR "papillomaviridae"[All Fields] OR ("human"[All Fields] AND "papilloma"[All Fields] AND "virus"[All Fields]) OR "human papilloma virus"[All Fields]

Web of Science

TS=(HPV) OR TS= (Human Papillomavirus) OR TS= (Human Papilloma virus) OR TS=(Papillomaviridae) AND TS=(South Africa)

2.4.4 Selection process and study risk of bias assessment

The full text of the relevant articles were read and analyzed for the objectives of the current study. Variables required to answer the study objectives were extracted and tabulated into their respective columns. Two authors independently reviewed the search and selection procedure for consistency. Most notably, the authors agreed that only those articles that strictly address the study inclusion criteria should be considered for further analysis. Un-reported variables required in certain articles were marked, and the authors agreed that such information should be labelled as "data was not reported".

© University of Venda

2.4.5 Data collection process

All records searched from both databases were then exported to Mendeley Desktop, a referencing tool and further processed to remove duplicates and screened for eligibility. The title and abstract were thoroughly screened in Mendeley Desktop. Later, full articles were downloaded and screened individually. This was undertaken by two authors who independently used the same tool.

2.4.6 Data items

Research articles focusing on cervical HPV infection in South Africa published between 1989 and 2021 were retrieved. Data extracted included: (1) relevant information on sample genotype (cervical cytology/cervicovaginal, biopsies) and from women; (2) sampling device and genotyping technique used; (3) study design, collection time period and age range; (4) study population and sample size; (5) study setting; and (6) data on cervical status. Following the PRISMA protocol (Moher et al., 2009), a total of 69 published articles were included for final analysis after screening. Figure 2.1 is a diagrammatic reflection of preferred reporting items for Systematic Reviews and Meta-Analyses (PRISMA).

© University of Venda

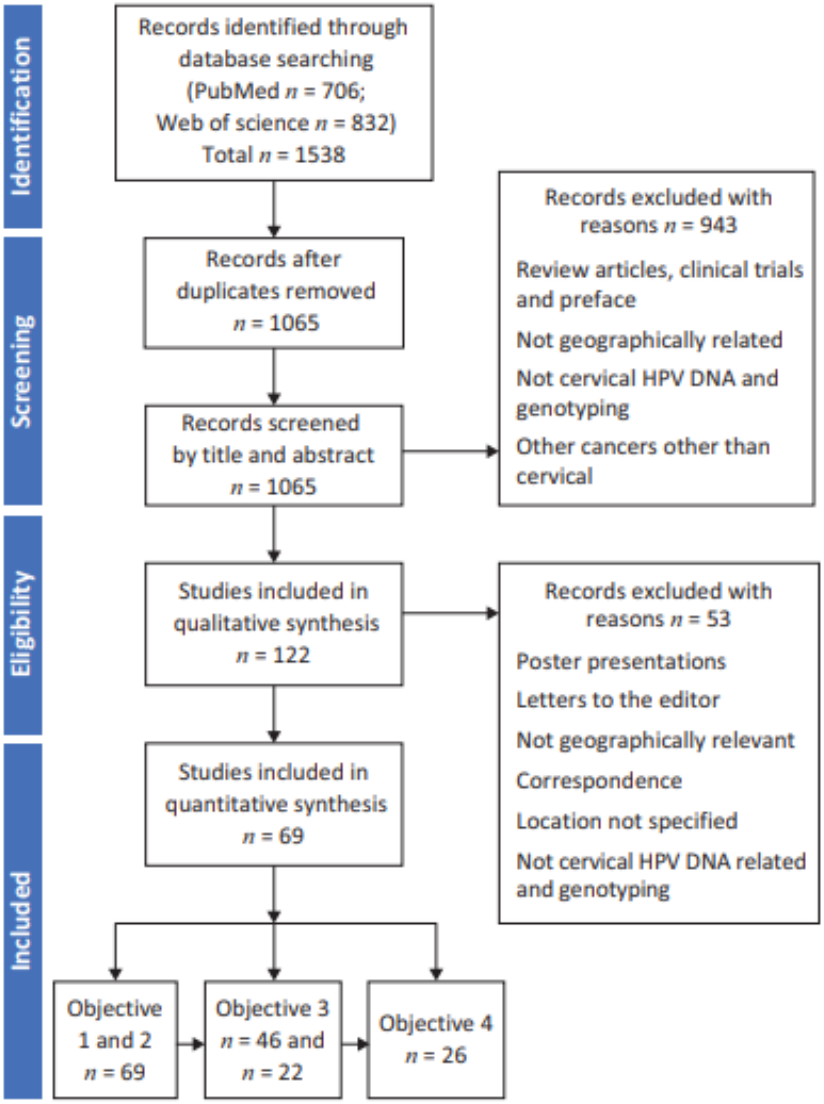


Figure 2.1: *Preferred reporting items for systematic reviews and meta-analyses (PRISMA) (Source: Moher D, Liberati A, Tetzlaff J, et al., 2009).*

2.4.7 Synthesis method

The full text of the relevant articles was read and analyzed for the objectives of the current study. These articles were grouped in terms of four objectives: (1) The distribution of cervical HPV studies across provinces in South Africa, (2) The detection methods used to identify HPV genotypes in South Africa, (3) The prevalence and distribution of hr-HPV genotypes among women in the general population and among those with normal and abnormal cervical cytology, (4) The prevalence and distribution of hr-lr-HPV genotypes by HIV status.

To achieve objectives 1 and 2, variables required to answer these objectives were extracted from individual records and tabulated into their respective columns using a Word file. The variables were: (1) author (year), and study title, (2) sample type, sampling device and genotyping technique, and (3) province in South Africa. It is worth noting that these objectives were analyzed without any emphasis on the HIV status of the recruited study participants,

© University of Venda

considering all the 69 studies that were included in the quantitative analysis. Records missing certain required information were identified and marked as “not reported”, (Table 2.1).

© University of Venda

Table 2.1: Characteristics of the 69 articles analyzed to achieve objective 1 and 2

Author (year) and study title	Sample type, sampling device and genotyping technique	Province in South Africa
1. Mbulawa et al. (2021) High human papillomavirus prevalence among females attending high school in the Eastern Cape Province of South Africa	Sample type comprised self-collected specimens collected using the Evalyn brush. HPV genotyping was conducted using Roche's Linear Array HPV Test	Eastern Cape
2. Taku et al. (2021) Distribution of Human Papillomavirus (HPV) Genotypes in HIV-Negative and HIV-Positive Women with Cervical Intraepithelial Lesions in the Eastern Cape Province, South Africa	Sample type comprised cervical specimens collected using a Viba-brush. HPV genotyping was conducted using HPV direct flow chip	Eastern Cape
3. Saidu et al. (2021) Performance of Xpert HPV on Self-collected Vaginal Samples for Cervical Cancer Screening Among Women in South Africa	Sample type comprised self-sampled specimens collected using a standard flock tip swab while cervical specimens were obtained using an extended tip plastic spatula and an endocervical cytobrush. HPV genotyping was conducted using Xpert HPV	Western-Cape
4. Taku et al. (2021) Detection of sexually transmitted pathogens and co-infection with human papillomavirus in women residing in rural Eastern Cape, South Africa	Sample type comprised cervical specimens collected using a cervical brush. HPV genotyping was conducted using Hybrid Capture 2	Eastern Cape
5. Chambuso et al. (2020) Age, absolute CD4 count, and CD4 percentage in relation to HPV infection and the stage of cervical disease in HIV-1-positive women	Sample type comprised biopsy specimens, collection device was not reported. HPV genotyping was conducted using Roche's Linear Array HPV Test	Western-Cape
6. Taku et al. (2020) Acceptability of self- collection for human papillomavirus detection in the Eastern Cape, South Africa	Sample type comprised cervical specimens collected using cervical brushes while self-collected specimens were collected using Viba-brushes. HPV genotyping was conducted using Digene Hybrid Capture 2 and hpVIR real-time PCR assay	Eastern Cape
7. Kuhn et al. (2020) Clinical evaluation of modifications to a human papillomavirus assay to optimise its utility for cervical cancer screening in low-resource settings: a diagnostic accuracy study	Sample type comprised cervical specimens were collected using plastic spatula with endocervical cytobrush. HPV genotyping was conducted using Xpert HPV	Western-Cape
8. Johnson et al. (2020) Selecting human papillomavirus genotypes to optimize the performance of screening tests among South African women	Sample type comprised cervical specimens collected using a cytobrush and plastic spatula. HPV genotyping was conducted using Roche's Linear Array HPV Test and Xpert HPV	Western-Cape
9. Taku et al. (2020) Human papillomavirus prevalence and risk factors among HIV-negative and HIV-positive women residing in rural Eastern Cape, South Africa	Sample type comprised cervical specimens collected using a cervical brush. HPV genotyping was conducted using Hybrid Capture 2	Eastern Cape
10. van de Wijgert et al. (2020) Human papillomavirus infection and cervical dysplasia in HIV-positive women: potential role of the vaginal microbiota	Sample type comprised cervical specimens collected using a Digene cervical sampler. HPV genotyping was conducted using INNO LiPA	Gauteng
11. Chikandiwa et al. (2018) Prevalence, incidence and correlates of low-risk HPV infection and anogenital warts in a cohort of women living with HIV in Burkina Faso and South Africa	Sample type comprised cervical specimens collected using Digene cervical samplers. HPV genotyping was conducted using INNO-LiPA	Burkina Faso and South Africa (Gauteng)
12. Mbulawa et al. (2018) High human papillomavirus (HPV) prevalence in South African adolescents and young women encourages expanded HPV vaccination campaigns	Sample type comprised cervical specimens collected using Digene cervical samplers. HPV genotyping was conducted using Roche's Linear Array Test	Western Cape
13. Mbatha et al. (2017) High-risk human papillomavirus types in HIV- infected and HIV-uninfected young women in KwaZulu-Natal, South Africa: implications for vaccination	Sample type comprised cervicovaginal specimen were collected by spraying 10 ml of saline on the cervix four times before drawing it back into the syringe and	KwaZulu-Natal

© University of Venda

	depositing it into the sterile tube. HPV genotyping was conducted using genotyped by EIA	
14. Mbatha et al. (2017) Self-sampling for human papillomavirus testing among rural young women of KwaZulu-Natal, South Africa	Sample type comprised self-sampled specimens collected using Dacron swab and Viba brush. HPV genotyping was conducted using an enzyme immunoassay (EIA).	KwaZulu-Natal
15. Kelly et al. (2017) Epidemiology of high-risk human papillomavirus and cervical lesions in African women living with HIV/AIDS: effect of anti-retroviral therapy	Sample type comprised cervical specimens collected using Digene cervical samplers. HPV genotyping was conducted using INNO-LiPA	Burkina Faso and South Africa (Gauteng)
16. Kelly et al. (2017) Associations of Human Papillomavirus (HPV) genotypes with high-grade cervical neoplasia (CIN2+) in a cohort of women living with HIV in Burkina Faso and South Africa	Sample type comprised cervical specimens collected using Digene cervical samplers and careHPV cervical sampler. HPV genotyping was conducted using INNO-LiPA	Burkina Faso and South Africa (Gauteng)
17. Dylla et al. (2017) Human papillomavirus clustering patterns among HIV-infected and HIV-uninfected adolescent females in South Africa	Sample type comprised self-sampled specimens collected using a Dacron swab. HPV genotyping was conducted using Roche's Linear Array HPV Test	Wester-Cape
18. Rad et al. (2017) HPV types in cervical cancer tissue in South Africa: A head-to-head comparison by mRNA and DNA tests	Sample type comprised biopsy specimens' collection device was not reported. HPV genotyping was conducted using reverse line blot (RLB) assay and PreTect HPV-proofer	Gauteng
19. Adler at al. (2016) Cumulative Impact of HIV and Multiple Concurrent Human Papillomavirus Infections on the Risk of Cervical Dysplasia	Sample type comprised self-collected specimens collected using a Dacron swab. HPV genotyping was conducted using Roche's Linear Array Test	Western Cape
20. Segondy et al. (2016) Performance of careHPV for detecting high-grade cervical intraepithelial neoplasia among women living with HIV-1 in Burkina Faso and South Africa: HARP study	Sample type comprised cervical specimens collected using a careHPV sample collection device. HPV genotyping was conducted using careHPV and INNO-LiPA	Burkina Faso and South Africa (Gauteng)
21. Ebrahim et al. (2016) High Burden of Human Papillomavirus (HPV) Infection among Young Women in KwaZulu- Natal, South Africa	Sample type comprised cervicovaginal specimens, the collection device was not reported. HPV genotyping was conducted using Roche's Linear Array Test	KwaZulu-Natal
22. Van Aardt et al. (2016) Oncogenic and incidental HPV types associated with histologically confirmed cervical intraepithelial neoplasia in HIV-positive and HIV-negative South African women	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using Roche's Linear Array HPV Test	Gauteng
23. Mbulawa et al. (2016) Xpert human papillomavirus test is a promising cervical cancer screening test for HIV-seropositive women	Sample type comprised cervical specimens collected using a Digene cervical sampler. HPV genotyping was conducted using Xpert HPV	Gauteng
24. Papasavvas et al. (2016) High-risk oncogenic HPV genotype infection associates with increased immune activation and T cell exhaustion in ART-suppressed HIV-1-infected women	Sample type comprised cervical specimens collected using a Digene DNA Collection Device (brush). HPV genotyping was conducted using Roche's Linear Array HPV Test	Gauteng
25. Mbatani et al. (2016) Performance of an Human Papillomavirus Test in Samples From Women With Histolopathologically Confirmed Invasive Cervical Cancer	Sample type comprised cervical specimens collected using a cervical brush. HPV genotyping was conducted using cobas HPV test, Roche's Linear Array HPV Test and Hybrid Capture 2	Western Cape
26. Mbulawa et al. (2015) Human papillomavirus prevalence in South African women and men according to age and human immunodeficiency virus status	Sample type comprised cervical specimens collected using Digene cervical samplers. HPV genotyping was conducted using Roche's Linear Array Test	Western Cape
27. van Aardt et al. (2015) Unique Human Papillomavirus Type Distribution in South African Women With Invasive Cervical Cancer and the Effect of Human Immunodeficiency Virus Infection	Sample type comprised biopsy specimens collected using a punch biopsy forceps. HPV genotyping was conducted using Roche's Linear Array HPV Test	Gauteng
28. Tayib et al. (2015)	Sample type comprised biopsy specimens, the collection device was not reported. HPV genotyping was conducted using Roche's Linear Array Test	Western Cape

© University of Venda

Human papillomavirus genotypes and clinical management of genital warts in women attending a colposcopy clinic in Cape Town, South Africa		
29. Zeier et al. (2015) Combination antiretroviral therapy reduces the detection risk of cervical human papilloma virus infection in women living with HIV	Sample type comprised cervical specimens collected using cervex brush. HPV genotyping was conducted using Roche's Linear Array Test	Province not reported
30. Adler et al. (2015) High Risk Human Papillomavirus Persistence Among HIV-infected Young Women in South Africa	Sample type comprised self-sampled specimens collected using a Dacron swab. HPV genotyping was conducted using Roche's Linear Array HPV Test	Western Cape
31. Lebelo et al. (2015) Detection, Genotyping and Quantitation of Multiple HPV Infections in South African Women With Cervical Squamous Cell Carcinoma	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using TaqMan-based quantitative qPCR	Gauteng
32. Ngou et al. (2015) Comparison of Analytical and Clinical Performances of the Digene HC2 HPV DNA Assay and the INNO-LiPA HPV Genotyping Assay for Detecting High-Risk HPV Infection and Cervical Neoplasia Among HIV-Positive African Women	Sample type comprised cervical specimens collected using Digene cervical samplers. HPV genotyping was conducted using Hybrid Capture 2 and the INNO-LiPA	Burkina Faso and South Africa (Gauteng)
33. Ameur et al. (2014) Comprehensive profiling of the vaginal microbiome in HIV positive women using massive parallel semiconductor sequencing	Sample type comprised cervical specimens collected using Digene cervical samplers. HPV genotyping was conducted using Roche's Linear Array Test, HPVIR real-time PCR, and Massively parallel DNA sequencing	Western Cape
34. McDonald et al. (2014) Distribution of human papillomavirus genotypes among HIV-positive and HIV-negative women in Cape Town, South Africa	Sample type comprised cervical specimens collected using a plastic spatula and cytobrush. HPV genotyping was conducted using Hybrid capture 2 and Roche's Linear Array Test	Western Cape
35. Adler et al. (2014) Cervical Dysplasia and High-Risk Human Papillomavirus Infections among HIV-Infected and HIV-Uninfected Adolescent Females in South Africa	Sample type comprised self-sampled specimens collected using a Dacron swab. HPV genotyping was conducted using Roche's Linear Array HPV Test	Western-Cape
36. Adler et al. (2013) High Rate of Multiple Concurrent Human Papillomavirus Infections among HIV Uninfected South African Adolescents	Sample type comprised cervical specimens, the collection device was not reported. HPV Genotyping was performed using Roche's Linear Array	Province not reported
37. Adler et al. (2013) A Viable and Simple Self-Sampling Method for Human Papillomavirus Detection among South African Adolescents	Sample type comprise self-sampled specimens collected using a Dacron swab. HPV genotyping was conducted using Roche's Linear Array Test	Gauteng
38. Denny et al. (2014) Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using Roche's Linear Array Test	Ghana, Nigeria and South Africa (Province not reported)
39. Richter et al. (2013) Age-specific prevalence of cervical human papillomavirus infection and cytological abnormalities in women in Gauteng Province, South Africa	Sample type comprised biopsy specimens collected using dry swabs and tampons. HPV genotyping was conducted using Roche's Linear Array Test	Gauteng
40. Ngou et al. (2013) Comparison of *care*HPV and Hybrid Capture 2 Assays for Detection of High-Risk Human Papillomavirus DNA in Cervical Samples from HIV-1-Infected African Women	Sample type comprised cervical specimens collected using Digene cervical sampler and *care*HPV sample collection device. HPV genotyping was conducted using *care*HPV and Hybrid capture 2 and INNO LiPA	Burkina Faso and South Africa (Gauteng)
41. Dols et al. (2012) PCR-Based Identification of Eight Lactobacillus species and 18 hr-HPV Genotypes in Fixed Cervical Samples of South African Women at Risk of HIV and BV	Sample type comprised cervical specimens collected using a standard sampling brush. HPV genotyping was conducted using INNO LiPA	Limpopo
42. Dols et al. (2012) HPV Type Distribution and Cervical Cytology among HIV-Positive Tanzanian and South African Women	Sample type comprised cervical specimens collected using a standard sampling brush. HPV genotyping was conducted using INNO LiPA	Tanzania and South Africa (Limpopo)

© University of Venda

43. McDonald et al. (2012) Distribution of High-Risk Human Papillomavirus Genotypes among HIV-Negative Women with and without Cervical Intraepithelial Neoplasia in South Africa	Sample type comprised cervical specimens collected using a plastic spatula and cytobrush. HPV genotyping was conducted using Hybrid capture 2 and Roche's Linear Array Test	Western Cape
44. Meiring et al. (2012) Next-generation sequencing of cervical DNA detects human papillomavirus types not detected by commercial kits	Sample type comprised cervical specimens collected using Digene cervical sampler. HPV genotyping was conducted using Roche's Linear Array Test and Illumina sequencing	Western Cape
45. Mbulawa et al. (2012) Impact of Human Immunodeficiency Virus on the Natural History of Human Papillomavirus Genital Infection in South African Men and Women	Sample type comprised cervical specimens collected using a Digene cervical sampler. HPV genotyping was conducted using Roche's Linear Array HPV Test	Western Cape
46. De Vuyst et al. (2012) Prevalence of human papillomavirus in women with invasive cervical carcinoma by HIV status in Kenya and South Africa	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using enzyme immunoassay (EIA)	Kenya and South Africa (KwaZulu-Natal)
47. Taylor et al. (2011) A comparison of human papillomavirus testing of clinician-collected and self-collected samples during follow-up after screen-and-treat	Sample type comprised self-collected specimens collected using a Dacron swab, while for cervical specimens, a plastic spatula and a cytobrush was used. HPV genotyping was conducted using Hybrid capture 2	Western Cape
48. Auvert et al. (2011) High-Risk Huan Papillomavirus Is Associated with HIV Acquisition among South African Female Sex Workers	Sample type comprised cervicovaginal specimens collection device was not reported. HPV genotyping was conducted using Roche's Linear Array Test	Gauteng and KwaZulu-Natal
49. Wang et al. (2011) Rapid Rise in Detection of Human Papillomavirus (HPV) Infection Soon After Incident HIV Infection Among South African Women	Sample type comprised cervical specimens collection device was not reported. HPV genotyping was conducted using Roche's Linear Array HPV Test and Hybrid capture 2	Western-Cape
50. Mbulawa et al. (2010) Influence of human immunodeficiency virus and CD4 count on the prevalence of human papillomavirus in heterosexual couples	Sample type comprised cervical specimens collected using a Digene cervical sampler. HPV genotyping was conducted using Roche's Linear Array HPV Test	Western Cape
51. Firnhaber et al. (2010) Association between cervical dysplasia and human papillomavirus in HIV seropositive women from Johannesburg South Africa	Sample type comprised cervical specimens collected using a using endocervical brush. HPV genotyping was conducted using Roche's Linear Array Test	Gauteng
52. Firnhaber et al. (2009) Diverse and High Prevalence of Human Papillomavirus Associated with a Significant High Rate of Cervical Dysplasia in Human Immunodeficiency Virus–Infected Women in Johannesburg, South Africa	Sample type comprised cervical specimens, collection device was not reported. HPV genotyping was conducted using Roche's Linear Array HPV Test	Gauteng
53. Mbulawa et al. (2009) Genital Human Papillomavirus Prevalence and Human Papillomavirus Concordance in Heterosexual Couples Are Positively Associated with Human Immunodeficiency Virus Coinfection	Sample type comprised cervical specimens collected using a Digene cervical samplers. HPV genotyping was conducted using Roche's Linear Array Test	Western Cape
54. Moodley et al. (2009) Human papillomavirus prevalence, viral load and pre-cancerous lesions of the cervix in women initiating highly active antiretroviral therapy in South Africa: a cross-sectional study	Sample type comprised cervical specimens collected using a Digene cervical samplers. HPV genotyping was conducted using Hybrid capture 2 and Roche's Linear Array Test	Western-Cape
55. Said et al. (2009) HPV genotypes in women with squamous intraepithelial lesions and normal cervixes participating in a community-based microbicide study in Pretoria, South Africa	Sample type comprised cervical specimens collected using a cytobrush. HPV genotyping was conducted using Roche's Linear Array Test	Gauteng
56. Richter et al. (2008) Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy	Sample type comprised cervical specimens collected using a Cytobrush Plus cell collector. HPV genotyping was conducted using Roche's Linear Array HPV Test	Gauteng

© University of Venda

57. Marais et al. (2008) Cervical and Oral Human Papillomavirus Types in HIV-1 Positive and Negative Women With Cervical Disease in South Africa	Sample type comprised cervical specimens collected using a Digene cervical samplers. HPV genotyping was conducted using Roche's Linear Array Test	Western-Cape
58. Marais et al. (2008) Cervical Human Papillomavirus (HPV) Infection and HPV Type 1 Antibodies in South African Women	Sample type comprised cervical scrapings (biopsy) specimens, collection device was not reported. HPV Genotyping was conducted using Roche's Linear Array Test	Western-Cape
59. Allan et al. (2008) Cervical Human Papillomavirus (HPV) Infection in South Africa Women: Implications for HPV Screening and Vaccine Strategies	Sample type comprised cervical specimens, the collection device was not reported. HPV genotyping was conducted using Roche's Linear Array Test	Western-Cape
60. Jones et al. (2007) Agreement between Self- and Clinician-Collected Specimen Results for Detection and Typing of High-Risk Human Papillomavirus in Specimens from Women in Gugulethu, South Africa\	Sample type comprised cervical specimens collected using a Digene cervical sampling brush, Self-collected swabs or tampons were also collected. HPV genotyping was conducted using Roche's Linear Array Test and Hybrid Capture 2	Western-Cape
61. Kay et al. (2003) High Prevalence of HPV 16 in South African Women With Cancer of the Cervix and Cervical Intraepithelial Neoplasia \	Sample type comprised biopsy specimens, collection device was not reported. Restriction fragment length polymorphism (RFLP) was employed, HPV genotyping was also done by direct sequencing	Western-Cape
62. Kuhn et al. (2000) Human Papillomavirus DNA Testing for Cervical Cancer Screening in Low-Re source Settings	Sample type comprised cervical specimens collected using an extended tip plastic spatula with endocervical cytobrush, HPV genotyping was conducted using Hybrid Capture I and HC2	Western-Cape
63. Wright et al. (2000) HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer	Sample type comprised self-sampled specimens collected using a Dacron swabs and cervical specimens used a special conically shaped brush. HPV Genotyping was conducted using Hybrid Capture 2	Western-Cape
64. Ramesar et al. (1996) Human papillomavirus in normal cervical smears from Cape Town	Sample type comprised biopsy specimens collected using a spatula. HPV genotyping was conducted using southern blot hybridisation	Western-Cape
65. Williamson et al. (1994) Typing of Human Papillomaviruses in Cervical Carcinoma Biopsies From Cape Town	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using southern blot hybridisation and sequencing	Western-Cape
66. Cooper et al. (1992) Integration of human papillomavirus types 16 and 18 in-cervical adenocarcinoma	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using NISH	Britain and South Africa (KwaZulu-Natal
67. Copper et al. (1991) In situ evidence for HPV 16,18,33 integration in cervical squamous cell cancer in Britain and South Africa	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using non-isotopic in situ hybridization (NISH)	Britain and South Africa (KwaZulu-Natal)
68. Cooper et al. (1991) In situ human papillomavirus (HPV) genotyping of cervical intraepithelial neoplasia in South African and British patients: Evidence for putative HPV integration in vivo	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using non-isotopic in situ hybridization (NISH)	Britain and South Africa (KwaZulu-Natal)
69. Williamson et al. (1989) Typing of Human Papillomaviruses in Cervical Intraepithelial Neoplasia Grade 3 Biopsies From Cape Town	Sample type comprised biopsy specimens collection device was not reported. HPV genotyping was conducted using Southern blotting hybridization	Western-Cape

© University of Venda

To achieve objective 3, data was first pooled from 46 of 69 studies to estimate the prevalence and distribution of hr-HPV genotypes among women in the general population, without any emphasis on the HIV status in the country (Table 2.2). Accordingly, the 69 records were further scrutinised, and the following categories of records were excluded: (1) reported the distribution of HPV genotypes based on the vaccine coverages, (2) reported HPV genotypes collectively as hr-lr-HPV genotypes and/or single/multiple infections instead of reporting on the individual genotypes across the study participants, (3) studies that updated data from the previously published data, (4) studies whose results were showed by graphs making it difficult to interpret the proportions based on the scales used, and (5) comparative studies which did not separate the distribution of the HPV DNA by country. In order to estimate the overall number of study participants, studies that used the same samples were only counted once. This was done to avoid over-representation of the population size. Furthermore, a sub-analysis was conducted from 22/46 studies that clearly documented the prevalence of hr-HPV genotypes among South African women according to their cervical cytology status (Table 2.2).

To achieve objective 4, which was to determine the prevalence and distribution of hr-lr-HPV genotypes by HIV status, an intra-search strategy using Mendeley was conducted again within the 46 studies. The following steps were taken: The upper right section of Mendeley page was clicked to help segregate the records by HIV status. Key words such as “Human Immunodeficiency Virus”, “HIV”, “HIV-infected/positive” and “HIV-uninfected/negative/HIV non-infected” and “HIV nonpositive” were entered into the search box interchangeably to search for HPV records in the library that speak of HIV status. The resulting 26 records that clearly specified the distribution of HPV genotypes according to HIV status in the country were then grouped into a sub-folder for analysis, Table 2.2. Of note, part of the excluded records included all studies that had unreported South African provinces, unspecified HIV status, and those that did not segregate the distribution of HPV genotypes by HIV status.

© University of Venda

Table 2.2: Human papillomavirus studies used to achieve objectives 3 and 4

Objective 3 n=46	Objective 3 n=22	Objective 4 (n=26)
1. Williamson et al., 1989	1. Williamson et al., 1989	1. Marais et al., 2008
2. Williamson et al., 1991	2. Williamson et al., 1991	2. Richter et al., 2008
3. Cooper et al., 1991	3. Cooper et al., 1991	3. Firnhaber et al., 2009
4. Cooper et al., 1991	4. Cooper et al., 1991	4. Moodley et al., 2009
5. Cooper et al., 1992	5. Cooper et al., 1992	5. Firnhaber et al., 2010
6. Ramesar et al., 1996	6. Ramesar et al., 1996	6. Mbulawa et al., 2010
7. Kay et al., 2003	7. Kay et al., 2003	7. Auvert et al., 2011
8. Jones et al., 2007	8. Jones et al., 2007	8. Wang et al., 2011
9. Allan et al., 2008	9. Allan et al., 2008	9. Dols et al., 2012
10. Marais et al., 2008	10. Marais et al., 2008	10. Dols et al., 2012
11. Richter et al., 2008	11. Richter et al., 2008	11. Richter et al., 2013
12. Firnhaber et al., 2009	12. Firnhaber et al., 2009	12. Adler et al., 2014
13. Mbulawa et al., 2009	13. Said et al., 2009	13. Ameur et al., 2014
14. Moodley et al., 2009	14. Moodley et al., 2009	14. McDonald et al., 2014
15. Said et al., 2009	15. McDonald et al., 2012	15. Tayib et al., 2015
16. Firnharber et al., 2010	16. Dols et al., 2012	16. Van Aardt et al., 2015
17. Mbulawa et al., 2010	17. McDonald et al., 2014	17. Ebrahim et al., 2016
18. Wang et al., 2011	18. Ameur et al., 2014	18. Van Aardt et al., 2016
19. Dols et al., 2012	19. Denny et al., 2014	19. Kelly et al., 2017
20. McDonald et al., 2012	20. Rad et al., 2017	20. Mbatha et al., 2017
21. Mbulawa et al., 2012	21. Taku et al., 2020	21. Rad et al., 2017
22. Adler et al., 2013	22. Taku et al., 2021	22. Chikandiwa et al., 2018
23. Richter et al., 2013		23. Mbulawa et al., 2018
24. Adler et al., 2014		24. Mbulawa et al., 2021
25. Ameur et al., 2014		25. Saidu et al., 2021
26. Denny et al., 2014		26. Taku et al., 2021
27. McDonald et al., 2014		
28. Tayib et al., 2015		
29. Van Aadrt et al., 2015		
30. Zeir et al., 2015		
31. Ebrahim et al., 2016		
32. Mbulawa et al., 2016		
33. Lebelo et al., 2015		
34. Van Aadrt et al., 2016		
35. Kelly et al., 2017		
36. Mbatha et al., 2017		
37. Rad et al., 2017		
38. Chikandiwa et al., 2018		
39. Mbulawa et al., 2021		
40. Saidu et al., 2021		
41. Segondy et al., 2016		
42. Mbulawa et al., 2018		
43. Johnson et al., 2020		
44. Taku et al., 2020		
45. Mbulawa et al., 2021		
46. Taku et al., 2021		

2.4.8 Analysis for human papillomavirus genotypes prevalence and distribution in South Africa

Eligible literature that reported for HPV genotypes, specimen genotypes, laboratory techniques used to identify HPV genotypes, and HIV status, were screened by provinces in South Africa. Furthermore, outcomes were tabulated according to author and year of publication, province of study, study population (women living with and without HIV), and study design. Extracted data was examined, across the country, for the prevalence of each of the 14 hr-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and 70) (Bouvard et al., 2009; Schiffman et

© University of Venda

al., 2009) regardless of the HIV status first and then classified according to HIV status with two lr-HPV genotype 6 and 11 included.

2.4.9 Statistical analysis

An online Statistical Package for Social Sciences (SPSS) (Social Science Statistics, 2022) tool using option p-value calculators was used to derive the p-values. A positive significant test was considered statistically significant at $p < 0.05$.

2.5 RESULTS

2.5.1 Characteristics of analyzed studies

According to our inclusion criteria, 69 studies were selected for analysis and represented only 5 of the 9 South African provinces, namely: the Western Cape, the Eastern Cape, Gauteng, KwaZulu-Natal, and Limpopo. Overall, a total of 38 049 women participated in these studies, of whom 9 955 and 20 747 were living with, and without HIV, respectively. Western Cape Province had the highest number of study participants (n = 27 779) followed by Gauteng Province (n = 5 817); Limpopo Province has been observed to have the least number (n = 100) of study participants. In order to avoid over-representation of the study participants, all studies that used the same sample size were counted once. The characteristics of all the 69 articles included in the quantitative analysis are shown in Table 2.1.

Fifty-two of the 69 studies were conducted uniquely in the Western Cape (n = 31), Gauteng (n = 12), Eastern Cape (n = 5), KwaZulu-Natal (n = 3), and Limpopo (n = 1). In two studies, participants were recruited from more than one province. One study comprised participants from KwaZulu-Natal Province and Gauteng Province, while the other study involved participants from the Western Cape and Gauteng provinces. In two studies, the province from which participants were recruited was not indicated. Furthermore, 13 comparative studies were conducted between South Africa and other countries. Three studies were reported for Britain and South Africa (KwaZulu-Natal), while seven studies were reported for Burkina Faso and South Africa (Gauteng), and only one study was conducted in three countries – Ghana, Nigeria, and South Africa. Lastly, one study each was conducted in Limpopo Province and Tanzania while the other one was in KwaZulu-Natal Province and Nairobi. These studies are represented in Table 2.1.

The 69 studies eligible for analysis comprised three study populations, namely: HIV non-infected (n = 6), HIV-positive (n = 17), and both HIV-positive and non-infected populations (n = 31). Fifteen studies contained unspecified HIV status. Overall, there were 48 studies that

© University of Venda

included HIV-positive populations, including those that comprised both HIV-positive and HIV uninfected individuals.

2.5.2 Sample type, collection mode, and human papillomavirus genotyping approaches

Of the 69 studies analyzed, we grouped specimens into cervical/cervicovaginal lavages, biopsies, self-collected, and both self-collected/cervical specimens. The most commonly used specimens included cervical/cervicovaginal lavages, reported in 40 studies, and cervical biopsies, reported in 17 studies. In addition, specimens in six studies were both cervical and self-collected, while self-collected specimens were used in another six studies. Of the devices and tools used to collect these specimens, brushes were the commonly used tool reported in 32 of the 69 studies. The use of a spatula was among the least used collection devices, (Table 2.1).

There is a fair degree of heterogeneity on the approaches used to genotype HPV. The Linear Array (LA) was the most commonly used method reported in 30 studies. This was followed by INNOLiPA (n = 6) and hybrid capture 2 (HC2) n = 4. Enzyme immuno assay (EIA), nonisotopic *in situ* hybridisation (NISH) and Xpert HPV were used in three studies each, while southern blot hybridisation was applied in two studies. The HPV direct flow chip and TaqMan based quantification polymerase chain reaction (PCR) were applied once, respectively. Of the studies that used more than one technique, HC2/LA was the most commonly used (n = 4), followed by HC2/INNO Lipa (n = 2). The least frequently used technique included LA/Xpert HPV, care HPV/INNO Lipa, reverse line blot/PreTeck HPV proofer, HC1/HC2, HC2/hpVIR, and cobas test/LA/HC2 which were used in one study each. In one comparative study, Illumina detected 16 HPV genotypes, including HPV 30, HPV 74, HPV 86, and HPV 90, not included in LA; and Sanger sequencing was seen to resolve HPV DNA (Deoxyribonucleic acid) untypable by Restriction Fragment Length Polymorphism (RFLP) in another study. Lastly, one study used southern blot hybridisation and sequencing, while the other study compared the genotypic outcomes obtained through massively parallel sequencing to HPVIR assay LA HPV genotyping test (Table 2.1).

2.5.3 High-risk human papillomavirus genotype distribution and human immunodeficiency virus status

The proportion of 14 hr-HPV genotypes was examined, irrespective of the HIV status of the women. Overall, 46 studies were thereafter examined, for the 14 hr-HPV genotypes, Table 2.2. Of the total 30 677 participants, HPV genotype 16 was the most frequent (8.80%), which was followed by genotypes 35 (4.86%), 18 (4.14%), 58 and 52 with the frequency of 3.65% and 3.62% individually. The least frequent was genotype HPV 70 (0.74%), Table2.3.

© University of Venda

Table 2.3: Estimated prevalence of 14 known hr-HPV genotypes across 46 studies regardless of HIV status in South Africa

HPV genotype	**16**	**18**	**31**	**33**	**35**	**39**	**45**	**51**	**52**	**56**	**58**	**59**	**68**	**70**
Total number of participants (n) who tested positive for HPV	2700	1270	795	829	1491	631	1045	991	1110	703	1120	656	637	226
Prevalence (%)	8,80	4,14	2.59	2,70	4,86	2,06	3,41	3,23	3,62	2,29	3,65	2,14	2,08	0,74

Furthermore, a sub-analysis of cytology data demonstrated that a total of 13 044 and 15 782 women had a normal and abnormal pap smear results, respectively (Table 2.2). Women with abnormal pap smear had higher prevalence of HPV genotypes when compared to their counterparts. Among women who had an abnormal pap smear, HPV 16 (6.60%), HPV 35 (2.43%), HPV 18 (2.24%), HPV 58 (1.70%), HPV 33 (1.62%), HPV 45 (1.57% each) and HPV 52 (1.52%) were the seven most prevalent genotypes in descending order of frequency. On the contrary, HPV genotypes 16 (2.03%), 35 (1.85%), 58 (1.58%), 45 (1.55%), 18 (1.40%), 52 (1.30%) and 68 (1.19%) were the most predominant genotypes among women who had a normal pap smear test.

From the 26 studies (Table 2.2) that documented the proportion of HPV genotype distribution according to HIV status, these studies were examined to estimate the frequency of 14 hr-HPV and two lr-HPV genotypes and to determine the statistical association between HPV genotype distribution and HIV status, also considering the genotypes included in the Gardasil-9 vaccine. The findings revealed that similar HPV genotypes were detected in both HIV-positive and HIV negative participants, with HPV 16, HPV 18, and HPV 35 being the most commonly detected genotypes in both HIV-positive and HIV-uninfected populations in the country. However, there was a variation in proportions. For these genotypes, the observed proportions were as follows: 0.135% and 0.047% (HPV 16); 0.065% and 0.028% (HPV 35); 0.068% and 0.024% for HPV 18 detected in women living with and without HIV, respectively. On the contrary, HPV 6, HPV 11, and HPV 70 were the least common genotypes, with the proportions ranging from 0.001% to 0.015%. Furthermore, statistical analysis depicted that genotypes HPV 6, HPV 11, HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 68, and HPV 70 were significantly more common among women living with HIV than those who were living without HIV. In contrast, HPV 59 was the only genotype observed to be significantly more common among women living without HIV. All these HPV genotypes were statistically significant at $p < 0.00001$ (Table 2.4).

© University of Venda

1451 **Table 2.4:** Estimated prevalence of known 14 hr-HPV and 2 lr-HPV genotypes (incorporated in Gardasil 9) with HIV status in South Africa

	HPV 45		**HPV 51**		**HPV 52**		**HPV 56**		**HPV 58**		**HPV 59**		**HPV 68**		**HPV 70**	
	HIV +	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**
Western Cape	213 (52,99%)	207 (72,63%)	133 (39,94%)	180 (64,29%)	175 (40,42%)	216 (71,76%)	92 (34,98%)	111 (71,61%)	230 (56,79%)	242 (73,78%)	81 (54.00%)	122 (67,40%)	122 (60.70%)	127 (79,38%)	43 (39,09%)	0
Gauteng	159 (39,55%)	26 (9,12%)	173 (51,95%)	23 (8,21%)	227 (52,42%)	25 (8,30%)	143 (54,37%)	3 (1,94%)	144 (35,55%)	25 (7,62%)	58 (38,67%)	11 (6,08%)	70 (34,83%)	5 (3,12%)	59 (53,64%)	0
KwaZulu-Natal	10 (2,49%)	42 (14,74%)	13 (3,90%)	72 (25,71%)	7 (1,62%)	49 (16,28%)	14 (5,32%)	38 (24,52%)	6 (1,48%)	50 (15,24%)	8 (5,33%)	46 (25,14%)	3 (1,49%)	26 (16,25%)	0	16 (84%)
Limpopo	0	1 (0)%	4 (1,20%)	2 (0,71%)	5 (1,15%)	4 (1,33%)	0	1 (0,65%)	4 (0,99%)	0	0	0	0	0	0	0
Eastern Cape	20 (4,98%)	9 (3,16%)	10 (3,00%)	3 (1,07%)	19 (4,39%)	7 (2,33%)	14 (5,32%)	2 (1,29%)	21 (5,19%)	11 (3,35%)	3 (2.00%)	2 (1,10%)	6 (2,99%)	2 (0,63%)	8 (7,27%)	3 (15,79%)
Total no: of HPV positives	402	285	333	280	433	301	263	155	405	328	150	181	201	160	110	19
Total no: of HIV ± participants	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066
Proportion (%) of HPV genotypes	0,055	0,018	0,046	0,017	0,059	0,019	0,036	0,010	0,056	0,020	0,021	0,011	0,028	0,010	0,015	0,001
Chi square	228,7107		147,1363		252,1913		190,488		188,9807		30,1523		98,5078		173,751	
P-value	< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001	

© University of Venda

Table 2.4: Continuation table of the estimated prevalence of known 14 hr-HPV and 2 lr-HPV genotypes (incorporated in Gardasil 9) with HIV status in South Africa

	HPV 6		HPV 11		HPV 16		HPV 18		HPV 31		HPV 33		HPV 35		HPV 39	
	HIV +	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**	**HIV +**	**HIV -**
Western Cape	47 (48,95%)	19 (100%)	59 (56,73%)	23 (100%)	335 (34,11%)	385 (50,72%)	213 (43,12%)	184 (47,92%)	98 (40,66%)	153 (74,73%)	115 (38,59%)	144 (67,61%)	191 (40,47%)	333 (73,03%)	101 (45,70%)	88 (64,71%)
Gauteng	49 (51,04%)	0	45 (43,27%)	0	563 (57,33%)	264 (34,78`%)	231 (46,76%)	136 (35,42%)	114 (47,30%)	17 (8,29%)	129 (43,29%)	23 (10,80%)	214 (45,34%)	55 (12,06%)	97 (43,89%)	3 (2,21%)
KwaZulu-Natal	0	0	0	0	25 (2,55%)	99 (13,04%)	21 (4,25%)	57 (14,84%)	9 (3,73%)	30 (14,63%)	14 (4,70%)	42 (19,72%)	15 (3,18%)	54 (11,84%)	9 (4,07%)	43 (31,62%)
Limpopo	0	0	0	0	27 (2,75%)	3 (0%)	17 (3,44%)	2 (0,52%)	2 (0,83%)	1 (0%)	17 (6,38%)	2 (0,94%)	19 (4,03%)	3 (0,66%)	3 (1,36%)	1 (0,74%)
Eastern Cape	0	0	0	0	32 (3,26%)	8 (1,05%)	12 (2,43%)	5 (1,30%)	18 (7,47%)	4 (1,95%)	23 (7,72%)	2 (0,94%)	33 (6,99%)	1 (0,22%)	11 (4,98%)	1 (0,74%)
Total no: of HPV positives	96	19	104	23	982	759	494	384	241	205	298	213	472	456	221	136
Total no: of HIV ± participants	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066	7289	16066
Proportion (%) of HPV genotypes	0,013	0,001	0,014	0,001	0,135	0,047	0,068	0,024	0,033	0,013	0,041	0,013	0,065	0,028	0,030	0,008
Chi square	144,978		150,399		465,4693		243,6381		105,4317		169,457		158,5202		153,0904	
P-value	< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001		< 0,00001	

© University of Venda

2.6 DISCUSSION

This review provides insights on the scope of HPV research on viral diversity and HIV status among South African women between 1989 and 2021. Primarily, it reveals that HPV research is limited to five of the nine South African provinces (Gauteng, KwaZulu-Natal, Limpopo, Western Cape and Eastern Cape). However, there are relatively few studies from each of the five provinces. Nevertheless, Western Cape Province had the highest number of studies among the five provinces. Factors such as poor access of primary healthcare systems, lack of viral HPV knowledge, lack of pap testing, misconceptions and conspiracy theories associated with the HPV infection at large, could have had an impact on the participation of the participants in the studies. Factors such as financial constraints involved in conducting the HPV diversity research studies, lack of HPV research expertise, difficulties in attaining permission (s) and approval (s) from relevant organisations might have been some of the reasons why other provinces lack data on HPV research.

Studies have shown that the distribution of HPV infection differs by province, technique used to detect it, HIV status of participants, specimen collection method and the age group of participants (Clifford et al., 2005a; Clifford et al., 2005b; Smith et al., 2008; Ebrahim et al., 2016). The study findings revealed that there are various methods used to identify HPV genotypes in the country, with Roche LA being the most commonly used technique, while sequencing is rarely used. Thus, more consistent methods for sample collection and genotyping procedures are necessary to systematically assess the prevalence of potentially carcinogenic genotypes of HPV.

Despite the higher cost involved in next generation sequencing (NGS) (Ari and Arikan, 2016), the genotyping tool has proved to be most specific (Nilyanimit et al., 2018) and sensitive (Barzon et al., 2011; Flores-Miramontes et al., 2015) compared to LA genotyping test kit that only detects the restricted number of 37 HPV genotypes incorporated in the kit, and has the possibility of missing mixed and minority infections. In Mexico Flores-Miramontes and colleagues genotyped HPV using both LA and NGS and detected that HPV genotypes 32, 44, 74, 102, and 114 were not incorporated in the LA (Flores-Miramontes et al., 2015). In South Africa, specifically in Cape-Town, Meiring and colleagues compared LA and NGS and found that NGS was able to detect four additional HPV genotypes (30, 74, 86 and 90) not covered by the 37 restricted genotypes of the LA (Meiring et al., 2012). This also implies that the differences on how the samples are genotyped have a great impact on the genotyping outcomes.

© University of Venda

Thus, the authors agree that more sensitive technologies such as NGS are significant at least at a research level to be used as a strategy to unravel the full spectrum of HPV genotypes in the South African population. Recent findings documented by Ardhaoui and colleagues depicted that NGS was able to detect genotypes of clinical importance that include HPV genotypes 31, 39 and 52 that were undetected by the reverse line hybridisation genotyping technique regardless of the presence of their probes embedded on the membrane (Ardhaoui et al., 2021). This raises a significant aspect of presumably under reporting of other HPV genotypes of clinical importance in certain cohorts. Of note, kits validation for accuracy purposes and in the management of the disease can be encouraged.

Despite the inconsistencies used in data reporting strategies across the studies, the overall HPV genotype distribution according to HIV status and in a general population depict that HPV 16, HPV 35 and HPV 18 were the most frequent genotypes in these populations. This encourages the need for routine hospital or clinic visits for regular check-ups among South African women, especially those who never received any vaccine. With evidence pointing to a significant increase of HPV more in women living with HIV (Clifford et al., 2006; Mbulawa et al., 2009; Mbulawa et al., 2012; Akarolo-Anthony et al., 2013; Mbulawa et al., 2013) and in the general population. The findings of the literature review conducted in 2021 by Kombe Kombe and colleagues highlighted that the prevalence of HPV genotype distribution varies across the world, although it does not change the associated diseases caused by these viral genotypes (Kombe Kombe et al., 2021). Furthermore, there are certain HPV genotypes that have been found to be present around the world (Kombe Kombe et al., 2021). Several review studies conducted across continents including Africa, revealed that hr-HPV genotypes such as 16, 18, 35 and 58 among others are the most frequent genotypes (Kombe Kombe et al., 2021), which is consistent with the findings of the current review.

HPV/HIV co-infection among women particularly in developing countries remains one of the principal public health challenges requiring closer intervention. Worldwide, in a general population HPV genotype 16, 18 and 45 are primarily linked with CC (Bouvard et al., 2009). However, the prevalence of HPV genotypes varies worldwide. In Asia, HPV 52 and HPV 45 are the most prevalent HPV genotypes. In the USA and Europe, HPV genotypes 45 and 33, respectively, are the most common genotypes, while genotypes 35 and 45 are more predominant in Africa (De Sanjose et al., 2010; Guan et al., 2012; Tjalma et al., 2013). All these genotypes were common after HPV 16 and HPV 18 in participants with invasive CC. Both HPV genotypes 35 and 45 are considered hr-HPV (Bouvard et al., 2009) for subsequent CC. Although several studies show that the bivalent vaccine does offer protection against these

© University of Venda

genotypes, cross-protection vaccines are less reliable compared to genotype specific and is of a limited duration (Joura and Pils, 2016). Neither of these are included in the currently available HPV vaccines in South Africa. To enrich our understanding of HPV genotypes, including detection of minority viral populations in South Africa, the application of sanger sequencing or next generation sequencing is strongly recommended (Di Resta and Ferrari, 2018), at least for research purposes. This is important to identify putative hr-HPV genotypes which may not be covered by the current detection approaches and vaccines.

The state of cervical cytology in the studied population plays a significant role in the distribution of HPV genotypes, be it single and/or multiple infections and the overall prevalence. Our findings revealed that HPV 16, HPV 35, HPV 58, HPV 45, HPV 18, HPV 52 and HPV 68, and HPV 16, HPV 35, HPV 18, HPV 58, HPV 33, HPV 45 and HPV 52 were the most prevalent genotypes in descending order among women with normal and abnormal pap smear, respectively. This was in accordance with a study conducted by Wolday and colleaques regarding the distribution of HPV genotype among Ethiopian women with normal and abnormal cervical cytology. Their findings revealed that HPV HPV 16, HPV 35, HPV 45, and HPV 18 were among the most prevalent HPV genotypes among women with normal cytology while most women with abnormal cervical cytology had HPV genotypes 16, 45, 31 and 35 (Wolday et al., 2018). Another similar findings were reported in Turkey (Muderris et al., 2019). In both studies, HPV genotype 16 was the most detected genotype among women with normal and abnormal cytology (Wolday et al., 2018; Muderris et al., 2019). A meta-analysis data by Obeid and colleagues demonstrated that HPV 16 and 58 were the most common genotypes among women with abnormal cervical cytology in the Middle East and North Africa (Obeid et al., 2020). Of note is that a normal cervical cytology test does not necessarily mean the absence of hr-HPV of clinical importance. Thus, the presence of hr-HPV genotypes among women with normal cytology raises a serious challenge among policymakers in South Africa. The study findings encourage closer routine check-ups and HPV screening should be prioritized among South African women.

Cervical cancer is responsible for significant mortality among women (Flores-Miramontes et al., 2015). Cervical cancer mortality among women living with HIV infection is twice greater than those living without HIV (Coghill et al., 2015; Strickler et al., 2015). Nonetheless, the availability of ART increases the life span of women living with HIV and raises the necessity for the prevention of CC in developing countries (Dryden-Peterson et al., 2016). To fully understand the distribution of HPV genotypes in a specific geographic location, genotyping samples from both individuals living with HIV and without HIV is highly recommended. In

© University of Venda

the absence of effective screening, CC might kill millions of women (Sankaranarayanan et al., 2001). Human papillomavirus genotyping can be both a secondary prevention technique employed to help in the screening and early detection of CC, as well as a tertiary prevention technique that also helps in the management of pre-cancerous cervical lesions (Choi and Park, 2016).

In addition, HPV genotyping technique can be used to further investigate women who test positive during cytology (Saslow et al., 2002). Possible recurrence of HPV infections can also be monitored using this method during patient follow-ups after their treatment of cervical intraepithelial neoplasia (CIN) (Paraskevaidis et al., 2004; Jones et al., 2011). Thus, understanding the facet of HPV genotypes would aid in the better strategies of prevention and management of CC (Choi and Park, 2016). Despite the late HPV vaccination rollout in South Africa, Western Cape, Gauteng and KwaZulu-Natal provinces have data depicting the vaccination coverages of first doses among the primary schools ranging between 64.0% and 99.7% (Moodley et al., 2013; Snyman et al., 2015; Department of Health, 2022). However, to the best of our knowledge, checking when the vaccination rollout programme commenced in the country and the target age groups, our study participants analyzed are highly unlikely to have been vaccinated or benefited from these programmes. Therefore, perhaps some of the circulating genotypes of clinical significance might have been prevented or lowered provided some of the study participants benefited from the vaccine.

This narrative review should be considered with some limitations. It was not possible to examine or investigate the differences in the distribution of HPV genotypes according to socio-economic and vaccine status of the participants due to the lack of data in the analyzed studies. It has been demonstrated that these two factors impact the carriage and genotype of HPV (Danis et al., 2010; Lefkowitz et al., 2014). Secondly, it would have been of interest to review the distribution of hr-HPV among women on antiretroviral therapy. Recent data suggests that ART may lead to clearance of HPV infection (Liu et al., 2018; Hidalgo-Tenorio et al., 2019). With South Africa's skewed and disparate urban and rural health and health outcomes, it would have been great to determine the HPV genotype distribution and prevalence in those two settings. However, out of 69 studies (Table 2.1) that met our inclusion criteria, only six (9%) were conducted specifically in rural areas. Thus, a comparison would be biased towards where more studies were done.

In conclusion, this review describes the prevalence and distribution of cervical HPV genotypes as reported in the literature which had data from only five of the nine provinces in South Africa.

© University of Venda

These regional variations highlight the necessity for improved geographical representation and national surveillance. The high prevalence of hr-HPV 16, HPV 18 and HPV 35 genotypes, regardless of HIV status is a public health concern, considering their significant contribution to the burden of CC. Routine HPV screening and prevention measures such as HPV vaccination for all individuals remain critical. Because current vaccines exclude HPV 35 targets, there is a need to consider this genotype in vaccine development approaches. Therefore, it is proposed that further research should be conducted in order to expand the scope of HPV genotypes, including the use of sensitive approaches such as NGS. This will augment to enhanced understanding regarding the patterns of HPV geo-diversity in South Africa. Such understanding will further help in decision-making regarding the study of geographically relevant vaccines and screening strategies.

© University of Venda

2.7 REFERENCES

1. Adler, D.H., Laher, F., Lazarus, E., Grzesik, K., Gray, G.E., Allan, B. and Williamson, A.L., 2013. A viable and simple self-sampling method for human papillomavirus detection among South African adolescents. *Journal of immunological techniques in infectious diseases*, 2(3), pp.10-4172.

2. Adler, D., Laher, F., Wallace, M., Grzesik, K., Jaspan, H., Bekker, L.G., Gray, G., Valley-Omar, Z., Allan, B. and Williamson, A.L., 2013. High rate of multiple concurrent Human Papillomavirus infections among HIV-uninfected South African Adolescents. *Journal of immunological techniques in infectious diseases*, 2(1), p.1000106.

3. Adler, D., Wallace, M., Bennie, T., Abar, B., Sadeghi, R., Meiring, T., Williamson, A.L. and Bekker, L.G., 2015. High risk human papillomavirus persistence among HIV-infected young women in South Africa. *International Journal of Infectious Diseases*, 33, pp.219-221.

4. Adler, D.H., Wallace, M., Bennie, T., Mrubata, M., Abar, B., Meiring, T.L., Williamson, A.L. and Bekker, L.G., 2014. Cervical Dysplasia and High-Risk Human Papillomavirus Infections among HIV-Infected and HIV-Uninfected Adolescent Females in South Africa. *Infectious diseases in obstetrics and gynecology*, 2014(1), p.498048.

5. Adler, D.H., Wallace, M., Bennie, T., Abar, B., Meiring, T.L., Williamson, A.L. and Bekker, L.G., 2016. Cumulative impact of HIV and multiple concurrent human papillomavirus infections on the risk of cervical dysplasia. *Advances in Virology*, 2016(1), p.7310894.

6. Akarolo-Anthony, S.N., Al-Mujtaba, M., Famooto, A.O., Dareng, E.O., Olaniyan, O.B., Offiong, R., Wheeler, C.M. and Adebamowo, C.A., 2013. HIV associated high-risk HPV infection among Nigerian women. *BMC infectious diseases*, 13, pp.1-6.

7. Allan, B., Marais, D.J., Hoffman, M., Shapiro, S. and Williamson, A.L., 2008. Cervical human papillomavirus (HPV) infection in South African women: implications for HPV screening and vaccine strategies. *Journal of clinical microbiology*, 46(2), pp.740-742.

8. Ameur, A., Meiring, T.L., Bunikis, I., Häggqvist, S., Lindau, C., Lindberg, J.H., Gustavsson, I., Mbulawa, Z.Z., Williamson, A.L. and Gyllensten, U., 2014. Comprehensive profiling of the vaginal microbiome in HIV positive women using massive parallel semiconductor sequencing. *Scientific reports*, 4(1), p.4398.

© University of Venda

9. Ardhaoui, M., Ennaifer, E., De Matos Salim, A.C., Gomez, F.M., Laasili, T., Boubaker, M.S. and Guizani, I., 2021. Nested PCR followed by NGS: Validation and application for HPV genotyping of Tunisian cervical samples. *PLos One*, 16(8), p.e0255914.

10. Ari, Ş. and Arikan, M., 2016. Next-generation sequencing: advantages, disadvantages, and future. Plant omics: *Trends and applications*, pp.109-135.

11. Auvert, B., Marais, D., Lissouba, P., Zarca, K., Ramjee, G. and Williamson, A.L., 2011. High-risk human papillomavirus is associated with HIV acquisition among South African female sex workers. *Infectious diseases in obstetrics and gynecology*, 2011(1), p.692012.

12. Barzon, L., Militello, V., Lavezzo, E., Franchin, E., Peta, E., Squarzon, L., Trevisan, M., Pagni, S., Dal Bello, F., Toppo, S. and Palù, G., 2011. Human papillomavirus genotyping by 454 next generation sequencing technology. *Journal of Clinical Virology*, 52(2), pp.93-97.

13. Bhatia, N., Lynde, C., Vender, R. and Bourcier, M., 2013. Understanding genital warts: epidemiology, pathogenesis, and burden of disease of human papillomavirus. *Journal of cutaneous medicine and surgery*, 17(6_suppl), pp.S47-S54.

14. Botha, M.H. and Richter, K.L., 2015. Cervical cancer prevention in South Africa: HPV vaccination and screening both essential to achieve and maintain a reduction in incidence. *South African medical journal*, *105*(1), pp.33-34.

15. Bouvard, V., Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L. and Cogliano, V., 2009. A review of human carcinogens—Part B: biological agents. *The lancet oncology*, 10(4), pp.321-322.

16. Bruni, L., Barrionuevo-Rosas, L., Albero, G., Aldea, M., Serrano, B., Valencia, S., Brotons, M., Mena, M., Cosano, R., Muñoz, J. and Bosch, F.X., 2015. ICO Information Centre on HPV and Cancer (HPV Information Centre). Human papillomavirus and related diseases in the world. *Summary Report*, pp.04-08.

17. Bruni, L., Albero, G., Serrano, B., et al., 2021. Human papillomavirus and related diseases in South Africa. Barcelona: ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). *Summary Report*.

18. Chambuso, R., Ramesar, R., Kaambo, E., Murahwa, A.T., Abdallah, M.O., De Sousa, M., Denny, L., Williamson, A.L. and Gray, C.M., 2020. Age, absolute CD4 count, and CD4

© University of Venda

percentage in relation to HPV infection and the stage of cervical disease in HIV-1-positive women. *Medicine*, 99(9), p.e19273.

19. Chikandiwa, A., Kelly, H., Sawadogo, B., Ngou, J., Pisa, P.T., Gibson, L., Didelot, M.N., Meda, N., Weiss, H.A., Segondy, M. and Mayaud, P., 2018. Prevalence, incidence and correlates of low risk HPV infection and anogenital warts in a cohort of women living with HIV in Burkina Faso and South Africa. *PLoS One*, 13(5), p.e0196018.

20. Choi, Y.J. and Park, J.S., 2016. Clinical significance of human papillomavirus genotyping. J Gynecol Oncol, 27(2), p.e21.

21. Clifford, G. and Franceschi, S., 2005. HPV in sub-Saharan Africa. *Papillomavirus Report*, 16(5), pp.322-326.

22. Clifford, G.M., Franceschi, S., Keiser, O., Schöni-Affolter, F., Lise, M., Dehler, S., Levi, F., Mousavi, M., Bouchardy, C., Wolfensberger, A. and Darling, K.E., 2016. Immunodeficiency and the risk of cervical intraepithelial neoplasia 2/3 and cervical cancer: a nested case-control study in the Swiss HIV cohort study. *International journal of cancer*, 138(7), pp.1732-1740.

23. Clifford, G.M., Gallus, S., Herrero, R., Munoz, N., Snijders, P.J., Vaccarella, S., Anh, P.T., Ferreccio, C., Hieu, N.T., Matos, E. and Molano, M., 2005. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *The Lancet*, 366(9490), pp.991-998.

24. Clifford, G.M., Goncalves, M.A.G., Franceschi, S. and HPV and HIV study group, 2006. Human papillomavirus types among women infected with HIV: a meta-analysis. *Aids*, 20(18), pp.2337-2344.

25. Coghill, A.E., Shiels, M.S., Suneja, G. and Engels, E.A., 2015. Elevated cancer-specific mortality among HIV-infected patients in the United States. *Journal of Clinical Oncology*, 33(21), pp.2376-2383.

26. Cooper, K., Herrington, C.S., Graham, A.K., Evans, M.F. and McGee, J.O., 1991. In situ evidence for HPV 16, 18, 33 integration in cervical squamous cell cancer in Britain and South Africa. *Journal of clinical pathology*, 44(5), pp.406-409.

© University of Venda

27. Cooper, K., Herrington, C.S., Graham, A.K., Evans, M.F. and McGee, J.O., 1991. In situ human papillomavirus (HPV) genotyping of cervical intraepithelial neoplasia in South African and British patients: evidence for putative HPV integration in vivo. *Journal of clinical pathology*, 44(5), pp.400-405.

28. Cooper, K., Herrington, C.S., Lo, E.S., Evans, M.F. and McGee, J.O., 1992. Integration of human papillomavirus types 16 and 18 in cervical adenocarcinoma. *Journal of clinical pathology*, 45(5), pp.382-384.

29. Danis, K., Georgakopoulou, T., Stavrou, T., Laggas, D. and Panagiotopoulos, T., 2010. Socioeconomic factors play a more important role in childhood vaccination coverage than parental perceptions: a cross-sectional study in Greece. *Vaccine*, 28(7), pp.1861-1869.

30. Delany-Moretlwe, S., Kelley, K.F., James, S., Scorgie, F., Subedar, H., Dlamini, N.R., Pillay, Y., Naidoo, N., Chikandiwa, A. and Rees, H., 2018. Human papillomavirus vaccine introduction in South Africa: implementation lessons from an evaluation of the national school-based vaccination campaign. Global Health: *Science and Practice*, 6(3), pp.425-438.

31. Denny, L., Adewole, I., Anorlu, R., Dreyer, G., Moodley, M., Smith, T., Snyman, L., Wiredu, E., Molijn, A., Quint, W. and Ramakrishnan, G., 2014. Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. *International journal of cancer*, 134(6), pp.1389-1398.

32. Department of Health (Western Cape Government), 2020. Vaccinations. [cited 2022 Jan 05]. Available from: https://www.westerncape.gov.za/general-publication/hpv-vaccinations

33. De Sanjosé, s., Serrano, B., Castellsagué, X., Brotons,., Muñoz, J., Bruni, L . and Bosch, F.X., 2012. Human papillomavirus (HPV) and related cancers in the Global Alliance for Vaccines and Immunization (GAVI) countries. A WHO/ICO HPV Information Centre Report. *Vaccine*, 30(4), pp. D1–D83.

34. De Sanjose, S., Quint, W.G., Alemany, L., Geraets, D.T., Klaustermeier, J.E., Lloveras, B., Tous, S., Felix, A., Bravo, L.E., Shin, H.R. and Vallejos, C.S., 2010. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *The lancet oncology,* 11(11), pp.1048-1056.

© University of Venda

35. De Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U. and Zur Hausen, H., 2004. Classification of papillomaviruses. *Virology*, 324(1), pp.17-27.

36. De Vuyst, H.D., Ndirangu, G., Moodley, M., Tenet, V., Estambale, B., Meijer, C.J., Snijders, P.J., Clifford, G. and Franceschi, S., 2012. Prevalence of human papillomavirus in women with invasive cervical carcinoma by HIV status in Kenya and South Africa. *International journal of cancer*, 131(4), pp.949-955

37. Di Resta, C. and Ferrari, M., 2018. Next generation sequencing: from research area to clinical practice. *Ejifcc*, 29(3), p.215.

38. Dols, J.A., Reid, G., Brown, J.M., Tempelman, H., Bontekoe, T.R., Quint, W.G. and Boon, M.E., 2012. HPV Type Distribution and Cervical Cytology among HIV-Positive Tanzanian and South African Women. *International Scholarly Research Notices*, 2012(1), p.514146.

39. Dols, J.A., Reid, G., Kort, R., Schuren, F.H., Tempelman, H., Bontekoe, T.R., Korporaal, H., Van der Veer, E.M., Smit, P.W. and Boon, M.E., 2012. PCR-based identification of eight Lactobacillus species and 18 hr-HPV genotypes in fixed cervical samples of South African women at risk of HIV and BV. *Diagnostic cytopathology*, 40(6), pp.472-477.

40. Dryden-Peterson, S., Bvochora-Nsingo, M., Suneja, G., Efstathiou, J.A., Grover, S., Chiyapo, S., Ramogola-Masire, D., Kebabonye-Pusoentsi, M., Clayman, R., Mapes, A.C. and Tapela, N., 2016. HIV infection and survival among women with cervical cancer. *Journal of Clinical Oncology*, 34(31), pp.3749-3757.

41. Dylla, L., Abar, B., Williamson, A.L., Meiring, T.L., Bekker, L.G. and Adler, D.H., 2017. Human papillomavirus clustering patterns among HIV-infected and HIV-uninfected adolescent females in South Africa. *Journal of AIDS and HIV research (Online)*, 9(10), p.202.

42. Ebrahim, S., Mndende, X.K., Kharsany, A.B., Mbulawa, Z.Z., Naranbhai, V., Frohlich, J., Werner, L., Samsunder, N., Karim, Q.A. and Williamson, A.L., 2016. High burden of human papillomavirus (HPV) infection among young women in KwaZulu-Natal, South Africa. *PloS one*, 11(1), p.e0146603.

43. Firnhaber, C., Van Le, H., Pettifor, A., Schulze, D., Michelow, P., Sanne, I.M., Lewis, D.A., Williamson, A.L., Allan, B., Williams, S. and Rinas, A., 2010. Association between cervical dysplasia and human papillomavirus in HIV seropositive women from Johannesburg South Africa. *Cancer Causes & Control*, 21, pp.433-443.

© University of Venda

44. Firnhaber, C., Zungu, K., Levin, S., Michelow, P., Montaner, L.J., McPhail, P., Williamson, A.L., Allan, B.R., Van der Horst, C., Rinas, A. and Sanne, I., 2009. Diverse and high prevalence of human papillomavirus associated with a significant high rate of cervical dysplasia in human immunodeficiency virus–infected women in Johannesburg, South Africa. *Acta cytologica*, 53(1), pp.10-17.

45. Flores-Miramontes, M.G., Torres-Reyes, L.A., Alvarado-Ruíz, L., Romero-Martínez, S.A., Ramírez-Rodríguez, V., Balderas-Peña, L.M.A., Vallejo-Ruíz, V., Piña-Sánchez, P., Cortés-Gutiérrez, E.I., Jave-Suárez, L.F. and Aguilar-Lemarroy, A., 2015. Human papillomavirus genotyping by Linear Array and Next-Generation Sequencing in cervical samples from Western Mexico. *Virology Journal*, 12, pp.1-11.

46. Guan, P., Howell-Jones, R., Li, N., Bruni, L., De Sanjosé, S., Franceschi, S. and Clifford, G.M., 2012. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. *International journal of cancer*, 131(10), pp.2349-2359.

47. Hidalgo-Tenorio, C., Gil-Anguita, C., López Ruz, M.A., Omar, M., López-Hidalgo, J. and Pasquau, J., 2019. ART is key to clearing oncogenic HPV genotypes (HR-HPV) in anal mucosa of HIV-positive MSM. *PloS one*, 14(10), p.e0224183.

48. Hogg, R.S., Lima, V. and Sterne, J.A.C., 2008. The ART Cohort Collaboration: Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet*, 372, pp.293-299.

49. Johnson, L.G., Saidu, R., Mbulawa, Z., Williamson, A.L., Boa, R., Tergas, A., Moodley, J., Persing, D., Campbell, S., Tsai, W.Y. and Wright, T.C., 2020. Selecting human papillomavirus genotypes to optimize the performance of screening tests among South African women. *Cancer Medicine*, 9(18), pp.6813-6824.

50. Jones, H.E., Allan, B.R., van De Wijgert, J.H., Altini, L., Taylor, S.M., de Kock, A., Coetzee, N. and Williamson, A.L., 2007. Agreement between self-and clinician-collected specimen results for detection and typing of high-risk human papillomavirus in specimens from women in Gugulethu, South Africa. *Journal of clinical microbiology*, 45(6), pp.1679-1683.

51. Jones, J., Saleem, A., Rai, N., Shylasree, T.S., Ashman, S., Gregory, K., Powell, N., Tristram, A., Fiander, A. and Hibbitts, S., 2011. Human Papillomavirus genotype testing

© University of Venda

combined with cytology as a 'test of cure'post treatment: the importance of a persistent viral infection. *Journal of Clinical Virology*, 52(2), pp.88-92.

52. Joura, E.A. and Pils, S., 2016. Vaccines against human papillomavirus infections: protection against cancer, genital warts or both?. *Clinical Microbiology and Infection*, 22, pp.S125-S127.

53. Karolinska Institutet., 2012. International human paillomavirus reference center. Karolinska University Hospital, Sweden: Huddinge. [cited 2021 July 07]. Available from: https://www.hpvcenter.se

54. Kay, P., Soeters, R., Nevin, J., Denny, L., Dehaeck, C.M. and Williamson, A.L., 2003. High prevalence of HPV 16 in South African women with cancer of the cervix and cervical intraepithelial neoplasia. *Journal of medical virology*, 71(2), pp.265-273.

55. Kelly, H.A., Ngou, J., Chikandiwa, A., Sawadogo, B., Gilham, C., Omar, T., Lompo, O., Doutre, S., Meda, N., Weiss, H.A. and Delany-Moretlwe, S., 2017. Associations of Human Papillomavirus (HPV) genotypes with high-grade cervical neoplasia (CIN2+) in a cohort of women living with HIV in Burkina Faso and South Africa. *PloS one*, 12(3), p.e0174117.

56. Kelly, H.A., Sawadogo, B., Chikandiwa, A., Segondy, M., Gilham, C., Lompo, O., Omar, T., Didelot, M.N., Nagot, N., Meda, N. and Weiss, H.A., 2017. Epidemiology of high-risk human papillomavirus and cervical lesions in African women living with HIV/AIDS: effect of anti-retroviral therapy. *Aids*, 31(2), pp.273-285.

57. Kombe Kombe, A.J., Li, B., Zahid, A., Mengist, H.M., Bounda, G.A., Zhou, Y. and Jin, T., 2021. Epidemiology and burden of human papillomavirus and related diseases, molecular pathogenesis, and vaccine evaluation. *Frontiers in public health*, 8, p.552028.

58. Kuhn, L., Denny, L., Pollack, A., Lorincz, A., Richart, R.M. and Wright, T.C., 2000. Human papillomavirus DNA testing for cervical cancer screening in low-resource settings. *Journal of the National Cancer Institute*, 92(10), pp.818-825.

59. Kuhn, L., Saidu, R., Boa, R., Tergas, A., Moodley, J., Persing, D., Campbell, S., Tsai, W.Y., Wright, T.C. and Denny, L., 2020. Clinical evaluation of modifications to a human papillomavirus assay to optimise its utility for cervical cancer screening in low-resource settings: a diagnostic accuracy study. *The Lancet Global Health*, 8(2), pp.e296-e304.

© University of Venda

60. Lebelo, R.L., Bogers, J.J., Thys, S., Depuydt, C., Benoy, I., Selabe, S.G., Bida, M.N. and Mphahlele, M.J., 2015. Detection, genotyping and quantitation of multiple hpv infections in south African women with cervical squamous cell carcinoma. *Journal of medical virology*, 87(9), pp.1594-1600.

61. Lefkowitz, E.S., Kelly, K.M., Vasilenko, S.A. and Maggs, J.L., 2014. Correlates of human papillomavirus vaccination among female university students. *Women & health*, 54(6), pp.487-501.

62. Leszczyszyn, J., Lebski, I., Lysenko, L., Hirnle, L. and Gerber, H., 2014. Anal warts (condylomata acuminata)-current issues and treatment modalities. Advances in Clinical and Experimental Medicine, 23(2), pp.307-11.

63. Lissouba, P., Van de Perre, P. and Auvert, B., 2013. Association of genital human papillomavirus infection with HIV acquisition: a systematic review and meta-analysis. *Sexually transmitted infections*, 89(5), pp.350-356.

64. Liu, G., Sharma, M., Tan, N. and Barnabas, R.V., 2018. HIV-positive women have higher risk of human papilloma virus infection, precancerous lesions, and cervical cancer. *Aids*, 32(6), pp.795-808.

65. Loke, A.Y., Kwan, M.L., Wong, Y.T. and Wong, A.K.Y., 2017. The uptake of human papillomavirus vaccination and its associated factors among adolescents: a systematic review. *Journal of primary care & community health*, 8(4), pp.349-362.

66. Luxembourg, A. and Moeller, E., 2017. 9-Valent human papillomavirus vaccine: a review of the clinical development program. *Expert review of vaccines*, *16*(11), pp.1119-1139.

67. Marais, D.J., Constant, D., Allan, B., Carrara, H., Hoffman, M., Shapiro, S., Morroni, C. and Williamson, A.L., 2008. Cervical human papillomavirus (HPV) infection and HPV type 16 antibodies in South African women. *Journal of clinical microbiology*, 46(2), pp.732-739.

68. Marais, D.J., Passmore, J.A.S., Denny, L., Sampson, C., Allan, B.R. and Williamson, A.L., 2008. Cervical and oral human papillomavirus types in HIV-1 positive and negative women with cervical disease in South Africa. *Journal of medical virology*, 80(6), pp.953-959.

69. Mbatani, N., Adams, T., van Wijk, L., Behrens, C., Tam, T., Wright Jr, T., Stoler, M. and Denny, L., 2016. Performance of an human papillomavirus test in samples from women

© University of Venda

with histolopathologically confirmed invasive cervical cancer. *Journal of Lower Genital Tract Disease*, 20(2), pp.151-153.

70. Mbatha, J.N., Taylor, M., Kleppa, E., Lillebo, K., Galapaththi-Arachchige, H.N., Singh, D., Kjetland, E.F., Baay, M.F. and Mkhize-Kwitshana, Z.L., 2017. High-risk human papillomavirus types in HIV-infected and HIV-uninfected young women in KwaZulu-Natal, South Africa: implications for vaccination. *Infectious Diseases*, 49(8), pp.601-608.

71. Mbatha, J.N., Galappaththi-Arachchige, H.N., Mtshali, A., Taylor, M., Ndhlovu, P.D., Kjetland, E.F., Baay, M.F.D. and Mkhize-Kwitshana, Z.L., 2017. Self-sampling for human papillomavirus testing among rural young women of KwaZulu-Natal, South Africa. *BMC research notes*, 10, pp.1-8.

72. Mbulawa, Z.Z., Coetzee, D., Marais, D.J., Kamupira, M., Zwane, E., Allan, B., Constant, D., Moodley, J.R., Hoffman, M. and Williamson, A.L., 2009. Genital human papillomavirus prevalence and human papillomavirus concordance in heterosexual couples are positively associated with human immunodeficiency virus coinfection. *The Journal of infectious diseases*, 199(10), pp.1514-1524.

73. Mbulawa, Z.Z., Coetzee, D. and Williamson, A.L., 2015. Human papillomavirus prevalence in South African women and men according to age and human immunodeficiency virus status. *BMC infectious diseases*, 15, pp.1-11.

74. Mbulawa, Z.Z., Marais, D.J., Johnson, L.F., Boulle, A., Coetzee, D. and Williamson, A.L., 2010. Influence of human immunodeficiency virus and CD4 count on the prevalence of human papillomavirus in heterosexual couples. *Journal of general virology*, 91(12), pp.3023-3031.

75. Mbulawa, Z.Z., Johnson, L.F., Marais, D.J., Coetzee, D. and Williamson, A.L., 2013. The impact of human immunodeficiency virus on human papillomavirus transmission in heterosexually active couples. *Journal of Infection*, 67(1), pp.51-58.

76. Mbulawa, Z.Z., Marais, D.J., Johnson, L.F., Coetzee, D. and Williamson, A.L., 2012. Impact of human immunodeficiency virus on the natural history of human papillomavirus genital infection in South African men and women. *The Journal of infectious diseases,* 206(1), pp.15-27.

77. Mbulawa, Z.Z., Johnson, L.F., Marais, D.J., Gustavsson, I., Moodley, J.R., Coetzee, D., Gyllensten, U. and Williamson, A.L., 2014. Increased alpha-9 human papillomavirus

© University of Venda

species viral load in human immunodeficiency virus positive women. *BMC Infectious Diseases*, 14, pp.1-7.

78. Mbulawa, Z.Z., Somdyala, N.I., Mabunda, S.A. and Williamson, A.L., 2021. High human papillomavirus prevalence among females attending high school in the Eastern Cape Province of South Africa. *PLoS One*, 16(6), p.e0253074.

79. Mbulawa, Z.Z., Van Schalkwyk, C., Hu, N.C., Meiring, T.L., Barnabas, S., Dabee, S., Jaspan, H., Kriek, J.M., Jaumdally, S.Z., Muller, E. and Bekker, L.G., 2018. High human papillomavirus (HPV) prevalence in South African adolescents and young women encourages expanded HPV vaccination campaigns. *PloS one*, 13(1), p.e0190166.

80. Mbulawa, Z.Z., Wilkin, T.J., Goeieman, B., Swarts, A., Williams, S., Levin, S., Faesen, M., Smith, J.S., Chibwesha, C.J., Williamson, A.L. and Firnhaber, C., 2016. Xpert human papillomavirus test is a promising cervical cancer screening test for HIV-seropositive women. *Papillomavirus Research*, 2, pp.56-60.

81. McDonald, A.C., Denny, L., Wang, C., Tsai, W.Y., Wright Jr, T.C. and Kuhn, L., 2012. Distribution of high-risk human papillomavirus genotypes among HIV-negative women with and without cervical intraepithelial neoplasia in South Africa. *Plos One*, 7(9), p.e44332.

82. McDonald, A.C., Tergas, A.I., Kuhn, L., Denny, L. and Wright Jr, T.C., 2014. Distribution of human papillomavirus genotypes among HIV-positive and HIV-negative women in Cape Town, South Africa. *Frontiers in oncology*, 4, p.48.

83. Meiring, T.L., Salimo, A.T., Coetzee, B., Maree, H.J., Moodley, J., Hitzeroth, I.I., Freeborough, M.J., Rybicki, E.P. and Williamson, A.L., 2012. Next-generation sequencing of cervical DNA detects human papillomavirus types not detected by commercial kits. V*irology journal*, 9, pp.1-10.

84. Moher, D., Liberati, A., Tetzlaff, J., et al., 2009. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine*. 6(7), p.e1000097.

85. Moodley, I., Tathiah, N., Mubaiwa, V. and Denny, L., 2013. High uptake of Gardasil vaccine among 9-12-yearold schoolgirls participating in an HPV vaccination demonstration project in KwaZulu-Natal, South Africa. *South African Medical Journal*, 103(5), pp.318-321.

© University of Venda

86. Moodley, J.R., Constant, D., Hoffman, M., Salimo, A., Allan, B., Rybicki, E., Hitzeroth, I. and Williamson, A.L., 2009. Human papillomavirus prevalence, viral load and pre-cancerous lesions of the cervix in women initiating highly active antiretroviral therapy in South Africa: a cross-sectional study. *BMC cancer*, 9, pp.1-8

87. Moodley, J.R., Hoffman, M., Carrara, H., Allan, B.R., Cooper, D.D., Rosenberg, L., Denny, L.E., Shapiro, S. and Williamson, A.L., 2006. HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study. *BMC cancer*, 6, pp.1-6.

88. Muderris, T., Afsar, I., Yıldız, A. and Varer, C.A., 2019. HPV genotype distribution among women with normal and abnormal cervical cytology in Turkey. *Revista Española de Quimioterapia*, 32(6), p.516.

89. Ngcobo, N.J., Burnett, R.J., Cooper, S. and Wiysonge, C.S., 2019. Human papillomavirus vaccination acceptance and hesitancy in South Africa: Research and policy agenda. *South African Medical Journal*, 109(1), pp.13-15.

90. Ngou, J., Gilham, C., Omar, T., Goumbri-Lompo, O., Doutre, S., Michelow, P., Kelly, H., Didelot, M.N., Chikandiwa, A., Sawadogo, B. and Delany-Moretlwe, S., 2015. Comparison of analytical and clinical performances of the digene HC2 HPV DNA assay and the INNO-LiPA HPV genotyping assay for detecting high-risk HPV infection and cervical neoplasia among HIV-positive African women. *Journal of Acquired Immune Deficiency Syndromes*, 68(2), pp.162-168.

91. Ngou, J., Magooa, M.P., Gilham, C., Djigma, F., Didelot, M.N., Kelly, H., Yonli, A., Sawadogo, B., Lewis, D.A., Delany-Moretlwe, S. and Mayaud, P., 2013. Comparison of care HPV and hybrid capture 2 assays for detection of high-risk human Papillomavirus DNA in cervical samples from HIV-1-infected African women. *Journal of clinical microbiology*, 51(12), pp.4240-4242.

92. Nilyanimit, P., Chansaenroj, J., Poomipak, W., Praianantathavorn, K., Payungporn, S. and Poovorawan, Y., 2018. Comparison of four human papillomavirus genotyping methods: next-generation sequencing, INNO-LiPA, electrochemical DNA Chip, and nested-PCR. *Annals of laboratory medicine*, 38(2), pp.139-146.

93. Obeid, D.A., Almatrrouk, S.A., Alfageeh, M.B., Al-Ahdal, M.N. and Alhamlan, F.S., 2020. Human papillomavirus epidemiology in populations with normal or abnormal cervical

© University of Venda

cytology or cervical cancer in the Middle East and North Africa: A systematic review and meta-analysis. *Journal of Infection and Public Health*, *13*(9), pp.1304-1313.

94. Palefsky, J., 2009. Human papillomavirus-related disease in people with HIV. *Current Opinion in HIV and AIDS*, *4*(1), pp.52-56.

95. Papasavvas, E., Surrey, L.F., Glencross, D.K., Azzoni, L., Joseph, J., Omar, T., Feldman, M.D., Williamson, A.L., Siminya, M., Swarts, A. and Yin, X., 2016. High-risk oncogenic HPV genotype infection associates with increased immune activation and T cell exhaustion in ART-suppressed HIV-1-infected women. *Oncoimmunology*, 5(5), p.e1128612.

96. Paraskevaidis, E., Arbyn, M., Sotiriadis, A., Diakomanolis, E., Martin-Hirsch, P., Koliopoulos, G., Makrydimas, G., Tofoski, J. and Roukos, D.H., 2004. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer treatment reviews*, 30(2), pp.205-211.

97. Rad, A., Sørbye, S.W., Dreyer, G., Hovland, S., Falang, B.M., Louw, M. and Skjeldestad, F.E., 2017. HPV types in cervical cancer tissue in South Africa: A head-to-head comparison by mRNA and DNA tests. *Medicine*, 96(47), p.e8752.

98. Ramesar, J.E., Dehaeck, C.M., Soeters, R. and Williamson, A.L., 1996. Human papillomavirus in normal cervical smears from Cape Town. *South African Medical Journal*, 86(11), pp.1402-1405.

99. Reid, R., Stanhope, C.R., Herschman, B.R., Booth, E., Phibbs, G.D. and Smith, J.P., 1982. Genital warts and cervical cancer. I. Evidence of an association between subclinical papillomavirus infection and cervical malignancy. *Cancer*, 50(2), pp.377-387.

100. Richter, K., Becker, P., Horton, A. and Dreyer, G., 2013. Age-specific prevalence of cervical human papillomavirus infection and cytological abnormalities in women in Gauteng Province, South Africa. *South African medical journal*, 103(5), pp.313-317.

101. Richter, K.L., Van Rensburg, E.J., Van Heerden, W.F. and Boy, S.C., 2008. Human papilloma virus types in the oral and cervical mucosa of HIV-positive South African women prior to antiretroviral therapy. *Journal of oral pathology & medicine*, 37(9), pp.555-559.

102. Said, H.M., Ahmed, K., Burnett, R., Allan, B.R., Williamson, A.L. and Hoosen, A.A., 2009. HPV genotypes in women with squamous intraepithelial lesions and normal cervixes

© University of Venda

participating in a community-based microbicide study in Pretoria, South Africa. *Journal of Clinical Virology*, 44(4), pp.318-321.

103. Saidu, R., Kuhn, L., Tergas, A., Boa, R., Moodley, J., Svanholm-Barrie, C., Persing, D., Campbell, S., Tsai, W.Y., Wright, T.C. and Denny, L., 2021. Performance of Xpert HPV on self-collected vaginal samples for cervical cancer screening among women in South Africa. *Journal of lower genital tract disease*, 25(1), pp.15-21.

104. Sankaranarayanan, R., Budukh, A.M. and Rajkumar, R., 2001. Effective screening programmes for cervical cancer in low-and middle-income developing countries. *Bulletin of the World Health Organization*, 79(10), pp.954-962.

105. Saslow, D., Runowicz, C.D., Solomon, D., Moscicki, A.B., Smith, R.A., Eyre, H.J. and Cohen, C., 2002. American Cancer Society guideline for the early detection of cervical neoplasia and cancer. CA: *a cancer journal for clinicians*, 52(6), pp.342-362.

106. Schiffman, M., Clifford, G. and Buonaguro, F.M., 2009. Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infectious agents and cancer*, 4, pp.1-8.

107. Segondy, M., Kelly, H., Magooa, M.P., Djigma, F., Ngou, J., Gilham, C., Omar, T., Goumbri-Lompo, O., Michelow, P., Doutre, S. and Clavero, O., 2016. Performance of careHPV for detecting high-grade cervical intraepithelial neoplasia among women living with HIV-1 in Burkina Faso and South Africa: HARP study. *British journal of cancer*, 115(4), pp.425-430.

108. Smith, J.S., Melendy, A., Rana, R.K. and Pimenta, J.M., 2008. Age-specific prevalence of infection with human papillomavirus in females: a global review. *Journal of Adolescent Health*, 43(4), pp.S5-e1.

109. Snyman, L.C., Dreyer, G., Visser, C., Botha, M.H. and Van der Merwe, F.H., 2015. The Vaccine and Cervical Cancer Screen project 2 (VACCS 2): Linking cervical cancer screening to a two-dose HPV vaccination schedule in the South-West District of Tshwane, Gauteng, South Africa. *South African Medical Journal*, 105(3), pp.191-194.

110. Statistics South Africa. Mid-year population estimates 2021 [homepage on the Internet]. [cited 2022 Feb 02] Available from: www.statssa.gov.za

© University of Venda

111. Strickler, H.D., Burk, R.D., Fazzari, M., Anastos, K., Minkoff, H., Massad, L.S., Hall, C., Bacon, M., Levine, A.M., Watts, D.H. and Silverberg, M.J., 2005. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus–positive women. *Journal of the National Cancer Institute*, 97(8), pp.577-586.

112. Taku, O., Brink, A., Meiring, T.L., Phohlo, K., Businge, C.B., Mbulawa, Z.Z. and Williamson, A.L., 2021. Detection of sexually transmitted pathogens and co-infection with human papillomavirus in women residing in rural Eastern Cape, South Africa. *PeerJ*, 9, p.e10793.

113. Taku, O., Businge, C.B., Mdaka, M.L., Phohlo, K., Basera, W., Garcia-Jardon, M., Meiring, T.L., Gyllensten, U., Williamson, A.L. and Mbulawa, Z.Z., 2020. Human papillomavirus prevalence and risk factors among HIV-negative and HIV-positive women residing in rural Eastern Cape, South Africa. *International Journal of Infectious Diseases*, 95, pp.176-182.

114. Taku, O., Mbulawa, Z.Z., Phohlo, K., Garcia-Jardon, M., Businge, C.B. and Williamson, A.L., 2021. Distribution of human papillomavirus (HPV) genotypes in HIV-negative and HIV-positive women with cervical intraepithelial lesions in the Eastern Cape Province, South Africa. *Viruses*, 13(2), p.280.

115. Taku, O., Meiring, T.L., Gustavsson, I., Phohlo, K., Garcia-Jardon, M., Mbulawa, Z.Z., Businge, C.B., Gyllensten, U. and Williamson, A.L., 2020. Acceptability of self-collection for human papillomavirus detection in the Eastern Cape, South Africa. PLoS One, 15(11), p.e0241781.

116. Tayib, S., Allan, B., Williamson, A.L. and Denny, L., 2015. Human papillomavirus genotypes and clinical management of genital warts in women attending a colposcopy clinic in Cape Town, South Africa. *South African Medical Journal*, 105(8), pp.679-684.

117. Taylor, S., Wang, C., Wright, T.C., Denny, L. and Kuhn, L., 2011. A comparison of human papillomavirus testing of clinician-collected and self-collected samples during follow-up after screen-and-treat. International journal of cancer, 129(4), pp.879-886.

118. Teixeira, M.F., Sabidó, M., Leturiondo, A.L., de Oliveira Ferreira, C., Torres, K.L. and Benzaken, A.S., 2018. High risk human papillomavirus prevalence and genotype distribution among women infected with HIV in Manaus, Amazonas. *Virology journal*, 15, pp.1-12.

© University of Venda

119. Tjalma, W.A., Fiander, A., Reich, O., Powell, N., Nowakowski, A.M., Kirschner, B., Koiss, R., O'Leary, J., Joura, E.A., Rosenlund, M. and Colau, B., 2013. Differences in human papillomavirus type distribution in high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europe. *International journal of cancer*, 132(4), pp.854-867.

120. Van Aardt, M.C., Dreyer, G., Pienaar, H.F., Karlsen, F., Hovland, S., Richter, K.L. and Becker, P., 2015. Unique human papillomavirus–type distribution in South African women with invasive cervical cancer and the effect of human immunodeficiency virus infection. *International Journal of Gynecologic Cancer*, 25(5), pp.919-925.

121. Van Aardt, M.C., Dreyer, G., Snyman, L.C., Richter, K.L., Becker, P. and Mojaki, S.M., 2016. Oncogenic and incidental HPV types associated with histologically confirmed cervical intraepithelial neoplasia in HIV-positive and HIV-negative South African women. *South African Medical Journal*, 106(6), pp.617-622.

122. van De Wijgert, J.H., Gill, A.C., Chikandiwa, A., Verwijs, M.C., Kelly, H.A., Omar, T., Delany-Moretlwe, S., Segondy, M., Francis, S., Darby, A.C. and Mayaud, P., 2020. Human papillomavirus infection and cervical dysplasia in HIV-positive women: potential role of the vaginal microbiota. *AIDS*, 34(1), pp.115-125.

123. Wang, C., Wright, T.C., Denny, L. and Kuhn, L., 2011. Rapid rise in detection of human papillomavirus (HPV) infection soon after incident HIV infection among South African women. *Journal of Infectious Diseases*, 203(4), pp.479-486.

124. World Health Organization, 2014. Human papillomavirus vaccines: WHO position paper, October 2014. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire*, *89*(43), pp.465-491.

125. Williamson, A.L., Brink, N.S., Dehaeck, C.M., Ovens, S., Soeters, R. and Rybicki, E.P., 1994. Typing of human papillomaviruses in cervical carcinoma biopsies from Cape Town. *Journal of medical virology*, 43(3), pp.231-237.

126. Williamson, A.L., Dehaeck, C.M. and Soeters, R., 1989. Typing of human papillomaviruses in cervical intraepithelial neoplasia grade 3 biopsies from Cape Town. *Journal of medical virology*, *28*(3), pp.146-149.

127. Wolday, D., Derese, M., Gebressellassie, S., Tsegaye, B., Ergete, W., Gebrehiwot, Y., Caplan, O., Wolf, D.G. and Maayan, S., 2018. HPV genotype distribution among women

© University of Venda

with normal and abnormal cervical cytology presenting in a tertiary gynecology referral Clinic in Ethiopia. *Infectious agents and cancer*, 13, pp.1-8.

128. Wright Jr, T.C., Denny, L., Kuhn, L., Pollack, A. and Lorincz, A., 2000. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. *Jama*, 283(1), pp.81-86.

129. Zeier, M.D., Botha, M.H., Engelbrecht, S., Machekano, R.N., Jacobs, G.B., Isaacs, S., Van Schalkwyk, M., Van Der Merwe, H., Mason, D. and Nachega, J.B., 2015. Combination antiretroviral therapy reduces the detection risk of cervical human papilloma virus infection in women living with HIV. *Aids*, 29(1), pp.59-66.

© University of Venda

CHAPTER THREE

PREVALENCE OF HPV DNA AND GENOTYPE DISTRIBUTION AMONG WOMEN LIVING WITH AND WITHOUT HIV IN SELECTED HEALTH FACILITIES IN LIMPOPO PROVINCE, SOUTH AFRICA

© University of Venda

ABSTRACT

Background and objective: Human papillomavirus (HPV) is a predominant causative agent of cervical cancer (CC). The prevalence of HPV DNA and genotype distribution was determined among women living with and without HIV recruited from selected health facilities in Limpopo Province, South Africa.

Methods: A total of 450 cervical specimens were collected from a group of women who self-reported to be living with, or without HIV. Qiagen DNA mini kit was utilized to extract DNA in accordance with the manufacturer's instructions. To determine HPV prevalence, a double nested polymerase chain reaction (PCR) strategy, comprising three sets of primers, was employed to amplify fragments of the L1 conserved gene of HPV. The amplification of HPV DNA was indicative of HPV infection. To determine HPV genotypes, DNA libraries were prepared from purified 450 bp PCR product using Nextera XT DNA library preparation kit and sequenced using the Illumina MiniSeq platform. Sequence reads were evaluated for quality before analysis. The Geneious Prime software version 2023.0.1, a bioinformatics analysis tool, was used to determine HPV genotypes. Statistical data was analyzed by means of R-studio, and a p-value of <0.05 was deemed statistically significant.

Results: HPV DNA was detected in cervical specimens from 147 (32.7%) of the 450 women. The HPV prevalence was significantly higher at 52.21%, (POR: 1.95, 95% CI: 1.26-3.02, p = 0.00) among women living with HIV (WLWH). The study detected 41 HPV genotypes that belonged to *Alphapapillomavirus* genus of the *Papillomaviridae* family from 48 participants. HPV 45 (16.7%) was the predominant genotype (16.7%) in the study population. In addition, HPV genotype 81 (18.8%) was the most common genotype among WLWH, while HPV genotype 56 (25.0%) was more common among women who were not living with HIV. Multiple infections and high-risk HPV genotypes were more common among WLWH.

Conclusion: The current study suggests that HPV infection is relatively high among the study participants and that women co-infected with HIV are more at risk for HPV infection, potentially due to immunosuppression. The implication is that WLWH ought to be prioritized for HPV vaccination and cervical HPV screening, a significant intervention to reduce CC. Moreover, strengthening integrated HPV and HIV screening programs and alongside ensuring regular monitoring of cervical health in WLWH remains critical for early detection and timely treatment.

Keywords: Human papillomavirus; Genotypes; Prevalence; HIV; Limpopo Province

© University of Venda

3.1 INTRODUCTION AND STUDY RATIONALE

Globally, cervical cancer (CC), caused by high-risk (hr-) human papillomaviruses (HPV) remains a public health concern (zur Hausen, 1989; Walboomers et al., 1999; Buck et al., 2013; Ogembo et al., 2015; Yang et al., 2022). Cervical cancer is the fourth most common malignancy among women (Bray et al., 2018), regardless of their immune status competency and ethnicity. Yearly, it is estimated that there are about 528 000 CC cases, half of which leads to death; with developing countries being the most affected populations (Pisani et al., 1999; Mboumba Bouassa et al., 2017). Human papillomavirus is a double-stranded DNA virus with a genome size of about 8 kilobases (kb) (Danos et al., 1982. The L1, a conserved gene, encodes for the major capsid protein of the virus (Buck et al., 2013). More than 200 genotypes of HPV have been identified globally (Martora et al., 2019), with about 40 of these reported to infect the ano-genital tract of women (Munoz et al., 2003). Based on their carcinogenic properties and probability for carcinogenicity HPV genotypes are further classified as hr-HPV, low-risk (lr-) HPV, probable (prb) or possible (psb) genotypes. High-risk HPV include genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; while lr- genotypes include HPV 6 and HPV 11 among others; prb HPV genotypes include type 68; and psb genotypes include genotypes 26, 30, 34, 53, 66, 67, 68, 69, 70, 73, 82, 85 and 87 (Burd, 2016). Against this background, Gardasil-9, one of the licensed vaccines is intended to provide protection for only 9 HPV genotypes belonging to the *Alphapapillomavirus* genus. This includes two lr-HPV genotypes 6 and 11, and seven hr-HPV genotypes 16, 18, 31, 33, 45, 52 and 58. Of note, Gardasil-9 is not yet available in South Africa; however, Gardasil-4 which targets HPV 6, HPV 11, HPV 16 and HPV 18 and Cervarix which offers protection against HPV 16 and HPV18 are registered and available in South Africa (Amponsah-Dacosta et al., 2022).

Common HPV genotyping kits are mostly PCR hybridization-based assays (Galan-Sanchez et al., 2009; Poljak and Kocjan, 2010). These genotyping kits, although useful, are limited in that they are designed to detect only known genotypes. Nevertheless, PCR remains important for HPV DNA detection since specific genes of interest can be amplified and further studied. In the current study, the L1 conserved region of HPV was amplified using a double-nested PCR approach, followed by Next-generation sequencing (NGS) to enhance the sensitivity and reliability for HPV detection (Meisal et al., 2017; Wagner et al., 2019; Ardhaoui et al., 2021; Basiletti et al., 2022).

Studies from the Western Cape, Eastern Cape, and KwaZulu-Natal Provinces of South Africa have documented HPV genotype distribution among women living with and without HIV using

© University of Venda

commercial kits, such as Hybrid capture 2, PCR-EIA and HPV direct flow (McDonald et al., 2014; Mbatha et al., 2017; Taku et al., 2020; Taku et al., 2021). However, data on HPV genotypes in South Africa based on double-nested PCR and NGS approaches are uncommon. Double-nested PCR enhances sensitivity and specificity in detecting DNA with a low viral copy number.

Cervical HPV DNA typing is a fundamental method to identify HPV genotypes of clinical importance. Using technologies such as NGS may lead to detection of viral genotypes existing in a minority population (Kireev et al., 2018; Knyazev et al., 2021). Therefore, the current study was designed to examine the prevalence of cervical HPV DNA among women living with and without HIV in selected health facilities in Limpopo Province, South Africa. Moreover, it further describes the HPV genotype distribution in the study population using NGS typing approach (Radford et al., 2012).

3.2 LITERATURE REVIEW

3.2.1 HPV and cervical cancer

Historically, it was thought that the cancers of the ano-genital tract and CC were caused by a sexually transmitted pathogen (Gagnon, 1950). Thereafter, a hypothesis prevailed that herpes simplex virus type 2 (HSV-2) was the causative agent of the tumor. The hypothesis was based on sero-epidemiological information, supported by nucleic acid hybridization and CC induction in animals (Rawls et al., 1969; Frenkel et al., 1972). There was successes in the utilization of the *in-situ* hybridization assays to detect Epstein-Bar virus (EBV) from nasopharyngeal specimens obtained from carcinomas (Zur Hausen et al., 1970; 1974; 1974a). However, there were also failures in the detection of HPV DNA in CC specimens using the same approach. As a result, a hypothesis developed on HPV in the etiology of CC (Zur Hausen et al, 1975; Zur Hausen, 1976). This hypothesis was premised on the belief that if the human cancerous cells are transformed by a viral pathogen, the cells would then contain viral genetic information integrated into the genome of the host. This “causality criteria” was then added to Koch’s postulate theory and its assertation that “the viral genome should be persistently present and transcriptionally active in the cancer” (Zur Hausen et al, 1975). Zur Hausen et al. (1976) further suggested that the DNA of the virus may be found in a non-productive state in the tumor.

3.2.2 Classification of human papillomavirus

Human papillomavirus is a heterogenous group of viruses and with over 200 HPV genotypes identified (Martora et al., 2019). These diverse groups of viruses belong to the

© University of Venda

papillomaviridae family. The family is further categorized into thirty-nine genera (De Villiers et al., 2004; Bernard et al., 2010) comprising both human and non-human papillomavirus genotypes, with HPV classified into five genera, namely: the *Gammapapillomaviruses*, the *Alphapapillomaviruses*, the *Betapillomaviruses,* the *Mupapillomaviruses* and the *Nupapillomavirus* (Figure 3.1). In 2012, the International Agency for Research on Cancer (IARC) classified HPV genotypes into groups, namely: group 1, which covers all the HPV genotypes known to have the carcinogenic potential to humans; groups 2A and 2B, which are individually defined as probably and possibly human carcinogens. The group 1 HPV include genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59; all of which are considered hr-HPV genotypes. Genotype 68 is assigned to group 2A since there is sparse data supporting it as a hr-genotype. Numerous literature sources show that about 96% of CC may be associated with either group 1 and 2A human carcinogens (Arbyn et al., 2014). Among others, Alpha HPV genotypes of group 2B, including HPV 26, HPV 34, HPV 53, HPV 66 and HPV 67 are possibly carcinogenic to humans; hence, they are tagged as of possible etiology.

© University of Venda

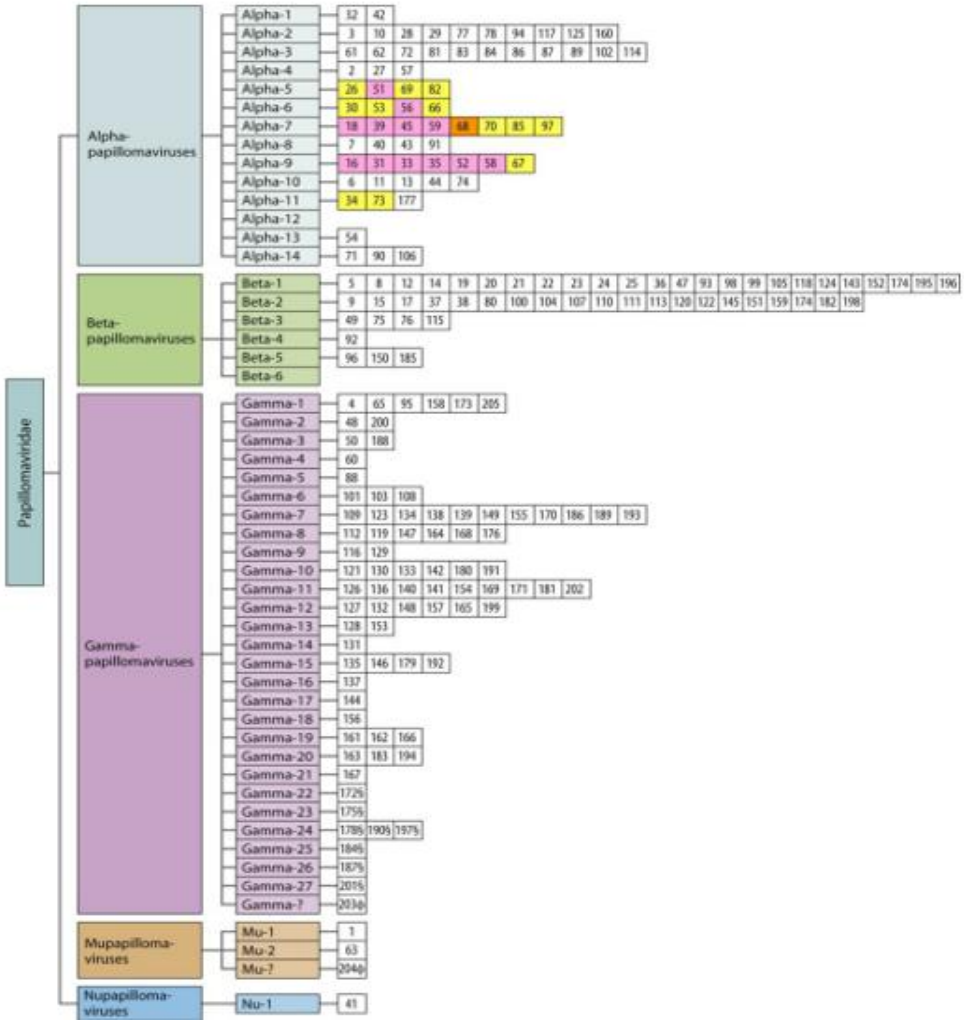


Figure 3.1: *Classification of human papillomavirus highlighting the 5 individual genera belonging to papillomaviridae family (Source: Burd, 2016). The HPV genotypes belonging to groups 1, 2A and 2B are represented by the purple, orange, and yellow colors, respectively.*

3.2.3 HPV virion, genome structure and organization

The genome of HPV is double-stranded circular DNA, of about 8000 bases (Graham, 2010). The genome encodes early and late proteins (Burley et al., 2020). The virus is non-enveloped, and specifically infects the squamous epithelia (Sibeko et al., 2024). The viral particle comprises an icosahedral capsid of approximately 60 nm in diameter (Knipe et al., 2013). The transcription process uses one strand of the genomic DNA as a template. The coding strand has three genomic fragments comprising nine open reading frames (ORFs) as illustrated in Figure 3.2. Polycistronic mRNAs are utilized to express several numbers of viral proteins (Favre et al., 1975; Zheng and Baker, 2006). The early (E) and late (L) genes genome comprise seven ORFs encoding viral regulatory proteins and 2 viral capsid proteins respectively (Burley et al., 2020). The long control region (LCR), also referred to as the upstream regulatory region (URR), a non-coding region, is the third region of the viral genome. This non-coding region

© University of Venda

comprises the origin of DNA replication, and transcription control sequences (Knipe et al., 2013).

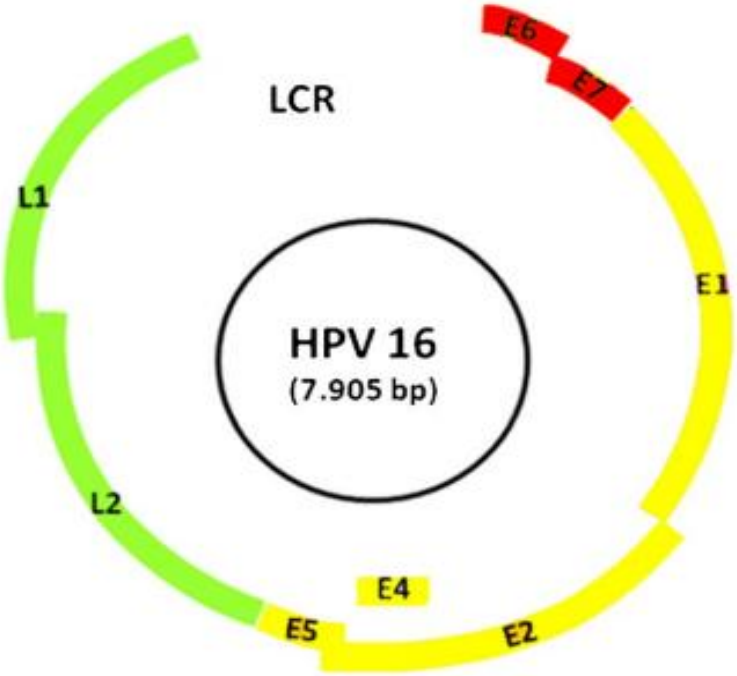


Figure 3. 2: *Illustration of HPV genotype 16 circular genome organization (Source: Faridi et al., 2011). L1 and L2: Late capsid proteins; LCR; Non-coding region; E1-2, E4-7: Early proteins.*

3.2.4 Viral proteins and their functions

The functions of HPV proteins are documented in Table 3.1

Table 3.1: Functions of HPV proteins.

Protein	Functions
E6	It inactivates many cellular proteins and is one of the major viral oncoprotein
E7	It inactivates many cellular proteins and is one of the major viral oncoprotein
E5	It stimulates mitogenic signals of growth factors
E4	It mediates the viral particle release by destabilizing cytokeratin network
E2	It regulates early gene promoter and together with E1 viral DNA replication
E1	It forms a heterodimer complex with E2 and controls viral replication
L2	It is the minor capsid protein
L1	It is the major capsid protein and is the component of the HPV prophylactic vaccine

(Source: Tommasino, 2014).

3.2.5 Epidemiology of HPV and cervical cancer

3.2.5.1 General population

In a 2011 global survey of HPV prevalence among women in a general population of women (n=576,281) screened for HPV DNA, the majority (67.9%) were reported to be gynecologically unhealthy, while only 32.1% were reported as gynecologically healthy (Vinodhini et al., 2012).

© University of Venda

In this general population, Africa and Asia were found to have the highest HPV prevalence of 29.6% and 45.5% respectively (Vinodhini et al., 2012).

3.2.5.2 Normal cervical cytology

The prevalence of HPV infection is increasing significantly around the world, especially in women, for whom it remains a major cause of cancer (IARC, 2018). Such a state of affairs presents HPV as a serious public health concern (Kombe Kombe et al., 2021). In 2017, De Sanjose et al. (2007) estimated that there were about 291 million women globally who were HPV DNA positive (De Sanjose et al., 2007; Kombe Kombe et al., 2021). Studies have shown that the prevalence of HPV among women with a negative cervical cytology was 10.4% (De Sanjose et al., 2007) and 11.7% (Bruni et al., 2010), and decreased to 9.9% in 2019 (Bruni et al., 2019). Other cervical HPV studies showed varying prevalences of 21.8% (Oceania), 21.1% (Africa), 14.2% (Europe), and 11.5% in America, with Asia having the least prevalence of 9,4% (Bruni et al., 2010; Bruni et al., 2019; De Sanjose et al., 2007).

3.2.5.3 Cutaneous warts

Human papillomavirus genotypes belonging to β and γ genera include HPV 4 and HPV 65 the major cause of cutaneous warts (King et al., 2014). A study conducted among Dutch children in 2015 revealed a prevalence of 92% of HPV DNA in cutaneous warts specimens (De Koning et al., 2015). Worldwide, HPVs are identified in commensal cutaneous flora of greater than 50% of healthy individuals (Asgari et al., 2008), especially among children who are less than four years old (Antonsson et al., 2003). In healthy individuals, the prevalence of cutaneous warts is less than 33%, and with an increased rate among males compared to females (Nindl et al., 2007; Weissenborn et al., 2009; Liu et al., 2018). Furthermore, it has been reported that in immunocompromised individuals, the transmission of cutaneous HPV on the skin is significant, with up to 90% positivity rate, which poses an increased risk of developing non-melanoma skin cancer in such populations (Nindl et al., 2007).

3.2.5.4 Ano-genital warts

Low-risk HPV 6 and HPV 11 are the major causes of ano-genital warts (Jamshidi et al., 2012; Zhu et al., 2019). Worldwide, these warts are known to be the most frequent clinical manifestation of HPV infections (Mann et al., 2019). In general, the morbidity linked with ano-genital warts seems to be minimal since infection by the lr-HPV genotypes is transient. Nonetheless, the cost of regular treatment and diagnosis is significant because of the common recurrences and increased transmission (Asiaf et al., 2014; Ginindza et al., 2017; Lee et al., 2019; Ong et al., 2019). Among children, the prevalence of ano-genital warts is low, nearly

© University of Venda

non-existent. Contrarily, other studies report a net rise in the anogenital warts prevalence in both children and adults, raising the hypothesis of sexual abuse among children (Marcoux et al., 2006), since HPV is considered a sexually transmitted infection (Kombe Kombe et al., 2021). However, there are numerous studies that are at variance with this hypothesis on the basis that the existence of ano-genital warts among children is also linked to non-sexual human papillomavirus transmission (Merckx et al., 2013; Costa-Silva et al., 2018), since it may be transmitted from mother to child (Merckx et al., 2013; Trottier et al., 2016). Regionally, the prevalence of ano-genital warts is slightly higher in Sub-Saharan Africa (De vuyst et al., 2013), particularly among individuals with multiple sexual partners, as well as those who have a compromised immune system (Banura et al., 2013; Carmago et al., 2018; Chikandiwa et al., 2019a; Chikandiwa et al., 2019b). On the other hand, developed countries seem to have a low prevalence (approximately 1.5% prevalence rate in the USA), could be attributable to the implementation of effective vaccine programs in such countries.

3.2.5.5 Cervical cancer (CC)

In 2018, approximately 56 9847 new cases of CC were reported as the cause of death to about 31 1365 women, with more than 85% of these cases taking place in developing countries alone (Arbyn et al., 2008; Bray et al., 2018; IARC, 2018; LeConte et al., 2018; Bruni et al., 2019). It is worth noting that the rate of deaths linked to CC will be greater than an estimated 443 000 by the year 2030, with 90% occurring only in Sub-Saharan Africa (Ferlay et al., 2015; WHO, 2015), in the event that no sanitary measure is established or set up (Kombe Kombe et al., 2021). Infection by hr-HPV genotypes has been associated with CC since the 1980s (Asiaf et al., 2014; De Martel et al., 2017). Moreover, literature reports that 85% of invasive cervical cancer (ICC) is linked with HPV, with almost 100% of CC deaths caused by hr-HPV DNA (De Sanjose et al., 2010; Forman et al., 2012). In 2018, the highest and lowest incidence rates of CC cases caused by HPV were observed in Africa, particularly in the Sub-Saharan African region (75.3/100.00) and Asia (10.2/100.000) respectively (Bruni et al., 2019). In addition to CC, HPV genotypes 6, 11, 18 and 33 were responsible for other ano-genital cancers, including vaginal (70%), penile (50%), anal (88%), vulvar (43%) and oropharyngeal (37%) cancers (De Martel et al., 2012; Forman et al., 2012; Buchanan et al., 2016).

3.2.6 Risk factors for HPV infection

A 2022 study conducted by Yang et al. (2022) showed that factors such as age at first sexual intercourse (≤ 19 years of age), multiple sexual partners, non-condom contraception, educational level (high school and below), were the possible risk factors for HPV infection in the studied population (Yang et al., 2022). Another study also found that having had multiple

© University of Venda

sexual partners in a lifetime and early age of sexual debut was associated with a higher risk of HPV infection (Yamaguchi et al., 2021). The same findings were also reported in a 2016 study by La Rosa and colleagues (La Rosa et al., 2016). Furthermore, the findings of a study by Taku et al. (2020) also supported that multiple sexual partners may influence the risk of HPV infection, and that a positive HIV status and a (current or previous) vaginal discharge were associated with HPV infection (Taku et al., 2020). Smoking was also considered a risk factor for HPV infection (Xi et al., 2009).

3.2.7 Routes of transmission

The primary transmission route of HPV infection is through skin to mucosa or skin-to-skin contact with an individual who is HPV DNA positive. Although there are studies whose findings show the non-sexual routes of HPV transmission, sexual transmission was found to be the most frequently reported transmission route (Petca et al., 2020).

3.2.7.1 Horizontal transmission

Human papillomavirus (HPV) horizontal transmission occurs through fingers, fomites, and skin contact (other than sexual) (Petca et al., 2020). These potential infection routes have been extensively discussed in several other studies. The contamination of equipment found in the gynaecological examination rooms has been an intriguing topic for investigating HPV transmission routes. It has been documented in literature that HPVs are very steady, resistant to heat, as well as drying, with 30% infectivity post 7 days of dehydration (Casalegno et al., 2012). These groups of diverse viruses can often survive for days on fomites, surfaces and clothing, as well as commonly used gynaecological equipment. It is noteworthy that several studies have collected HPV DNA specimens to investigate these concerns (Petca et al., 2020).

A study conducted by Gallay et al. (2016) reported a 17.9% HPV DNA prevalence rate among specimens collected from gynaecological equipment; with 11.8% positivity in a hospital setting relative to a 27.5% found in private offices (Gallay et al., 2016). This highlighted a contamination rate of 2.7 times greater in private offices, compared to hospital offices. Another study also reported that approximately 10% of the gloves were contaminated, and that rooms used for colposcopy had a higher prevalence of HPV DNA than the outpatient offices (Gallay et al., 2016). Furthermore, due to the asymptomatic nature of HPV infection, most individuals are unaware of their HPV positive status. Therefore, it is imperative for women to visit their nearby clinics and hospitals for routine check-ups. Data also shows that HPV DNA can be detected from fingernails and underwear of individuals who have genital warts (Gallay et al., 2016).

© University of Venda

3.2.7.2 Vertical transmission

Vertical transmission is another route through which HPV infection can be transmitted, particularly from a mother to her child. Many research studies have emphasised on the possibility of HPV infection through the placenta, amniotic fluid, as well as contact with the mucosa of the mother during natural birth (Petca et al., 2020). The potential route of HPV vertical transmission has been a perennial issue since the early 1950s, as reflected in a study on infantile anogenital warts, congenital conjunctival papilloma and juvenile laryngeal papillomatosis (Hong et al., 2013).

3.2.8 Vaccines for HPV

To date, there are only two vaccines available to help fight against HPV infection. These vaccines are Cervarix® (manufactured by GlaxoSmithKline) and Gardasil (manufactured by Merk Frost). Furthermore, these two vaccines contain virus-like particles (VLPs) which are created through a recombinant DNA platform. Furthermore, both Cervarix and Gardasil possess the viral capsids L1 protein component (Katz and Wright, 2006). It is well established that these kind of VLPs generate strong reactions from the immune system and produce the antibody titer that are higher than those which are prompted by natural infection (Dawar et al., 2007).

3.2.8.1 South Africa and HPV vaccination

There are three globally licensed HPV vaccines namely: Cervarix, Gardasil-4 and Gardasil-9. Gardasil-9 vaccine targets seven hr-HPV genotypes (16, 18, 31, 33, 45, 52 and 58) with the addition of two lr-HPV genotypes 11 and 6. Moreover, HPV 6, HPV 11, HPV 16 and HPV 18 are found within the Gardasil-4 vaccine, while the Cervarix HPV vaccine does not cater for any of the low-risk genotypes. Instead, it offers protection against two hr-HPV genotypes 16 and 18. However, the Gardasil-9 HPV vaccine has not yet been introduced in South Africa, only Cervarix and Gardasil-4. Figure 3.3 is a representative of the HPV genotypes and their estimated percentage of CC and genital warts cases covered by HPV vaccines.

© University of Venda

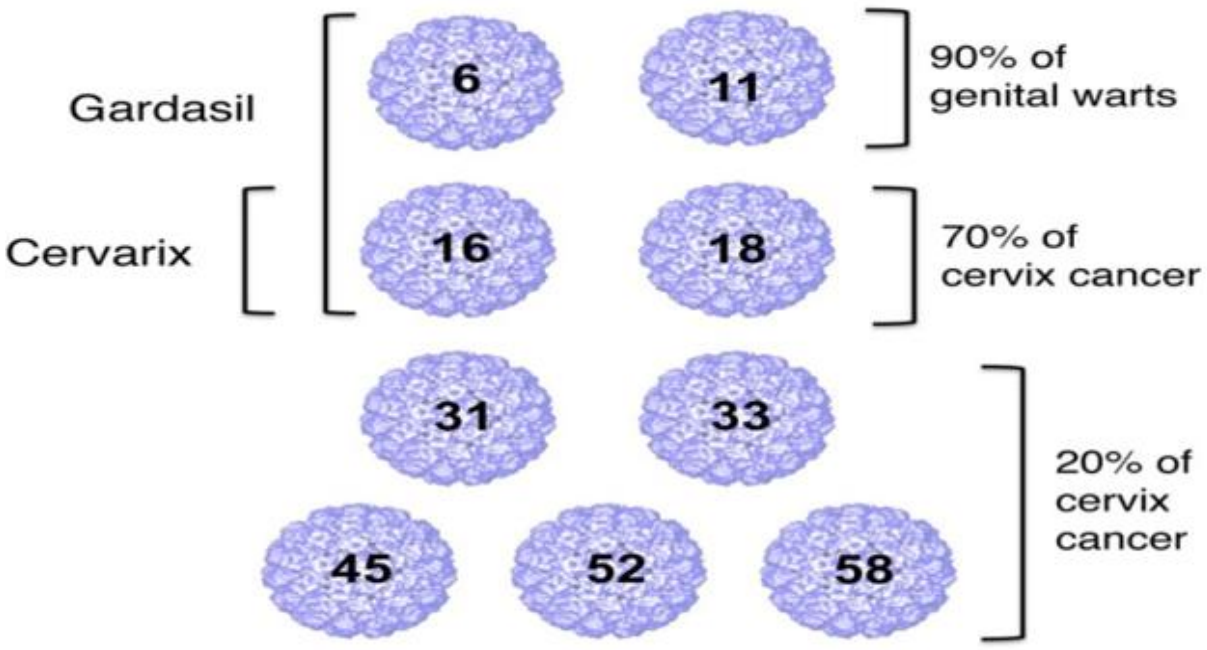


Figure 3.3: *The HPV genotypes and their estimated percentage of CC and genital warts cases covered by Cervarix and Gardasil-4 (Source Lowy, 2016).*

3.2.8.2 The HPV vaccination rollout programs in South Africa

The availability of HPV vaccines, particularly in developed regions of the world dates back to 2006 (Amponsah-Dacosta et al., 2022). However, the initial regional HPV vaccination rollout in Sub-Saharan Africa dates back to 2011, marking the 13th year (in 2023) since its initial availability. Gardasil-4 and Cervarix HPV were first permitted for use in South Africa in 2008, but were only available at a cost through the private health sector (Amponsah-Dacosta et al., 2022). It was only in April of 2014 that free nationwide vaccination rollout programs were introduced in South Africa, strictly targeting young girls in Grade 4 across all public schools in the country. It is worth noting that only girls aged 9 years and above were eligible for inclusion in the free nationwide vaccination program (Delany-Moretlwe et al., 2018). This initiative was intended to become the most successful and cost-effective South African strategy for distributing HPV vaccines to the eligible young girls (Moodley et al., 2013; Moodley et al., 2016).

A total 419 520 (83.3%) of the 503 507 young and eligible Grade 4 girls were successfully vaccinated with Cervarix first dose during the first rollout of the school-based vaccination program in 2014 (WHO, 2021). However, there was a 65% decline in the rollout about six months after the second-round vaccine administration on the eligible Grade 4 girls. Since then, coverage and dosage completion of the national HPV program have been noted to drastically decrease in both the first and second doses. However, disparities still exist in HPV vaccines coverage's within and across various South African districts and provinces.

© University of Venda

In their 2018 report, Delany-Moretlwe and colleagues noticed a low vaccine coverage in South Africa during the assessment of the first round of the vaccination programme. The coverage rate was below 50% each in the sub-districts of Mpumalanga and KwaZulu-Natal Provinces (Delany-Moretlwe et al., 2018). It is also important to note that these rates are twice lower than those attained during the first dose vaccination rollout in 2014 (Delany-Moretlwe et al., 2018). There should be very close monitoring of the discrepancies in the vaccine coverage rates between the last and first dose of HPV vaccination, which points to a rising dropout rate over the time, which ranged from 18% in 2014 to a higher rate of 26% in 2018 and 2019. There are contributing factors which have been suggested as having an impact on the completion of HPV vaccine among young girls from low to middle income countries (LMICs). The contributory factors include: insufficient tracking devices needed to trace those who might had missed their doses, as well as insufficient vaccinator refresher trainings before the rollout of the second dose vaccinations (Tsu et al., 2021).

3.2.9 The HPV detection methods

The amplification-based techniques do vary in terms of their specifications. For example, they vary according to the primers employed, reagents used, targeted genes and lengths, number of amplification cycles, number of genotypes covered, and overall reaction time, among other factors. The detailed list of the HPV assays is highlighted in Figure 3.4.

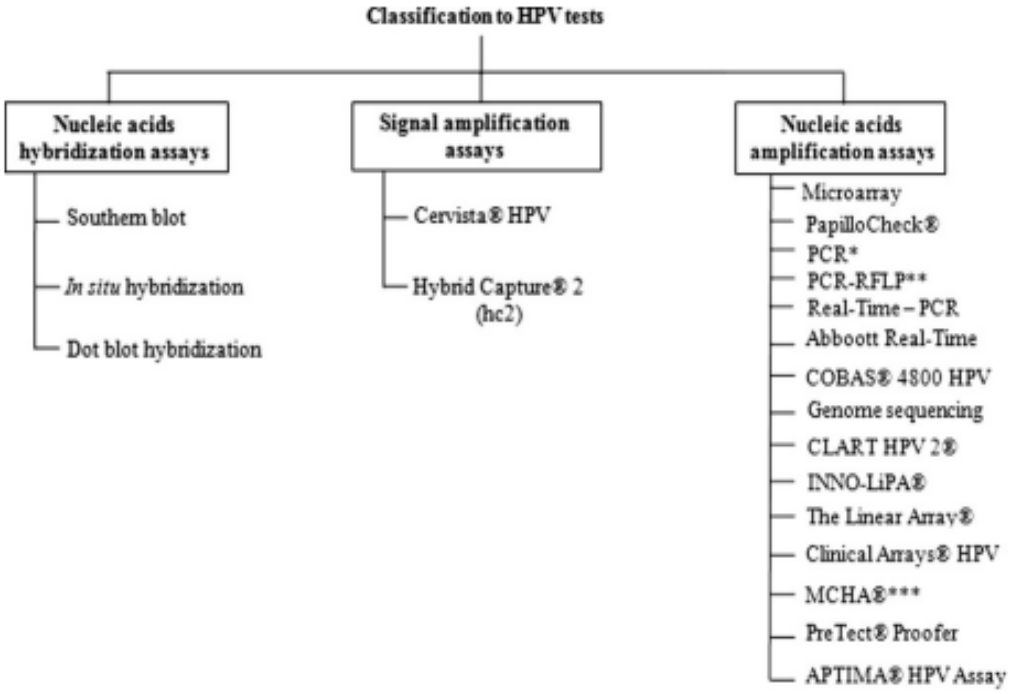


Figure 3.4: *Illustration of the HPV classification test utilized to HPV detection (Source: Abreu et al., 2012).*

© University of Venda

3.2.9.1 Nucleic acid-hybridization assays

Southern blotting, dot blot as well as *in situ* hybridization techniques utilized radio labelled nucleic acid hybridization approaches to identify HPV DNA from cervical specimens. The assay drawbacks include low sensitivity and requirement for high volumes of purified DNA as a time-consuming input. However, it should be noted that such tests have the ability to produce data of high quality (Villa and Denny, 2006).

3.2.9.2 Signal-amplification assays

This category includes two assays, namely: Cervista® HPV HR assay (manufactured by Hologic, Inc. in the USA) and hybrid capture® 2 (HC2) (manufactured by Digene Corp., USA). The hybrid capture 2 (HC2) kit has been designed to detect 5 lr-HPV (genotypes, 6, 11, 42, 43 and 44) and 13 hr-HPV (genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) genotypes (Bozzetti et al., 2000). It should be noted that this technology is a non-radioactive signal amplification assay which is based on the hybridization of the target HPV DNA to labelled probes of RNA in solution (Hwang and Shroyer, 2012). On the contrary, Cervista, detects the presence of 14 hr-HPV genotypes covered by the HC2 technology with the addition of HPV genotype 66 (Hwang and Shroyer, 2012). This technique also uses target HPV DNA. The strengths of Cervista over HC2 is based in its high specificity and sensitivity in typing hr-HPV genotypes 18/16, as well as the lower rate of detecting the false positives (Einstein et al., 2010; Bartholomew et al., 2011). In addition, the assay is highly sensitive in detecting CIN II and CIN III with 98% and 100% detection rates respectively (Johnson et al., 2008).

3.2.9.3 Nucleic acid-amplification assays

3.2.9.3.1 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a commonly used technique, known for its higher specificity and sensitivity properties. During the conventional PCR, a thermostable DNA polymerase is utilized to recognize and extend the pair PCR primers (oligonucleotides) which flank the fragment of interest. At the end of the reaction, up to a billion viral copies can be generated from a single double-stranded molecule after 30 cycles of amplification (Zaravinos et al., 2009). Furthermore, the HPV-PCR based methods use consensus primers, including GP5+/6+ and PGMY09/11 for amplifying a pool of HPV genotypes in a single reaction. The PCR primers target the genes of the viral genome that are conserved, including the L1 capsid gene, which is the widely amplified region of HPV (Camargo et al., 2011).

Post amplification typing of HPV-specific genotypes is carried out separately using assays such as direct sequencing, restriction-fragment length polymorphism (RFLP), and linear probe

© University of Venda

methods (Coser et al., 2011). It should be noted that other studies have employed the genotype-specific PCR targeting other regions such as the LCR, E6 and E7 (Carvalho et al., 2010). The competition of reagents renders employment of the PCR technique disadvantageous. Thus, false negative results particularly in samples with multiple infections of low viral copy numbers. As a result of this challenge, not all the mixed infections may be detected in certain samples. Sometimes, more labour-intensive work such as sequencing is required (Coser et al., 2011).

3.2.9.3.2 The linear array® (LA) genotyping method

The Linear array (LA) genotyping kit (Roche Molecular Systems, Inc., Branchburg, NJ) is a PCR-hybridization based test kit that is restricted to the identification of 37 hr-lr-undetermined-HPV genotypes. These include 15 hr- genotypes (82, 73, 68, 59, 58, 56, 52, 51, 45, 39, 35, 33, 31, 18 and 16), with the addition of 10 lr-HPV genotypes (CP6108, 81, 72, 70, 61, 54, 42, 40, 11 and 6); 9 undetermined HPV genotypes (IS39, 84, 83, 71, 69, 67, 64, 62 and 55), as well as three probable hr-HPV genotypes (66, 53 and 26). The LA test targets the 450 bp L1 fragment of the HPV genome. Notably, the kit also has a co-amplification of human beta globin gene of 268 bp in length, which serves to control and measure the adequacy of DNA extraction and PCR efficiency. The gel visualization step is not compulsory, and may be bypassed, while the positively amplified specimens may be identified during the typing step. Meanwhile, the typing of results is straightforward and easy to interpret. The blue-colored signals on the typing strip (s) are read manually, while the LA reference guide is utilized for data interpretation.

3.2.9.3.3 Real-time PCR and Abbott real-time PCR

This is a specific, sensitive, and reliable method for screening and typing HPV genotypes in cellular and tissue specimens (Kocjan et al., 2011). The advantages of real-time PCR are its ability to detect the nucleic acids even when present in low concentrations; it is fast and highly reproducible; applicable to clinical specimens; as well as its detection of the viral load and ability to amplify multiple targets (Roberts et al., 2008) in a single reaction. On the other hand, Abbott Real-Time HR-HPV test detection is limited to 14 hr-HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. (Kocjan et al., 2011).

3.2.9.3.4 INNO-Lipa

The 14 hr-HPV genotypes covered by real-time are all incorporated and genotyped using this assay (Kocjan et al., 2011). This PCR-based assay targets the 65 bp of L1 HPV gene which is co-amplified with the 270 bp (human HLA-DP1 gene) utilizing the SPF10 biotinylated primers, then followed by typing of the HPV genotypes (van Hamont et al., 2006; Sukasem et al., 2011).

© University of Venda

Specimens collected using tampons, lavages, brushes and swabs can be amplified and genotyped using this commercial kit (Lenselink et al., 2009; Schmeink et al., 2011).

3.2.9.4 HPV sequencing

Sanger sequencing is a dideoxy chain termination-based technology (Sanger et al., 1992). The first genome sequenced using this technique was described more than 31 years ago (Sanger et al., 1992). Nucleotides that are labelled fluorescently are incorporated into Sanger sequencing, which helped in the development of advanced techniques of increased quality and thorough sequencing (Smith et al., 1986; Lee et al., 1992).

3.2.10 Summary of HPV research over the years

In the past seven decades, great advances have been made in understanding HPV molecular biology and the significance of viral typing. The first HPV viral particles were visualized around the 1940s. In the late 1990s, it was then revealed that hr-HPV DNA genotypes were the main risk factor for CC development. Consequently, numerous HPV typing assays were developed, while three vaccines were approved by the Food and Drug Administration (FDA) between 2006 through 2014. These measures offer enhance protection against specific HPV genotypes of clinical importance. The significance of HPV typing includes CC prevention. The diagrammatic evidence in Figure 3.5 shows that concerted investigations and studies on the human papillomavirus that have been ongoing since 1949.

© University of Venda

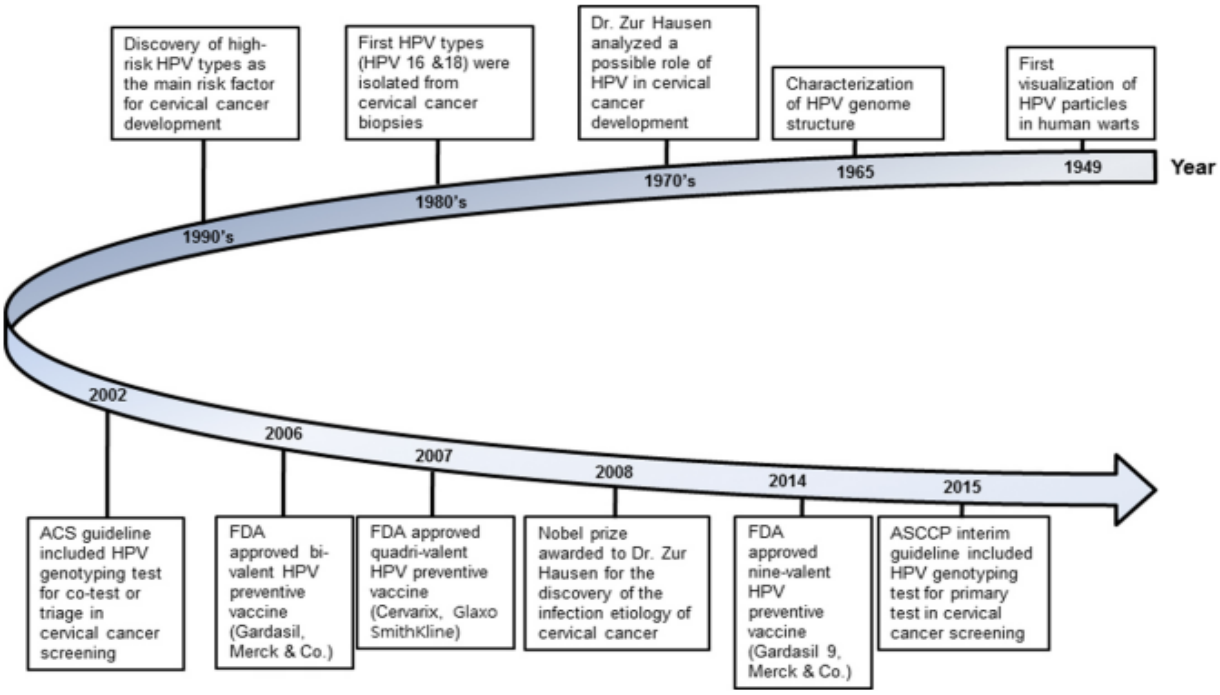


Figure 3.5: *Illustration of human papillomavirus research over time (Source: Choi and Park, 2016).*

3.3 HYPOTHESIS

- There is a high variety of cervical HPV genotypes among women living with HIV (WLWH) in selected health facilities in Limpopo Province, South Africa.

3.4 RESEARCH QUESTIONS

1. What is the prevalence of cervical HPV infection among women living with and without HIV in selected health facilities in Limpopo Province, South Africa?
2. Which HPV genotypes are present in the study population?

3.5 MAIN OBJECTIVE

The main objective of this study was to identify and characterize HPV among women with and without HIV in selected health facilities in Limpopo Province, South Africa.

3.6 SPECIFIC OBJECTIVES

1. To determine the prevalence of cervical HPV infection among women living with and without in selected health facilities in Limpopo Province, South Africa.
2. To describe HPV genotypes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.

© University of Venda

3.7 MATERIALS AND METHODS

3.7.1 Study design and sample size

The Analytical cross-sectional study was done among 450 women who self-reported to be living with and without HIV. To estimate the study's minimum sample size, the following formula (Pourhoseingholi et al., 2013) was used.

$$n = \frac{Z^2 P (1-P)}{d^2}$$

n= Sample size
Z=Statistic corresponding to the level of confidence (CI of 95%, which is 1.96)
P= Estimated prevalence
d= Error margin considered as 5%

To estimate the study's minimum sample size, a previous study conducted by Taku et al. (2020) which reported an overall prevalence 28.5% was used.

$$\text{Sample (n)} = \frac{(1.96)^2\, 0.285\, (1-0.285)}{(0.05)^2}$$

$$n = \frac{3.8416^*\, 0.285\, (0.715)}{(0.0025)}$$

$$n = \frac{0.78282204}{0.0025}$$

n=313

The completeness of data was then estimated at 80%, thus, 313/0.8=391.
The estimated minimum sample size of this study was 391, however, a total 450 cervical specimens were randomly collected.

3.7.2 Study area, study population and participant recruitment

The study participants were recruited from four selected public health facilities located in the Vhembe (Thohoyandou Health Centre and Donald Frazer Hospital) and Capricorn (Rethabile Health Care Centre and Seshego Clinic) districts of Limpopo Province, South Africa (Figure 3.6) among women living with and without HIV. These participants included those visiting health facilities for HIV treatment and others as part of routine healthcare services. The recruitment process was carried out by qualified healthcare professionals. In 2017, the estimated populations of the Vhembe and Capricorn districts were 740 000 and 1.29 million, respectively (Government Provinces, 2019).

© University of Venda

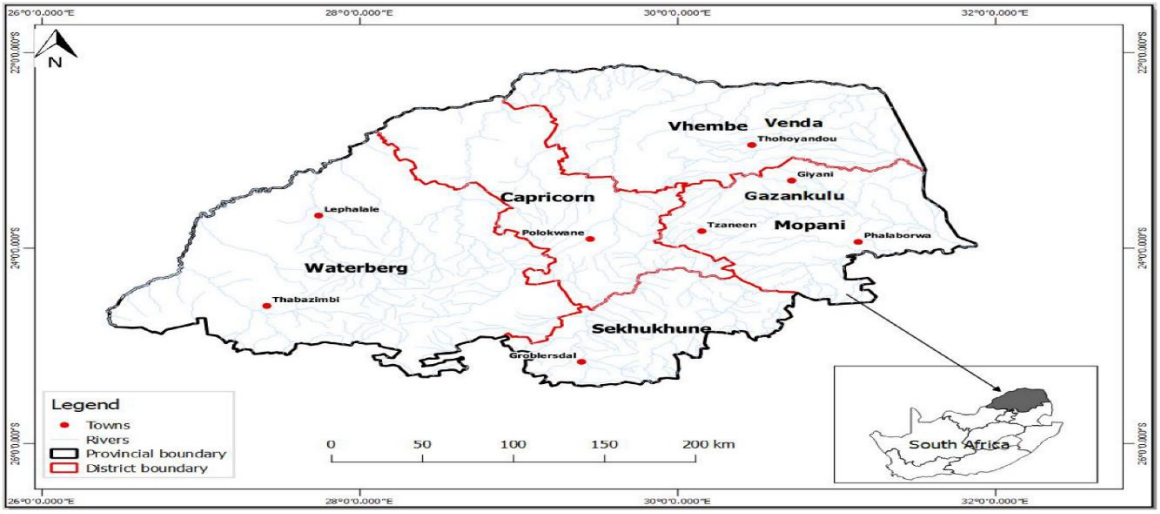


Figure 3.6: *Map indicating Vhembe and Capricorn districts (Serote et al., 2023).*

3.7.3 Inclusion and exclusion criteria

The inclusion criteria included 1) Women who were ≥18 years old, 2) willingness to provide voluntary informed consent, 3) being a resident of Limpopo Province, and 4) having no history of a positive CC diagnosis. The excluded criteria included women who were: 1) Pregnant, 2) had a history of hysterectomy, 3) undergoing treatment for a sexually transmitted infection, and 4) experiencing any form of vaginal bleeding for discharge.

3.7.4 Data collection

The study utilized a structured questionnaire to collect participants' demographic and clinical information. The demographic data included age, income level, marital status, and educational level, among others. Clinical information such as HIV and HPV vaccination status were also collected. Data was entered into Microsoft Excel. Two independent individuals verified the collected information for accuracy and consistency after the data was captured.

3.7.5 Specimen collection and processing

Cervical swab specimens were collected using Aptima Cervical Collection and Transport Kit according to the protocol of the manufacturer (Hologic, San Diego, USA). Specimens were strictly collected by a qualified clinician and stored in the transport medium. To retain a cervical pellet, specimens were centrifuged for 10 minutes at 13 000 rpm.

3.7.6 DNA extraction

Total DNA from cervical pellets was extracted using a QIAamp DNA mini kit (Qiagen in Hilden, Germany) in accordance with the manufacturer's instructions. However, in the DNA elution step, 100µl of eluent was used instead of 200 µl. The presence of total DNA purified

© University of Venda

was ascertained by a 2.5% gel electrophoresis. Purified DNA was stored at -20^0 C until used for downstream applications.

3.7.7 Detection of HPV DNA by double nested PCR

A double-nested PCR amplification strategy was applied, to enhance the chance of detection. Three different primer pairs (Table 3.2) were used to amplify the PCR products from the L1 gene of the HPV genome.

Table 3.2: Oligonucleotide specifications and target sizes

Primer Name	Sequence Arrangement (5'-3')	Target Size
SB01/02 (First round PCR)	5'-CAAWTRTTYAATAARCCWTATTGG/AAAAAYTTYCGWCCMARRGG-3'	~495 bp
MY09/11 (Nested PCR)	5'-CGTCCMARRGGAWACTGATC/GCMCAGGGWCATAAYAATGG-3'	~450 bp
GP5/6 (Double-nested PCR)	5'-GAACACCAAGCATCACTGGA/GAAATCAAATACTGATCTGTTGC-3'	~150 bp

The DNA extract was first amplified through PCR using SB01/02, a forward and reverse primer, respectively, for the amplification of ~495 bp of the HPV L1 fragment. A volume of 10 µl of cervical DNA extract was added into the PCR reaction mixture which contained: 3.5 mM of $MgCl_2$, 0.4 µM of each primer, 2X PCR buffer, 0.2 mM dNTPs, 0.08 U/µl of FastStart Taq DNA Polymerase and 3.6 µl PCR water into a final reaction volume of 25 µl. The cycling conditions used are shown in Table 3.3.

Table 3.3: Cycling conditions used during the first round of PCR

Cycling Conditions		
Process	**Temperature (^{0}C)**	**Duration and Cycles**
Pre-denaturation	95	5 minutes } 1
Denaturation	95	1 minute
Annealing	42	1 minute } 10
Extension	72	1 minute
Denaturation	95	20 seconds
Annealing	42	40 seconds } 30
Extension	72	1 minute
Final extension	72	10 minutes } 1

Nested PCR was defined as a two consecutive amplification reaction whereby a PCR product of the first reaction was used as a template for the second reaction. The first nested reaction is expected to yield an HPV DNA product of ~450 bp. Briefly, the reaction comprised: 5 µl DNA template, 3.5 mM $MgCl_2$, 0.4 µM of each reverse and forward primer, 0.2 mM dNTPs, 0.08 U/µl of FastStart Taq DNA Polymerase, 2X PCR buffer and 8.6 µl PCR water to achieve a final volume of 25 µl. The cycling conditions employed were: Pre-denaturation for 5 minutes

© University of Venda

at 95 ^{0}C, denaturation for 15 seconds at 95 ^{0}C, annealing for 1 minute at 50 ^{0}C, extension for 1 minute at 72 ^{0}C, and final extension for 10 minutes at 72 ^{0}C.

Double-nested PCR was defined as an additional amplification reaction in which a template obtained from a nested PCR was used as an input. Negative reactions from the first nested reaction were subjected to another nested reaction with an expected HPV DNA product of ~150 bp. This reaction constituted the same final PCR reaction mixture as the first nested reaction. The cycling conditions employed were Pre-denaturation: 95 ^{0}C for 10 minutes, denaturation: 40 cycles consisting of 95 ^{0}C for 30 seconds, annealing: 50 ^{0}C for 40 seconds, extension: 72 ^{0}C for 30 seconds, and a final hold at 72 ^{0}C for 10 minutes. For the expected band size verification, the amplified PCR products were ascertained by a 2.5% agarose gel electrophoresis, stained with ethidium bromide. The prevalence of HPV DNA in the study population was determined by adding the number of DNA detections in the first and second nested reactions and then expressed as a percentage.

3.7.8 Next generation sequencing with the Illumina Miniseq platform for HPV genotyping

The detected HPV DNA were cleaned up using AMPure XP beads according to the manufacturer's instructions (Beckman Coulter, California, USA). DNA libraries were then prepared using Nextera XT DNA library preparation kit (Illumina San Diego, California, USA). Libraries were then sequenced using the Illumina MiniSeq platform, according to the manufacturer's instructions. Briefly, a 2 ng initial genomic input was used to prepare libraries, and a 1.8 pM final concentration of the pooled and denatured libraries was sequenced using a local run manager option.

3.7.9 Sequences control check

Prior to sequence analysis, all the generated FastQ raw sequencing data from the MiniSeq sequencer (Illumina, San Diego, CA, USA) was downloaded into the Apple MacBook Pro laptop with the following specifications: Intel i5 quad-core processor at 2.3 GHz and 8 GB RAM. The data was imported into FastQC tool version 0.11.8 for analysis. FastQC is the most frequently used tool for checking the quality control of sequencing data (Andrews, 2010). This tool performs analytic functions such as calculating the per base sequence quality, per sequence quality scores, per base sequence content, per base GC content, per sequence GC content, sequence length distribution, sequence duplication levels, and over-represented sequences.

© University of Venda

3.7.10 Data analysis

3.7.10.1 Statistical analysis

Statistical analysis was performed using R software version 4.4.1 (R Core Team, 2023). The majority of the data was categorical. Data was double-checked for errors and consistency prior to analysis. When calculating the data as proportions of the total sample, undisclosed data was excluded from the denominator. A bivariate regression model was employed to identify risk factors associated with HPV DNA positivity. Factors with a cut-off p-value of less than 20% in the bivariate analysis were included in the final multivariate model. Briefly, a logistic regression was employed to examine the relationship between HPV status (the dependent variable) and a set of predictor variables, including age group, marital status, level of education, and HIV status. Logistic regression is appropriate here because the outcome variable, HPV status, is binary (i.e., having HPV or not). This method models the probability of an event occurring (in this case, the presence of HPV) as a function of the predictor variables.

The logistic regression model estimates the log-odds of having HPV based on each predictor, with the following general form:

Where P is the probability of being HPV positive, and are the parameters of the model which represent the impact of each predictor on the likelihood of HPV. A positive coefficient indicates that an increase in the corresponding variable raises the odds of having HPV, while a negative coefficient implies a decrease in the odds. For instance, a significant positive coefficient for HIV status would suggest that being HIV-positive is associated with higher odds of HPV. A cut-off p-value of 0.05 was deemed statistically significant. Notably, logistic regression models were not computed to estimate the risk factors associated with hr-HPV genotypes due to a small sample size.

3.7.10.2 HPV Genotyping

FastQ sequences of quality were imported into Geneious Prime software version 2023.0.1 (Geneious Prime, 2023). Short-read sequences of low or poor quality were filtered and trimmed off for each specimen. All the HPV reference genomes were downloaded from the PaVE database, accessed on the 30th of May 2022. The sequences were subsequently mapped to various HPV genotypes to identify the genotype (s) of the test sequence. The identified genotypes were then examined for their distribution in the study population. The detected genotypes were further classified according to their carcinogenicity using the known 25 carcinogenic HPV genotypes of the International Agency for Research on Cancer. The identified genotypes were also examined for coverage by Gardasil-9, as it contained genotypes

© University of Venda

targeted by Gardasil-4 and Cervarix vaccines. The study methodology is summarized in Figure 3.7.

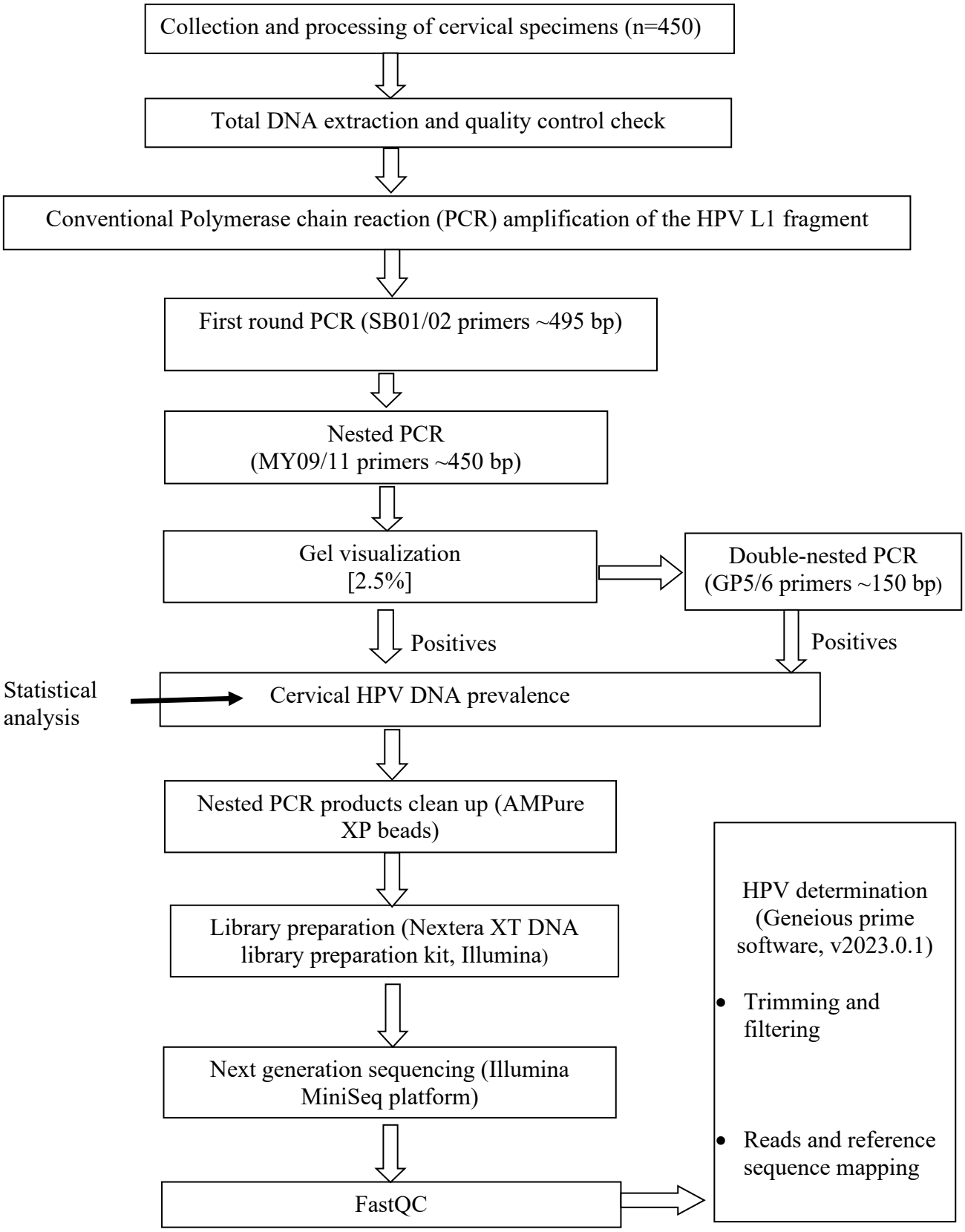


Figure 3.7: *Illustration of the study methodology, from sample collection through sequence analysis.*

© University of Venda

3.8 RESULTS

3.8.1 Characteristics of the study population

Table 3.4 details the socio-economic and clinical characteristics of the study participants. A total of 450 participants were enrolled in the current study and screened for HPV DNA. Demographic data were available for 356 participants with an age range of 18-84 years old, with the majority (115/356, 32.30%) ranging between the ages of 39-48 years. The mean age was 41.51 (SD± =12.03). Additionally, most women (317/356, 89.04%) identified themselves as heterosexual and were predominantly (206/356, 57.87%) living without HIV.

© University of Venda

Table 3.4: Socio-demographic and clinical characteristics of the study population

Characteristics	Frequency, n (%)
N = 356	
Age (years)	
Mean age 41.51 (±12.0 Standard deviation)	
18-28	53 (14.89%)
29-38	103 (28.93%)
39-48	115 (32.30%)
49-58	47 (13.20%)
≥59	38 (10.67%)
Level of education	
No educational background	15 (4.21%)
Primary level (grade 1-7)	44 (12.36%)
Secondary level (grade 8-12)	245 (68.82%)
Post grade 12 studies	39 (10.96%)
Marital status	
Single	161 (45.22%)
Married	165 (46.35%)
Divorced	3 (0.84%)
Widow	27 (7.58%)
Employment status	
Not disclosed/specified	11 (3.09%)
Employed	142 (39.89%)
Unemployed	180 (50.56)
Pensioner	23 (6.46%)
Income (Rands)	
Not disclosed/specified	219 (61.52%)
<R3000	73 (20.51%)
Between R3000-R10000	29 (8.15%)
Greater than R10000	35 (9.83%)
HIV status	
Positive	150 (42.13%)
Negative	206 (57.87%)
Tobacco users	
Not disclosed/specified	2 (0.56%)
Smoker	10 (2.81%)
Non-smoker	344 (96.63%)
Vaccination status	
Not disclosed/specified	24 (6.74%)
Not vaccinated	332 (93.26%)
Age at first sexual intercourse	
Not disclosed/specified	30 (8.43%)
11-20	275 (77.25%)
21-30	50 (14.04%)
31-40	1 (0.28%)
Type of sexual partner	
Not disclosed/specified	38 (10.67%)
Female	1 (0.28%)
Male	317 (89.04%)
Number of sexual partners	
Not disclosed/specified	30 (8.43%)

© University of Venda

0 or celibate	12 (3.37%)
1-2	310 (87.08%)
3-4	3 (0.84%)
Widow	1 (0.28%)

3.8.2 Prevalence of HPV DNA

The prevalence of cervical HPV DNA detected by any of the nested PCR approaches was 32.7% (147/450). HPV DNA was detected in more specimens by the second nested PCR (n=95) compared to the first nested reaction (n=52). Representative gels of the first and second nested reactions are shown in Figures 3.8 and 3.9, respectively. A total of 136/147 participants had demographic data. Women who were living with HIV had significantly higher HPV positivity rate (52.21%, n=71), compared to women living without HIV (47.79%, n=65), p= 0.00 (POR: 1.95, 95% CI: 1.26-3.02).

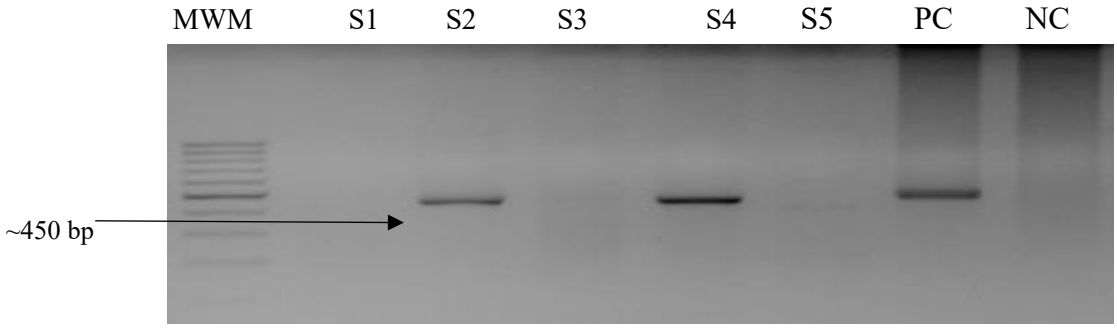


Figure 3.8: *Representation of the gel UV visualization of the nested PCR amplicons on 2.5% agarose gel. MWM: Molecular weight marker (100bp); S1, S3 and S5: Negative samples; S2 and S4: Positive samples; PC: Positive control; and NC: Negative control.*

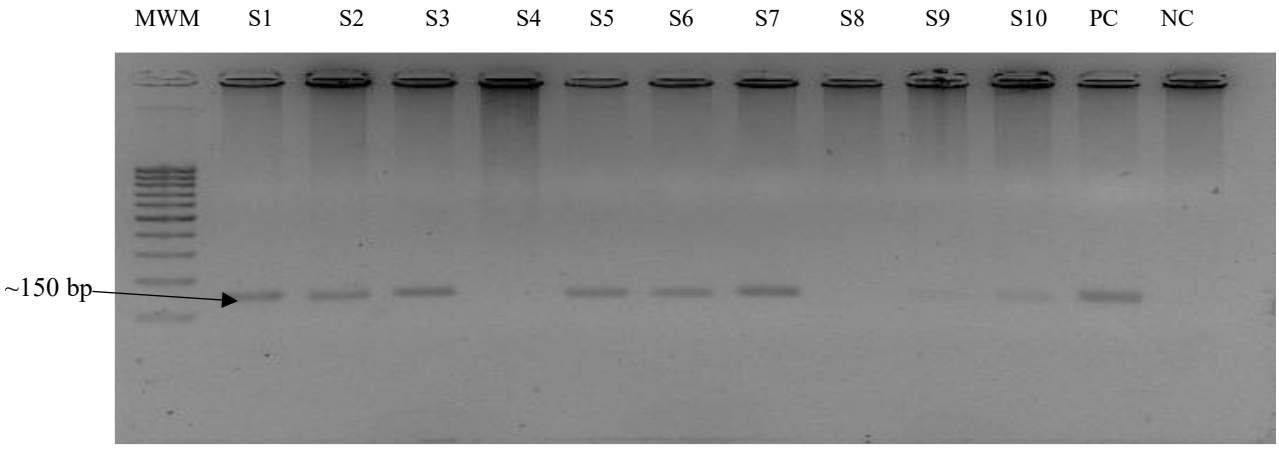


Figure 3.9: *Representation of the gel UV visualization of the double-nested PCR amplicons on 2.5% agarose gel. MWM: Molecular weight marker (100bp); S4 and S8: Negative samples; S1-S3, S5-S7, S9 and S10: Positive samples; PC: Positive control; and NC: Negative control.*

© University of Venda

3.8.3 Distribution of HPV genotypes

Of the 52 sequenced data, 48 sequences were of acceptable quality. Of these, 32 and 12 participants were living with and without HIV, respectively. Consequently, a total of 41 HPV genotypes, all belonging to *alphapapillomavirus* genus of *papillomaviridae* family were identified in the study population. The most commonly detected HPV genotype was HPV 45 (16.7%), followed by HPV 81, HPV 56, HPV 53 and HPV 6, with a frequency of 14.6% each. Additionally, rarely studied genotypes such as HPV 81 (14.6%), HPV 90 (6.3%), HPV 102 (6.6%) and HPV 114 (4.2%), were also identified (Figure 3.10).

Figure 3.11 illustrates the distribution of the detected HPV genotypes according to HIV status. Among women living with HIV, the most frequently detected HPV genotype was HPV 81 (18.8%), followed by HPV 6, HPV 45, HPV 53, HPV 58, HPV 62 and HPV 66 (15.6% each). In constrast, the participants not living with HIV were more frequently infected with HPV 6, HPV 16, HPV 42, HPV 45, HPV 54, HPV 61 (16.7% each) and 56 (25.0%). A higher variety of HPV genotypes (n=38 vs n=20), hr-HPV genotypes and multiple infections (59.4%) were more frequent among women living with HIV than their counterparts.

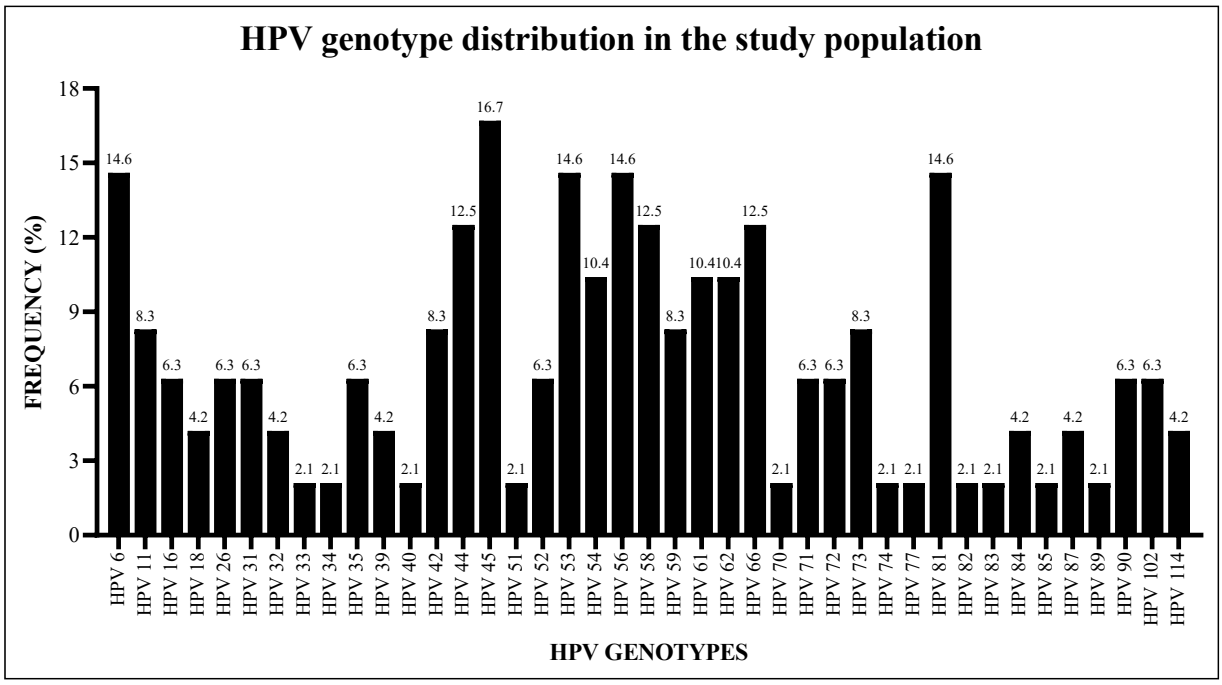


Figure 3.10: *The distribution of 41 HPV genotypes detected in a study population.*

© University of Venda

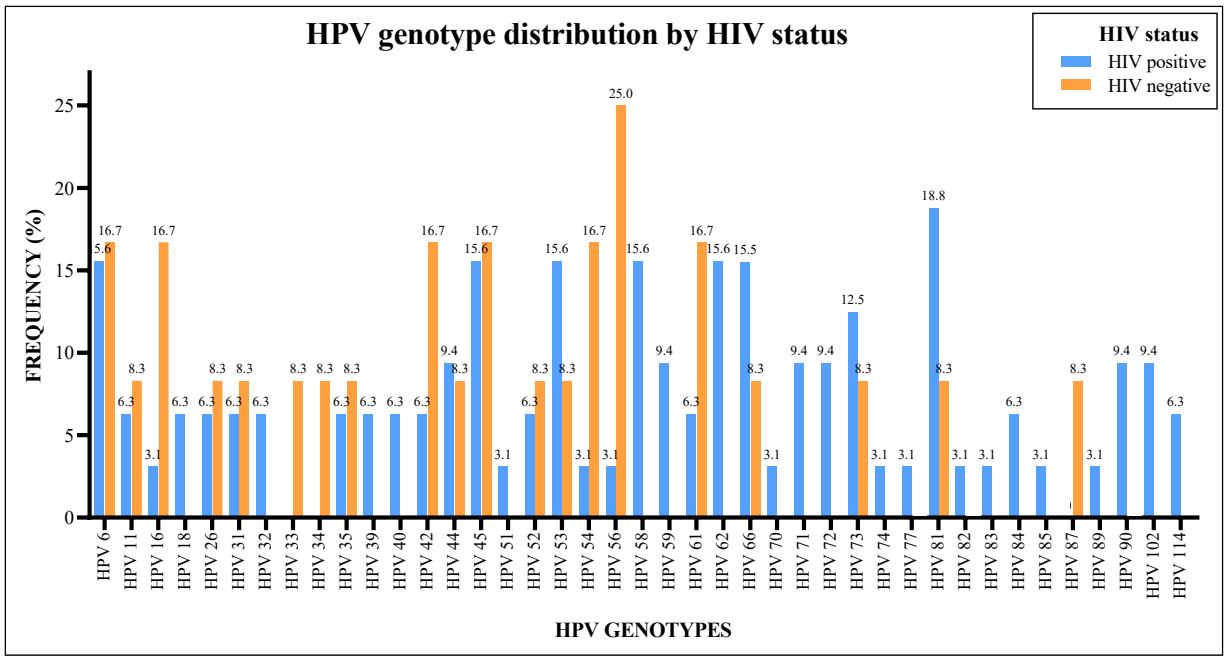


Figure 3.11: *The frequency distribution of HPV genotypes by HIV status.*

3.8.3.1 Prevalence and distribution of known carcinogenic HPV genotypes

A sub-analysis on the distribution of carcinogenic HPV genotypes showed that there were 20 of 25 known carcinogenic HPV genotypes present in the study population (Figure 3.12). In this study, five carcinogenic genotypes namely: HPV 30, HPV 67, HPV 68, HPV 69 and HPV 97 were not detected. As a consequence, 79.2% (38/48) of participants were infected with at least one carcinogenic HPV genotype. The predominant carcinogenic HPV genotype was HPV 45 (21.1%), followed by HPV 53 and HPV 56 (18.4% each). The majority of women living with HIV were infected with HPV 45, HPV 53, HPV 58 and HPV 66, with a frequency of 19.2% each. HPV 16 (25.0%) and HPV 45 (25.0%) were more prevalent among women not living with HIV, Figure 3.12.

© University of Venda

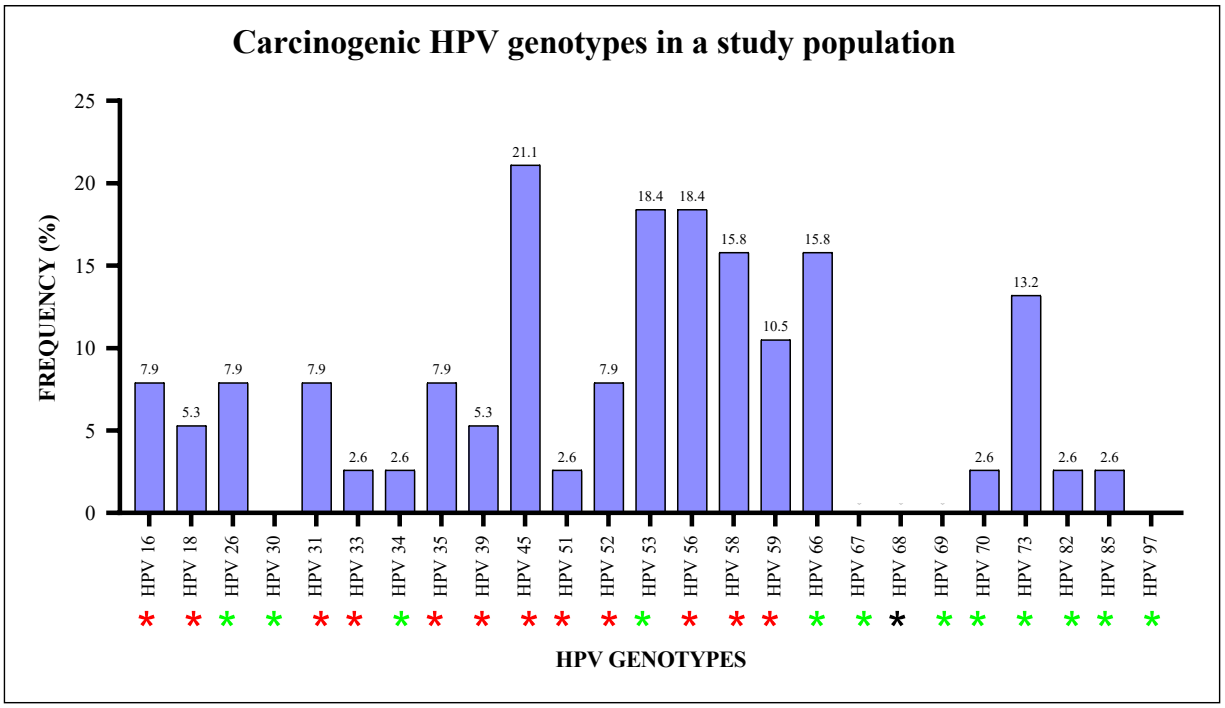


Figure 3.12: *The distribution of carcinogenic HPV genotypes (x-axis) and their frequencies (y-axis) in a study population. The red, black, and green asterisks designate the high-risk, probable and possible carcinogenic HPV genotypes, respectively.*

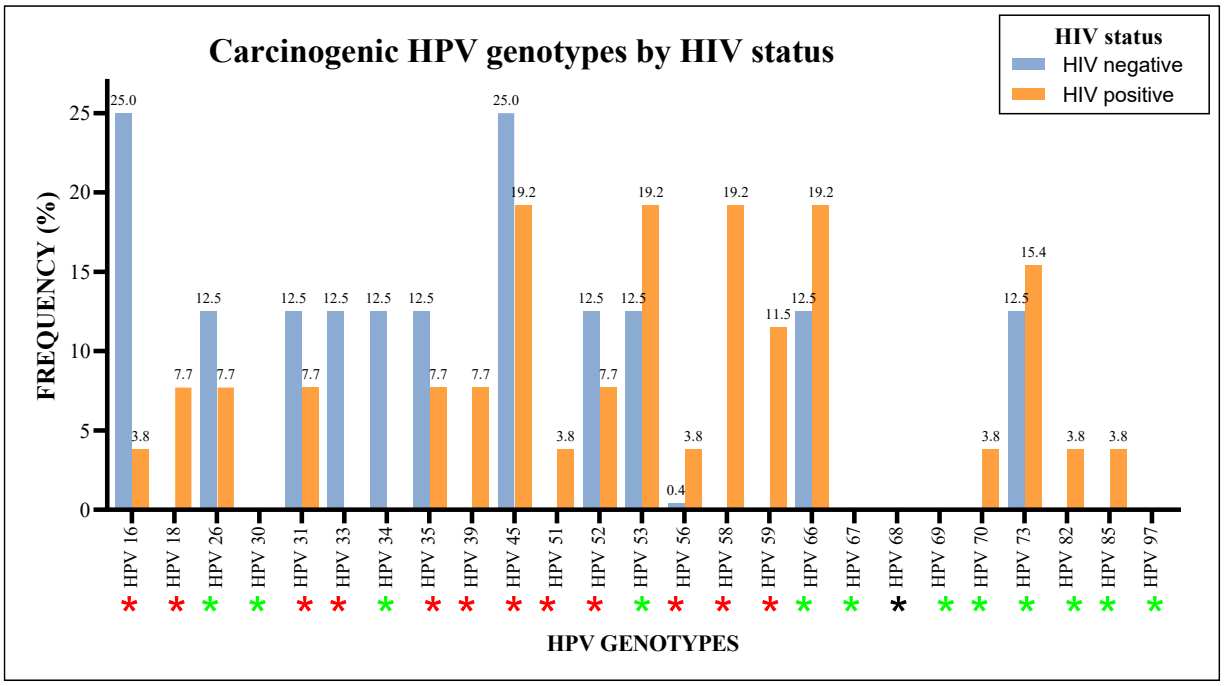


Figure 3.13: *The carcinogenic HPV genotype distribution among the HIV negative (blue) and HIV positive (brown) group. The asterisks designated by the red, black and green colors represent the high-risk, probable and possible carcinogenic HPV genotypes respectively.*

© University of Venda

3.8.3.2 Prevalence and distribution of HPV genotypes incorporated in the Gardasil-9 vaccine

More than half of the participants (n=25/48, 52.1%) tested positive for at least one of the HPV genotypes incorporated in the Gardasil-9 vaccine, not in use in South Africa. The most frequently detected genotype was HPV 45 (32.0%), followed by HPV 6 (28.0%), as shown in Figure 3.14. The most frequently detected Gardasil-9 incorporated HPV genotypes among women living with HIV were HPV 45 and HPV 6 (33.0% each). Women living without HIV were more likely to be infected with HPV 6, HPV 16, HPV 45, HPV 52 and HPV 58, each with a frequency of 33.3%. Figure 3.15 shows the frequency of occurrence of HPV genotypes observed in the study population, which are incorporated in Gardasil-9 vaccine according to HIV status.

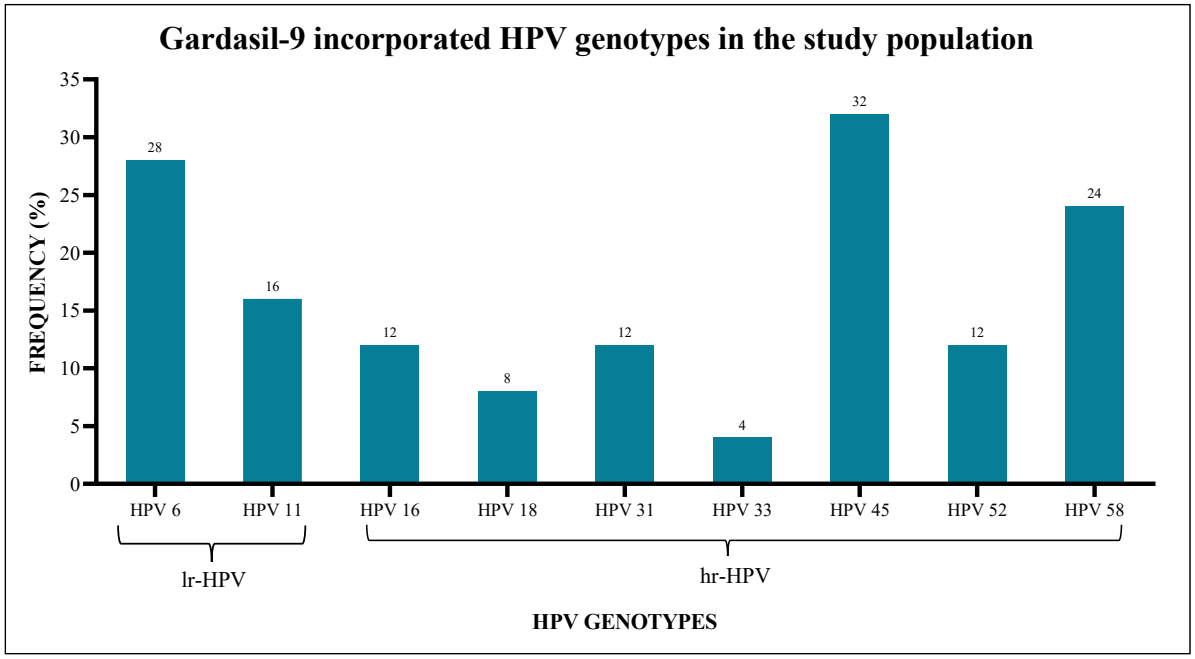


Figure 3.14: *The occurrence of HPV genotypes covered by the Gardasil-9 HPV vaccine among women in a study population.*

© University of Venda

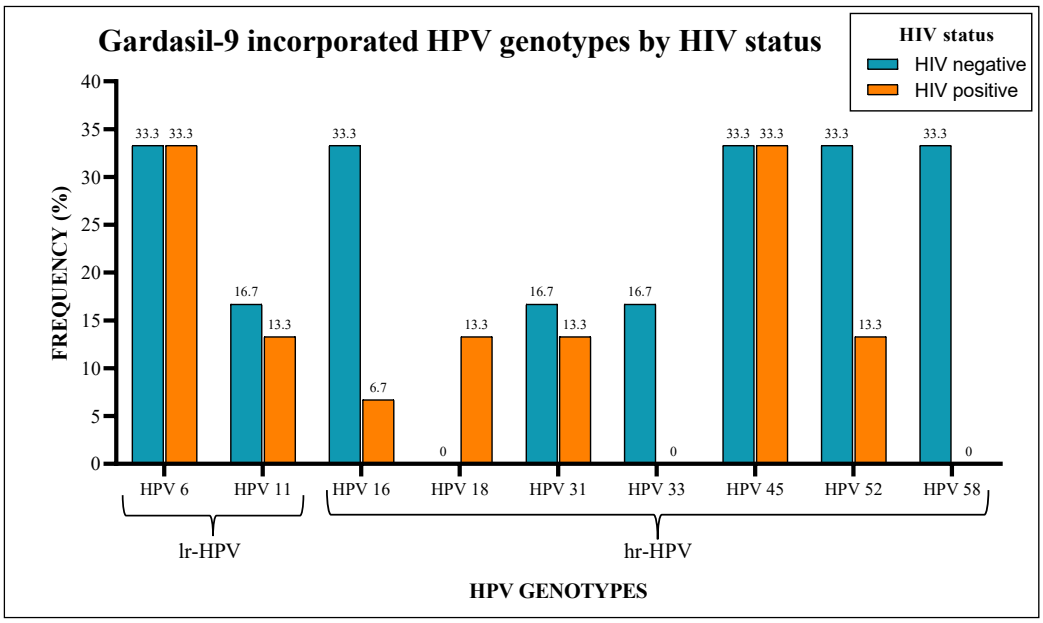


Figure 3.15: *The distribution of HPV genotypes incorporated in Gardasil-9 vaccine among women living with and without HIV in the study population.*

3.8.4 Risk factors associated with HPV infection

Table 3.5 shows the unadjusted and adjusted prevalence odds ratios (OR) along with their corresponding confidence intervals (CIs) and p-values for various socio-demographic and clinical factors potentially associated with HPV infection. HIV status was significantly associated with HPV status. In the bivariate analysis, the risk of being HPV positive among WLWH was approximately two-folds as compared to those living without HIV (OR = 1.95, 95% CI: 1.26–3.02, p = 0.00). Women aged 49-to-58 years were also significantly more likely to be HPV positive than those aged 18-to-28 years (OR = 2.56, 95% CI: 1.14–5.91, p = 0.03). Additionally, women with primary level education or less had a significantly lower risk of HPV positivity compared to those with tertiary education (OR = 0.38, 95% CI: 0.16–0.89, p = 0.02). Variables such as employment, marital status and income level were not significantly associated with HPV positivity. Even after multivariate adjustment, living with HIV remained a significant risk factor for HPV positivity, with approximately twice the risk compared to women without HIV (OR =1.97, 95% CI: 1.24–3.16, p = 0.00). Similarly, women aged 49-to-58 years continued to show a significantly higher risk of being HPV positive compared to those aged 18-to-28 years (reference group) (OR = 2.71, 95% CI, 1.14-6.63, p = 0.03). No other age group showed a significant difference relative to the reference group. Notably, there was no statistical association between the other variables and HPV infection in the multivariate model. Factors such as vaccination status and type of sexual partner were excluded from the analysis due to data skewness.

© University of Venda

Table 3.5: Risk factors associated with HPV infection (bivariate and multivariate analysis)

Variable (category)	Unadjusted p-value	Unadjusted POR (95% CI)	Adjusted p-value	Adjusted OR (95% CI)
Employment	0.13			
Employed **(ref)**	-	-	-	-
Unemployed	0.31	0.79 (0.50-1.24)	0.71	0.91 (0.56-1.48)
Pensioner	0.07	0.38 (0.12-1.01)	0.10	0.21 (0.03-1.20)
HIV status	0.00			
Negative **(ref)**	-	-	-	-
Positive	0.00	1.95 (1.26-3.02)	0.00	1.97 (1.24-3.16)
Age	0.13			
18-28 **(ref)**	-	-	-	-
29-38	0.27	1.49 (0.74-3.08)	0.31	1.47 (0.71-1.34)
39-48	0.41	1.34 (0.67-2.75)	0.38	1.39 (0.67-2.79)
49-58	0.03	2.56 (1.14-5.91)	0.03	2.71 (1.14-6.63)
59+	0.85	0.92 (0.36-2.28)	0.33	1.86 (0.51-6.49)
Age at first sexual intercourse	0.80			
11-19 **(ref)**	-	-	-	-
16-20	0.86	0.94 (0.48-1.89)		
21-25	0.82	1.10 (0.47-2.61)		
25+	0.42	0.39 (0.02-2.94)		
Education	0.08			
Tertiary level **(ref)**	-	-	-	-
Primary and below	0.02	0.38 (0.16-0.89)	0.08	0.44 (0.17-1.11)
Secondary level	0.56	0.56 (0.28-1.11)	0.16	0.60 (0.29-1.23)
Marital status	0.98			
Married **(ref)**	-	-	-	-
Never married	0.95	1.01 (0.65-1.59)		
Widow/Divorced	0.88	0.94 (0.41-2.07)		
Income	0.79			
Between R3000-R10 000 **(ref)**	-	-	-	-
Greater than R10 000	0.75	0.85 (0.30-2.40)		
Less than R3000	0.79	1.14 (0.48-2.82)		
Tobacco users	0.90			
Non-smoker **(ref)**	-	-	-	-
Smoker	0.90	1.08 (0.27-3.87)		

© University of Venda

3.9 DISCUSSION

Infection with high-risk (hr-) HPV is a global public health concern because of the greater risk of developing cervical cancer (CC). Data on the prevalence and genotype distribution of HPV are scarce in the Limpopo Province of South Africa. The current cross-sectional study determined the prevalence of cervical HPV infection and genotype distribution among 450 women who were living with and without HIV from selected health facilities in Limpopo Province, South Africa. Data on HPV prevalence and genotype distribution is crucial in evaluating the burden of viral infection in the studied population and assessing the effectiveness of prevention policies (Zinzendorf et al., 2022). To the best of our knowledge, this is the first study to investigate HPV infection among women living with and without HIV in Limpopo Province, South Africa.

Compared to a single-nested PCR approach, a double-nested reaction significantly enhanced the detection rate of HPV DNA in the study population. The study reports an overall prevalence of 32.7%. Subsequently, this finding is congruent with findings from Central/Eastern Italy (Tartaglia et al., 2017). A slightly similar prevalence of 28.5% and much higher prevalences of 76.0% and 84.2% were reported in South Africa (Taku et al, 2020; Mbulawa et al., 2021; Mbulawa et al., 2022). In Africa and elsewhere, HPV prevalences also vary (Si-Mohamed et al., 2005; Agarossi et al., 2009; Pimentel et al., 2013; Joshi et al., 2014; Teixeira et al., 2018; Aziz et al., 2023), contributing factors to the variability may include cervical cytology status, genotyping techniques (Ali et al., 2021), and study population variations (Taku et al., 2021). The high rates of HPV prevalence and ineffective cervical screening programs in South Africa, where the rates of HIV are high remain a significant public health concern (Taku et al., 2020). Therefore, implementation of effective preventative measures such as HPV vaccinations could benefit many in this population. Literature report increased rates of HPV infection among WLWH (McDonald et al., 2014; Mbulawa et al., 2015; Tartaglia et al., 2017; Taku et al., 2021; Mcharo et al., 2021), which aligns with our study finding. The current study further showed that WLWH were more susceptible to being infected with multiple infections and hr-HPV genotypes. Other studies have reported an increased risk of multiple infections, higher viral persistence and development to CC among WLWH (Firnhaber et al., 2011; Liu et al., 2018; McDonald et al., 2014; Ebrahim et al., 2016; Mbulawa et al., 2018; Taku et al., 2020; Hopkins et al., 2021). It is thought that HIV impacts the immune competence of the vaginal mucosa, thus promoting susceptibility to high-risk infections such as HPV (Kelly et al., 2017). Therefore, the higher prevalence among WLWH might be due to the fact that

© University of Venda

immunocompromised individuals do not clear off HPV infections easily compared to women living without HIV.

In general, hr-HPV 45 (16.7%) emerged as the predominant genotype in our study population. This is similar to findings from KwaZulu-Natal Province, where HPV 45 (7.6%) was among the most commonly detected genotypes in a generalized population (Mbatha et al., 2017). Worldwide, hr-HPV 45 accounts for approximately 5% of all CC cases, with varying prevalences across regions in Eastern Asia (3%) and Africa (9%) (Li et al., 2011). Additionally, studies including South Africa are HPV 45 among women with abnormal cytology (Andall-Brereton et al., 2011; Haghshenas et al., 2013; Taku et al., 2021; Mbulawa et al., 2022). The prevalence of hr-HPV 45 is thought to increase with the severity of cervical disease (Rad et al., 2017). Despite the cervical cytology status of the study participants being unknown, the high prevalence of hr-HPV 45 is concerning mainly because the Cervarix and Gardasil-4 vaccines currently in use in South Africa do not target this genotype. Therefore, without adequate prevention or control measures, there is a potential for continued transmission of HPV 45, leading to an increased risk of CC. Furthermore, besides HPV 45, the distribution of the most frequently detected HPV genotypes varied between women living with HIV and women not living with HIV. This finding is similar to reports from a study in Kigali, Rwanda (Veldhuijzen et al., 2011). However, the current study is inconsistent with a study conducted in Cape Town, South Africa, that reported similar common HPV genotypes in both HIV positive and negative populations with varying prevalences (McDonald et al., 2014). The application of NGS could have influenced the rate of detection and the types of HPV genotypes. Moreover, HPV 53, HPV 54, HPV 56, HPV 62, HPV 66 and HPV 81, not included in currently used vaccines, were among the most common HPV genotypes detected in the study population. Therefore, including these genotypes in the future HPV vaccines could enhance preventive benefits.

Several studies have predominantly reported HPV 16 among WLWH compared to women living without HIV (Palefsky et al., 1999; Ginindza et al., 2017; Mbulawa et al., 2024). However, the current study observed a higher prevalence of HPV 16 among women living without HIV compared to those living with HIV. Nonetheless, studies have reported mixed findings regarding the association between highly active antiretroviral therapy (HAART) and HPV clearance, with some showing a significant association and others showing no significant association (Minkoff et al., 2010; Blitz et al., 2013; Mane et al., 2017). A study by Paramsothy and colleagues observed a significantly higher prevalence of HPV clearance among WLWH (Paramsoth et al., 2009). The current findings could be that WLWH may be on HAART, and

© University of Venda

over time, the immune system clears off the infection, thus a higher prevalence of HPV 16 among HIV-negative than HIV-positive population.

Studies show that the Gardasil-9 vaccine has been highly effective in combating infection by HPV and cancers, with efficacy rates from 90% to 100% (Joura et al., 2015; Huh et al., 2017; Garland et al., 2018; Ruiz-Sternberg et al., 2018). However, Gardasil-9 vaccine is not yet available in South Africa, and over 50% of study participants in the current study had at least one of the Gardasil-9 incorporated HPV genotypes. Previous sentinel surveillance studies conducted among South African women have also reported high rates of Gardasil-9 genotypes (Kularatne et al., 2017; Mbulawa et at., 2018). The current study finding raises the importance of further engagement among policymakers to prioritize the Gardasil-9 vaccine rollout to reduce the transmission in South Africa. Moreover, the study findings could inform vaccination campaign in Limpopo Province, where data on HPV epidemiology is limited.

A report on the evaluation of carcinogenic risks to humans showed that sexual related behaviours are major risk factor for infection by HPV (IARC, 2007). The current study also found a significant association between HIV status and HPV infection, specifically among women who were living with HIV. The study finding is consistent with studies conducted by Dartell et al. (2012), McDonald et al. (2012), Ginindza et al. (2017), Taku et al. (2020a), Taku et al. (2020b) and Travill et al. (2024), who reported a significant association between HIV and HPV infection. HIV infection has a negative impact on the function of the immune system, thus promoting increased rates of HPV infection, viral persistence of hr-HPV and reactivation of latent HPV (Strickler et al., 2005; Mbulawa et al., 2012). A study conducted in the Western Cape Province reported that women living with HIV were 3 times more likely to test positive for new HPV infection compared to their counterparts (Mbulawa et al., 2012). Both HPV and HIV are STIs. Therefore, educational campaigns in communities, including schools and healthcare centers, can help raise awareness about HPV infection and contribute to reducing its burden. While several studies have reported that the risk of HPV infection decreases with increasing age (Kjaer et al., 2000; Cuschieri et al., 2004; Sanjosé et al., 2007; Smith et al., 2008; Forman et al., 2012), the current study observed a significant association between HPV infection and age particularly those who were 48-to-59 years old. This pattern may reflect the reactivation of latent HPV infections than new infections, as supported by existing literature (Gravitt, 2012), and could be relevant to the population studied. These findings support the HPV screening, even among older women. Other risk factors associated with HPV infection have been reported elsewhere (Schluterman et al., 2013; Ribeiro et al., 2015; Niyazi et al., 2016).

© University of Venda

The strengths of this study should be considered in the context of some limitations. This include the lack of clinical information (example; cervical cytology, CD4 counts and antiretroviral therapy status), and a small sample size for HPV genotyping. Participants were enrolled from only four healthcare facilities in Limpopo Province. Thus, the findings are an important indication, but do not represent the general population in Limpopo Province. Additional geographical inclusivity to understand the HPV genotype distribution on a broader scale would expand the scope and add more data to the current findings. Additional geographical inclusivity to understand the HPV genotype distribution on a broader scale would expand the scope and add more data to the current findings. Furthermore, the detection of less-well-known genotypes, including HPV 81, HPV 90, HPV 102 and HPV 114, in the study population is very interesting. However, their clinical relevance requires to be determined.

In conclusion, the findings in this study suggests a relatively high prevalence of HPV infection and WLWH were more prone to HPV infections compared to those living without HIV, probably due to immunosuppression. Considering the high rate of co-infection between HIV and HPV observed in this study and other studies conducted in South Africa, prioritizing women living with HIV for cervical HPV screening and prevention strategies such as vaccination is recommended. Strengthening integrated HIV and HPV screening services, along with ensuring regular follow-up of cervical health in WLWH remains important for early detection and timely treatment. Additionally, it was also observed that women living with and without HIV may have different profiling in terms of HPV infections which is significant in intervention strategies, example development of targeted HPV screening strategies. Both HIV and HPV are sexually transmitted infections; consequently, promoting tailored public health programs such as clinic and school-based education focusing on the prevention of STIs, practising safe sex, and HPV awareness may help reduce the burden of HPV infection in the study population. Considering limited data on HPV epidemiology in Limpopo Province, the current results moreover encourage national-level surveillance with the inclusion of expanded regional coverage.

© University of Venda

3.10 REFERENCES

1. Abreu, A.L., Souza, R.P., Gimenes, F. and Consolaro, M.E., 2012. A review of methods for detect human papillomavirus infection. *Virology Journal*, *9*(1), pp.1-9.

2. Agarossi, A., Ferrazzi, E., Parazzini, F., Perno, C.F. and Ghisoni, L., 2009. Prevalence and genotype distribution of high-risk human papillomavirus infection in women undergoing voluntary cervical cancer screening in Italy. *Journal of Medical Virology*, *81*(3), pp.529-535

3. Ali, M.J., Godwin, I.E., Achenbach, C., Sagay, A.S., Daru, P.H., Ocheke, A.N., Afolaranmi, T., Hou, L., Murphy, R.L. and Musa, J., 2021. Prevalence and distribution of high-risk human papillomavirus genogenotypes in HIV positive and HIV negative women with Abnormal cervical cytology. *Tropical Journal of Obstetrics and Gynaecology*, *38*(2), pp.144-153.

4. Amponsah-Dacosta, E., Blose, N., Nkwinika, V.V. and Chepkurui, V., 2022. Human papillomavirus vaccination in South Africa: programmatic challenges and opportunities for integration with other adolescent health services? *Frontiers in Public Health*, *10*, p.799984.

5. Andall-Brereton, G.M., Hosein, F., Salas, R.A., Mohammed, W., Monteil, M.A., Goleski, V., Severini, A., Quesnel, S.M., Carrington, C.V., Boodram, L.L. and Boisson, E., 2011. Human papillomavirus genotypes and their prevalence in a cohort of women in Trinidad. *Revista Panamericana de Salud Pública*, *29*(4), pp.220-226.

6. Antonsson, A., Karanfilovska, S., Lindqvist, P.G. and Hansson, B.G., 2003. General acquisition of human papillomavirus infections of skin occurs in early infancy. *Journal of Clinical Microbiology*, *41*(6), pp.2509-2514.

7. Arbyn, M., Castellsagué, X., de Sanjosé, S., Bruni, L., Saraiya, M., Bray, F. and Ferlay, J., 2011. Worldwide burden of cervical cancer in 2008. *Annals of Oncology*, *22*(12), pp. 2675-2686

8. Arbyn, M., Tommasino, M., Depuydt, C. and Dillner, J., 2014. Are 20 human papillomavirus types causing cervical cancer? *The Journal of Pathology*, *234*(4), pp.431-435.

© University of Venda

9. Ardhaoui, M., Ennaifer, E., De Matos Salim, A.C., Gomez, F.M., Laasili, T., Boubaker, M.S. and Guizani, I., 2021. Nested PCR followed by NGS: Validation and application for HPV genotyping of Tunisian cervical samples. *Plos one*, *16*(8), p.e0255914.

10. Asgari, M.M., Kiviat, N.B., Critchlow, C.W., Stern, J.E., Argenyi, Z.B., Raugi, G.J., Berg, D., Odland, P.B., Hawes, S.E. and de Villiers, E.M., 2008. Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals. *Journal of Investigative Dermatology*, *128*(6), pp.1409-1417.

11. Asiaf, A., Ahmad, S.T., Mohammad, S.O. and Zargar, M.A., 2014. Review of the current knowledge on the epidemiology, pathogenesis, and prevention of human papillomavirus infection. *European Journal of Cancer Prevention*, *23*(3), pp.206-224.

12. Aziz, H., Sattar, A.A., Mahmood, H., Fatima, S., Khurshid, M. and Faheem, M., 2023. Prevalence of HPV types in HIV-positive and negative females with normal cervical cytology or dysplasia. *Journal of Clinical Laboratory Analysis*, *37*(4), p.e24851.

13. Banura, C., Mirembe, F.M., Orem, J., Mbonye, A.K., Kasasa, S. and Mbidde, E.K., 2013. Prevalence, incidence and risk factors for anogenital warts in Sub Saharan Africa: a systematic review and meta analysis. *Infectious agents and cancer*, *8*, pp.1-10.

14. Basiletti, J.A., Valls, J., Poklépovich, T., Fellner, M.D., Rol, M., Alonso, R., Correa, R.M., Colucci, M.C., Rodriguez de la Pena, M., Falabella, P.G. and Saíno, A., 2022. Human papillomavirus genotyping using next generation sequencing (NGS) in cervical lesions: Genotypes by histologic grade and their relative proportion in multiple infections. *Plos one*, *17*(11), p.e0278117.

15. Bernard, H.U., Burk, R.D., Chen, Z., Van Doorslaer, K., Zur Hausen, H. and De Villiers, E.M., 2010. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology*, *401*(1), pp.70-79.

16. Bozzetti, M.C., Nonnenmacher, B., Mielzinska, I., Villa, L.L., Lorincz, A.T., Breitenbach, V. and Prolla, J.C., 2000. Comparison between hybrid capture II and polymerase chain reaction results among women at low risk for cervical cancer. *Annals of epidemiology*, *10*(7), p.466.

17. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A. and Jemal, A., 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA. A Cancer Journal for Clinicians*, *68*(6), pp. 394-424.

© University of Venda

18. Bruni, L., Albero, G., Serrano, B., Mena, M., Gómez, D., Muñoz, J., Bosch, F.X. and de Sanjosé, S., 2019. ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human papillomavirus and related diseases in the world. Summary Report 17 June 2019 (Accessed January 15, 2019).

19. Bruni, L., Diaz, M., Castellsagué, M., Ferrer, E., Bosch, F.X. and de Sanjosé, S., 2010. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *Journal of Infectious Diseases*, *202*(12), pp.1789-1799.

20. Buchanan, T.R., Graybill, W.S. and Pierce, J.Y., 2016. Morbidity and mortality of vulvar and vaginal cancers: Impact of 2-, 4-, and 9-valent HPV vaccines. *Human Vaccines & Immunotherapeutics*, *12*(6), pp.1352-1356.

21. Burd, E.M., 2016. Human papillomavirus laboratory testing: the changing paradigm. *Clinical microbiology reviews*, *29*(2), pp. 291-319

22. Buck, C.B., Day, P.M. and Trus, B.L., 2013. The papillomavirus major capsid protein L1. *Virology*, *445*(1-2), pp.169-174.

23. Burley, M., Roberts, S. and Parish, J.L., 2020, April. Epigenetic regulation of human papillomavirus transcription in the productive virus life cycle. In *Seminars in immunopathology*, 42(2), pp.159-171.

24. Camargo, M., Del Río-Ospina, L., Soto-De León, S.C., Sánchez, R., Pineda-Peña, A.C., Sussmann, O., Patarroyo, M.E. and Patarroyo, M.A., 2018. Association of HIV status with infection by multiple HPV types. *Tropical Medicine & International Health*, *23*(11), pp.1259-1268.

25. Camargo, M., Soto-De Leon, S., Sanchez, R., Munoz, M., Vega, E., Beltran, M., Perez-Prados, A., Patarroyo, M.E. and Patarroyo, M.A., 2011. Detection by PCR of human papillomavirus in Colombia: Comparison of GP5+/6+ and MY09/11 primer sets. *Journal of Virological Methods*, *178*(1-2), pp.68-74.

26. Carvalho, N.D.O., del Castillo, D.M., Perone, C., Januário, J.N., Melo, V.H.D. and Brasileiro Filho, G., 2010. Comparison of HPV genotyping by type-specific PCR and sequencing. *Memórias do Instituto Oswaldo Cruz*, *105*, pp.73-78.

© University of Venda

27. Casalegno, J.S., Le Bail Carval, K., Eibach, D., Valdeyron, M.L., Lamblin, G., Jacquemoud, H., Mellier, G., Lina, B., Gaucherand, P., Mathevet, P. and Mekki, Y., 2012. High-risk HPV contamination of endocavity vaginal ultrasound probes: An underestimated route of nosocomial infection? *PloS One*, *7*(10), p.e48137.

28. Chikandiwa, A., Pisa, P.T., Muller, E.E., Tamalet, C., Mayaud, P., Chersich, M.F. and Delany-Moretlwe, S., 2019. Incidence, persistence, clearance, and correlates of genital human papillomavirus infection and anogenital warts in a cohort of men living with human immunodeficiency virus in South Africa. *Sexually Transmitted Diseases*, *46*(5), p.347.

29. Chikandiwa, A., Pisa, P.T., Tamalet, C., Muller, E.E., Michelow, P., Chersich, M.F., Mayaud, P. and Delany-Moretlwe, S., 2019. Prevalent, persistent anal HPV infection and squamous intraepithelial lesions: Findings from a cohort of men living with HIV in South Africa. *PloS One*, *14*(12), p.e0225571.

30. Choi, Y.J. and Park, J.S., 2016. Clinical significance of human papillomavirus genotyping. *J Gynecol Oncol*, *27*(2), p.e21.

31. Coser, J., da Rocha Boeira, T., Fonseca, A.S.K., Ikuta, N. and Lunge, V.R., 2011. Human papillomavirus detection and typing using a nested-PCR-RFLP assay. *The Brazilian Journal of Infectious Diseases*, *15*(5), pp.467-472.

32. Costa-Silva, M., Azevedo, F. and Lisboa, C., 2018. Anogenital warts in children: Analysis of a cohort of 34 prepubertal children. *Pediatric Dermatology*, *35*(5), pp.e325-e327.

33. Crosbie, E.J., Einstein, M.H., Franceschi, S. and Kitchener, H.C., 2013. Human papillomavirus and cervical cancer. *The Lancet*, *382*(9895), pp.889-899.

34. Cuschieri, K.S., Cubie, H.A., Whitley, M.W., Seagar, A.L., Arends, M.J., Moore, C., Gilkisson, G. and McGoogan, E., 2004. Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *Journal of clinical pathology*, *57*(1), pp.68-72.

35. Danos, O., Katinka, M. and Yaniv, M., 1982. Human papillomavirus 1a complete DNA sequence: a novel type of genome organization among papovaviridae. *The EMBO journal*, *1*(2), pp.231-236.

36. Dartell, M., Rasch, V., Kahesa, C., Mwaiselage, J., Ngoma, T., Junge, J., Gernow, A., Ejlersen, S.F., Munk, C., Iftner, T. and Kjaer, S.K., 2012. Human papillomavirus

© University of Venda

prevalence and type distribution in 3603 HIV-positive and HIV-negative women in the general population of Tanzania: the PROTECT study. *Sexually transmitted diseases*, *39*(3), pp.201-208.

37. Dawar, M., Deeks, S. and Dobson, S., 2007. Human papillomavirus vaccines launch a new era in cervical cancer prevention. *Cmaj*, *177*(5), pp.456-461.

38. De Koning, M.N.C., Quint, K.D., Bruggink, S.C., Gussekloo, J., Bouwes Bavinck, J.N., Feltkamp, M.C.W., Quint, W.G.V. and Eekhof, J.A.H., 2015. High prevalence of cutaneous warts in elementary school children and the ubiquitous presence of wart-associated human papillomavirus on clinically normal skin. *British Journal of Dermatology*, *132.72*(1), pp.196-201.

39. Delany-Moretlwe, S., Kelley, K.F., James, S., Scorgie, F., Subedar, H., Dlamini, N.R., Pillay, Y., Naidoo, N., Chikandiwa, A. and Rees, H., 2018. Human papillomavirus vaccine introduction in South Africa: Implementation lessons from an evaluation of the national school-based vaccination campaign. *Global Health: Science and Practice*, *6*(3), pp.425-438.

40. De Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D. and Plummer, M., 2012. Global burden of cancers attributable to infections in 2008: A review and synthetic analysis. *The Lancet Oncology*, *13*(6), pp.607-615.

41. De Martel, C., Plummer, M., Vignat, J. and Franceschi, S., 2017. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *International Journal of Cancer*, *141*(4), pp.664-670.

42. De Sanjosé, S., Diaz, M., Castellsagué, X., Clifford, G., Bruni, L., Muñoz, N. and Bosch, F.X., 2007. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: A meta-analysis. *The Lancet Infectious Diseases*, *7*(7), pp.453-459.

43. De Sanjose, S., Quint, W.G., Alemany, L., Geraets, D.T., Klaustermeier, J.E., Lloveras, B., Tous, S., Felix, A., Bravo, L.E., Shin, H.R. and Vallejos, C.S., 2010. Human papillomavirus genotype attribution in invasive cervical cancer: A retrospective cross-sectional worldwide study. *The Lancet Oncology*, *11*(11), pp.1048-1056.

44. De Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U. and Zur Hausen, H., 2004. Classification of papillomaviruses. *Virology*, *324*(1), pp.17-27.

© University of Venda

45. De Vuyst, H., Alemany, L., Lacey, C., Chibwesha, C.J., Sahasrabuddhe, V., Banura, C., Denny, L. and Parham, G.P., 2013. The burden of human papillomavirus infections and related diseases in Sub-Saharan Africa. *Vaccine*, *31*, pp.F32-F46.

46. Ebrahim, S., Mndende, X.K., Kharsany, A.B., Mbulawa, Z.Z., Naranbhai, V., Frohlich, J., Werner, L., Samsunder, N., Karim, Q.A. and Williamson, A.L., 2016. High burden of human papillomavirus (HPV) infection among young women in KwaZulu-Natal, South Africa. *PloS one*, *11*(1), p.e0146603.

47. Einstein, M.H., Martens, M.G., Garcia, F.A., Ferris, D.G., Mitchell, A.L., Day, S.P. and Olson, M.C., 2010. Clinical validation of the Cervista® HPV HR and 16/18 genotyping tests for use in women with ASC-US cytology. *Gynecologic Oncology*, *118*(2), pp.116-122.

48. Faridi, R., Zahra, A., Khan, K. and Idrees, M., 2011. Oncogenic potential of Human Papillomavirus (HPV) and its relation with cervical cancer. *Virology Journal*, *8*(1), pp.1-8.

49. Favre, M., Orth, G., Croissant, O. and Yaniv, M., 1975. Human papillomavirus DNA: physical map. *Proceedings of the National Academy of Sciences*, *72*(12), pp. 4810-4814.

50. Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D. and Bray, F., 2015. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer*, *136*(5), pp.E359-E386.

51. Firnhaber, C., Evans, D., Friedman-Khalili, R., Willliams, S., Michelow, P., Matlhagela, K., Wester, C., Grinsztejn, B. and Lockman, S., 2011. Seroprevalence of HPV vaccine types 6, 11, 16 and 18 in HIV-infected women from South Africa, Brazil and Botswana. *Journal of clinical virology*, *52*(3), pp.265-268.

52. Forman, D., de Martel, C., Lacey, C.J., Soerjomataram, I., Lortet-Tieulent, J., Bruni, L., Vignat, J., Ferlay, J., Bray, F., Plummer, M. and Franceschi, S., 2012. Global burden of human papillomavirus and related diseases. *Vaccine*, *30*, pp.F12-F23.

53. Frenkel, N., Roizman, B., Cassai, E. and Nahmias, A., 1972. A DNA fragment of herpes simplex 2 and its transcription in human cervical cancer tissue. *Proceedings of the National Academy of Sciences*, *69*(12), pp.3784-3789.

© University of Venda

54. Gagnon, F., 1950. Contribution to the study of the etiology and prevention of cancer of the cervix of the uterus. *American Journal of Obstetrics and Gynecology*, *60*(3), pp.516-522.

55. Galan-Sanchez, F.A.T.I.M.A. and Rodriguez-Iglesias, M.A., 2009. Comparison of human papillomavirus genotyping using commercial assays based on PCR and reverse hybridization methods. *Journal of Pathology, Microbiology and Immunology*, *117*(10), pp.708-715.

56. Gallay, C., Miranda, E., Schaefer, S., Catarino, R., Jacot-Guillarmod, M., Menoud, P.A., Guerry, F., Achtari, C., Sahli, R., Vassilakos, P. and Petignat, P., 2016. Human papillomavirus (HPV) contamination of gynaecological equipment. *Sexually Transmitted Infections*, *92*(1), pp.19-23.

57. Garland, S.M., Pitisuttithum, P., Ngan, H.Y.S., Cho, C.H., Lee, C.Y., Chen, C.A., Yang, Y.C., Chu, T.Y., Twu, N.F., Samakoses, R. and Takeuchi, Y., 2018. Efficacy, immunogenicity, and safety of a 9-valent human papillomavirus vaccine: subgroup analysis of participants from Asian countries. *The Journal of infectious diseases*, *218*(1), pp.95-108.

58. Geneious Prime 2023.0.1 (2023) Biomatters Ltd. Available at: 0 (Accessed: 30 July 2024).

59. Ginindza, T.G., Dlamini, X., Almonte, M., Herrero, R., Jolly, P.E., Tsoka-Gwegweni, J.M., Weiderpass, E., Broutet, N. and Sartorius, B., 2017. Prevalence of and associated risk factors for high risk human papillomavirus among sexually active women, Swaziland. *PloS one*, *12*(1), p.e0170189.

60. Ginindza, T.G., Sartorius, B., Dlamini, X. and Östensson, E., 2017. Cost analysis of Human Papillomavirus-related cervical diseases and genital warts in Swaziland. *Plos One*, *12*(5), p.e0177762.

61. Government provinces. Limpopo socio-economic review and outlook 2018/19. Polokwane: provincial treasury, 2019

62. Graham, S.V., 2010. Human papillomavirus: gene expression, regulation and prospects for novel diagnostic methods and antiviral therapies. *Future microbiology*, *5*(10), pp.1493-1506.

63. Gravitt, P.E., 2012. Evidence and impact of human papillomavirus latency. *The open virology journal*, *6*, p.198.

© University of Venda

64. Haghshenas, M., Golini-Moghaddam, T., Rafiei, A., Emadeian, O., Shykhpour, A. and Ashrafi, G.H., 2013. Prevalence and type distribution of high-risk human papillomavirus in patients with cervical cancer: a population-based study. *Infectious agents and cancer*, *8*, pp.1-6.

65. Hong, Y., Li, S.Q., Hu, Y.L. and Wang, Z.Q., 2013. Survey of human papillomavirus types and their vertical transmission in pregnant women. *BMC Infectious Diseases*, *13*(1), pp.1-7.

66. Hopkins, K.L., Jaffer, M., Hlongwane, K.E., Otwombe, K., Dietrich, J., Cheyip, M., Olivier, J., Doherty, T. and Gray, G.E., 2021. Assessing national cervical cancer screening guidelines: Results from an HIV testing clinic also screening for cervical cancer and HPV in Soweto, South Africa. *Plos one*, *16*(7), p.e0255124.

67. Huh, W.K., Joura, E.A., Giuliano, A.R., Iversen, O.E., de Andrade, R.P., Ault, K.A., Bartholomew, D., Cestero, R.M., Fedrizzi, E.N., Hirschberg, A.L. and Mayrand, M.H., 2017. Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16–26 years: a randomised, double-blind trial. *The Lancet*, *390*(10108), pp.2143-2159.

68. Hwang, S.J. and Shroyer, K.R., 2012. Biomarkers of cervical dysplasia and carcinoma. *Journal of Oncology*, *2012*(1), p.507286.

69. International Agency for Research on Cancer (IARC). 2018. Cancer attributable to infections. Available online at: https://gco.iarc.fr/ (accessed January 2023).

70. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Meeting and International Agency for Research on Cancer, 2007. *Human papillomaviruses* (Vol. 90). World Health Organization.

71. Jamshidi, M., Shekari, M., Nejatizadeh, A.A., Malekzadeh, K., Baghershiroodi, M., Davudian, P., Dehghan, F. and Jamshidi, F., 2012. The impact of human papillomavirus (HPV) types 6, 11 in women with genital warts. *Archives of Gynecology and Obstetrics*, *286*(5), pp.1261-1267.

72. Johnson, L.R., Starkey, C.R., Palmer, J., Taylor, J., Stout, S., Holt, S., Hendren, R., Bock, B., Waibel, E., Tyree, G. and Miller, G.C., 2008. A comparison of two methods to determine the presence of high-risk HPV cervical infections. *American Journal of Clinical Pathology*, *130*(3), pp.401-408.

© University of Venda

73. Joshi, S., Babu, J.M., Jayalakshmi, D., Kulkarni, V., Divate, U., Muwonge, R., Gheit, T., Tommasino, M., Sankaranarayanan, R. and Pillai, M.R., 2014. Human papillomavirus infection among human immunodeficiency virus-infected women in Maharashtra, India. *Vaccine*, *32*(9), pp.1079-1085.

74. Joura, E.A., Giuliano, A.R., Iversen, O.E., Bouchard, C., Mao, C., Mehlsen, J., Moreira Jr, E.D., Ngan, Y., Petersen, L.K., Lazcano-Ponce, E. and Pitisuttithum, P., 2015. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. *New England Journal of Medicine*, *372*(8), pp.711-723.

75. Katz, I.T. and Wright, A.A., 2006. Preventing cervical cancer in the developing world. *New England Journal of Medicine*, *354*(11), pp.1110-1110.

76. Kelly, H.A., Sawadogo, B., Chikandiwa, A., Segondy, M., Gilham, C., Lompo, O., Omar, T., Didelot, M.N., Nagot, N., Meda, N. and Weiss, H.A., 2017. Epidemiology of high-risk human papillomavirus and cervical lesions in African women living with HIV/AIDS: effect of anti-retroviral therapy. *Aids*, *31*(2), pp.273-285.

77. King, C.M., Johnston, J.S., Ofili, K., Tam, M., Palefsky, J., Da Costa, M., Mathur, Y. and Barbosa, P., 2014. Human papillomavirus types 2, 27, and 57 Identified in plantar verrucae from HIV-positive and HIV-negative individuals. *Journal of the American Podiatric Medical Association*, *104*(2), pp.141-146.

78. Kireev, D.E., Lopatukhin, A.E., Murzakova, A.V., Pimkina, E.V., Speranskaya, A.S., Neverov, A.D., Fedonin, G.G., Fantin, Y.S. and Shipulin, G.A., 2018. Evaluating the accuracy and sensitivity of detecting minority HIV-1 populations by Illumina next-generation sequencing. *Journal of virological methods*, *261*, pp.40-45.

79. Kjaer, S.K., Svare, E.I., Worm, A.M., Walboomers, J.M., Meijer, C.J. and Van den Brule, A.J., 2000. Human papillomavirus infection in Danish female sex workers: decreasing prevalence with age despite continuously high sexual activity. *Sexually transmitted diseases*, *27*(8), pp.438-445.

80. Knyazev, S., Tsyvina, V., Shankar, A., Melnyk, A., Artyomenko, A., Malygina, T., Porozov, Y.B., Campbell, E.M., Switzer, W.M., Skums, P. and Mangul, S., 2021. Accurate assembly of minority viral haplotypes from next-generation sequencing through efficient noise reduction. *Nucleic acids research*, *49*(17), pp.e102-e102.

© University of Venda

81. Knipe, D., Howley, P., Griffin, D., Lamb, R., Martin, M., Roizman, B. and Straus. S., 2013. *Fields virology, volumes 1 and 2*. Philadelphia, PA, USA, 2013.

82. Kocjan, B.J., Seme, K. and Poljak, M., 2011. Comparison of the Abbott RealTime high-risk HPV test and INNO-LiPA HPV Genotyping Extra test for the detection of human papillomaviruses in formalin-fixed, paraffin-embedded cervical cancer specimens. *Journal of Virological Methods*, *175*(1), pp.117-119.

83. Kombe Kombe, A.J., Li, B., Zahid, A., Mengist, H.M., Bounda, G.A., Zhou, Y. and Jin, T., 2021. Epidemiology and burden of human papillomavirus and related diseases, molecular pathogenesis, and vaccine evaluation. *Frontiers in Public Health*, *8*, p.552028.

84. Kularatne, R., Radebe, F., Kufa-Chakezha, T., Mbulawa, Z. and Lewis, D., 2017. Sentinel surveillance of sexually transmitted infection syndrome aetiologies and HPV genotypes among patients attending primary health care facilities in South Africa, April 2014–September 2015. *National Institute of Communicable Diseases*.

85. La Rosa, G., 2016. Papillomavirus. *Global Water Pathogen Project. Rose JB and Jiménez-Cisneros B (eds). (Meschke JS and Girones R (eds) Part 3 Viruses).* Michigan State University: E. Lansing, MI, Unesco.

86. LeConte, B.A., Szaniszlo, P., Fennewald, S.M., Lou, D.I., Qiu, S., Chen, N.W., Lee, J.H. and Resto, V.A., 2018. Differences in the viral genome between HPV-positive cervical and oropharyngeal cancer. *Plos One*, *13*(8), p.e0203403.

87. Lee, L.G., Connell, C.R., Woo, S.L., Cheng, R.D., McArdle, B.F., Fuller, C.W., Halloran, N.D. and Wilson, R.K., 1992. DNA sequencing with dye-labeled terminators and T7 DNA polymerase: effect of dyes and dNTPs on incorporation of dye-terminators and probability analysis of termination fragments. *Nucleic acids research*, *20*(10), pp.2471-2483.

88. Lee, T.S., Kothari-Talwar, S., Singhal, P.K., Yee, K., Kulkarni, A., Lara, N., Roset, M., Giuliano, A.R., Garland, S.M. and Ju, W., 2019. Cross-sectional study estimating the psychosocial impact of genital warts and other anogenital diseases in South Korea. *BMJ Open*, *9*(3), p.e025035.

89. Lenselink, C.H., de Bie, R.P., van Hamont, D., Bakkers, J.M., Quint, W.G., Massuger, L.F., Bekkers, R.L. and Melchers, W.J., 2009. Detection and genotyping of human papillomavirus in self-obtained cervicovaginal samples by using the FTA cartridge: new

© University of Venda

possibilities for cervical cancer screening. *Journal of Clinical Microbiology*, *47*(8), pp. 2564-2570.

90. Li, N., Franceschi, S., Howell-Jones, R., Snijders, P.J. and Clifford, G.M., 2011. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *International journal of cancer*, *128*(4), pp.927-935.

91. Liu, G., Sharma, M., Tan, N. and Barnabas, R.V., 2018. HIV-positive women have higher risk of human papilloma virus infection, precancerous lesions, and cervical cancer. *Aids*, *32*(6), pp.795-808.

92. Lowy, D.R., 2016. HPV vaccination to prevent cervical cancer and other HPV-associated disease: From basic science to effective interventions. *The Journal of Clinical Investigation*, *126*(1), pp.5-11.

93. Mann, L.M., Llata, E., Flagg, E.W., Hong, J., Asbel, L., Carlos-Henderson, J., Kerani, R.P., Kohn, R., Pathela, P., Schumacher, C. and Torrone, E.A., 2019. Trends in the prevalence of anogenital warts among patients at sexually transmitted disease clinics - Sexually transmitted disease surveillance network, United States, 2010–2016. *The Journal of Infectious Diseases*, *219*(9), pp.1389-1397.

94. Marcoux, D., Nadeau, K., McCuaig, C., Powell, J. and Oligny, L.L., 2006. Pediatric anogenital warts: A 7-year review of children referred to a tertiary-care hospital in Montreal, Canada. *Pediatric Dermatology*, *23*(3), pp.199-207.

95. Martora, F., Della Pepa, M.E., Grimaldi, E., Franci, G., Folliero, V., Petrillo, A., Schettino, M.T., De Franciscis, P., Galdiero, M. and Galdiero, M., 2019. Seven years prevalence and distribution of high and low risk HPV genotypes in women living in the metropolitan area of Naples. *Cancer Epidemiology*, *63*, p.101625.

96. Mbatha, J.N., Taylor, M., Kleppa, E., Lillebo, K., Galapaththi-Arachchige, H.N., Singh, D., Kjetland, E.F., Baay, M.F. and Mkhize-Kwitshana, Z.L., 2017. High-risk human papillomavirus genotypes in HIV-infected and HIV-uninfected young women in KwaZulu-Natal, South Africa: implications for vaccination. *Infectious Diseases*, *49*(8), pp.601-608.

97. Mboumba Bouassa, R.S., Prazuck, T., Lethu, T., Jenabian, M.A., Meye, J.F. and Bélec, L., 2017. Cervical cancer in sub-Saharan Africa: A preventable noncommunicable disease. *Expert review of anti-infective therapy*, *15*(6), pp.613-627.

© University of Venda

98. Mbulawa, Z.Z., Coetzee, D. and Williamson, A.L., 2015. Human papillomavirus prevalence in South African women and men according to age and human immunodeficiency virus status. *BMC infectious diseases*, 15, pp.1-11.

99. Mbulawa, Z.Z., Kondlo, S., Toni, S., Faye, L.M. and Businge, C.B., 2024. Prevalence, Characteristics, and Distribution of Human Papillomavirus According to Age and HIV Status in Women of Eastern Cape Province, South Africa. *Viruses*, *16*(11), p.1751.

100. Mbulawa, Z.Z., Kufa-Chakezha, T., Williamson, A.L. and Kularatne, R., 2018. Sentinel surveillance of human papillomavirus genotypes among young women attending public healthcare facilities in South Africa, 2017. *Public health surveillance*, p.91.

101. Mbulawa, Z.Z., Marais, D.J., Johnson, L.F., Coetzee, D. and Williamson, A.L., 2012. Impact of human immunodeficiency virus on the natural history of human papillomavirus genital infection in South African men and women. *The Journal of infectious diseases*, *206*(1), pp.15-27.

102. Mbulawa, Z.Z., Phohlo, K., Garcia-Jardon, M., Williamson, A.L. and Businge, C.B., 2022. High human papillomavirus (HPV)-35 prevalence among South African women with cervical intraepithelial neoplasia warrants attention. *Plos one*, *17*(3), p.e0264498.

103. Mbulawa, Z.Z., Somdyala, N.I., Mabunda, S.A. and Williamson, A.L., 2021. High human papillomavirus prevalence among females attending high school in the Eastern Cape Province of South Africa. *PLoS One*, *16*(6), p.e0253074.

104. Mbulawa, Z.Z., Van Schalkwyk, C., Hu, N.C., Meiring, T.L., Barnabas, S., Dabee, S., Jaspan, H., Kriek, J.M., Jaumdally, S.Z., Muller, E. and Bekker, L.G., 2018. High human papillomavirus (HPV) prevalence in South African adolescents and young women encourages expanded HPV vaccination campaigns. *PloS one*, 13(1), p.e0190166.

105. McDonald, A.C., Tergas, A.I., Kuhn, L., Denny, L. and Wright Jr, T.C., 2014. Distribution of human papillomavirus genogenotypes among HIV-positive and HIV-negative women in Cape Town, South Africa. *Frontiers in oncology*, *4*, p.48.

106. Meisal, R., Rounge, T.B., Christiansen, I.K., Eieland, A.K., Worren, M.M., Molden, T.F., Kommedal, Ø., Hovig, E., Leegaard, T.M. and Ambur, O.H., 2017. HPV genotyping of modified general primer-amplicons is more analytically sensitive and specific by sequencing than by hybridization. *PloS one*, *12*(1), p.e0169074.

© University of Venda

107. Merckx, M., Liesbeth, W.V.W., Arbyn, M., Meys, J., Weyers, S., Temmerman, M. and Broeck, D.V., 2013. Transmission of carcinogenic human papillomavirus types from mother to child. *European Journal of Cancer Prevention*, *22*(3), pp.277-285.

108. Moodley, I., Tathiah, N., Mubaiwa, V. and Denny, L., 2013. High uptake of Gardasil vaccine among 9-12-yearold schoolgirls participating in an HPV vaccination demonstration project in KwaZulu-Natal, South Africa. *South African Medical Journal*, *103*(5), pp.318-321.

109. Moodley, I., Tathiah, N. and Sartorius, B., 2016. The costs of delivering human papillomavirus vaccination to Grade 4 learners in KwaZulu-Natal, South Africa. *South African Medical Journal*, *106*(5), pp.497-501.

110. Muñoz, N., Bosch, F.X., De Sanjosé, S., Herrero, R., Castellsagué, X., Shah, K.V., Snijders, P.J. and Meijer, C.J., 2003. Epidemiologic classification of human papillomavirus genotypes associated with cervical cancer. *New England Journal of Medicine*, *348*(6), pp.518-527.

111. Nindl, I., Köhler, A., Gottschling, M., Forschner, T., Lehmann, M., Meijer, C.J., Snijders, P.J. and Stockfleth, E., 2007. Extension of the typing in a general-primer-PCR reverse-line-blotting system to detect all 25 cutaneous beta human papillomaviruses. *Journal of Virological Methods*, *146*(1-2), pp.1-4.

112. Niyazi, M., Husaiyin, S., Han, L., Mamat, H., Husaiyin, K. and Wang, L., 2016. Prevalence of and risk factors for high-risk human papillomavirus infection: a population-based study from Hetian, Xinjiang, China. *Bosnian journal of basic medical sciences*, *16*(1), p.46.

113. . Ogembo, R.K., Gona, P.N., Seymour, A.J., Park, H.S.M., Bain, P.A., Maranda, L. and Ogembo, J.G., 2015. Prevalence of human papillomavirus genotypes among African women with normal cervical cytology and neoplasia: a systematic review and meta-analysis. *PloS one*, *10*(4), p.e0122488.

114. Ong, K.J., Checchi, M., Burns, L., Pavitt, C., Postma, M.J. and Jit, M., 2019. Systematic review and evidence synthesis of non-cervical human papillomavirus-related disease health system costs and quality of life estimates. *Sexually Transmitted Infections*, *95*(1), pp.28-35.

© University of Venda

115. Palefsky, J.M., Minkoff, H., Kalish, L.A., Levine, A., Sacks, H.S., Garcia, P., Young, M., Melnick, S., Miotti, P. and Burk, R., 1999. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *Journal of the National Cancer Institute*, *91*(3), pp.226-236.

116. Petca, A., Borislavschi, A., Zvanca, M.E., Petca, R.C., Sandru, F. and Dumitrascu, M.C., 2020. Non-sexual HPV transmission and role of vaccination for a better future. *Experimental and Therapeutic Medicine*, *20*(6), p.1.

117. Pimentel, V.M., Jiang, X., Mandavilli, S., Nwana, C.U. and Schnatz, P.F., 2013. Prevalence of high-risk cervical human papillomavirus and squamous intraepithelial lesion in Nigeria. *Journal of lower genital tract disease*, *17*(2), pp.203-209.

118. Pisani, P., Parkin, D.M., Bray, F. and Ferlay, J., 1999. Estimates of the worldwide mortality from 25 cancers in 1990. *International journal of cancer*, *83*(1), pp.18-29.

119. Poljak, M. and Kocjan, B.J., 2010. Commercially available assays for multiplex detection of alpha human papillomaviruses. *Expert review of anti-infective therapy*, *8*(10), pp.1139-1162.

120. Pourhoseingholi, M.A., Vahedi, M. and Rahimzadeh, M., 2013. Sample size calculation in medical studies. *Gastroenterology and Hepatology from bed to bench*, *6*(1), p.14.

121. Rad, A., Sørbye, S.W., Dreyer, G., Hovland, S., Falang, B.M., Louw, M. and Skjeldestad, F.E., 2017. HPV types in cervical cancer tissue in South Africa: A head-to-head comparison by mRNA and DNA tests. *Medicine*, *96*(47), p.e8752.

122. Radford, A.D., Chapman, D., Dixon, L., Chantrey, J., Darby, A.C. and Hall, N., 2012. Application of next-generation sequencing technologies in virology. *Journal of General Virology*, *93*(9), pp.1853-1868.

123. Rawls, W.E., Tompkins, W.A.F. and Melnick, J.L., 1969. The association of herpesvirus type 2 and carcinoma of the uterine cervix. *American Journal of Epidemiology*, *89*(5), pp.547-554.

124. R Core Team (2023) *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/ (Accessed: [June -09-2024]).

© University of Venda

125. Ribeiro, A.A., Costa, M.C., Alves, R.R.F., Villa, L.L., Saddi, V.A., Carneiro, M.A.D.S., Zeferino, L.C. and Rabelo-Santos, S.H., 2015. HPV infection and cervical neoplasia: associated risk factors. *Infectious agents and cancer*, *10*, pp.1-7.

126. Roberts, I., Ng, G., Foster, N., Stanley, M., Herdman, M.T., Pett, M.R., Teschendorff, A. and Coleman, N., 2008. Critical evaluation of HPV16 gene copy number quantification by SYBR green PCR. *BMC Biotechnology*, *8*(1), pp.1-14.

127. Ruiz-Sternberg, Á.M., Moreira Jr, E.D., Restrepo, J.A., Lazcano-Ponce, E., Cabello, R., Silva, A., Andrade, R., Revollo, F., Uscanga, S., Victoria, A. and Guevara, A.M., 2018. Efficacy, immunogenicity, and safety of a 9-valent human papillomavirus vaccine in Latin American girls, boys, and young women. *Papillomavirus Research*, *5*, pp.63-74.

128. Sanger, F., Nicklen, S. and Coulson, A.R., 1992. DNA sequencing with chain-terminating inhibitors. 1977. *Biotechnology (Reading, Mass.)*, *24*, pp.104-108.

129. Schluterman, N.H., Sow, S.O., Traore, C.B., Bakarou, K., Dembelé, R., Sacko, F., Gravitt, P.E. and Tracy, J.K., 2013. Differences in patterns of high-risk human papillomavirus infection between urban and rural low-resource settings: cross-sectional findings from Mali. *BMC women's health*, *13*, pp.1-9.

130. Schmeink, C.E., Bekkers, R.L., Massuger, L.F. and Melchers, W.J., 2011. The potential role of self-sampling for high-risk human papillomavirus detection in cervical cancer screening. *Reviews in Medical Virology*, *21*(3), pp.139-153.

131. Serrano, B., Alemany, L., de Ruiz, P.A., Tous, S., Lima, M.A., Bruni, L., Jain, A., Clifford, G.M., Qiao, Y.L., Weiss, T. and Bosch, F.X., 2014. Potential impact of a 9-valent HPV vaccine in HPV-related cervical disease in 4 emerging countries (Brazil, Mexico, India and China). *Cancer epidemiology*, *38*(6), pp.748-756.

132. Serote, B., Mokgehle, S., Senyolo, G., du Plooy, C., Hlophe-Ginindza, S., Mpandeli, S., Nhamo, L. and Araya, H., 2023. Exploring the barriers to the adoption of climate-smart irrigation technologies for sustainable crop productivity by smallholder farmers: Evidence from South Africa. *Agriculture*, *13*(2), p.246.

133. Sibeko, S., Sanderson, M., Moyo, S. and Botha, M.H., 2024. Role of the epithelium in human papillomavirus and human immunodeficiency virus infections in the female genital tract. *Frontiers in Reproductive Health*, *6*, p.1408198.

© University of Venda

134. Si-Mohamed, A., Ndjoyi-Mbiguino, A., Cuschieri, K., Onas, I.N., Colombet, I., Ozouaki, F., Goff, J.L., Cubie, H. and Bélec, L., 2005. High prevalence of high-risk oncogenic human papillomaviruses harboring atypical distribution in women of childbearing age living in Libreville, Gabon. *Journal of Medical Virology*, *77*(3), pp. 430-438.

135. Smith, J.S., Melendy, A., Rana, R.K. and Pimenta, J.M., 2008. Age-specific prevalence of infection with human papillomavirus in females: a global review. *Journal of Adolescent Health*, *43*(4), pp.S5-e1.

136. Smith, L.M., Sanders, J.Z., Kaiser, R.J., Hughes, P., Dodd, C., Connell, C.R., Heiner, C., Kent, S.B. and Hood, L.E., 1986. Fluorescence detection in automated DNA sequence analysis. *Nature*, *321*(6071), pp.674-679.

137. Strickler, H.D., Burk, R.D., Fazzari, M., Anastos, K., Minkoff, H., Massad, L.S., Hall, C., Bacon, M., Levine, A.M., Watts, D.H. and Silverberg, M.J., 2005. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus–positive women. *Journal of the National Cancer Institute*, 97(8), pp.577-586.

138. Sukasem, C., Pairoj, W., Saekang, N., Pombubpha, H., Srichunrasami, C., Pongtippan, A., Junyangdikul, P. and Chantratita, W., 2011. Molecular epidemiology of human papillomavirus genotype in women with high-grade squamous intraepithelial lesion and cervical cancer: Will a quadrivalent vaccine be necessary in Thailand? *Journal of Medical Virology*, *83*(1), pp.119-126.

139. Taku, O., Businge, C.B., Mdaka, M.L., Phohlo, K., Basera, W., Garcia-Jardon, M., Meiring, T.L., Gyllensten, U., Williamson, A.L. and Mbulawa, Z.Z., 2020. Human papillomavirus prevalence and risk factors among HIV-negative and HIV-positive women residing in rural Eastern Cape, South Africa. *International Journal of Infectious Diseases*, *95*, pp.176-182.

140. Taku, O., Mbulawa, Z.Z., Phohlo, K., Garcia-Jardon, M., Businge, C.B. and Williamson, A.L., 2021. Distribution of human papillomavirus (HPV) genogenotypes in HIV-negative and HIV-positive women with cervical intraepithelial lesions in the Eastern Cape Province, South Africa. *Viruses*, *13*(2), p.280.

141. Taku, O., Meiring, T.L., Gustavsson, I., Phohlo, K., Garcia-Jardon, M., Mbulawa, Z.Z., Businge, C.B., Gyllensten, U. and Williamson, A.L., 2020. Acceptability of self-collection

© University of Venda

for human papillomavirus detection in the Eastern Cape, South Africa. *PLoS One*, *15*(11), p.e0241781.

142. Tartaglia, E., Falasca, K., Vecchiet, J., Sabusco, G.P., Picciano, G., Di Marco, R. and Ucciferri, C., 2017. Prevalence of HPV infection among HIV-positive and HIV-negative women in Central/Eastern Italy: Strategies of prevention. *Oncology Letters*, *14*(6), pp. 7629-7635.

143. Teixeira, M.F., Sabidó, M., Leturiondo, A.L., de Oliveira Ferreira, C., Torres, K.L. and Benzaken, A.S., 2018. human papillomavirus prevalence and genogenotype distribution among women infected with HIV in Manaus, Amazonas. *Virology Journal*, *15*(1), pp.1-12.

144. Travill, D.I., Machalek, D.A., Rees, H., Mbulawa, Z., Chikandiwa, A., Munthali, R., Petoumenos, K., Kaldor, J.M. and Delany-Moretlwe, S., 2024. High prevalence of human papillomavirus (HPV) in unvaccinated adolescent girls in South Africa, particularly those living with HIV. *Vaccine*, *42*(26), p.126442.

145. Trottier, H., Mayrand, M.H., Coutlée, F., Monnier, P., Laporte, L., Niyibizi, J., Carceller, A.M., Fraser, W.D., Brassard, P., Lacroix, J. and Francoeur, D., 2016. Human papillomavirus (HPV) perinatal transmission and risk of HPV persistence among children: Design, methods and preliminary results of the heritage study. *Papillomavirus Research*, *2*, pp.145-152.

146. Tsu, V.D., LaMontagne, D.S., Atuhebwe, P., Bloem, P.N. and Ndiaye, C., 2021. National implementation of HPV vaccination programs in low-resource countries: Lessons, challenges, and future prospects. *Preventive Medicine*, *144*, p.106335.

147. Tommasino, M., 2014. The human papillomavirus family and its role in carcinogenesis. In *Seminars in Cancer Biology*, 26, pp.13-21.

148. van Hamont, D., van Ham, M.A., Bakkers, J.M., Massuger, L.F. and Melchers, W.J., 2006. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. *Journal of Clinical Microbiology*, *44*(9), pp.3122-3129.

149. Veldhuijzen, N.J., Braunstein, S.L., Vyankandondera, J., Ingabire, C., Ntirushwa, J., Kestelyn, E., Tuijn, C., Wit, F.W., Umutoni, A., Uwineza, M. and Crucitti, T., 2011. The epidemiology of human papillomavirus infection in HIV-positive and HIV-negative high-risk women in Kigali, Rwanda. *BMC infectious diseases*, *11*, pp.1-14.

© University of Venda

150. Villa, L.L. and Denny, L., 2006. CHAPTER 7 Methods for detection of HPV infection and its clinical utility. *International Journal of Gynecology & Obstetrics*, *94*, pp.S71-S80.

151. Vinodhini, K., Shanmughapriya, S., Das, B.C. and Natarajaseenivasan, K., 2012. Prevalence and risk factors of HPV infection among women from various provinces of the world. *Archives of Gynecology and Obstetrics*, *285*(3), pp.771-777.

152. Wagner, S., Roberson, D., Boland, J., Kreimer, A.R., Yeager, M., Cullen, M., Mirabello, L., Dunn, S.T., Walker, J., Zuna, R. and Porras, C., 2019. Evaluation of TypeSeq, a novel high-throughput, low-cost, next-generation sequencing-based assay for detection of 51 human papillomavirus genotypes. *The Journal of infectious diseases*, *220*(10), pp.1609-1619.

153. Walboomers, J.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J., Peto, J., Meijer, C.J. and Muñoz, N., 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*, *189*(1), pp.12-19.

154. Weissenborn, S.J., De Koning, M.N.C., Wieland, U., Quint, W.G.V. and Pfister, H.J., 2009. Intrafamilial transmission and family-specific spectra of cutaneous betapapillomaviruses. *Journal of Virology*, *83*(2), pp.811-816.

155. World Health Organization, 2015. *Trends in maternal mortality: 1990-2015: Estimates from WHO, UNICEF, UNFPA, World Bank Group and the United Nations Population Division*. World Health Organization.

156. World health organization. Human papillomavirus (HPV) vaccination coverage. (2021). Available online at: https://immunizationdata.who.int/pages/coverage/hpv.html?code=ZAF&ANTIGEN=&YEAR= (Accessed January 15, 2023).

157. Xi, L.F., Koutsky, L.A., Castle, P.E., Edelstein, Z.R., Meyers, C., Ho, J. and Schiffman, M., 2009. Relationship between cigarette smoking and human papilloma virus types 16 and 18 DNA load. *Cancer Epidemiology, Biomarkers & Prevention*, *18*(12), pp.3490-3496.

158. Yamaguchi, M., Sekine, M., Hanley, S.J., Kudo, R., Hara, M., Adachi, S., Ueda, Y., Miyagi, E. and Enomoto, T., 2021. Risk factors for HPV infection and high-grade cervical disease in sexually active Japanese women. *Scientific Reports*, *11*(1), pp.1-9.

© University of Venda

159. Yang, D., Zhang, J., Cui, X., Ma, J., Wang, C. and Piao, H., 2022. Risk factors associated with human papillomavirus infection, cervical cancer, and precancerous lesions in large-scale population screening. *Frontiers in Microbiology*, *13*, p.914516.

160. Zaravinos, A., Mammas, I.N., Sourvinos, G. and Spandidos, D.A., 2009. Molecular detection methods of human papillomavirus (HPV). *The International Journal of Biological Markers*, *24*(4), pp.215-222.

161. Zheng, Z.M. and Baker, C.C., 2006. Papillomavirus genome structure, expression, and post-transcriptional regulation. *Frontiers in Bioscience: A Journal and Virtual Library*, *11*, p.2286.

162. Zinzendorf, N.Y., Asher, C.M., Sandrine, Z.F., Sandrine, A.T., Pierre, B.K., Timotée, O., Agathe, D., Joseph, L.S. and Thérèse, A.K.B., 2022. Prevalence and Human Papillomavirus Genotypes Distribution among HIV-Positive and -Negative Young Women Aged 20 to 37-Years-Old with Cervical Cancer in Abidjan, Côte d'Ivoire. *International Journal of Virology and AIDS*, 9(1), p.080.

163. Zhu, C., Wang, Y., Mao, W., Zhang, H. and Ma, J., 2019. Prevalence and distribution of HPV types in genital warts in Xi'an, China: A prospective study. *BMJ Open*, *9*(5), p. e023897.

164. Zur Hausen, H., 1976. Condylomata acuminata and human genital cancer. *Cancer Research*, *36*(2_Part_2), pp.794-794.

165. Zur Hausen, H., 1989. Papillomaviruses in anogenital cancer as a model to understand the role of viruses in human cancers. *Cancer Research*, *49*(17), pp.4677-4681.

166. Zur Hausen, H., Schulte-Holthausen, H., Klein, G., Henle, W., Henle, G., Clifford, P. and Santesson, L., 1970. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature*, *228*, p. 1056.

167. Zur Hausen, H., Meinhof, W., Scheiber, W. and Bornkamm, G.W., 1974. Attempts to detect virus-specific DNA in human tumors. I. Nucleic acid hybridizations with complementary RNA of human wart virus. *International Journal of Cancer*, *13*(5), pp. 650-656.

© University of Venda

168. Zur Hausen, H., Gissmann, L., Steiner, W., Dippold, W. and Dreger, I., 1975. Human papilloma viruses and cancer. *Bibliotheca Haematologica*, (43), pp. 569-571.

© University of Venda

CHAPTER FOUR

CHARACTERIZATION OF WHOLE GENOMES OF CERVICAL HUMAN PAPILLOMAVIRUS AMONG WOMEN LIVING WITH AND WITHOUT HIV IN SELECTED HEALTH FACILITIES IN LIMPOPO PROVINCE, SOUTH AFRICA

© University of Venda

ABSTRACT

Introduction and objective: Factors such as HPV persistence and non-synonymous variants may influence cervical cancer (CC) progression and development. Therefore, it is significant to generate and characterize HPV genomes that could serve as a resource for the improvement and development of vaccines or diagnostics.

Methods: To generate and characterize HPV genomes, rolling circle amplification (RCA) was used to enrich DNA extracted from cervical samples of 100 participants. The enriched DNA was purified using AMPure XP beads, quantified using Qubit, and then sequenced on Illumina MiniSeq technology. Sequence reads were quality assured and analyzed for whole genomes. Sequence analysis was performed through the Geneious Prime software version 2023.0.1. The phylogenetic trees were bootstrapped using 1000 replicates.

Results: Rolling circle amplification successfully enriched DNA from 22 of the 100 samples. Following deep sequence analysis, six whole cervical HPV genomes comprising of genotypes 34, 35, 42, 90 and two of genotype 45 were successfully generated and classified for their lineages and or sub-lineages from six samples. The sequences of these genomes have been deposited with the National Center for Biotechnology Information (NCBI) in the Sequence Read Archive (SRA) database.

Conclusion: The study successfully characterized the first six whole cervical HPV genomes from women in the study population, including well-known and less-reported HPV genotypes and their associated sub-lineages. These findings may underscore the need to strengthen HPV vaccine coverage, routine cervical HPV screening, and genomic monitoring in underrepresented areas such as Limpopo Province. Additionally, this data supports the development of risk stratification models that could help identify women at high-risk for persistent infection and progression to CC, enabling early, tailored interventions that can reduce the HPV disease burden. The genome sequences described herein can be used as references for further research studies.

Keywords: Rolling circle amplification; Genomes; Next generation sequencing; Human papillomavirus; Lineages

© University of Venda

4.1 INTRODUCTION AND STUDY RATIONALE

Interestingly, rolling circle amplification (RCA) could amplify the circular genomes randomly, with double-stranded viruses belonging to the *papillomaviridae* family included (Nelson et al., 2002). On the other hand, the next generation sequencing (NGS) is an approach that could enhance the detection of known and or novel variants of HPV(Arroyo et al., 2013; Kocjan et al., 2013; Muhr et al., 2015; Siqueira et al., 2016; Murahwa et al, 2018; Galati et al., 2020; Poljak et al, 2020). Thus, RCA-NGS could also be a great approach to study HPV infection at a genomic level. The African continent is home to one of the most varied populations. Yet only a limited amount of genomic HPV information is available from the region required for decision-making (Okeke, 2024).

Genomic data is foundational to future studies on the evolution of sequences, variants, their epidemiology, and pathogenicity to inform effective interventions such as screening. However, there's a lack of data on whole genome characterization in Limpopo Province, South Africa. The current study therefore, aimed at characterizing complete or near full-length HPV genomes from women residing in Limpopo Province, South Africa, utilizing RCA-NGS approach.

4.2 LITERATURE REVIEW

4.2.1 Genome sequencing of HPV

Sanger sequencing is known for its ability to produce high-quality sequences of HPV genome (Nelson and Mirabello, 2023). Nonetheless, it is labor-intensive, expensive, and slow (Nelson and Mirabello, 2023). Furthermore, Sanger sequencing technology is primers dependant. Thus, the technology is limited to sequencing known HPV genotypes and restricted to investigating within-host variation, including HPV minor populations of HPV within a single host (Nelson and Mirabello, 2023). Regardless of these limitations, this technology remains the "gold standard'' and continues to provide insights that are significant (Weele et al., 2017; Weele et al., 2018) in the science community in understanding the biology of HPV infections. The availability of NGS technology has immensely increased the number of available HPV whole genomes (Nelson and Mirabello, 2023). In 2015, a type of NGS called an amplicon-based ion torrent technology or assay was introduced (Cullen et al ., 2015) and has been the major contributor to this increase of HPV genomes, producing more than 5000 genomes of HPV 16 by 2017 (Mirabello et al., 2017), and substantially increasing the numbers of HPV genotypes such HPV 35 (Pinheiro et al., 2020) and HPV 31 (Pinheiro et al., 2021). Compared to Illumina, Ion Torrent assay has fewer single base errors, yet with high indel errors due to its difficulty in sequencing homopolymers, including AAAAA (Rothberg et al., 2011; Pereira et al., 2016).

© University of Venda

The error rates per base between the first generation (~0.00431–0.0110) (Rothberg et al., 2011) and the improved new chemistry (~0.000129) (Pereira et al., 2016) vary. Nevertheless, the chemistry of the old technology (HPV Ion Torrent sequencing assay) shows 99.97% (standard deviation [sd] = 0.07%) agreement between duplicates of a specimen and Sanger sequencing (99.97%, sd = 0.13) (Cullen et al., 2015). Another approach utilizes RCA followed by sequencing (Illumina) (Kukimoto et al., 2013; Hirose et al., 2018). To date, the full circle PCR has generated hundreds of genomes with a low error rate (~0.000076) per base; this method is mainly utilized to deep sequence within-host specimens (Hirose et al., 2018). Hundreds of new cutaneous HPV genotypes have been identified through the use of Shotgun metagenomics in conjunction with sequencing (Illumina) among immunocompromised individuals, nearly doubling the number of the existing HPV genotypes in recent years (Pastrana et al., 2018; Tirosh et al., 2018).

4.2.2 HPV lineages and sub-lineages

Alphanumeric nomenclature is utilized to classify sequences of HPV within a genotype (Nelson and Mirabello, 2023). At the highest level, lineages and sub-lineages vary from each other across the HPV full genome by ~1.0-10% and 0.5-1.0%, respectively (Burk et al., 2013). Lineages are represented by an uppercase letter (eg., A), while sub-lineages are represented with a number (eg., A1) (Burk et al., 2013). HPV genotype 16 comprises 4 lineages (A-D) and is further classified into 16 sub-lineages such as A1-4, B1-4, C1-4, and D1-4. Furthermore, lineage and sub-lineage classifications are also available for HPV 45 (Chen et al., 2009) and HPV 35 (Burk et al., 2013), among other genotypes. For example, A1 and A2 are assigned HPV 35, while HPV 45 is classified into A1-A3 and B1-B2. However, not all HPV genotypes have available lineages and or sublineages. It is significant to note that the classification of lineages and sub-lineages depends on the analysis of the full genomes since the L1 gene alone is not variable enough to resolve within genotype differences (Burk et al., 2013; Harari et al., 2014).

4.3 RESEARCH QUESTION

- What are the characteristics of HPV genomes in the study population?

4.4 MAIN OBJECTIVE

- To characterize HPV full genomes in selected health facilities in Limpopo Province, South Africa.

© University of Venda

4.4.1 Specific objectives

1. To generate and characterize HPV genomes for their genotypes among women residing in Limpopo Province, South Africa.
2. To identify HPV lineages and or sub-lineages in the study population.

4.5 MATERIALS AND METHODS

4.5.1 Study specimens

This descriptive cross-sectional study used a subset of one hundred cervical specimens, positive for HPV DNA, used in the previous chapter. Briefly, a double nested PCR was employed to amplify the highly conserved L1 gene of HPV, as described in section 3.7.7 of Chapter three.

4.5.2 Specimen collection, DNA enrichment and sequencing

The extraction process of total DNA and storage procedures are described in Chapter Three, 3.7.6. Following the manufacturer's instructions, the RCA approach was utilized to enrich the sample circular double-stranded DNA. The technique used the TempliPhi Amplification Kit for 100 reactions (GE Healthcare Life Sciences, Piscataway, NY). The RCA products were visualized under a 2.5% agarose gel electrophoresis stained with ethidium bromide, with a resultant band size of approximately 40 kb. These products were further subjected to a clean-up step and normalized into a 2 ng concentration, which was used as an initial genomic DNA input. Libraries were prepared by means of the Nextera XT DNA Sample Preparation Kit (Illumina Inc., San Diego, CA). Each sample was subsequently indexed with the i5 and i7 indexing primers. The libraries were pooled into a 1nM concentration, denatured, and diluted into a 1.8 pM final concentration and added into a sequencer, which was run using the local run manager option. Sequencing by synthesis was carried out through the use of the Illumina Miniseq technology (2 X 150 paired-end run).

4.6 DATA ANALYSIS

4.6.1 Genome characterization

The FastQC tool (Babraham Bioinformatics, Cambridge, UK) was used to assess the quality of the generated fastQ study reads. To characterize individual HPV genomes for their genotypes, high-quality reads were analyzed using Geneious Prime software version 2023.0.1 (Geneious Prime, 2023). Briefly, multiple sequence mapping was performed on the generated reads against over 200 reference sequences of HPV genotypes downloaded from Papillomavirus Episteme (PAVE) database. The resultant consensus sequences covering at least >97% of the HPV genome or near full HPV genome were characterized for their

© University of Venda

genotypes, annotated, and documented. Furthermore, phylogenetic analysis was used as a confirmatory method to verify the classification of the generated genotypes of HPV genomes.

4.6.2 HPV lineages or sub-lineages determination

Guided by the identified HPV genotypes, reference sequence genomes of HPV lineages and or sub-lineages when available were retrieved from GeneBank. The Molecular Evolutionary Genetics Analysis (MEGA) 11 software and muscle DNA alignment were used to build the alignments (Tamura et al., 2021). Lineages/sub-lineages were determined by phylogenetic analysis with neighbor-joining protocol through the 1000 bootstrap replications. However, it should be noted that such analysis was not conducted when the lineages/sub-lineages reference genomes for that genotype were not yet available on the database.

4.7 RESULTS

4.7.1 Generated and characterized HPV genomes

Of the 100 PCR-positive samples, 22 (22%) were successfully enriched by RCA. Of the 22 samples successfully enriched by RCA and sequenced, HPV full genomes were obtained for six samples (6/22; 27%). These six HPV whole genomes are described and characterized below.

From the six samples, five known HPV genotypes were determined; these include genotype 34 (n=1/6; 16.7%), 35 (n=1/6; 16.7%), 42 (n=1/6; 16.7%), 45 (n=2/6; 33.3%), and 90 (n=1/6; 16.7%), with HPV 45 being the most frequent genotype. These full genomes ranged from 7,747 - 8,063 bp in size. For all six sequences, good read coverage was observed throughout the HPV genome. A representation of read distribution and genome coverage is shown in Figure 4.1. Characterization of each full genome is detailed in the subsequent sections.

© University of Venda

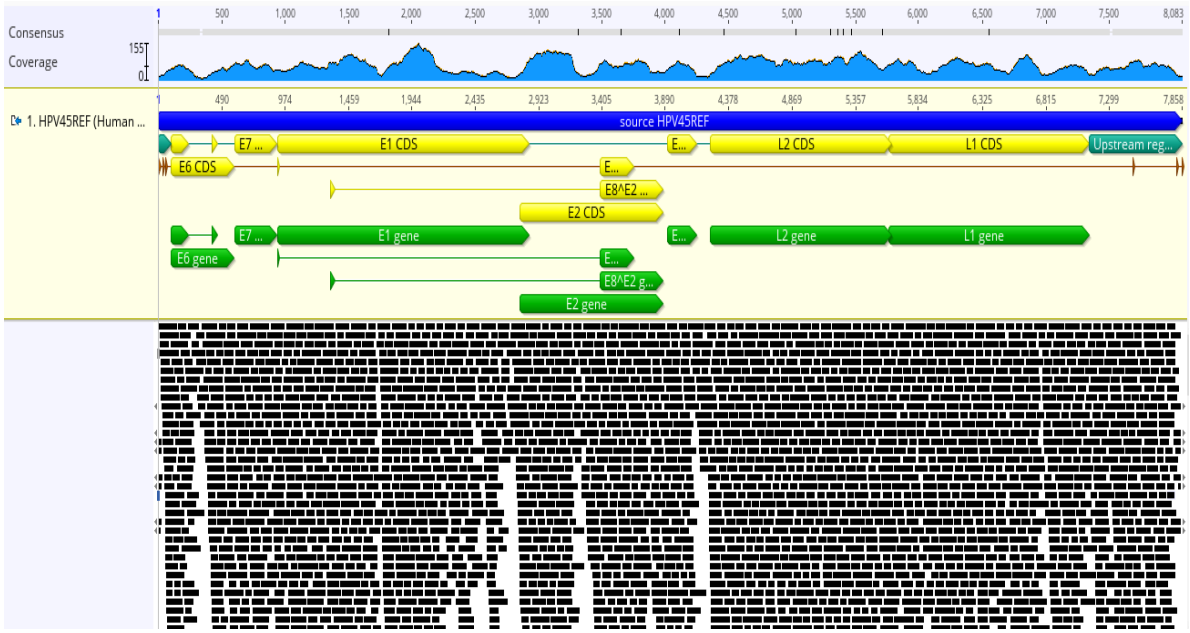


Figure 4.1: *A representative of read distribution and coverage in study sequence. Reads distribution across HPV genotype 45, mapped to a downloaded HPV 45 reference genome (X74479). Dark blue color: reference sequence, yellow color: coding sequences and green color: HPV genes.*

4.7.1.1 Characterized HPV 35 genome and associated lineages/sub-lineages

The generated HPV 35 genome was 7,880 bp in size, obtained from a total of 2,422 reads (Figure 4.2). Phylogenetic analysis determined this HPV 35 genotype to be of the A1 sub-lineage, and clustered with an A1 sub-lineage from Costa Rica, see Figure 4.3.

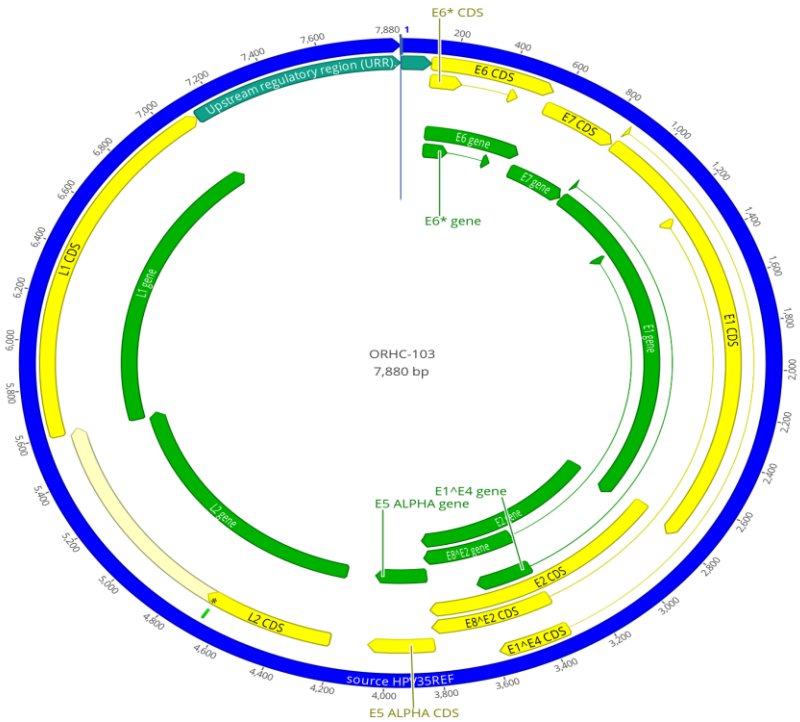


Figure 4.2: *A circular representation of HPV 35 genome (7,880 bp), sample ORHC-103. The blue color: HPV genotype; Yellow color: Coding sequences; and Green color: Viral genes.*

© University of Venda

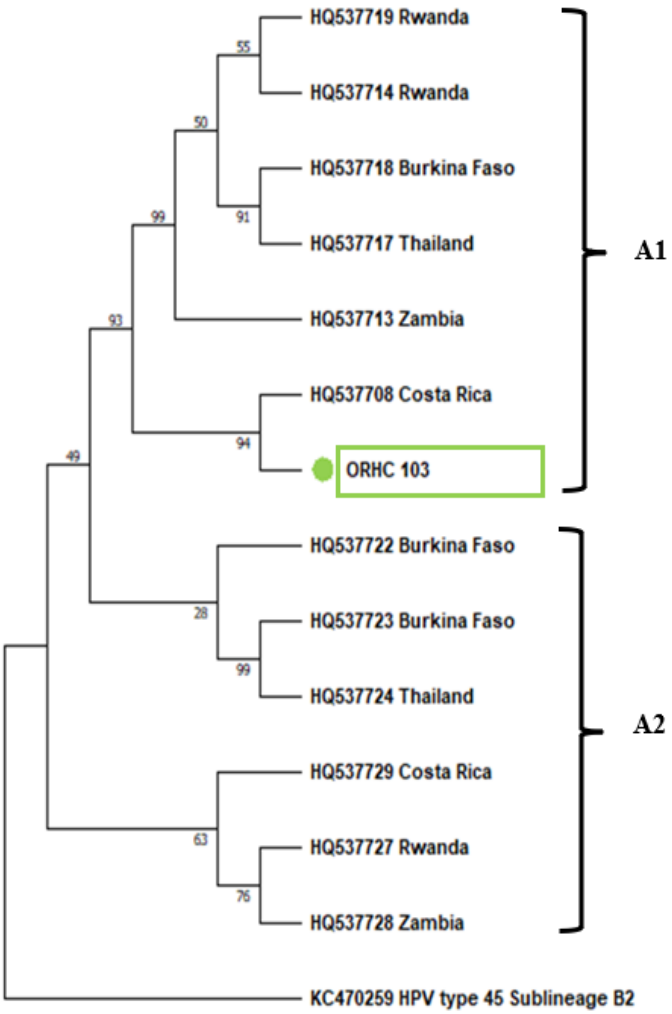


Figure 4.3: *A rooted phylogenetic tree between study consensus sequence genome (indicated with a green color) and reference sequences. The reference sequences belonged to sub-lineage A1 and A2. Sub-lineage B2 of HPV 45 was used an outgroup. The tree was constructed using 1000 replicates of the bootstrap.*

4.7.1.2 Characterized HPV 45 genome and associated lineages/sub-lineages

Of the six successfully sequenced samples, two belonged to HPV 45, and were of sizes 7,859 bp and 7,858 bp, with reads distribution of 7,646 and 5,077, respectively (see Figures 4.4. and 4.5). Both study sequences were classified as sub-lineage A1, see Figure 4.6.

© University of Venda

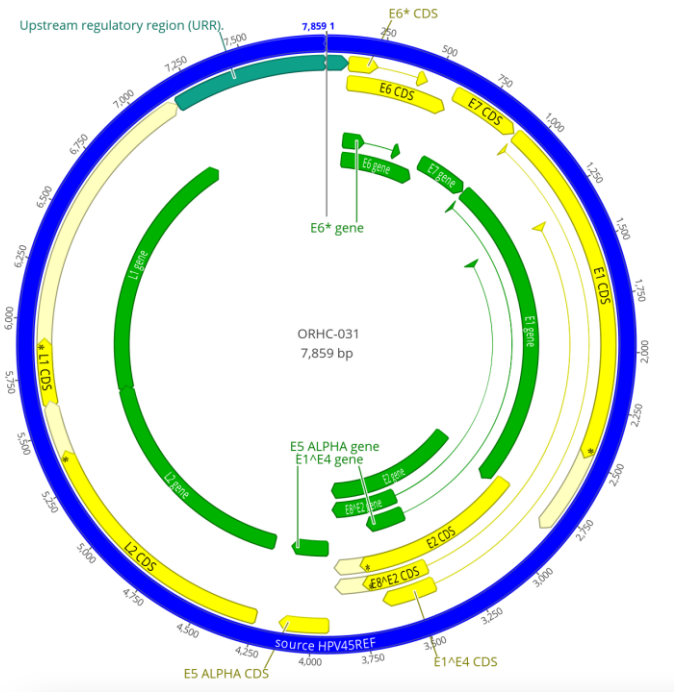


Figure 4.4: *A circular representation of HPV 45 genome (7,859 bp), sample ORHC-031. The blue color: HPV genotype; Yellow color: Coding sequences; and Green color: Viral genes.*

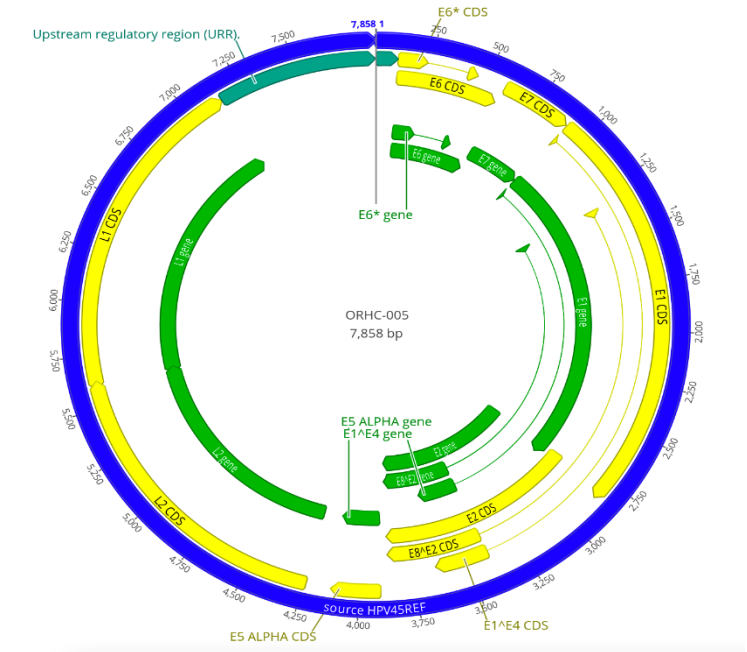


Figure 4.5: *A circular representation of HPV 45 genome (7,858 bp), sample ORHC-005. The blue color: HPV genotype; Yellow color: Coding sequences; and Green color: Viral genes.*

© University of Venda

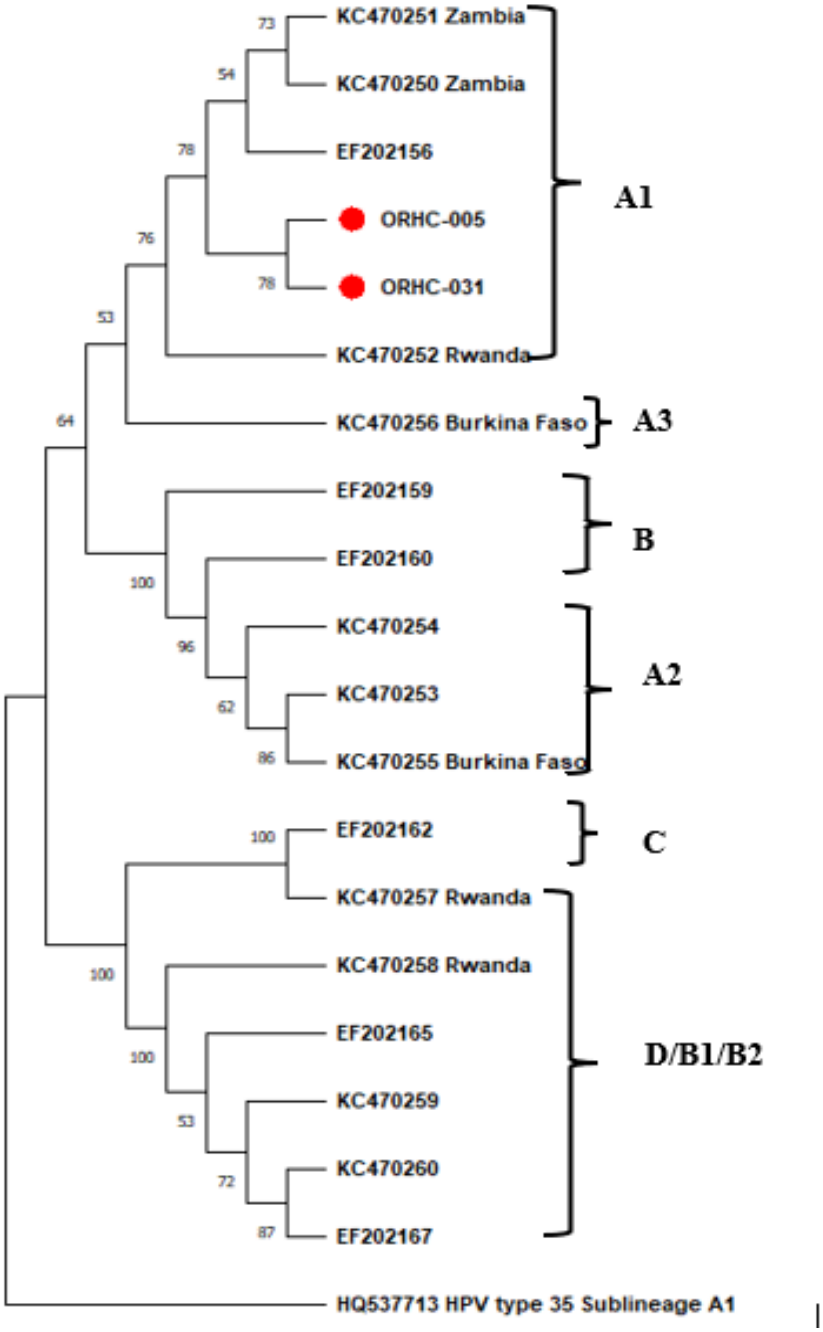


Figure 4.6: *A rooted phylogenetic tree showing a relationship between HPV 45 study sequences (indicated with red dots) and downloaded reference sequences. Downloaded reference sequences belonged to lineages B, C, and D, and sub-lineages A1, A2, B1, and B2. Sub-lineage A1 of HPV 35 was used as an outlier. This tree was constructed using 1000 bootstrap replicates.*

4.7.1.3 Characterized HPV 34 genome and associated lineages/sub-lineages

A total of 1,155 reads generated a full genome of HPV 34 of 7,747 bp (Figure 4.7). The study consensus sequence genome was best classified as sub-lineage C2, Figure 4.8.

© University of Venda

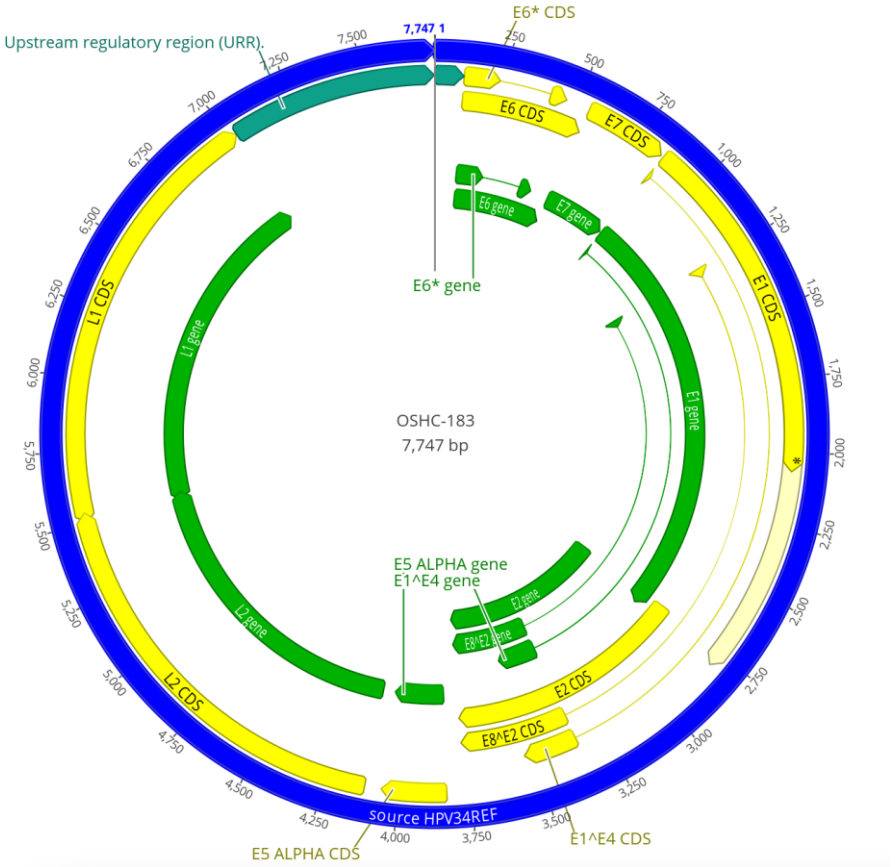


Figure 4.7: *A circular representation of HPV 34 genome (7,747 bp), sample OSHC-183. The blue color: HPV genotype; Yellow color: Coding sequences; and Green color: Viral genes.*

© University of Venda

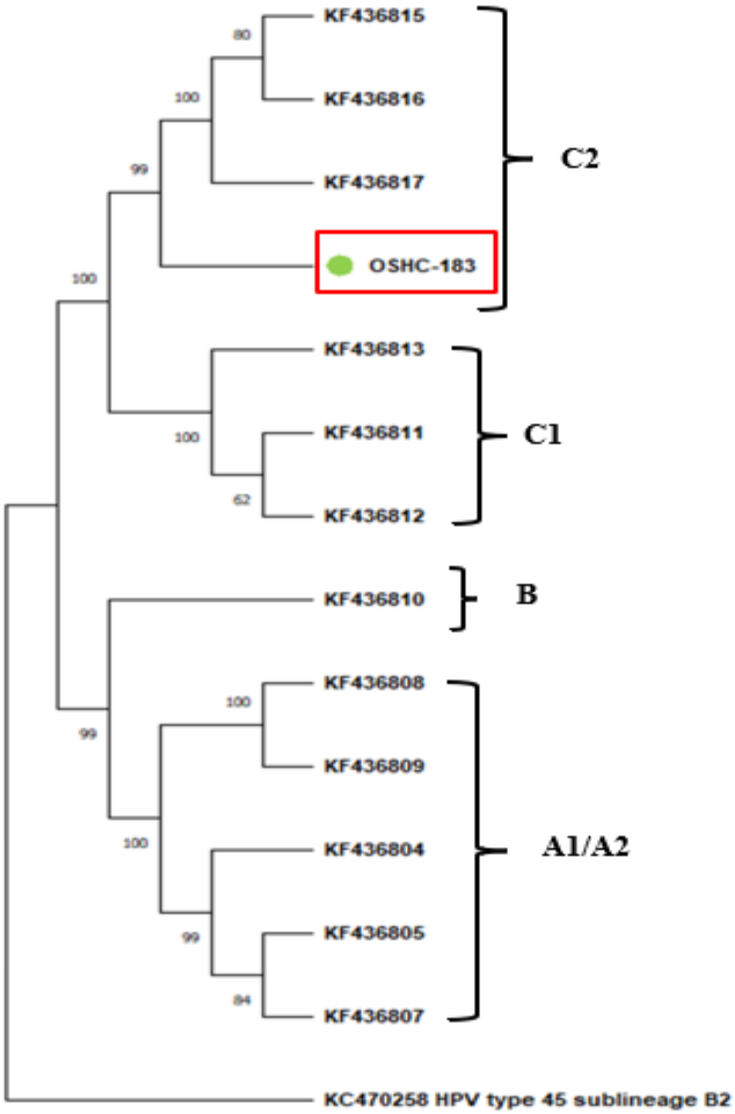


Figure 4.8: *A phylogenetic tree illustrating a relationship between HPV 34 study sequence (indicated with a green dot) and downloaded reference sequences. Downloaded reference sequences belonged to lineage B and sub-lineages A1, A2, C1 and C2. Sub-lineage B2 of HPV 45 was used as an outgroup. This tree was bootstrapped 1000 times.*

4.7.1.4 Characterized HPV 42 and 90 genomes and associated lineages/sub-lineages

The generated HPV 42 and HPV 90 genomes were 7,894 bp and 8,063 bp in size, respectively. The genomes composed of 976 and 4,487 reads (Figure 4.9 and Figure 4.10). Lineage and/or sub-lineages of HPV 42 and HPV 90 were not determined due to the unavailability of reference sequences in GeneBank.

© University of Venda

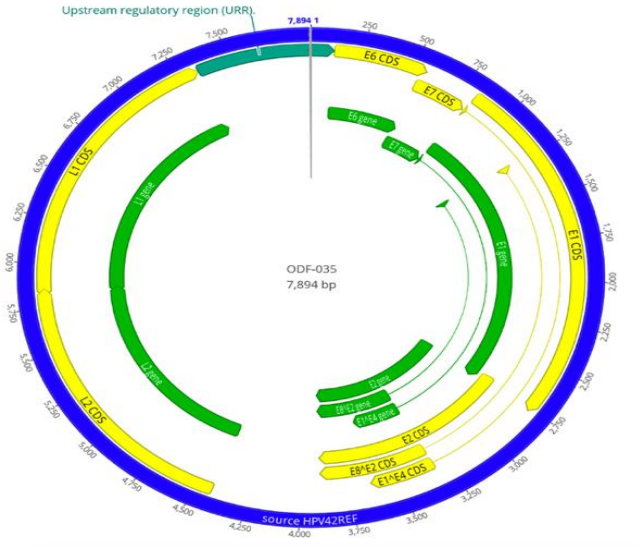


Figure 4.9: *A circular representation of HPV 42 genome (7,894 bp), sample ODF-035.The blue color: HPV genotype; Yellow color: Coding sequences; and Green color: Viral genes*

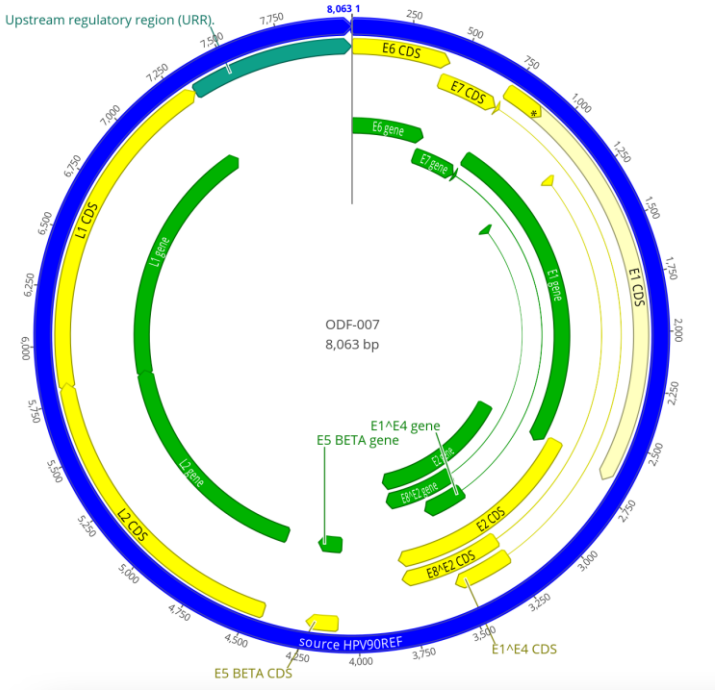


Figure 4.10*: A circular representation of HPV 90 genome (8,063 bp), sample ODF-007.The blue color: HPV genotype; Yellow color: Coding sequences; and Green color: Viral genes.*

4.8 PHYLOGENETIC ANALYSIS OF GENERATED HPV FULL GENOMES

The phylogenetic analysis confirmed the classification of the six detected HPV genomes in the study population, showing 100% concordance with Geneious prime results. Figure 4.11 indicates phylogenetic relationship between classified HPV genotypes from the study

© University of Venda

sequences and reference sequences downloaded from PAVE database. All the described genomes are submitted to the National Center for Biotechnology Information (NCBI) using sequence read archive (SRA), a built-in submission portal. The genomes are available online under the following accession numbers: SRX19523647 (ODF-035, genotype 42), SRX19523376 (OSHC-183, genotype 34), SRX19523146 (ODF-007, genotype 90), SRX19522368 (ORHC-103, genotype 35), SRX19522115 (ORHC-031, genotype 45) and SXR19520230 (ORHC-005, genotype 45).

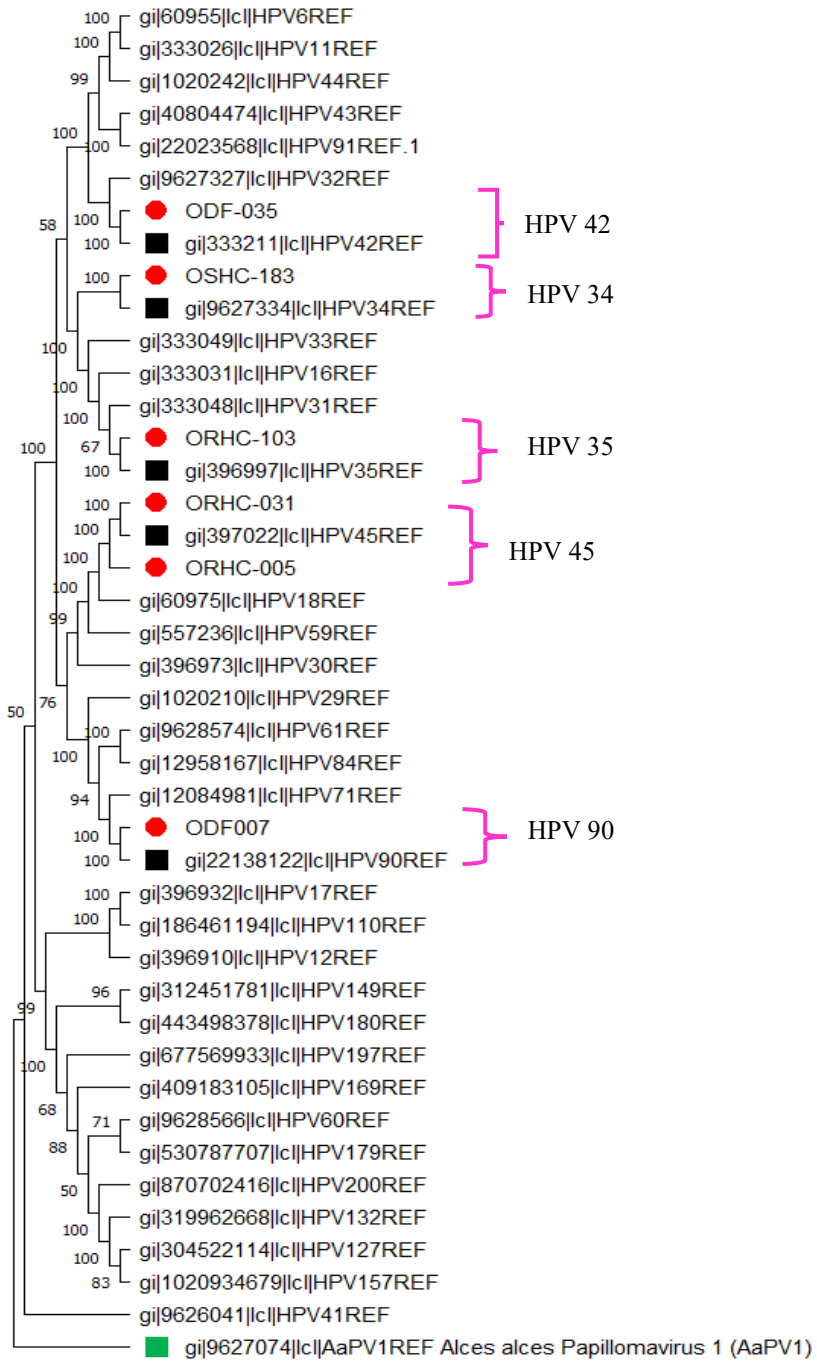


Figure 4.11: *Phylogenetic analysis of HPV genomes of genotypes 34, 45, 42, 45 and 90 (indicated with red dots) and HPV reference sequences. Using the neighbor-joining algorithm, the tree was bootstrapped 1000 times. The green square denotes the outlier.*

© University of Venda

4.9 DISCUSSION

Worldwide, approximately 99.7% of cervical cancer (CC) cases are associated with infection by human papillomavirus (HPV) (Walboomers et al., 1999; Hou et al., 2012). Due to the high sensitivity and specificity of next-generation sequencing (NGS), the technology can identify HPV infection with low viral copies, novel HPV genotypes, and various lineages of the same HPV genotype in one specimen (Barzon et al., 2011; Johansson et al., 2013; Bzhalava et al., 2014). In South Africa, data on cervical HPV whole genome analysis is limited to only one study that was conducted in Cape-Town Province more than 10 years ago (Meiring et al., 2012). This shows the gaps in the country's shortage of in-depth studies that may be necessary to inform vaccine campaigns. The current study has successfully produced valuable new data on the characterization of whole genomes of HPV (Namely: HPV 34, HPV 35, HPV 42, HPV 45, and HPV 90) from selected health facilities in Limpopo Province, South Africa, a region where there is a dearth of information on HPV epidemiology and characterization.

In Africa, HPV genotype 35 is reported among the most frequent hr-HPV genotypes (De Sanjose et al., 2010; Zhao et al., 2024), particularly in women with cervical dysplasia and invasive cervical cancer (ICC) (Burd, 2003). The latter assertion is supported by a study conducted in the Eastern Cape Province, South Africa, which reported HPV 35 among the six predominate genotypes in women with cervical intraepithelial neoplasia (CIN) (Mbulawa et al., 2022). A sentinel surveillance study, conducted in the Free-State, Western-Cape, Gauteng and Eastern-Cape Provinces, investigated the prevalence of HPV and genotype distribution also substantiated a high prevalence of HPV 35 among South African women (Mbulawa et al., 2018). Two reviews conducted in South Africa also detected HPV 35 among the top three detected genotypes (Rikhotso et al., 2022; Maswanganye et al., 2024). This makes HPV 35 a public health concern requiring closer monitoring. Of note, none of the available HPV vaccines are targeting this hr-HPV genotype. In the current study, HPV 35 was also detected, and characterized. Although few genomes were generated, this may give an idea of what may be circulating in the population. Further studies are needed to confirm the predominant circulating HPV genotypes in this population. Such information could be relevant for stakeholders for inclusion of HPV 35 in the cocktail of next generation HPV vaccines, which could inform public health policies.

The HPV 35 genome generated from this study clustered with an A1 sub-lineage. Compared to the A2 sub-lineage, the A1 sub-lineage of HPV 35 is associated with a greater risk of viral persistence and development of cervical interepithelial neoplasia (CIN) 3+ (Schiffman et al.,

© University of Venda

2010). Therefore, this could suggest that lineages may serve as excellent biological markers in assessing viral persistence and the risk of CC. Studies conducted in China, America, Mexico, Belgium, Brazil, Norway and Chad have also reported the circulation of the A1 lineage (Calleja-Macias et al., 2004; Chen et al., 2011; Basto et al., 2017; Mboumba Bouassa et al., 2024; Yuan et al., 2025). The A1 sub-lineage observed in this study was seen clustering with an A1 sub-lineage from Costa Rica (Figure 4.3), which could imply similar origins. Such phylogenetic patterns have also been reported from a study conducted among Zimbabwean women, where some of their test samples clustered with A1 sub-lineages from Rwanda, Zambia and Thailand (Fitzpatrick et al., 2020). Geographical immigration patterns including international travel and tourism, globalization and trade as well as social and cultural behaviours may serve as drivers of spread of HPV sub-lineages. Such observation further signifies that lineages/sub-lineages spread between countries is possible.

The current study provided further characterization of HPV 45, a high-risk genotype frequently detected among South African women diagnosed with CIN (Taku et al., 2020; Mbulawa et al., 2022). Our study finding also reports sub-lineage A1 of HPV 45. While data on the detection of this specific sub-lineage in South Africa is currently lacking, previous studies have reported its presence in other countries, including Iran and Luxembourg (Hosseini et al., 2021; Latsuzbaia et al., 2020). However, literature shows that the distribution of HPV lineages may differ across geographical locations (Chen et al., 2014). Additionally, Chen and colleagues reported that the A1 sub-lineage of HPV 45 is mostly common in Africa compared to other continents. A1 sub-lineage accounts for 9% of cases in Africa and 3% of cases in Eastern Asia (Li et al., 2011). Contributing factors may include ethnicity, vaccination coverage and geographical origin (Burk et al., 2013; Chen et al., 2015; Xu et al., 2018; Rahangdale et al., 2022). Due to the association of hr-HPV 45 (A1 sub-lineage) and CC, the study underscores the need for implementing targeted control measures of HPV 45, including enhanced surveillance efforts. Moreover, health authorities may consider expanding the vaccine access such as the introduction of Gardasil-9 which targets HPV 45.

HPV 34 is another hr-HPV genotype that was detected in this study, alongside sub-lineage C2. This genotype is rarely detected among women living with HIV. Reports on HPV 34 circulation and lineage/sub-lineage organization have mostly been from studies outside South Africa. A recent study in Botswana found hr-HPV 34 as the second common genotype among WLWH (Grover et al., 2023). By the time this study was reported (February 2025), information on HPV

© University of Venda

34 prevalence in South Africa was scarce. In a country such as South Africa, with high numbers of people living with HIV, there is a need for investigating its presence in the population.

The current study further characterized HPV genomes of rare genotypes such as HPV 42 and HPV 90, through NGS. One study conducted in Cape Town, South Africa reported identifying additional HPV genotypes, including HPV 90, which is not targeted by Linear array genotyping kit (Meiring et al., 2012). This finding aligns with the current study, which applied a deep sequencing method for the identification of a lr-HPV genotype that is not commonly reported. The underreporting of certain HPV genotypes may be influenced by the genotyping techniques used to identify HPV genotypes. Next generation sequencing can be an excellent method to study the wider spectrum of HPV genotypes including those occurring at minority level. This lr-HPV genotype has also been reported in the United States of America (Quiroga-Garza et al., 2013).

The current study should be considered with some limitations. Firstly, the characterization of the whole genomes was only achieved in a few samples. Thus, it may not be an accurate representation of the genotypes present in the general population. Additionally, the scope of the study was limited to generating and characterizing the HPV genomes for their genotypes and lineages. However, the strength of the current study is its focus in Limpopo, a Province where HPV genomic studies are underrepresented. Comparatively, most HPV genotyping studies used partial sequences for determining HPV lineages/sub-lineages occurring in a specific study population (Zehbe et al., 1998; Sichero et al., 2007; Xi et al., 2007; Cornet et al., 2013).

It is recommended that future studies should be undertaken to investigate the variant profiles among different HPV genotypes to understand their molecular epidemiology, evolution and pathogenicity using a larger population size. Further scientific data is also needed to correlate the genomic variants with clinical parameters such as the CIN stage, persistence, clearance, effects of anti-retroviral therapy (ART), and CC in the studied population. Additionally, further research should be recommended to explore the HPV polymorphisms targeting all the genes of the HPV genome for a comprehensive understanding of the variants profiling, also considering broader geographical inclusivity. Continuous surveillance is needed for monitoring circulating HPV genotypes of clinical importance, as well as identification of new HPV genotypes, which may arise in the population. This information could contribute to rapid public health intervention, and vaccine development strategies.

© University of Venda

In conclusion, this study provides the first six whole HPV genomes from the study population. These genomes include well-known and less frequently reported genotypes. The genome sequences described herein can be used as references for further research studies. Notably, the detection of A1 variant sub-lineages of hr-HPV 35 and hr-HPV 45 has been previously associated with the progression and development of CC. The study findings therefore underscore the need to strengthen HPV vaccine coverage, routine cervical screening, and genomic monitoring in underrepresented areas such as Limpopo Province. Moreover, this information supports the development of risk stratification models that could help identify women at high-risk for persistent infection and progression to CC, enabling early, tailored interventions that can reduce the HPV disease burden. Notably, the clustering relationship between the study's HPV 35 genome sequence with an A1 sub-lineage from Costa Rica suggests a shared evolutionary history or migration-associated transmission patterns, highlighting the importance of continued genomic surveillance to comprehensively understand viral evolution and geographical distribution which may aid in designing and developing effective future HPV vaccines that may be geographically inclusive.

© University of Venda

4.10 REFERENCES

1. Arroyo, L.S., Smelov, V., Bzhalava, D., Eklund, C., Hultin, E. and Dillner, J., 2013. Next generation sequencing for human papillomavirus genotyping. *Journal of Clinical Virology*, *58*(2), pp.437-442.3.

2. Barzon, L., Militello, V., Lavezzo, E., Franchin, E., Peta, E., Squarzon, L., Trevisan, M., Pagni, S., Dal Bello, F., Toppo, S. and Palù, G., 2011. Human papillomavirus genotyping by 454 next generation sequencing technology. *Journal of Clinical Virology*, *52*(2), pp.93-97.

3. Basto, D.L., Vidal, J.P., Pontes, V.B., Felix, S.P., Pinto, L.C., Soares, B.M., Martins, L.F., Correa, F.M., Montenegro, R.C., Chaves, C.B.P. and Almeida, L.M., 2017. Genetic diversity of human papillomavirus types 35, 45 and 58 in cervical cancer in Brazil. Archives of Virology, 162, pp.2855-2860.

4. Burk, R.D., Harari, A. and Chen, Z., 2013. Human papillomavirus genome variants. *Virology*, *445*(1-2), pp.232-243.

5. Bzhalava, D., Mühr, L.S.A., Lagheden, C., Ekström, J., Forslund, O., Dillner, J. and Hultin, E., 2014. Deep sequencing extends the diversity of human papillomaviruses in human skin. *Scientific reports*, *4*(1), pp.1-7.

6. Chen, A.A., Heidman, D.A., Boon, D., Gheit, T., Snijders, P.J., Tommasino, M., Franceschi, S. and Clifford, G.M., 2014. Human papillomavirus 45 genetic variation and cervical cancer risk worldwide. *Journal of virology*, *88*(8), pp.4514-4521.5.

7. Chen, Z., DeSalle, R., Schiffman, M., Herrero, R. and Burk, R.D., 2009. Evolutionary dynamics of variant genomes of human papillomavirus types 18, 45, and 97. *Journal of virology*, *83*(3), pp.1443-1455.

8. Calleja-Macias, I.E., Kalantari, M., Huh, J., Ortiz-Lopez, R., Rojas-Martinez, A., Gonzalez-Guerrero, J.F., Williamson, A.L., Hagmar, B., Wiley, D.J., Villarreal, L. and Bernard, H.U., 2004. Genomic diversity of human papillomavirus-16, 18, 31, and 35 isolates in a Mexican population and relationship to European, African, and Native American variants. *Virology*, *319*(2), pp.315-323.

© University of Venda

9. Chen, Z., De Freitas, L.B. and Burk, R.D., 2015. Evolution and classification of oncogenic human papillomavirus types and variants associated with cervical cancer. *Cervical Cancer: Methods and Protocols*, pp.3-26.

10. Chen, A.A., Heideman, D.A., Boon, D., Chen, Z., Burk, R.D., De Vuyst, H., Gheit, T., Snijders, P.J., Tommasino, M., Franceschi, S. and Clifford, G.M., 2014. Human papillomavirus 33 worldwide genetic variation and associated risk of cervical cancer. *Virology*, *448*, pp.356-362.

11. Chen, Z., Schiffman, M., Herrero, R., DeSalle, R., Anastos, K., Segondy, M., Sahasrabuddhe, V.V., Gravitt, P.E., Hsing, A.W. and Burk, R.D., 2011. Evolution and taxonomic classification of human papillomavirus 16 (HPV16)-related variant genomes: HPV31, HPV33, HPV35, HPV52, HPV58 and HPV67. *PloS one*, *6*(5), p.e20183.

12. Cornet, I., Gheit, T., Iannacone, M.R., Vignat, J., Sylla, B.S., Del Mistro, A., Franceschi, S., Tommasino, M. and Clifford, G.M., 2013. HPV16 genetic variation and the development of cervical cancer worldwide. *British journal of cancer*, *108*(1), pp.240-244.

13. Cullen, M., Boland, J.F., Schiffman, M., Zhang, X., Wentzensen, N., Yang, Q., Chen, Z., Yu, K., Mitchell, J., Roberson, D. and Bass, S., 2015. Deep sequencing of HPV16 genomes: A new high-throughput tool for exploring the carcinogenicity and natural history of HPV16 infection. *Papillomavirus research*, *1*, pp.3-11.

14. De Sanjose, S., Quint, W.G., Alemany, L., Geraets, D.T., Klaustermeier, J.E., Lloveras, B., Tous, S., Felix, A., Bravo, L.E., Shin, H.R. and Vallejos, C.S., 2010. Human papillomavirus genotype attribution in invasive cervical cancer: A retrospective cross-sectional worldwide study. *The Lancet Oncology*, *11*(11), pp. 1048-1056.

15. Galati, L., Brancaccio, R.N., Robitaille, A., Cuenin, C., Luzi, F., Fiorucci, G., Chiantore, M.V., Marascio, N., Matera, G., Liberto, M.C. and Donà, M.G., 2020. Detection of human papillomaviruses in paired healthy skin and actinic Grover, S., Seckar, T., Gao, L., Bhatia, R., Lin, X., Zetola, N., Ramogola-Masire, D. and Robertson, E., 2023. Characterization of HPV subtypes in invasive cervical cancer in Botswana patients using a pan-pathogen microarray technology. *Tumour Virus Research*, *15*, p.200262.

16. Harari, A., Chen, Z. and Burk, R.D., 2014. Human papillomavirus genomics: past, present and future. In *Human papillomavirus,* 45, pp. 1-18.

© University of Venda

17. Hirose, Y., Onuki, M., Tenjimbayashi, Y., Mori, S., Ishii, Y., Takeuchi, T., Tasaka, N., Satoh, T., Morisada, T., Iwata, T. and Miyamoto, S., 2018. Within-host variations of human papillomavirus reveal APOBEC signature mutagenesis in the viral genome. *Journal of Virology*, *92*(12), pp.10-1128.

18. Hosseini, N., Shoja, Z., Younesi, S., Shafiei-Jandaghi, N.Z. and Jalilvand, S., 2021. Lineage analysis of human papillomavirus types 31 and 45 in cervical samples of Iranian women. *Journal of Medical Virology*, *93*(6), pp.3857-3864.

19. Hou, R., Xu, C., Zhang, S., Wu, M. and Zhang, W., 2012. Distribution of human papillomavirus genotype and cervical neoplasia among women with abnormal cytology in Beijing, China. *International Journal of Gynecology & Obstetrics*, *119*(3), pp.257-261.

20. Johansson, H., Bzhalava, D., Ekström, J., Hultin, E., Dillner, J. and Forslund, O., 2013. Metagenomic sequencing of "HPV-negative" condylomas detects novel putative HPV types. *Virology*, *440*(1), pp.1-7.encing technology. *Journal of Clinical Virology*, *52*(2), pp.93-97.

21. Kocjan, B.J., Steyer, A., Sagadin, M., Hošnjak, L. and Poljak, M., 2013. Novel human papillomavirus type 174 from a cutaneous squamous cell carcinoma. *Genome Announcements*, *1*(4), pp.e00445-13.

22. Kukimoto, I., Maehama, T., Sekizuka, T., Ogasawara, Y., Kondo, K., Kusumoto-Matsuo, R., Mori, S., Ishii, Y., Takeuchi, T., Yamaji, T. and Takeuchi, F., 2013. Genetic variation of human papillomavirus type 16 in individual clinical specimens revealed by deep sequencing. *PLoS One*, *8*(11), p.e80583.

23. Latsuzbaia, A., Wienecke-Baldacchino, A., Tapp, J., Arbyn, M., Karabegović, I., Chen, Z., Fischer, M., Mühlschlegel, F., Weyers, S., Pesch, P. and Mossong, J., 2020. Characterization and diversity of 243 complete human papillomavirus genomes in cervical swabs using next generation sequencing. *Viruses*, *12*(12), p.1437.

24. Li, N., Franceschi, S., Howell-Jones, R., Snijders, P.J. and Clifford, G.M., 2011. Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication. *International journal of cancer*, *128*(4), pp.927-935.

25. Londesborough, P., Ho, L., Terry, G., Cuzick, J., Wheeler, C. and Singer, A., 1996. Human papillomavirus genotype as a predictor of persistence and development of high-grade

© University of Venda

lesions in women with minor cervical abnormalities. *International journal of cancer*, *69*(5), pp.364-368

26. Maswanganye, C.K., Mkhize, P.P. and Matume, N.D., 2024. Mapping the HPV Landscape in South African Women: A Systematic Review and Meta-Analysis of Viral Genotypes, Microbiota, and Immune Signals. *Viruses*, *16*(12), p.1893.

27. Mboumba Bouassa, R.S., Avala Ntsigouaye, J., Lemba Tsimba, P.C., Nodjikouambaye, Z.A., Sadjoli, D., Mbeko Simaleko, M., Camengo, S.P., Longo, J.D.D., Grésenguet, G., Veyer, D. and Péré, H., 2024. Genetic diversity of HPV35 in Chad and the Central African Republic, two landlocked countries of Central Africa: A cross-sectional study. *Plos one*, *19*(1), p.e0297054.

28. Mbulawa, Z., Kufa-Chakezha, T., Williamson, A.L. and Kularatne, R., 2018. Sentinel surveillance of human papillomavirus genotypes among young women attending public healthcare facilities in South Africa, 2017. *Public health surveillance*, p.91.

29. Mbulawa, Z.Z., Phohlo, K., Garcia-Jardon, M., Williamson, A.L. and Businge, C.B., 2022. High human papillomavirus (HPV)-35 prevalence among South African women with cervical intraepithelial neoplasia warrants attention. *Plos One*, *17*(3), p.e0264498.

30. Mbulawa, Z.Z., Van Schalkwyk, C., Hu, N.C., Meiring, T.L., Barnabas, S., Dabee, S., Jaspan, H., Kriek, J.M., Jaumdally, S.Z., Muller, E. and Bekker, L.G., 2018. High human papillomavirus (HPV) prevalence in South African adolescents and young women encourages expanded HPV vaccination campaigns. *PloS One*, *13*(1), p.e0190166.

31. Meiring, T.L., Salimo, A.T., Coetzee, B., Maree, H.J., Moodley, J., Hitzeroth, I.I., Freeborough, M.J., Rybicki, E.P. and Williamson, A.L., 2012. Next-generation sequencing of cervical DNA detects human papillomavirus types not detected by commercial kits. *Virology Journal*, *9*(1), pp.1-10.

32. Mühr, L.S.A., Bzhalava, D., Lagheden, C., Eklund, C., Johansson, H., Forslund, O., Dillner, J. and Hultin, E., 2015. Does human papillomavirus-negative condylomata exist? *Virology*, *485*, pp.283-288.

33. Mirabello, L., Yeager, M., Yu, K., Clifford, G.M., Xiao, Y., Zhu, B., Cullen, M., Boland, J.F., Wentzensen, N., Nelson, C.W. and Raine-Bennett, T., 2017. HPV16 E7 genetic conservation is critical to carcinogenesis. *Cell*, *170*(6), pp.1164-1174.

© University of Venda

34. Murahwa, A.T., Meiring, T.L., Mbulawa, Z.Z. and Williamson, A.L., 2018. Complete genome sequences of four novel human Gamma papillomavirus genotypes, HPV-219, HPV-220, HPV-221, and HPV-222, isolated from penile skin swabs from South African men. *Genome Announcements*, *6*(25), pp.e00584-18.

35. Nelson, C.W. and Mirabello, L., 2023. Human papillomavirus genomics: Understanding carcinogenicity. Tumour Virus Res. 2023; 15: 200258.Nelson, J.R., Cai, Y.C., Giesler, T.L., Farchaus, J.W., Sundaram, S.T., Ortiz-Rivera, M., Hosta, L.P., Hewitt, P.L., Mamone, J.A., Palaniappan, C. and Fuller, C.W., 2002. TempliPhi, ϕ 29 DNA Polymerase Based Rolling Circle Amplification of Templates for DNA Sequencing. *Biotechniques*, *32*(sup), pp.S44-S47.

36. Okeke, S.U., 2024. Fighting cervical cancer in Africa: Taking a closer look at human papillomavirus 35. *African Journal of Laboratory Medicine*, *13*(1), pp.1-3.

37. Papillomavirus Episteme. Available online: https://pave.niaid.nih.gov (accessed on 23 July 2022).

38. Pastrana, D.V., Peretti, A., Welch, N.L., Borgogna, C., Olivero, C., Badolato, R., Notarangelo, L.D., Gariglio, M., FitzGerald, P.C., McIntosh, C.E. and Reeves, J., 2018. Metagenomic discovery of 83 new human papillomavirus types in patients with immunodeficiency. *Msphere*, *3*(6), pp.10-1128.

39. Pereira, F.L., Soares, S.C., Dorella, F.A., Leal, C.A. and Figueiredo, H.C., 2016. Evaluating the efficacy of the new Ion PGM Hi-Q Sequencing Kit applied to bacterial genomes. *Genomics*, *107*(5), pp.189-198.

40. Pinheiro, M., Gage, J.C., Clifford, G.M., Demarco, M., Cheung, L.C., Chen, Z., Yeager, M., Cullen, M., Boland, J.F., Chen, X. and Raine-Bennett, T., 2020. Association of HPV35 with cervical carcinogenesis among women of African ancestry: evidence of viral-host interaction with implications for disease intervention. *International journal of cancer*, *147*(10), pp.2677-2686.

41. Pinheiro, M., Harari, A., Schiffman, M., Clifford, G.M., Chen, Z., Yeager, M., Cullen, M., Boland, J.F., Raine-Bennett, T., Steinberg, M. and Bass, S., 2021. Phylogenomic analysis of human papillomavirus type 31 and cervical carcinogenesis: a study of 2093 viral genomes. *Viruses*, *13*(10), p.1948.

© University of Venda

42. Poljak, M., Valenčak, A.O., Domjanič, G.G., Xu, L. and Arbyn, M., 2020. Commercially available molecular tests for human papillomaviruses: A global overview. *Clinical Microbiology and Infection*, *26*(9), pp.1144-1150.

43. Quiroga-Garza, G., Zhou, H., Mody, D.R., Schwartz, M.R. and Ge, Y., 2013. Unexpected high prevalence of HPV 90 infection in an underserved population: is it really a low-risk genotype?. *Archives of Pathology and laboratory medicine*, *137*(11), pp.1569-1573.

44. Rahangdale, L., Mungo, C., O'Connor, S., Chibwesha, C.J. and Brewer, N.T., 2022. Human papillomavirus vaccination and cervical cancer risk. *Bmj*, *379*, pe070115.

45. Rikhotso, R.R., Mitchell, E.M., Wilson, D.T., Doede, A., Matume, N.D. and Bessong, P.O., 2022. Prevalence and distribution of selected cervical human papillomavirus types in HIV-infected and HIV-uninfected women in South Africa, 1989–2021: A narrative review. *Southern African Journal of Infectious Diseases*, *37*(1), p.10.

46. Rothberg, J.M., Hinz, W., Rearick, T.M., Schultz, J., Mileski, W., Davey, M., Leamon, J.H., Johnson, K., Milgrew, M.J., Edwards, M. and Hoon, J., 2011. An integrated semiconductor device enabling non-optical genome sequencing. *Nature*, *475*(7356), pp.348-352.

47. Schiffman, M., Rodriguez, A.C., Chen, Z., Wacholder, S., Herrero, R., Hildesheim, A., Desalle, R., Befano, B., Yu, K., Safaeian, M. and Sherman, M.E., 2010. A population-based prospective study of carcinogenic human papillomavirus variant lineages, viral persistence, and cervical neoplasia HPV variants and cervical neoplasia. *Cancer Research*, *70*(8), pp. 3159-3169.

48. Sichero, L., Ferreira, S., Trottier, H., Duarte-Franco, E., Ferenczy, A., Franco, E.L. and Villa, L.L., 2007. High grade cervical lesions are caused preferentially by non-European variants of HPVs 16 and 18. *International journal of cancer*, *120*(8), pp.1763-1768.

49. Siqueira, J.D., Alves, B.M., Prellwitz, I.M., Furtado, C., Meyrelles, Â.R., Machado, E.S., Seuánez, H.N., Soares, M.A. and Soares, E.A., 2016. Identification of novel human papillomavirus lineages and sublineages in HIV/HPV-coinfected pregnant women by next-generation sequencing. *Virology*, *493*, pp.202-208.

50. Taku, O., Mbulawa, Z.Z., Phohlo, K., Garcia-Jardon, M., Businge, C.B. and Williamson, A.L., 2021. Distribution of human papillomavirus (HPV) genotypes in HIV-negative and

© University of Venda

HIV-positive women with cervical intraepithelial lesions in the Eastern Cape Province, South Africa. *Viruses*, *13*(2), p.280.

51. Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, *38*(7), pp.3022-3027.

52. Tirosh, O., Conlan, S., Deming, C., Lee-Lin, S.Q., Huang, X., Su, H.C., Freeman, A.F., Segre, J.A. and Kong, H.H., 2018. Expanded skin virome in DOCK8-deficient patients. *Nature medicine*, *24*(12), pp.1815-1821.

53. Walboomers, J.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J., Peto, J., Meijer, C.J. and Muñoz, N., 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*, *189*(1), pp.12-19.

54. Weele, P.V.D., Meijer, C.J. and King, A.J., 2017. Whole-genome sequencing and variant analysis of human papillomavirus 16 infections. *Journal of virology*, *91*(19), pp.10-1128.

55. Weele, P.V.D., Meijer, C.J. and King, A.J., 2018. High whole-genome sequence diversity of human papillomavirus type 18 isolates. *Viruses*, *10*(2), p.68.

56. Xi, L.F., Koutsky, L.A., Hildesheim, A., Galloway, D.A., Wheeler, C.M., Winer, R.L., Ho, J. and Kiviat, N.B., 2007. Risk for high-grade cervical intraepithelial neoplasia associated with variants of human papillomavirus types 16 and 18. *Cancer Epidemiology Biomarkers & Prevention*, *16*(1), pp.4-10.

57. Xi, L.F., Schiffman, M., Koutsky, L.A., Hughes, J.P., Winer, R.L., Mao, C., Hulbert, A., Lee, S.K., Shen, Z. and Kiviat, N.B., 2014. Lineages of oncogenic human papillomavirus types other than type 16 and 18 and risk for cervical intraepithelial neoplasia. *Journal of the National Cancer Institute*, *106*(10), p.dju270.

58. Xu, H.H., Wang, K., Feng, X.J., Dong, S.S., Lin, A., Zheng, L.Z. and Yan, W.H., 2018. Prevalence of human papillomavirus genotypes and relative risk of cervical cancer in China: a systematic review and meta-analysis. *Oncotarget*, *9*(20), p.15386.

59. Yamada, T., Wheeler, C.M., Halpern, A.L., Stewart, A.C., Hildesheim, A. and Jenison, S.A., 1995. Human papillomavirus type 16 variant lineages in United States populations characterized by nucleotide sequence analysis of the E6, L2, and L1 coding segments. *Journal of virology*, *69*(12), pp.7743-7753.

© University of Venda

60. Yuan, H.B., Yu, J.H., Gan, J., Qiu, Y., Yan, Z.Y. and Xu, H.H., 2025. Genetic variations and carcinogenicity analysis of E6/E7 oncogenes in HPV31 and HPV35 in Taizhou, China. *Virology Journal*, *22*(1), pp.1-10.

61. Zhao, M., Kang, P., Zhu, L., Zhou, D., Cui, M., Zhang, M., Jia, J. and Luo, L., 2024. Global pattern of persistent human papillomavirus infection in female genital tract: An update system review and meta-analysis. Iscience, 27(10), p.110991.

62. Zehbe, I., Wilander, E., Delius, H. and Tommasino, M., 1998. Human papillomavirus 16 E6 variants are more prevalent in invasive cervical carcinoma than the prototype. *Cancer Research*, *58*(4), pp.829-833.

© University of Venda

CHAPTER FIVE

SYNTHESIS OF THE STUDY AND RECOMMENDATIONS

In this study four objectives were examined: (1) To provide a narrative review on the prevalence and distribution of selected cervical HPV genotypes among women living with and without HIV in South Africa, (2) To determine the prevalence of cervical HPV infection among women living with and without HIV in selected health facilities in Limpopo Province, South Africa, (3) To describe HPV genotypes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa, and (4) To generate and characterize HPV full genomes among women living with and without HIV in selected health facilities in Limpopo Province, South Africa.

The narrative review showed significant gaps in the prevalence and genotype distribution of cervical HPV infection in South Africa between 1989-2021, with data available for only five of the nine provinces in the country. These regional differences highlight the need for improved geographical coverage and national surveillance. Moreover, of the 69 reviewed studies, only four employed sequencing as a genotyping approach. Considering integrating HPV genotyping, particularly sequencing-based technologies (example, next-generation sequencing) to systematically assess the predominant HPV genotypes, at least for research purposes may be of clinical importance due to its specificity and sensitivity. This may include, monitoring emerging HPV genotypes, which may aid to inform the clinical practice in terms of future designing of targeted prevention programs, guiding screening strategies and systematically monitor the viral epidemiological trends. HPV 16, HPV 18 and HPV 35 were the predominant genotypes among women living with and without HIV. Monitoring of these most common HPV genotypes may aid in early intervention considering their interaction with cervical cancer. Furthermore, the circulation of HPV 35, is of public health concern since it is not included in the available vaccines including the latest vaccine, Gardasil-9, suggesting a potential gap in vaccine coverage. This data encourages the need to consider this genotype in future vaccine development efforts.

The current analytical cross-sectional study employed advanced molecular techniques such as NGS, instead of commonly used commercial kits to investigate cervical HPV infection in an underrepresented region with limited data, such as Limpopo Province, South Africa. We

© University of Venda

observed a relatively high prevalence of HPV infection among the study participants, which signified the burden of HPV in the province. The HPV prevalence was significantly higher among women living with HIV (WLWH), including multiple infections and high-risk HPV genotypes as compared to women not living with HIV, possibly due to immunosuppression. Considering the high rate of HPV-HIV co-infection observed in this study and other studies in South Africa, prioritizing women living with HIV for cervical HPV screening and vaccination is a public health imperative. Strengthening integrated HPV and HIV screening programs and ensuring regular monitoring of cervical health among WLWH remains critical in for early detection, treatment and reducing the CC burden in high risk population. Moreover, the study also showed that women living with and without HIV may have different profiling in terms of HPV infections which is important in intervention strategies, example development of targeted HPV screening strategies. The current study also fills a significant gap of limited data by providing baseline information on the prevalence and genotype distribution of cervical HPV infection among women living with and without HIV in the study area. The study findings further encourage national-level surveillance with the inclusion of expanded regional representation.

This study further reports the first six complete cervical HPV genomes from Limpopo Province, South Africa. Employing the RCA-NGS approach, the study identified both lr-HPV and hr-HPV genomes, including rarely reported genotypes such as HPV 34 and HPV 90, which may be overlooked by available commercial kits. The study findings add scientific data in a region where genomic data is limited. The generated genome sequences could serve as a significant baseline resource in virology, and perhaps in the identification of epitopes for vaccine trials and vaccine improvement efforts. Additionally, the identification of non-vaccine HPV genotypes, including high-risk HPV 35, which is predominant in Africa, including South Africa, further highlights the potential gaps in current vaccine coverage, necessitating the need for continuous genomic surveillance to monitor circulating strains, ensuring that vaccine and treatment approaches stay relevant with the viral evolution. The study adds new knowledge, providing the first datasets of HPV in the country, specifically in an underrepresented region such as Limpopo province. The identification of HPV sub-lineages in this study may support the development of risk stratification models that could help identify women at high-risk for persistent infection and progression to CC, thereby enabling early, tailored interventions that can reduce the HPV related disease burden.

Finally, the study confirms our hypothesis of a high variety of HPV genotypes among women living with HIV in selected health facilities in Limpopo Province, South Africa.

© University of Venda

RECOMMENDATIONS

1. Due to the high burden of HPV in the study population, HPV vaccination, routine cervical HPV screening, and HPV genotyping should be prioritized, particularly for women living with HIV. This could facilitate the early detection and intervention of HPV infections and associated diseases.

2. A double-nested PCR approach increased the detection of HPV infection and demonstrated improved sensitivity. Therefore, this method is recommended for detecting low-copy infections that might be missed by a first-nested PCR.

3. Next-generation sequencing is also recommended because it has the capacity to detect HPV genotypes that are not covered by currently available test kits.

4. All the Gardasil-9 incorporated HPV genotypes were detected in the study population, which encourages the introduction of this vaccine, which may benefit many.

5. It is recommended that the most common HPV genotypes, including HPV 35, should be considered in the next generation of HPV vaccines.

6. Furthermore, raising awareness is essential. Therefore, HPV campaigns should be implemented and intensified in the study population. These campaigns should emphasise HPV prevention, screening, and safe sex practices.

FURTHER STUDIES ON HPV IN LIMPOPO PROVINCE

1. Women living with HIV were the most vulnerable population to be infected with HPV including multiple infections and high-risk genotypes. As such, a longitudinal cohort study to monitor the impact of key clinical parameters such as antiretroviral therapy, CD4 counts and HIV viral load on HPV persistence, clearance, lesions development and CC progression will be vital in understanding host-virus interactions among immunocompromised individuals.

2. The current study focused on HPV/HIV as co-infections. Consequently, investigating how the composition of the vaginal and cervical microbiome, may influence susceptibility to HPV infection and persistence will also be significant.

© University of Venda

3. The current study focused on HPV prevalence and genotype distribution. Further studies, such as the cost associated with the burden of HPV-related conditions in the provinces, will be vital. This will help in health policy planning and resource allocation.

4. Additionally, a projection study to estimate the age-standardized incidence rates of HPV-related cancers such as cervical cancer, head and neck is needed in such an HIV-prevalent area.

5. The expansion of HPV vaccination programs and the development of targeted screening strategies for women living with HIV, and further studies to explore the long-term impact of HPV-HIV co-infection on cervical cancer rates are recommended.

© University of Venda