Universidade de Aveiro Departamento de Biologia
2016

Bibiana de Fátima Correia da Costa

HPV virus genotyping in a sampling of Angolan origin- Pilot study

Genotipagem do vírus HPV numa amostragem de origem Angolana- Estudo piloto

DECLARAÇÃO

Declaro que este relatório é integralmente da minha autoria, estando devidamente referenciadas as fontes e obras consultadas, bem como identificadas de modo claro as citações dessas obras. Não contém, por isso, qualquer tipo de plágio quer de textos publicados, qualquer que seja o meio dessa publicação, incluindo meios eletrónicos, quer de trabalhos académicos.

Universidade de Aveiro Departamento de Biologia
2016

Bibiana de Fátima Correia da Costa

HPV virus genotyping in a sampling of Angolan origin- Pilot study

Genotipagem do vírus HPV numa amostragem de origem Angolana- Estudo piloto

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Microbiologia, realizada sob a orientação científica da Doutora Sónia Mendo, professora assistente com habilitações do Centro de Estudos do Ambiente e do Mar, Departamento de Biologia da Universidade de Aveiro e do Doutor Rui Medeiros, IPO-Porto serviço de Virologia.

.

Dedico este trabalho a meus pais.

“The good thing about science is that it’s true whether or not you believe in it.”

Neil deGrasse Tyson

o júri

presidente	Prof.ª Doutora Ângela Cunha Professora Auxiliar, Universidade de Aveiro
orientador	Prof. Doutor Rui Medeiros Professor Associado Convidado C/Agregação, Universidade do Porto-ICBAS
arguente	Prof.ª Doutora Fátima Cerqueira Professora Auxiliar, Universidade Fernando Pessoa

agradecimentos

Ao longo deste último ano, a colaboração de certas pessoas foi fulcral para a este projeto. Deste modo queria agradecer a todos os que deram o seu contributo para a realização do projeto.

Em primeiro lugar ao Dr. Rui Medeiros pela disponibilidade com que me recebeu no Instituto Português de Oncologia do Porto, orientação e apoio demonstrado.

À Jani Silva pela paciência, dedicação, compreensão, disponibilidade e transmissão de conhecimentos, orientando-me sempre da melhor forma, os meus mais sinceros agradecimentos.

À Professora Doutora Sónia Mendo não só pela coorientação institucional como também por toda a disponibilidade demonstrada bem como pela transmissão de conhecimentos.

Aos meus pais, José Costa e Rosa Correia, pelo apoio incondicional, carinho, educação e ajuda que me proporcionaram durante toda a minha vida, sem os quais não me seria possível atingir os meus objetivos. Muito obrigada por sereis umas pessoas maravilhosas e graças a vocês estou a transformar-me na pessoa que sempre quis ser.

À Monica Oliveira, ao Shabeer Mohammed por todo o apoio, ajuda, boa disposição, amizade e muita paciência. Bem como a todos meus amigos, pela compreensão, carinho e amizade, o meu muito obrigada!

palavras-chave Vírus do papiloma humano, cancro do colo do útero, Angola, genótipos, vacinação.

resumo

O HPV é o vírus sexualmente transmissível mais comum em todo o mundo, tendo uma forte relação causal com o cancro do colo do útero. A infeção por HPV é a causa necessária, mas não suficiente do cancro do colo do útero, em todo o mundo. Países em desenvolvimento têm maior taxa de infeção por HPV e cancros relacionados sendo que a prevalência da infeção por HPV global varia por país, região dentro do mesmo país e subgrupo da população sendo que os genótipos de HPV podem apresentar diferentes distribuições de acordo com a região geográfica. Mulheres africanas são desproporcionalmente afetadas com HPV e têm uma maior taxa de morbidade de cancro do colo do útero. Devido à falta de conhecimento sobre o HPV, são necessários rastreios, medidas preventivas relacionadas com cancros do colo do útero, programas de tratamento, acompanhamento posterior e imunização com vacinas contra o HPV.

O objetivo deste trabalho foi determinar a prevalência da infeção por HPV e caracterizar a frequência dos vários genótipos de HPV numa população de mulheres angolanas, usando a *Polymerase Chain Reaction* (PCR), *Restriction Fragment Length Polymorphism* (RFLP) e realizar uma revisão sistemática sobre a prevalência e distribuição genotípica de HPV no continente africano.

Os resultados mostram que a prevalência de HPV em mulheres angolanas foi 20,9% (14/67), que foi muito baixa em comparação com outros países da África Central. Os genótipos mais prevalentes foram HPV-61 (35.7%), HPV-16 (14.3%), HPV-33, -56, -58a, -58b, -70c, -72, -84 (7,1%). Também se verificou que dentro de regiões geográficas africanas podemos esperar diferentes taxas de eficácia resultantes de uma vacinação das populações utilizando as vacinas atualmente existentes.

Em conclusão, este estudo fornece as primeiras estimativas da prevalência de HPV e sua distribuição entre as mulheres angolanas, demonstra que a epidemiologia da infeção por HPV em Angola é diferente de outras regiões do mundo. Sendo que vacinação específica para cada área geográfica é necessária, para evitar doenças relacionadas com o cancro do colo do útero e outras doenças relacionadas com o HPV. Os diferentes dados observados entre nosso estudo e os estudos utilizados para comparar os resultados podem refletir a diferenças na distribuição dos tipos HPV em diferentes populações ou podem ser por causa de diferenças entre a sensibilidade dos métodos utilizados.

keywords

Human papillomavirus, cervical cancer, Angola, genotypes, vaccination.

abstract

Worldwide HPV is the most common sexually transmitted virus that has a strong causal relationship with cervical cancer (CC). Persistent HPV infection is the necessary but non-sufficient cause of CC worldwide. Developing countries have the highest burden of HPV infection and related cancers and the prevalence of HPV infection overall varies by country, region within country, population subgroup and HPV genotypes may exhibit differing distributions according to geographic region. African women are disproportionately impacted with HPV and have a higher rate of morbidity of cervical cancer. Due to lack of knowledge about HPV, smears and cervical cancer-related preventive measures, treatment adherence and follow-up and immunization programs of HPV vaccines are needed.

The aim of this work was determine the prevalence of HPV infection and characterize the frequency of multiple HPV genotypes in Angola using *Polymerase Chain Reaction* (PCR) and *Restriction Fragment Length Polymorphism (*RFLP) and to perform a systematic review on the prevalence and genotypic distribution of HPV in the African continent.

The results show that HPV prevalence in Angolan women was 20.9% (14/67) which was very low compared with other countries of Central Africa. The most prevalent genotypes were HPV-61(35.7%), HPV-16(14.3%), HPV-33, 56, 58ª, 58b, 70c, 72, 84 (7.1%). It also shows that within African geographic regions we may expect different rates of efficacy resulting from a putative vaccination of populations using the currently existing vaccines.

In conclusion, this study provides the first estimates of the prevalence of HPV and distribution among women from Angola and demonstrates that the epidemiology of HPV infection in Angola is different from that of other world regions. Specific area vaccinations are needed to prevent cervical cancer and the other HPV- related diseases. The observed different data between our study and the studies used to compare the results might reflect true differences in the distribution of HPV types in different populations or might be because of differences in the sensitivity of the methods used.

INDEX

TABLE INDEX

FIGURE INDEX

ABBREVIATIONS

9Vhpv	Ninevalent HPV vaccine
A	Adenine
Bhpv	Bivalent HPV vaccine
bp	Base pair
CC	Cervical cancer
CcaPV1	*Capreolus capreolus*
CcPV1	*Caretta caretta*
C	Citosine
cSCC	Cutaneous squamous cell carcinoma
DdeI	Restriction enzyme
DNA	Deoxyribonucleic acid
dntp	Deoxyribonucleoside triphosphate
dsDNA	Double stranded deoxyribonucleic acid
E1	Early region 1
E2	Early region 2
E3	Early region 3
E4	Early region 4
E5	Early region 5
E6	Early region 6
E7	Early region 7
EGF	Epidermal growth factor
ERK ½	Extracellular-signal-regulated kinase 1/2
EV	Epidermodysplasia Verruciformis
G	Guanine
GAVI	Global aliance for vacines and immunization
HaeIII	Restriction enzyme
HC2	Hybrid capture second-generation
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HR	High risk
IBSCC	International Biological Study on Cervical Cancer
ICTV	International Committee on Taxonomy of Viruses
L1	Late region 1
L2	Late region 2
LEEP	Loop electrosurgical excision procedure
LR	Low risk
MAP	Mitogen-activated protein
$MgCl_2$	Magnesium Clorette
Mu-HPV	Mupapillomaviruses
MY09/11	MY amplicon
NMSC	Non-melanoma skin cancer
Nu-HPV	Nupapillomavirus
ORF	Open Reading frame
p105	Protein 105
p107	Protein 107
p130	Protein 130

p38	Protein 38
PBS	Phosphate buffered saline
PCO_3	Primer
PCR	Polymerase Chain Reaction
PHR	Probable high risk
pRb	Retinoblastoma protein
PstI	Restriction enzyme
PV	Papillomavirus
PV's	Papilomaviruses
qhpv	Quadrivalent HPV vaccine
Rb	Retinoblastoma
RFLP	Restriction fragment length polymorphism
RRP	Respiratory papillomatosis
RsaI	Restriction enzyme
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesions
STD's	Sexual transmitted diseases
STI	Sexual transmited infection
STI's	Sexual transmitted infections
T	Timine
UR	Undertermined risk
URR	Upstream Regulatory Region
VIA	Visual inspection with acetic acid
VILI	Visual inspection with lugol
VLP's	Virus like particles
WHO	World health organization
α- HPV	Alphapapillomaviruses
β- HPV	Betapapillomaviruses
γ- HPV	Gammapapillomaviruses

1. INTRODUCTION

1.1. Brief notes on Angola

Angola is the largest country in southern Africa (Paixão *et al.*, 2014), the Capital and largest city is Luanda with 5.068 million habitants. The official language it's the Portuguese but people also speaks Bantu and other African languages. The President of the country, José Eduardo dos Santos was elected in 1979.

In 2015, WHO reported that the population in 2013 was 21472 thousand with a growth rate of 2.78%, birth rate of 38.97/1000, infant mortality rate of 79.99/1000 and life expectancy of 55.29. The literacy rate was 70.4%.

As for the Ethnicity/race Angola had Ovimbundu (37%), Kimbundu (25%), Bakongo (13%), mixed European and Native African (2%), European (1%) and other (22%). Once Angola is a very religious country they also had Indigenous (47%), Roman Catholic (38%), and Protestant (15%) ("World Health Organization", 2016; "Infoplease-Angola", 2016.; "Our Africa- angola", 2016).

1.1.1. Geographical and hydrographic features

Located at 8° 50' S, 13° 14' E (Latitude/Longitude) (Fig.1) ("Our Africa- angola", 2016), in the southwestern part of Africa, both in the eastern and southern hemispheres, is located south of the Equator. The Republic of Angola covers 1,246,700 square kilometers of land and 1600 km of coastline along the South Atlantic in southwest Africa making it the 23rd largest nation in terms of land area ("Worldatlas-Angola Geography", 2016). Having a population density of 15.23 per sq km, the nation has a density of 14 people per square kilometer ("Our Africa- angola", 2016). It has a Horizontal Width of 528.54 miles (850.61 km) from Luanda east to Saurimo and a Vertical Length of 898.78 miles (1446.45 km) from M'banza south to Ondjiva.

Figure 1 - Map of geographic location of Angola (adapted from "Worldatlas-Angola Geography", 2016).

1.1.2. Cultural characteristics

It is estimated that 68% of Angolan population lives below the poverty line, and15% in extreme poverty conditions. The situation is particularly serious in rural areas, where 94% of households can be categorized as poor. In addition, child and maternal mortality rates are among the highest in the world, and are almost 70% higher in rural than in urban areas. Because of the widespread malnutrition, more than a quarter of children are physically hill. Malaria, diarrhea, respiratory infections and neonatal diseases hit by low birth weight are major killers of children, bleedings and obstetric infections, blocked works account for 80% of the deaths of women during pregnancy or immediately after birth. Within this context, biological features offered by the environment may act as a safety net in the livelihoods of poor people by providing food, medicine and other resources (Urso *et al.*, 2016).

There are more than 90 different ethnic groups in Angola, talking about a variety of Bantu languages. The largest groups are located in the Centre and in the South (who speak Umbundu) which are the Ovimbundu, in the North West the Bakongo (Kikongo language variants), North and Lunda and Chokwe the Mbundu (Kimbundu-speaking), in the East Nganguela peoples.

While the majority of Angolans speak a local language as their mother tongue, Portuguese is the official language. Children learn Portuguese from an early age. Most Angolans are Roman Catholics. Portuguese missionaries came to the country since the end of the years 1500 and the King of the Kingdom of Kongo converted to Catholicism.

Musicians play traditional African instruments such as the marimba, kissange and ngoma drums. Angola is also known for its traditional, ethnic art. The masks and statues of the Chokwe. Pottery, basketry and textiles are also forms of art well practiced. During the 19th century, Portuguese-educated Angolans began writing articles, novels and poems that explored the Angolan history and folklore. Agostinho Neto was famous through the world of Portuguese language for his poetry.

Sports, especially football and basketball, which best combines the Angolans. Capoeira is also very popular among young people in Angola. It is said that originated among the Angolan slaves who were brought to Brazil. Here, slaves practiced this unusual combination of dance and martial arts as a way to channel the aggression and express themselves ("Our Africa-angola", 2016).

1.1.3. Natural resources

With a wealth of human and natural resources, rural areas have a great potential for growth that would benefit not only the rural poor people, but also national economies.

The potential for improvement in family farm offers the opportunity to reduce rural poverty and stimulate broad-based growth.

Angola is one of the richest countries in Africa, resources representing the second largest oil producer in sub-Saharan Africa and the fourth largest producer (in value) of diamonds. The country also is fortunate to have a wealth of other natural resources, including forestry, minerals, fisheries and water.

Despite the increasingly strong expansion in the non-oil sectors in recent years due to public investment programs, oil still represents 95% of all exports and accounts for 79.5% of fiscal revenues.

About two-thirds of the population depends on agriculture for food, income and employment, with women providing the majority of the workforce. It is estimated that 80% of farmers is small producers, in general, producing little or no supplies, with very low productivity. Lack of access to agricultural inputs is a major obstacle to production.

Currently fish represent the main source of animal protein in Angola with an estimated annual consumption per capita of 18.7 kg. However, the marine fisheries along the 1,650 kilometers of coastline has diminished drastically, a trend attributed to large-scale fishing by foreign fleets and low capacity of the authorities to survey and to intercept illegal fishing and impose quota limits. Sea fishing, however, is thought to have good potential for private development with the prospects for the internal and external markets ("Investing in rural people", 2016).

1.2. Human Papillomavirus

1.2.1. Historical note

Among the ancient Greeks and Romans, skin and genital warts were well known. Mainly genital warts were found as a result of sexual promiscuity and so considered potentially infectious. The first unequivocal demonstration of infectious nature of human warts resulted from Ciuffo cell-free transmission experiments.

Within early 1920 the infectious nature of genital warts and laryngeal Papillomatosis was also confirmed (Zur Hausen, 2009). In 1930, Rous and his associates did the first experiments to relate Human Papilloma Virus (HPV) infections to cancer development and to study interactions with other carcinogenic factors (Zur Hausen, 1996). Electron microscopic demonstration of viral particles was reached in 1949 (Zur Hausen, 2009) and the papillomavirus (PV) genome structure was unveiled by Crawford and Crawford in 1963(Zur Hausen, 1996).

In 1965, the first reports featuring a circular double stranded DNA from human papillomavirus appeared (Zur Hausen, 2009).

In the second part of the decade of 1970 interest in papillomavirus evolved, this developed in part from the assumption that the papillomavirus can play a significant role in the etiology of cervical cancer (Zur Hausen, 1996). In the decade of 1980, the situation changed abruptly once the isolation of new HPV types -6 and -11, from genital warts and HPV types -16 and -18 from cervical cancer biopsies resulted in a rapid expansion of the experimental work and also the first epidemiological approaches (Zur Hausen, 2002).

The complete genome of HPV-1 was cloned in 1980, thanks to the development of cloning methodology, followed by application of the first DNA sequencing methods available to generate the sequence of DNA HPV-1. HPV genome organization was established by initial collinear DNA comparisons and consecutive DNA sequencing of type 1 of the bovine papillomavirus (BPV1) (De Villiers, 2013). Today the main interest shifted to mechanisms of carcinogenesis by HPV (Zur Hausen, 1996).

PV are highly diverse (De Villiers *et al.*, 2004) and specific (Lavezzo *et al.*, 2016), the genomes were isolated and characterized from reptiles (Burk *et al.*, 2013), birds (De Villiers *et al.*, 2004), marsupials and several other species of mammals (Burk *et al.*, 2013; Doorbar *et al.*, 2012; Lavezzo *et al.*, 2016) with the possible exception of lab rats (De Villiers *et al.*, 2004), physical proximity, can be a prerequisite for PV types occasionally cross host species barriers (De Villiers *et al.*, 2004), suggesting an evolutionary history more than 300 million years (Burk *et al.*, 2013).

The existence of various types of HPV was initially recognized by digestion of dsDNA genomes using a very restricted number of restriction enzymes that were available. DNA was purified from PV particles obtained from warts. As so they were able to create physical maps of generated genome fragment lengths (De Villiers, 2013).

HPV evolved over millions of years to survive in a great variety of animal species, including humans (Doorbar *et al.*, 2012), the single host intensively studied (De Villiers *et al.*, 2004). Typical from viruses that evolved in conjunction with their hosts, many PV produce only chronic, unnoticeable infections and produce virions from the surface of infected epithelium without apparent damage to the host (Doorbar *et al.*, 2012).

It is recognized that viral variants of HPV-16 and HPV-18 are associated with human population migrations and the continent of origin (Burk *et al.*, 2013).

Many types of HPV have been shown to be ubiquitous and distributed worldwide (De Villiers *et al.*, 2004).

Reference centers in France, United States and Germany were initially planned, but only the reference center in Heidelberg, Germany came to exist (De Villiers, 2013).

1.2.2. Global epidemiological situation

HPV is the most common sexually transmitted virus that has a strong causal relationship with cervical cancer globally (Awolude *et al.*, 2013). Persistent HPV infection is the necessary but non-sufficient cause of cervical cancer worldwide. Developing countries have the highest burden of HPV infection and related cancers (Sudenga *et al.*, 2015; Yang *et al.*, 2016).

HPV is a highly prevalent STI worldwide and the infection with multiples genotypes is common (Gallagher *et al.*, 2016). Virtually, all cases of cervical cancer are attributable to HPV infections (Niyazi *et al.*, 2016).

High cervical cancer incidence in developing countries (>85% of cases) is due to the lack of resources and infrastructure for routine Pap screening for high grade cervical intraepithelial lesions and intervention to remove or ablate this precursor lesion increases the HPV infection incidence. (Jiang *et al.*, 2016).

The prevalence of multiple HPV infections in women with precancerous lesions varies greatly from study to study in the USA and Europe, but both agrees that these are rare in women with invasive cancer worldwide (La Ruche *et al.*, 1998). As a matter of fact, the prevalence of HPV infection overall varies by country, region within country, population subgroup (Fig. 2) (Keita *et al.*, 2009) and HPV genotypes may exhibit differing distributions according to geographic region (Niyazi *et al.*, 2016).

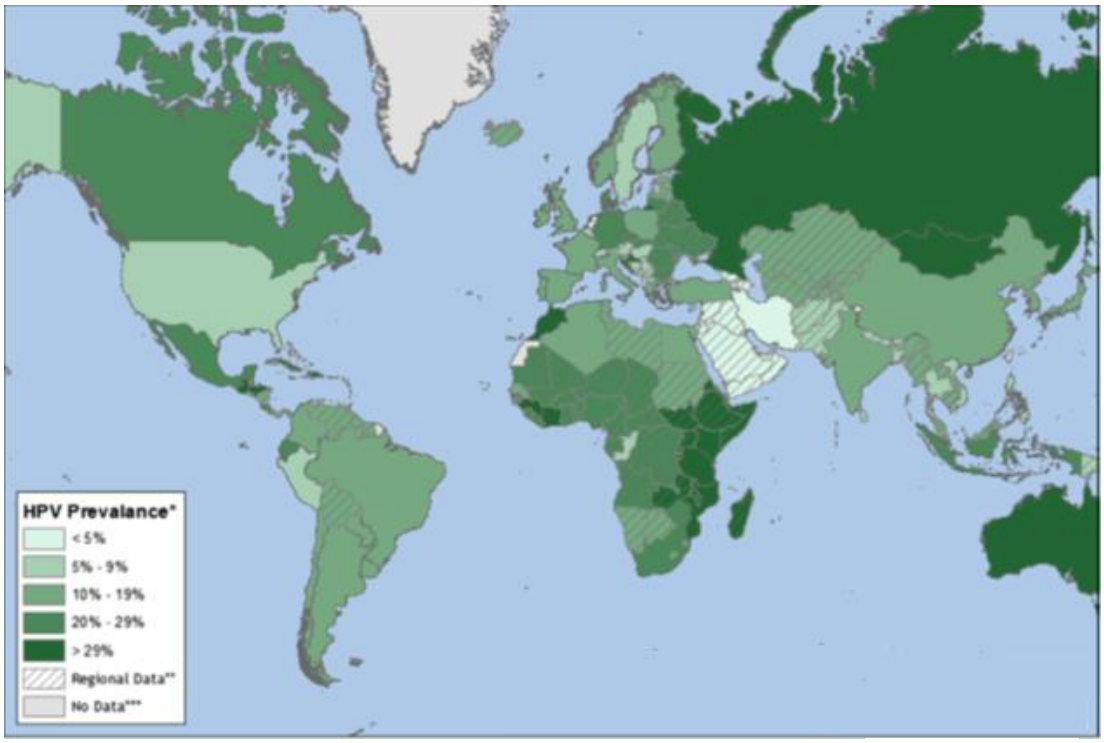

Figure 2 - HPV prevalence among women aged 15–49 years by country or region (Schelar *et al.*, 2015).

Epidemiological studies have shown that the association of HPV with genital cervical cancer is strong, independent of other risk factors and consistent in various countries.

The International Biological Study on Cervical Cancer (IBSCC), reported that HPV-DNA was detected in 93% of the tumors, without significant variation of HPV positivity among countries. HPV 16 was present in 50% of the samples, HPV-18 in 14%, HPV-45 in 8% and HPV-31 in 5%. The role of genital HPV that are sexually transmitted, as the central etiological factor in cervical cancer worldwide (Ntekim, 2012). In more than 99% of cases, cervical cancer is associated with persistent infection with one or more high-risk (HR) HPV genotype.

In many developed countries, the incidence and mortality have decreased over the past decades, since the introduction of cervical cancer screening programs (Tanton *et al.*, 2015). Studies of cervical cancer have shown that women infected with multiple HPV types run a greater risk of developing cervical cancer and cervical squamous intraepithelial lesions (SIL) (Moodley *et al.*, 2009).

Either in women with normal cervical cytology and cervical lesions or cancer, HPV-16 is the most common type of HPV, followed for HPV-18, in Europe, Central and South America, for HPV-52 and HPV-58 in Asia and for HPV-53 and HPV-52 in North America (Fig.3). These differences in the pattern of HPV type distribution in countries and regions may be related to different sexual habits and migrations (Piras *et al.*, 2011) as the implementation of vaccination. The addition of HPV vaccine in the portfolio of new vaccines offered by GAVI to GAVI eligible countries vastly improved the chances of introducing HPV vaccination.

Knowledge of the causes and co-factors that increase the risk of disease is vital for effective primary prevention strategies (Sankaranarayanan *et al.*, 2013).

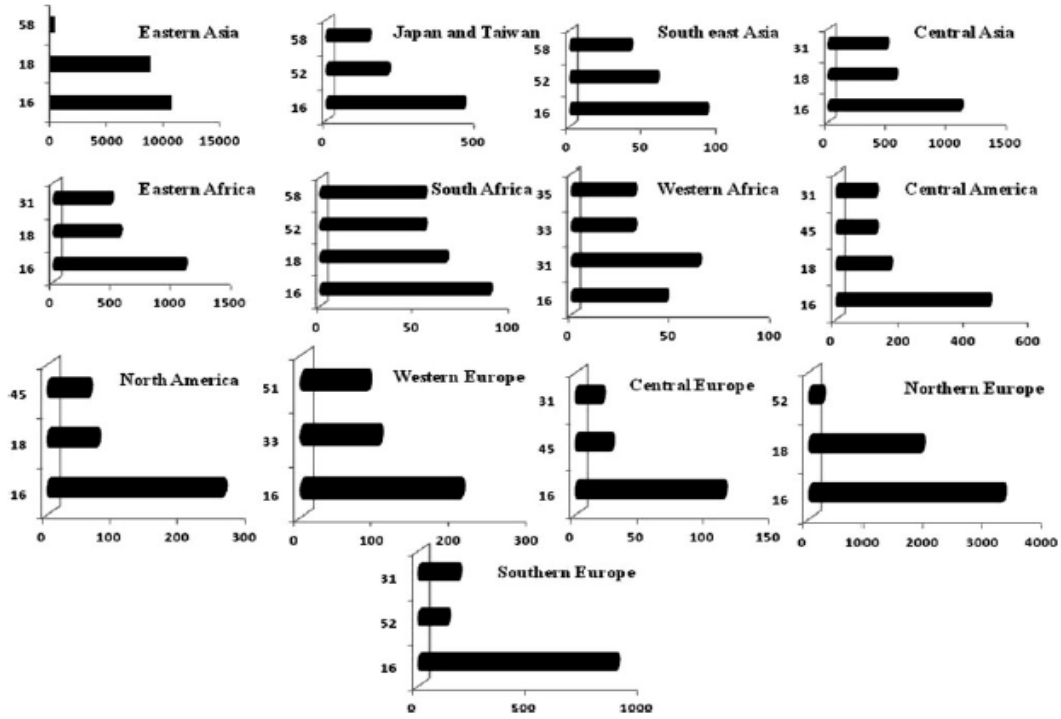

Figure 3 - Ranking of the three most common HPV types among women within world regions. The prevalent type has been given in *Y* axis and the number of women positive for HPV as *X* axis (Keita *et al.*, 2009).

1.2.3. Epidemiological situation in Angola

Compared with the world average of 1.7%, support for science in Angola is less than 0.1% of its gross domestic product. Therefore, it would be needed a more serious commitment to build local and national capacity to accelerate the improvement of training of human resources for health research. The reconstruction of the national health system and policy will

benefit from the knowledge of the factors that influence the success of some interventions and measure their impact on the populations. As so, the health research will contribute to increase the efficiency and effectiveness of health policies and decision-making processes in Angola in programs and practices (Sambo *et al.*, 2015).

Due to the absence of national policies for early detection of cervical neoplasia, only a small percentage of women performed cervical cancer screening and usually with a delay of several years (Horo *et al.*, 2015).

HPV infection is common, yet its prevalence and distribution in women with normal cytology throughout the world is heterogeneous. Women with normal cytology have 10.4% of co-existing detectable HPV DNA, but women under 25 years of age and women in the least developed countries have higher prevalence, ranging from 15-45% (Luckett and Feldman, 2015).

Tests for HPV detection in cervical samples, estimated that about of 9.8% of women in the general population will have cervical infection of HPV-16 at any given time, in Angola (Table 1) (Bruni *et al.*, 2015).

Table 1 - Key statistics on Angola (Bruni *et al.*, 2015)

Population		
Women at risk for cervical cancer (Female population aged >=15 yrs)		6.67 millions
Burden of cervical cancer and other HPV-related cancers		
Annual number of cervical cancer cases		2,072
Annual number of cervical cancer deaths		1,141
Crude incidence rates per 100,000 population and year ‡:	Male	Female
Cervical cancer	-	20.4
Anal cancer	-	-
Vulvar cancer	-	-
Vaginal cancer	-	-
Penile cancer	-	-
Pharynx cancer (excluding nasopharynx)	0.5	0.3
Burden of cervical HPV infection		
Prevalence (%) of HPV 16 and/or HPV 18 among women with:		
Normal cytology		-
Low-grade cervical lesions (LSIL/CIN-1)		12.5†
High-grade cervical lesions (HSIL/CIN-2/CIN-3/CIS)		34.2†
Cervical cancer		-
Other factors contributing to cervical cancer		
Smoking prevalence (%), women		-
Total fertility rate (live births per women)		5.8
Oral contraceptive use (%) among women		2.2
HIV prevalence (%), adults (15-49 years)		2.4 [1.7 - 3.3]

‡Range of crude incidence rates of the following registries: -.
† The data is the subregion Middle Africa

African women are disproportionately impacted with HPV and have a higher rate of morbidity of cervical cancer. Due to lack of knowledge about HPV, smears and cervical cancer-related preventive measures, treatment adherence, follow-up and immunization programs of HPV, vaccines are needed (Hurst *et al., 2015)*.

Immunization is a proven public health strategy, cost-effective that drastically decreased childhood morbidity and mortality worldwide. Immunization programs recorded notable successes in the past four decades, contributing to the eradication of smallpox, bringing polio eradication, measles control and significantly reduce the incidence of vaccine-preventable diseases (Mihigo *et al., 2015)*. However, currently, it isn't easy to implement the HPV vaccines available in sub-Saharan African countries due to the cost, low-resource laboratory infrastructure, smear methods and the need for trained technicians to conduct HPV tests. So far the careHPV tests and other viable, fast and affordable tests are widely available for use in African countries, establishing the visual tracking VIA service platforms and VILI, cryotherapy

and LEEP is an important option for cervical cancer to provide prevention services in these countries (Muwonge *et al., 2010)*.

In January of 2016 started the vaccination program, of a three-dose vaccine for girls aged between 9 and 14 years of age, although on the contrary of what happens in other places around the world like Portugal this vaccine will have the cost of 9000 kwanza each, which is still an issue for many people to have their children vaccinated.

1.2.4. Taxonomy

PV had originally been lumped together with the polyomaviruses in one family, the *Papovaviridae*. This was based on similar non-enveloped capsids and the genomes of circular dsDNA. Later it was recognized that the two groups of virus have different genome sizes, genome organization completely different and no main nucleotide or amino acid sequence similarities. They are now officially recognized by the International Committee on Taxonomy of Viruses (ICTV) as two distinct families, *Papillomaviridae* and *Polyomaviridae* (De Villiers *et al.*, 2004).

Classification of PV at the species level and above is determined by the ICTV based on the recommendations of the Study Group of Papillomaviruses (Bzhalava *et al.*, 2015; Chen *et al.*, 2015). Below the level of species, new HPV types are given a unique number, only after the entire genome was cloned and deposited in the International HPV Reference Center (Bzhalava *et al.*, 2015).

Taxonomic criteria used by the ICTV for species demarcation were based on genome sequence affinity, host range, pathogenicity, tissue and cellular tropism, cytopathology, mode of transmission, as well as the physio chemical and antigenic properties. PV was an exception to classical rules applied for the establishment of a system of classification.

The current system of HPV type number designation, which is based only on L1 sequences, it was agreed in 1995 at the papillomavirus Workshop held in Quebec (Bzhalava *et al.*, 2015).

The rapid increase in the number of isolates of PV demonstrated the need for a taxonomic classification within the family *Papillomaviridae*. Such classification must have at least three objectives: (i) it must establish the relationship between types of PV; (ii) must compare the term PV type against the taxonomic "species" and "genus", which are used for the systematic of all biological organisms and often applied in Virology, (iii) to investigate the relationship between taxonomic classification biological and pathological properties of the virus (De Villiers *et al.*, 2004).

PV taxa are defined based on sequence identities (Panatto *et al.*, 2015) of L1 nucleotide and their topological position within a phylogenetic tree of PV (Burk *et al.*, 2013; Chen *et al.*, 2015).

The use of complete genomes for taxonomy provides a simple mechanism for HPV researchers to discuss the properties of HPV variants without having to describe sets of nucleotide changes to define a group of HPV variants (Burk *et al.*, 2013).

The gene L1 is useful for the classification and construction of phylogenetic trees because it is fairly well conserved within the genome (Bernard *et al.*, 2010 and De Villiers *et al.*, 2004) and can be aligned for all known PV. In addition, PV doesn't elicit antibody responses, which prevented a classification based on "serotype" denominations. As a result, the classification of PV types based predominantly on nucleotide sequence similarities with some biological and medical properties served as the basis for a formal nomenclature (Bernard *et al.*, 2010).

Genera of PV are designated using the Greek alphabet (e.g. *Alphapapillomavirus*). The prefix dyo is added to the Greek letter to encompass the expanding genera of PV. Species within genera are named by a number (e.g. *Alphapapillomavirus 9*).

Researchers have developed a nomenclature of papillomavirus that has been widely embraced and extremely useful in epidemiological studies. A different PV type is established when the nucleotide sequence of the L1 gene from a cloned virus differs from any other

characterized types by >10 %. PV types are named based on the scientific name of the host from which the genome of PV was isolated, using the designation of genus and species from the host. In case of overlap, a third letter is added to give each PV type a unique name (e.g. *Caretta caretta* PV became CcPV1 while the *Capreolus capreolus* PV became CcaPV1). However, some historical names were kept, like HPV (with H standing for human or Homo without entering the name of the species "sapiens") (Chen *et al.*, 2015).

Approximately 40 anogenital HPV (Jiang *et al.*, 2016; Luckett and Feldman, 2015) types of α-genus are divided by oncogenic activity in four groups: high-risk (HR), probable high-risk (PHR), low-risk (LR) and undetermined-risk (UR) HPV's (Table 2) (Nobre *et al.*, 2008).

Table 2 - Classification of Human Papillomaviruses in High and Low Risk Groups HPV (Nobre *et al.*, 2008)

Classification	HPV types
High-risk HPV	-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59
Probable high-risk HPV	-26, -53, -66, -68, -73, -82
Low-risk HPV	6, -11, -13, -40, -42, -43, -44, -54, -61, -70 -72, -81, -89
Undetermined-risk HPV	-30, -32, -34, -62, -67, -69, -71, -74, -83, -84, -85, -86, -87, -90, -91

Ranging from the HPV1 to HPV205 were recognized officially: 65 Alphapapillomaviruses, 51 Betapapillomaviruses, 81 Gammapapillomaviruses, 2 Mupapillomaviruses and 1 Nupapillomavirus (Fig.4) (Poljak *et al.*, 2015).

A highly conserved residue of amino acid was contained in a 291bp segment of the ORF L1. This was used to construct phylogenetic trees, resulting in taxonomic Super Groups A to E. Different taxonomic levels were recognized by dividing each super group into groups. Super-group A (genital HPV's) contained 11 groups and super-group B (EV HPV types) 2 groups. HPV-1, HPV-63 and HPV-41 classified super-group E and along with some animal papillomavirus (De Villiers, 2013).

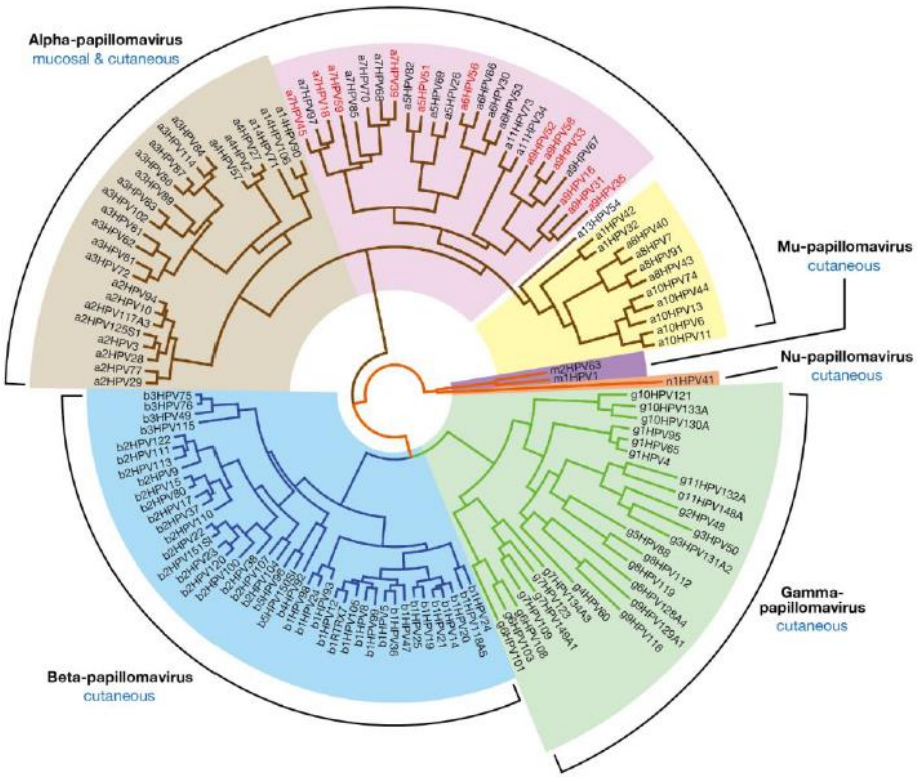

Figure 4 - Evolutionary Relationship between Human Papillomaviruses (Doorbar *et al.*, 2012).

1.2.5. Morphology and biology

PV are a heterogeneous group of small (Burk *et al.*, 2013; Lavezzo *et al.*, 2016; Poljak *et al.*, 2015) non-enveloped (Luckett and Feldman, 2015; Panatto *et al.*, 2015; Poljak *et al.*, 2015) virus with genomes of double-stranded circular DNA about 8 KB nucleotides of size (Chen *et al.*, 2015; Jiang *et al.*, 2016), features an icosahedral viral capsid composed of 72 capsomeres (Fig.5) (Doorbar *et al.*, 2015).

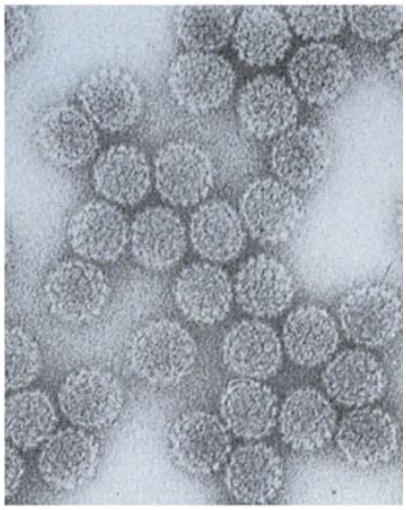

Figure 5 - Electron micrograph of negatively stained papillomavirus particles (Doorbar *et al.*, 2015).

Given the fact that it doesn't have a membrane, virions are poorly immunogenic (Egawa *et al.*, 2015), highly specific and preferentially infect the basal layer of skin and mucosal epithelia (Burk *et al.*, 2013; Lavezzo *et al.*, 2016), that can be mucosotropic (HPV 6, 11, 13, 16, 18, 30–35, 39, 41–45, 51–56) or epitheliotropic/cutaneotropic (1–5, 7–10, 12, 14–15, 17, 19–29, 36, 46–50), specifically infect stratified squamous, cutaneous cells or the mucosa of the genitals and upper-respiratory tract (Panatto *et al.*, 2015).

PV's genomes include three general regions: (1) an Upstream Regulatory Region (URR), that contains sequences that control viral replication and transcription; (2) an early region, containing Open Reading Frames (ORFs; for example, E1, E2, E4, E5, E6 and E7) involved in trans-activation of transcription, transformation, viral replication and adaptation to different cellular milieus (Chen *et al.*,2015). Three oncogenes, E5, E6 and E7, modulate the process of transformation, two regulatory proteins E1 and E2, modulate transcription and replication, and two structural proteins (De Villiers *et al.*, 2004) and (3) a Late region, which codes for the capsid proteins L1 and L2 that form the structure of the virion and facilitates the packaging of the viral DNA and maturation (Chen *et al.*, 2015). The respective functions of each viral ORF are listed in Table 3 (De Villiers *et al.*, 2004).

All PV described so far contain an E1, E2, E4, L1, L2 and some contain E6 and E7 (Chen *et al.*,2015). L1 encodes the main capsid protein which is necessary and sufficient to produce virus-like particles (VLP's) used for vaccines (Bernard *et al.*, 2010). L1 and L2, make up the viral capsid (Fig. 6) (De Villiers *et al.*, 2004). Although all share a common genetic organization, the size and position of the major ORFs can vary (Doorbar *et al.*, 2015).

Table 3 - Function of viral proteins (Egawa *et al.*, 2015)

HPV protein	Functional roles
L1	Major capsid protein
L2	Minor capsid protein Viral trafficking Encapsidation of viral DNA
E1	Viral genome replication
E2	Coactivator of viral genome replication through the recruitment of E1 to the viral replication origin. Transcription factor of E6 and E7
E3	Unknown function
E4	Binds to cytokeratin filaments and disrupts their structure Viral release and transmission
E5	Small transmembrane protein Interference with EGF/EGFR pathways Inhibition of apoptosis and immune response Beta-, Gamma- and Mu-PV's lack of this gene
E6	Oncoprotein; interaction with p53 Immunoescape Inhibition of cell differentiation Some Gamma-PV'S lack this gene
E7	Oncoprotein; interaction with pRb protein and pocket proteins (p107 and p130) Protein degradation, necessary for cell transformation Chromosomal and genomic instability,

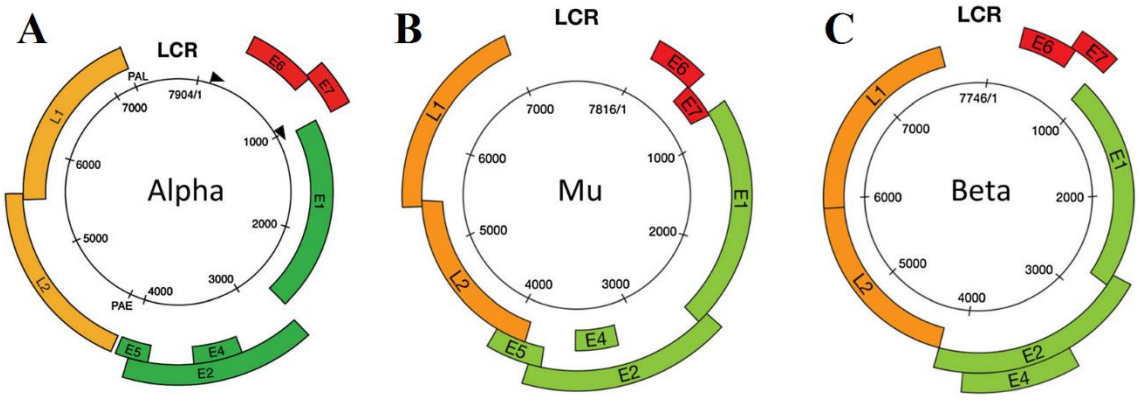

Figure 6 - Schematic structure of genome organization of high-risk Alpha (A), Mu (B), and Beta(C) (Doorbar *et al.*, 2015).

HPV101, HPV103 and HPV108 (γ6) genomic organization differ from all other HPV types that do not have a gene E6. In addition, currently unknown HPV types can exist that has other genome organizations. HPV-1 and HPV-63 (Mu-HPV), as well as HPV-41 (Nu-HPV), remain the only representatives of these respective genres harboring HPV types. It's extremely unlikely, based on present knowledge of HPV types, that these genera contain only 1 or 2 papillomavirus types. (De Villiers, 2013).

1.2.6. Transmission and life cycle

It is widely accepted that papillomavirus infection occurs once virus particles have access to the epithelial basal cells or stem cells, which in many cases involves some level of

epithelial trauma (Egawa *et al.,* 2015), followed by the infection of an epithelial basal stem cell, with the longevity of these cells underlying lesion persistence (Doorbar *et al.,* 2015).

Whether a productive life-cycle is or is not completed depends on the nature of the epithelial site where infection occurs, the local microenvironment (Doorbar *et al.,* 2015), as well as on the presence of external factors such as hormones and cytokines (Doorbar *et al.,* 2012).

Structural changes in the virion capsid, which is necessary for virus internalization and subsequent transfer of the viral genome to the nucleus (Doorbar *et al.,* 2012).

HPV are usually internalized through an endocytic mechanism, although some types of HPV can use alternative routes. The process of internalization may be dependent on the type of HPV and few factors of the human host take part in the process of internalization (Panatto *et al.,* 2015).

After PV infection, an initial phase of genome amplification takes place prior to maintenance of the viral episome at low copy number in the infected basal cells. E2 has an established role in genome partitioning, replication and viral gene expression (Egawa *et al.,* 2015). E6 may be carefully regulated during the virus life-cycle. Functional differences between the high- and low-risk E7 proteins center to a large extent on their differential ability to associate with members of the Retinoblastoma (Rb) protein (pRb) family, with the high-risk E7 proteins being able to bind and degrade both p105 and p107, which control cell cycle entry in the basal layer, as well as p130, which is involved in cell cycle re-entry in the upper epithelial layers (Doorbar *et al.,* 2012).

The essential role of E6 and E7 in the viral lifecycle is primarily to modify the cellular environment to allow viral genome amplification (Egawa *et al.,* 2015).

During HPV life cycle, E5 also contributes to viral genome amplification as a result of its ability to stabilize epidermal growth factor (EGF) receptor and to enhance mitogen-activated protein (MAP) kinase activity. E5 also modulates both extracellular-signal-regulated kinase 1/2 (ERK 1/2) and p38 independently of EGF receptor. In the upper layers of the epithelium, amplified viral genomes are packaged into virus particles produced from L1 and L2 virus coat proteins. The E4 protein, which accumulates at very high levels in cells supporting virus synthesis, appears to have a primary function in virus release and/or transmission, but also acts to optimize the success of virus genome amplification (Fig.7).

The completion of the HPV life cycle ultimately involves the expression L2, the exit of the cell from the cell cycle, and the expression of L1 to allow genome packaging. Genome encapsulation involves the recruitment of L2 to regions of replication via E2, prior to the expression of L1 and the assembly of the icosahedral capsid in the nucleus (Doorbar *et al.,* 2012).

In general, HPV infections evade both the adaptive and innate immune response, with the life cycle being totally intra-epithelial, without viremia, cell lysis or inflammation.

Cells supporting viral late gene expression, and which may contain high levels of viral proteins, are shed from the surface of the epithelium away from immune surveillance. In general, a failure to develop an effective host immune response correlates with persistent infection and an increased probability of progression toward invasive cancer (Egawa *et al.,* 2015).

Usually, the virus remains latent in an episomal status and is usually cleared by human's natural defenses such as defensins (Panatto *et al.,* 2015).

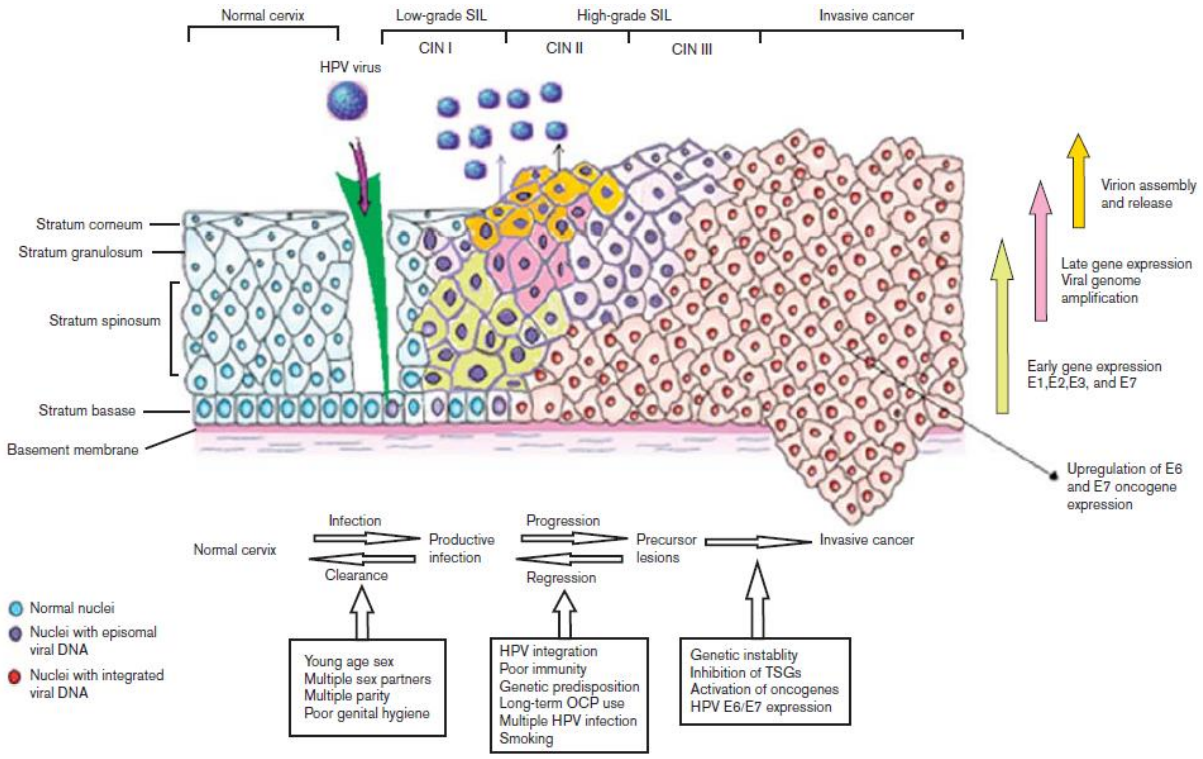

Figure 7 - Scheme of HPV lifecycle and their integration into the host genome (Asiaf *et al.*, 2014).

1.2.7. Pathophysiology

HPV cause a wide range of diseases from benign lesions to invasive tumors. The HPV mucous oncogenic types are the leading cause of cervical cancer, as well as anal, vulvar, vaginal, penile and oral cancers. There are also benign HPV types of the genus Alphapapillomavirus (α- HPV) (Bzhalava *et al.*,2015) that infect epithelial cells of genital mucosa (Bzhalava *et al.*,2013) which cause benign genital condylomas (Bzhalava *et al.*,2015) and all five genera infect oral mucosa or skin (Bzhalava *et al.*,2013).

α- HPV include low-risk mucous types that cause genital warts, the high-risk types that can cause cancer and cervical neoplasia. Though the cutaneous HPV types Alpha, Beta and Gamma aren't usually associated with cancers (Doorbar *et al.*, 2012).

The identification of HPV as an etiological factor for HPV-associated malignancies creates the opportunity for the control of these diseases through immunization and other targeted therapies (Yang *et al.*, 2016).

PV can cause benign tumors (warts, papillomas) in their natural hosts and occasionally in related species. Papillomas are induced on the skin and mucosal epithelium, often in specific locations of the body. Some papillomas proliferation, induced by specific types of PV support a high risk for malignant progression. The infection often leads to micro lesions, which are lightly or not visible at all without optical aid. (De Villiers *et al.*, 2004).

HPV types that cause visible papillomas are usually of greater concern for the individual, especially when they occur in oral or genital sites and are persistent. Approximately 1/3 of individuals who present for treatment with genital warts will still have their injuries 3 months later, with relapse after treatment, being this a significant problem. The low-risk Alpha types that cause these lesions are also involved in the development of respiratory papillomatosis (RRP). (Doorbar *et al.*, 2012).

Reports on a high prevalence of HPV types relative unknown on the surface of the skin and smaller reports studies investigating the role of PV in the pathogenesis of non-melanoma skin cancer, led to a general change in interest in research into PV (De Villiers, 2013).

HPV is often detected in oral mucosa that appears clinically healthy. Although oral HPV infections are common, affecting up to 47% of children and about 5-12% of the adult population (Haukjoja *et al.*, 2014).

Relatively to UV radiation, there is an emerging pathogenic role for HPV of the β- HPV genus in cutaneous squamous cell carcinoma (cSCC) initiation phase. (Hufbauer *et al.*, 2015).

The β-HPV genus infects the skin and HPV-5 and -8 were first isolated from papillomas of patients with rare hereditary syndrome Epidermodysplasia Verruciformis (EV). EV patients develop warts and macular lesions that persist and ranging the 40 years of age, 30-60% develop multifocal carcinoma in situ or SCC. β-HPV can also be the causal agent for non-melanoma skin cancer (NMSC) in solid organ transplant recipients, of which 90% develop skin warts and 40% develop NMSC after 15 years of their transplant (Doorbar *et al.*, 2012; Jiang *et al.*, 2016).

1.2.8. Risk Factors

The unplanned and unsafe sexual activity may result in unexpected pregnancy and may be responsible for an increased risk of sexually transmitted diseases (STDs), including HPV infections. Risky sexual behaviors have been acknowledged as the most important risk factor for acquiring HPV infections and for the development of HPV-related cancers (Marek *et al.*, 2016).

The most frequent risk factors correlated with the infection and persistence of HPV in the population are listed in Table 4.

Women who begin to have sexual intercourse before the age of 16 are more vulnerable to HPV infection because during puberty the cervix undergoes cellular changes at the transformation zone (Ribeiro *et al.*, 2015), making the epithelium more susceptible to viral entry and persistence (Bahmanyar *et al.*, 2012).

The risk of infection also increases with each new sexual partner, underscoring the ease of transmission via sexual acts. Early age at first sexual intercourse, which may be a marker for the total number of sexual partners, is also associated with the risk of progression of the disease among HPV-positive individuals. An active HPV infection is likely to be dependent on recent sexual activity, hence acquired recently, whereas latent or persistent infection could have been influenced by past sexual behavior. (Ribeiro *et al.*, 2015).

Oral sex has been associated with oral HPV infections incidents (Haukjoja *et al.*, 2014).

Smoking was also associated with higher incidence and prevalence rates for HPV infection (Kaderli *et al.*, 2014 and Namujju *et al.*, 2014)., it has deleterious effects on systemic and local immunity, as it suppresses both cell-mediated and humoral immune responses, which might lead to the present finding of increased susceptibility to HPV infection and development of anogenital warts. The prevalence of HPV infection seems to decrease in patients who quit smoking, but the period after which nonsmoker levels are reached is not yet clear (Kaderli *et al.*, 2014, Namujju *et al.*, 2014).

Alcohol consumption and drug use predisposes women to oncogenic HPV infections and thus contributes indirectly to cervical carcinogenesis and associated to a significantly higher probability of sexual intercourse, and also with engagement in sexual risk-taking behaviors (Marek *et al.*, 2016).

Bahmanyar *et al.*, 2012 found that longer duration of hormonal contraception use was associated with increased risk of HPV infection.

History of *Chlamydia trachomatis* infection and other STIs (but to a lesser extent) also increases the risk of infection for HPV (Bahmanyar *et al.*, 2012; de Sanjosé and Alemany, 2015).

Although the efficacy of the condom in the prevention of HPV infection and cervical cancer remains controversial, it is the generally accepted view of the scientific community, that although condoms may not give 100% protection, their use is highly recommended as they may

reduce the risk of contracting HPV infection, protect against other STDs (Boccalini *et al.,* 2012; Marek *et al.,* 2016).

The presence of vulvovaginal ulcers and vulvovaginal inflammation were associated with HR-HPV infection (Kaderli *et al.,* 2014; Niyazi *et al.,* 2016).

Immunodeficiency may also predispose animals to develop papillomas (De Villiers *et al.,* 2004), as so HPV is more likely to persist and to progress to cancer in HIV positive and immunosuppressed women (Gallagher *et al.,* 2016 and Kaderli *et al.,* 2014).

Table 4 - Risk factors of HPV infection

Risk factor	References
Age of sexual activity initiation	Bahmanyar et al., 2012; Boccalini et al., 2012; Kaderli et al., 2014; Marek et al., 2016; Niyazi et al., 2016; Panatto et al., 2012; Ribeiro et al., 2015; Tran et al., 2015
Number of life-time sexual partners	Bahmanyar et al., 2012; Kaderli et al., 2014; Marek et al., 2016; Niyazi et al., 2016; Panatto et al., 2012; Ribeiro et al., 2015; Tran et al., 2015
Frequency of sex or other intimate skin-to-skin contact	Haukjoja et al., 2014 and Panatto et al., 2012
Behaviors of sexual partners	Kaderli et al., 2014; Panatto et al., 2012; Ribeiro et al., 2015
Smoking	Bahmanyar et al., 2012; Boccalini et al., 2012; Haukjoja et al., 2014; Kaderli et al., 2014; Panatto et al., 2012
Alcohol consumption/drug use	Kaderli et al., 2014; Marek et al., 2016; Panatto et al., 2012
Use of oral contraceptives	Bahmanyar et al., 2012; Boccalini et al., 2012; Haukjoja et al., 2014; Niyazi et al., 2016; Panatto et al., 2012; Ribeiro et al., 2015; Tran et al., 2015
History of STI	Bahmanyar et al., 2012; Kaderli et al., 2014
Low condom use rates	Bahmanyar et al., 2012; De Sanjosé and Alemany, 2015; Haukjoja et al., 2014; Kaderli et al., 2014; Panatto et al., 2012; Tran et al., 2015
Parity	Panatto et al., 2012; Ribeiro et al., 2015; Tran et al., 2015
Multiple pregnancies, poor genital hygiene, malnutrition, lack of HPV awareness, low socioeconomic status, history of other HPV-mediated neoplasia	Kaderli et al., 2014; Ribeiro et al., 2015
Diet and conditions of immune suppression	Ribeiro *et al.,* 2015

1.2.9. General diagnosis

In the past few years, there was an advancement in the field of biomolecular diagnostics, as so new sensitive tests can detect HPV in different sites, thus challenging the common view of HPVs as exclusively cutaneotropic or epitheliotropic and mucosotropic viruses. However, until now, evidence collected are still inconclusive and it may be that HPV-positive tumors differ from those HPV-negative in terms of natural history and prognosis. The diagnose methods that were developed so far can be consulted on Table 5 (Panatto *et al.,* 2015), however not all are implemented.

Primary high risk (hrHPV) screening is an important scientific and clinical advance in cervical cancer screening (Fig.8). Primary hrHPV screening should only be initiated prior to 25

years of age as so the re-screening after a negative primary hrHPV screen should occur no sooner than every 3 years. (Huh *et al.*, 2015).

Table 5 - Available Technologies for Human Papillomavirus Detection (Panatto *et al.*, 2015)

Test for HPV Detection
AMPLICOR HPV Test (Roche)
APTIMA® HPV Test (Gen-Probe)
CareHPV™ Test (Qiagen)
Cervista HPV-16/18 (Hologic)
Cervista HPV HR (Hologic)
Clinical Arrays HPV® (Genomica)
Cobas® 4800 HPV Test (Roche Molecular Systems)
Colposcopy
Digene® HPV Genotyping PS Test (Digene/Qiagen)
Digene® Hybrid Capture II (HC2) HPV DNA Test (Digene/Qiagen)
Digene® Hybrid Capture II (HC2) HR HPV DNA Test (Digene/Qiagen)
DuoPap® (Bi-tech)
EIA kit HPV GP HR (Diassay)
GenoID Real-Time HPV Assay (GenoID)
HPV Genotyping Chips (Biomedlab Company)
INNO-LiPA HPV Genotyping Extra (Innogenetics)
LINEAR ARRAY HPV Genotyping Test (Roche)
Multiplex HPV Genotyping Kit (Multimetrix)
NucliSENS EasyQ® HPV (Biomérieux)
Papanicolau (pap smear)
PapilloCheck (Greiner Bio-One)
PreTect HPV-Proofer (NorChip)
RealTime High Risk HPV test (Abbott Molecular)
Reverse Line Blot (Roche)
Virapap/ViraType

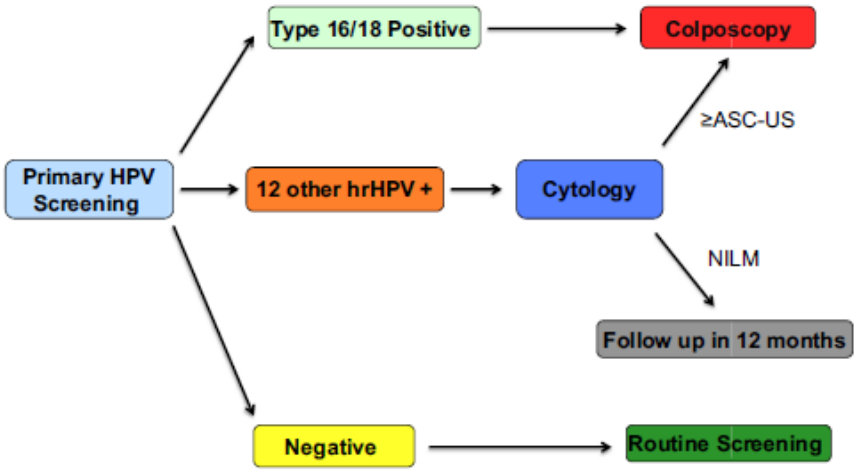

Figure 8 - Recommended primary HPV screening algorithm (Huh *et al.*, 2015).

HPV molecular detection has been implemented in several countries and it was already proposed as the primary test for screening by replacing the Pap smear test. However, HPV genotyping can be considered a methodology more important than detection only, since it helps

to identify the persistent infections that are predictive of outcome of HPV infection. Methods of molecular pathology have been shown important in improving triage, diagnosis, or monitoring cancer and genetic or viral-associated diseases.

Alternatives to cytology-based screening, including HPV-DNA testing and visual inspection of the cervix, using acetic acid (VIA), has demonstrated to be effective and potentially cost-effective in low-resource settings, allowing less follow-up visits and automated processing of laboratory samples, reducing resource requirements and quality in control (La Ruche *et al.*, 1998).

Visual inspection with 3 – 5% dilute VIA and HPV test are the two alternative screening methods that have been most studied in cross-sectional and random clinical trials. Its effectiveness in reducing cervical cancer is likely to be significantly lower than the HPV test. Effective cervical cancer prevention, especially in resource-poor settings, must be focused on reaching women for screening at the time of peak risk of precancerous condition treatable because of persistent HPV infection and before the middle ages in which invasive cancers occur. On the other hand, tests based on Visual inspection screening methods are affordable for developing countries. Also the use of self-collected samples, where no speculum examination is needed, showed proper sensitivity. A test (careHPVTM; QIAGEN, Inc., Gaithersburg, MD, USA formerly Digene Corp.) can detect, in about 2.5 hours,14 high-risk types of HPV that can cause cancer, to screen women in developing regions for cervical neoplasia and is expected to be commercially available in the near future. This test has proven to be fast, accurate and promising for the HPV test implementation in resource-poor settings. Rapid tests for HPV may also facilitate same day test and treat cervical cancer prevention strategies (Sankaranarayanan *et al.*, 2013).

1.3. Integrated prevention and control of HPV

Population-based data for HPV type distribution is a prerequisite to the development of new HPV screening tests to predict the potential benefits of HPV vaccination and to monitor the impact of vaccination on HPV type replacement. Unfortunately, these data are quite limited or missing in many regions (Piras *et al.*, 2011).

The etiologic connection between HPV-16 and -18 drove the development of prophylactic HPV vaccines using a cocktail of VLP's (Jiang *et al.*, 2016 and Yang *et al.*, 2016).
The introduction of vaccination against HPV infection in many countries reduced the prevalence of cervical cancer and cervical intra-epithelial neoplasia. In developing countries, where screening programs are not effectively implemented, HPV vaccination may be difficult to introduce and to evaluate because epidemiologic data on the distribution of HPV infection and the cervical cancer burden prior to HPV vaccine are scanty, but will be particularly attractive and cost effective (Guettiti *et al.*, 2014 and Sudenga *et al.*, 2015).

Vaccines are yet to reach women in developing countries where incidence and mortality rates are much higher. The price of HPV vaccine has been the major barrier to worldwide use (De Sanjosé *et al.*, 2012).

Vaccination is now a recognized primary prevention approach against HPV infection and at the moment (Awolude *et al.*, 2013), the use of HPV vaccines in young women for the primary prevention of cervical cancer and some other HPV related diseases has been endorsed by the European Medicines Agency (EMA), in 2006 with the introduction of the quadrivalent HPV vaccine (qHPV) (HPV6/11/16/18) and in 2007 with bivalent vaccine (bHPV) (HPV 16/18) (von Karsa *et al.*, 2015). Though both commercially available. HPV vaccines were originally approved only for use in girls and women, the quadrivalent vaccine (Gardasil) was approved by the US Food and Drug Administration in 2009 for use in boys and men (Ryser *et al.*, 2015).

The HPV vaccination is recommended by the World Health Organization (WHO) (De Sanjosé *et al.*, 2012) for girls aged between 9 and 13 years, comprised, before their sexual debut (Sankaranarayanan *et al.*, 2013). Both vaccines are prophylactic but not therapeutic, so cervical

screening programs are still needed for women already infected or unimmunized with the potential for future infection (De Sanjosé *et al.*, 2012).

These two licensed prophylactic HPV vaccines, that were evaluated for the quality, safety and efficacy, by WHO (Sankaranarayanan *et al.*, 2013) and are highly efficacious in preventing around 70% of cervical cases among women through protection against HPV-16 and HPV-18 infections targeted by first-generation HPV vaccines (Gardasil® and Cervarix®) (Wagner *et al.*, 2015) they also show evidence of cross-protection against non-vaccine types (particularly HPV 31, 33 and 45) (Awolude *et al.*, 2013; Houlihan *et al.*, 2012). In 2014 a 9-valent HPV vaccine (9vHPV), that includes 5 additional oncogenic HPV types compared with the qHPV (Van Damme *et al*.,2015), was licensed in the United States under the name Gardasil 9 (Merck & Co., Inc, Kenilworth, NJ) which increases prevention of cervical disease to 90% through the protection from HPV types -16, -18, -31, -33, -45, -52, and -58 targeted by the 9vHPV (Gardasil® 9) (Van Damme *et al*.,2015 and Wagner *et al.*, 2015). Prophylactic administration of a 3-dose regimen of 9vHPV vaccine to HPV-naive women aged between 16 and 26 years is highly efficacious against HPV infection and disease. Efficacy and effectiveness of the three commercialized vaccines can be found in Table 6.

Table 6 - Comparison of efficacy and effectiveness of HPV vaccines (Luckett and Feldman, 2015)

HPV vaccine		Bivalent	Quadrivalent	Nonavalent
HPV types		-16, -18	-6, -11, -16, -18	-6, -11, -16, -18, -31, -33, -45, -52, -58
Efficacy in HPV naïve Women *	Prevention of vaccine specific HPV type infection	94.3% (HPV16/18) at 3.6y 87.9% (HPV16/18) at 4y	97% (HPV16) at 3.7y 100% (HPV18) at 3.7y	96% (HPV16/18) at 4.5y
Efficacy in all women (including HPV-exposed)	Prevention of vaccine specific HPV type infection	76.4% at 4y	42% (HPV16) at 3.7y 79% (HPV18) at 3.7y	80.2% at 4.5y
Cross-protection	Efficacy in preventing CIN2 lesions associated with HPV types -31, -33, -45, -52, -58	31.5% at 3.6y (all women) 51.3% at 4y (HPV naïve, and only -31, -33,-45)	NS$	N/A
Immunogenicity	Vaccine-specific HPV types	100% at 3.6y 99% at 4.5y	100% (HPV16) at 3y 76% (HPV18) at 3y	NSD from quadrivalente at 7m
HPV 16/18 change in prevalence pre- and post-vaccination		19.1% → 6.5% in 16-18 y/o	11.5% → 5.1% in 14-19 y/o	N/A

* HPV - naïve in the bivalent vaccine efficacy trial included women who were naïve to 14 high-risk HPV types, including -16 and -18. HPV-naïve in the quadrivalent vaccine efficacy trial included women who were naïve to HPV 16/18. HPV-naïve in the nonavalent vaccine efficacy trial included women who were naïve to HPV -6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, - 59.
$ When lesions co-infected with HPV -16 and -18 are excluded.

Licensed HPV vaccines have been introduced in the national vaccination programs of more than 40 countries, the licensure status of HPV vaccination in GAVI. Although the age range of the primary target age group varies by country, all of these programs target young adolescents. The uptake of the vaccine is of major public health importance, especially in countries that have no or poor cervical cancer screening programs. Clinical efficacy of HPV vaccine trials requires longer follow-up to determine prevention of a persistent HPV infection (Sudenga *et al.*, 2015).

Even though prophylactic vaccines have been proven effective in preventing vaccinated, healthy patients from acquiring HPV infections or previously infected patients who

do not have active infection from being re-infected by the same HPV type, there are no strong evidence to demonstrate the therapeutic effects of these prophylactic vaccines in treating and clearing established HPV infections and HPV-associated lesions. It remains an important need to develop effective treatments for established HPV infections and associated diseases. One potential treatment method involves the use of therapeutic vaccines that, unlike the preventive ones, aim to stimulate cell-mediated immune responses to specifically target and kill the infected cells (Yang *et al.*, 2016).

2. AIMS

Determine the prevalence of HPV infection and characterize the frequency of HPV genotypes in Angolan women.

To perform a systematic review comparing the available published data on the prevalence and genotypic distribution of HPV in the African continent based on highly sensitive HPV detection techniques and its implication for vaccination.

3. MATERIAL AND METHODS

The following protocol was performed according described by Silva, 2011.

3.1. Study population

3.1.1. Ethical aspects

The study has been followed the recommendations of the Helsinki Declaration to the elaboration of research projects.

3.2. Processing of biological samples

3.2.1. Conservation and processing of cervico-vaginal samples

Cervical samples were collected in PreservCyt (Cytyc Corporation, Boxborough, MA, USA), a liquid cytology medium, and centrifuged at 4500 rpm for 15 min. The pelleted cells were suspended in 1000 µL of phosphate buffered saline (PBS), concentrated in 200µL of PBS for later extraction of nucleic acids.

3.2.2. Isolation of nucleic acids

The extraction of DNA from the cervical samples was carried out with commercial kit QiAamp DNA Blood mini Kit (Qiagen, Hilden, Germany) in accordance with the instructions of the manufacturer (Qiagen, 2010).

3.2.3. Polymerase Chain Reaction

DNA of HPV positive samples of Virology Service of Portuguese Institute of Oncology of Porto was used as positive control. A negative control containing all PCR reagents, except DNA, was added to monitor contamination.

The amplification reactions were carried out in the thermocycler Bio-Rad MyCycler™ (Bio-Rad, Hercules, United States of America) in a total volume of 25 µL.

3.2.4. Control of DNA extraction method

To test the efficiency of the harvest method and DNA extraction, was tested the presence of the reference gene, beta globin, using PCR technique, so we would get a fragment of 175 base pair (bp), with the PCO_3 primers and BGII (Table 7). The amplification contained 10 ng of DNA, 1U Taq DNA Polymerase and its respective reaction buffer 1 X, 4 mM $MgCl_2$, 0.2 mM of deoxynucleotide triphosphate (dNTP) and 0.3 µM of each primer. Amplification conditions were: pre-denaturation for 3 min at 95 ºC; 35 cycles of 1 min at 94 ºC, 1 min at 55 °C and 1 min at 72 ºC, with a final extension step of 10 min at 72 ºC.

3.2.5. HPV DNA detection

The presence of viral DNA was evaluated using the PCR technique, degenerate primers MY09/11 (Table 7) that amplify a region relatively stable of the HPV L1 gene with 450 bp.

The reaction with primers MY09/11 contained 10 ng of DNA, 1U Taq DNA Polymerase and its reaction buffer 1X, 4 mM $MgCl_2$, 0.2 mM dNTPs and 0.4 µM of each

primer. Amplification conditions were: pre-denaturation for 3 min at 95 ºC; 40 cycles of 45s at 94 ºC, 45 to 55 ºC and 1 min at 72 ºC, with a final extension step of 5 min at 72 ºC.

Table 7 - Sequence of the used *primers.*

Sequence of *primers*		
Beta globin	PCO_3 BGII	5´-ACA CAA CTG TGT TCA TAG C-3´ 5´-GTC TCC TTA AAC CTG TCT TG-3´
HPV	MY 09 MY 11	5´-CGT CCM ARR GGA WAC TGA TC-3´ 5´-GCM CAG GGW CAT AAY AAT GG-3´

A=Adenine, T=Thymine, G=Guanine and C=Cytosine. The MY09/11 *primers* are degenerated and use modified nucleotides, in which M=A or C, R=A or G, W=A or T and Y=C or T.

Agarose gel electrophoresis of the amplified fragments

To check the amplification of DNA fragments, 15 µl of PCR products were analyzed by electrophoresis in 1.5%, agarose gel stained with SYBR® Safe (Thermo Fisher Scientific). Then, gels were visualized using a transilluminator (Quantity one, Bio-Rad) of UV light and with the support of the computer program.

3.2.6. HPV genotyping by RFLP

Samples positive by PCR with the *primers* MY09/11 were genotyped by RFLP method, as described by Nobre and co-workers (Nobre *et al*., 2008). This method allows the differentiation of various HPV genotypes, for examining DNA cleavage.

The genotypes of HPV divided into four groups based on their oncogenic activity: high risk HPV (HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58 and -59), likely high risk HPV (-26, -53, -66, -68, -73, -82), low-risk HPV (-6, -11, -13, -40, -42, -43, -44, -54, -55, -61, -70, -72, -81 and -89) and undetermined risk HPV (-30, -32, -34, -62, -64, -67, -69, -71, -74, -83, -84, -85, -86, -87, -90, -91, -97, -102 and -106) (Munoz et al., 2006; Nobre et al., 2008).

About 5 µL of PCR products have undergone digestion in four independent reactions, in a total volume of 20 µL, which contained, 2µL of 10X buffer of each enzyme and 10U from the following restriction enzymes: PstI (New England BioLabs, R0140S), HaeIII (New England BioLabs, R0108S) DdeI (New England BioLabs, R0175L) and RsaI (New England BioLabs, R0167S). The digestions occurred during 5 hours at 37 °C.

Agarose gel electrophoresis of the fragments obtained by RFLP

Fragments obtained by RFLP were analyzed by electrophoresis in 3% agarose gel, stained with SYBR® Safe (Thermo Fisher Scientific) and visualized under UV light. The identification of the types of HPV was made following the algorithm proposed by Nobre et al. (2008).

3.3. Data extraction and analysis for the systematic review

We conducted a systematic review of peer-reviewed literature published in English in PubMed ⁄ MEDLINE databases, text words were used alone or in combination: ‘HPV’, ‘HPV genotypes’, ‘HPV prevalence’, ‘HPV vaccination’ and ‘African countries’.

This review summarizes the evidence from recent meta-analyses, narrative reviews and epidemiological studies.

Studies were limited to those that provided data on the region inside a country, age of the participants, HPV prevalence and HPV genotypes detected and this pilot study in Angola. Unpublished manuscripts were rejected. References cited in retrieved articles were also evaluated and included if appropriate. Inclusion criteria comprised the use of polymerase chain reaction (PCR) or Hybrid Capture 2 (HC2) techniques for HPV detection.

HPV prevalence was expressed as the proportion of women positive for a given HPV type among all women tested for this type. Evaluation of types was always based on the ability of the assay to detect them. Each HPV type was evaluated independently of others, estimations show the presence of a given type either as a single type multiple infections (Clifford *et al.*, 2005).

For each study, the following information was extracted: authors, region and country of study population, study period, study type, age of sample, sample size and prevalence of HPV in total (overall high risk and low risk types). In total, more than 130 studies were evaluated from which 61 studies were included in the final analysis.

The unit of geographical evaluation was the African continent and regions inside its countries. All studies were grouped into geographical regions based on United Nations classification, which categorizes Africa in five geographical areas (Eastern, Northern, Central, Western and Southern Africa), being the countries included in this regions the following: North Africa(Algeria, Egypt, Libya, Morocco, Sudan, Tunisia), West Africa(Benin, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Mali, Nigeria, Senegal), Central Africa(Angola, Cameroon, Democratic Republic of Congo, Equatorial Guinea, Gabon, Republic of Congo), East Africa(Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Rwanda, Tanzania, Uganda, Zambia, Zimbabwe) and Southern Africa(Botswana, Namibia, South Africa).

4. RESULTS

4.1. HPV prevalence/ genotypes

A total of 67 women were included in the analysis, being the overall HPV prevalence of 20.9% (14/67).

Table 8 - HPV frequency in 67 Angolan women

Sample number	HPV result
22	-
28	-
29	+
30	-
31	+
32	+
33	+
34	-
35	-
36	-
37	-
39	-
40	-
41	-
42	-
43	-
44	+
45	-
46	-
48	-
49	-
51	-
52	-
53	-
54	-
55	-
56	+
57	-
58	+
59	-
60	-
61	-
62	-
63	-
64	-
65	+

66	-
67	-
68	+
70	-
71	-
72	-
73	-
74	-
75	-
76	-
77	+
78	+
79	-
80	-
81	-
82	-
83	-
84	-
85	-
86	-
87	-
89	-
90	-
93	+
94	-
95	-
96	+
97	-
98	+
99	-
100	-

There were detected 9 HPV types (Table 9), - 4 high risk (HPV-16, HPV33, HPV-56, HPV-58a/b), 3 low risk (HPV-61, HPV-70c, HPV-72) and 1 undetermined risk (HPV-84).

Table 9 - HPV genotypes of the 14 HPV positive women

Sample number	HPV genotype
29	HPV58b
31	HPV56
32	HPV16
33	HPV72
44	HPV33
56	HPV16
58	HPV84
65	HPV70c
68	HPV58a
77	HPV61

78	HPV61
93	HPV61
96	HPV61
98	HPV61

High-risk genotypes, low-risk genotypes, undetermined- risk genotypes.

In order of highest to lowest prevalence the genotypes found were: HPV-61 35.7% (5/14), HPV-16 14.3% (2/14), HPV-33, HPV-56, HPV-58a, HPV-58b, HPV-70c, HPV-72, HPV-84 7.1% (1/14) as shown in Figure 9.

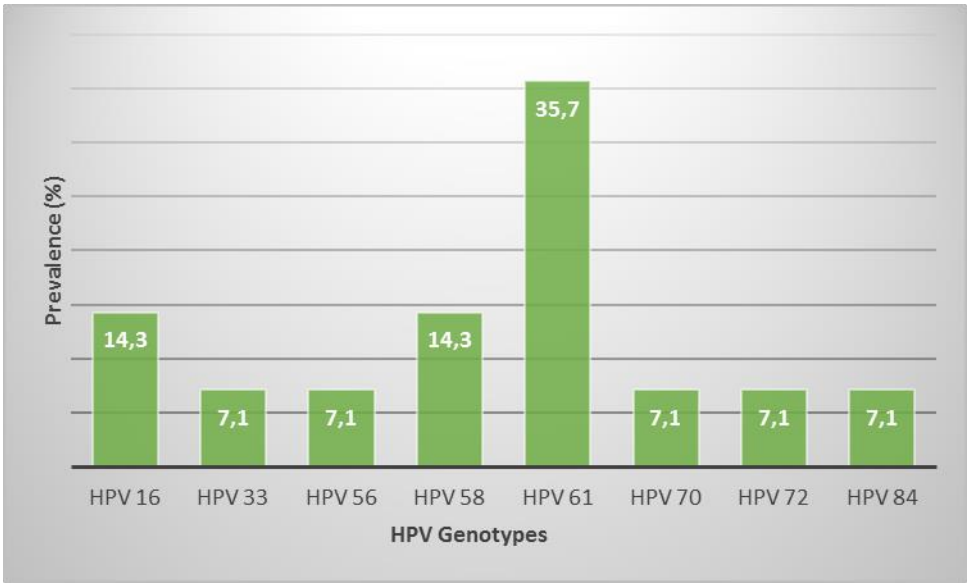

Figure 9 - Prevalence of HPV genotypes in women from Angola.

4.2. Genotypic distribution of HPV in the African continent (systematic review article)

The estimated crude HPV prevalence among women from Africa was 31.7%. Northern Africa (33.1%), Western Africa (33.4%), Central Africa (21.7%), Eastern Africa (35.2%) and Southern Africa (26.9%).

Figure 10 shows point estimates of HPV prevalence by geographical region.

The most common HPV type, in either single or multiple infections, was HPV-16, followed by HPV-18, HPV-52, HPV-35, HPV-58, HPV-31, HPV-45, HPV-33, HPV-51 and HPV-6. HPV-16 was present in 36.1%, 10.4%, 41.7%, 22% and 13.8% of HPV-positive women from North, West, Central, East and South Africa, respectively.

Heterogeneity was significant between regions for HPV52 (which was particularly prevalent in East Africa compared with other regions) (Clifford *et al.*, 2005). HPV-6 was the most frequent low-risk type in North, Central and East Africa (2.4%, 1.9% and 4.4%, respectively) but was less common in West (HPV-53 3.6%) and South Africa (HPV-66 0.5%). In Africa, HPV-6 had an estimated prevalence of 2.7%. Compared with other types, HPV-52 was especially frequent in East Africa (12.9%), and HPV-39 was especially frequent in Southern Africa (0.6%). HPV-18 was second after HPV-16 in the overall estimate except for Eastern Africa where it only appears in 3rd being the 2nd place occupied by HPV-52. Interestingly, the proportion of HPV-positive women infected with HPV-18 was similar across regions.

Of the global HPV burden, 20.2% of HPV infections were estimated to be produced by HPV-16. A significant inverse correlation was observed between overall HPV prevalence

and the contribution of HPV-16, with the lowest HPV-16 proportions in the regions with the highest prevalence. Some African regions had the lowest HPV-16 contributions estimates (10.4%, 22%, and 13.8% for Western, Eastern and Southern Africa, respectively) and Northern Africa (36.1%) and Central Africa (41.7%) had the highest (Bruni *et al.*, 2010).

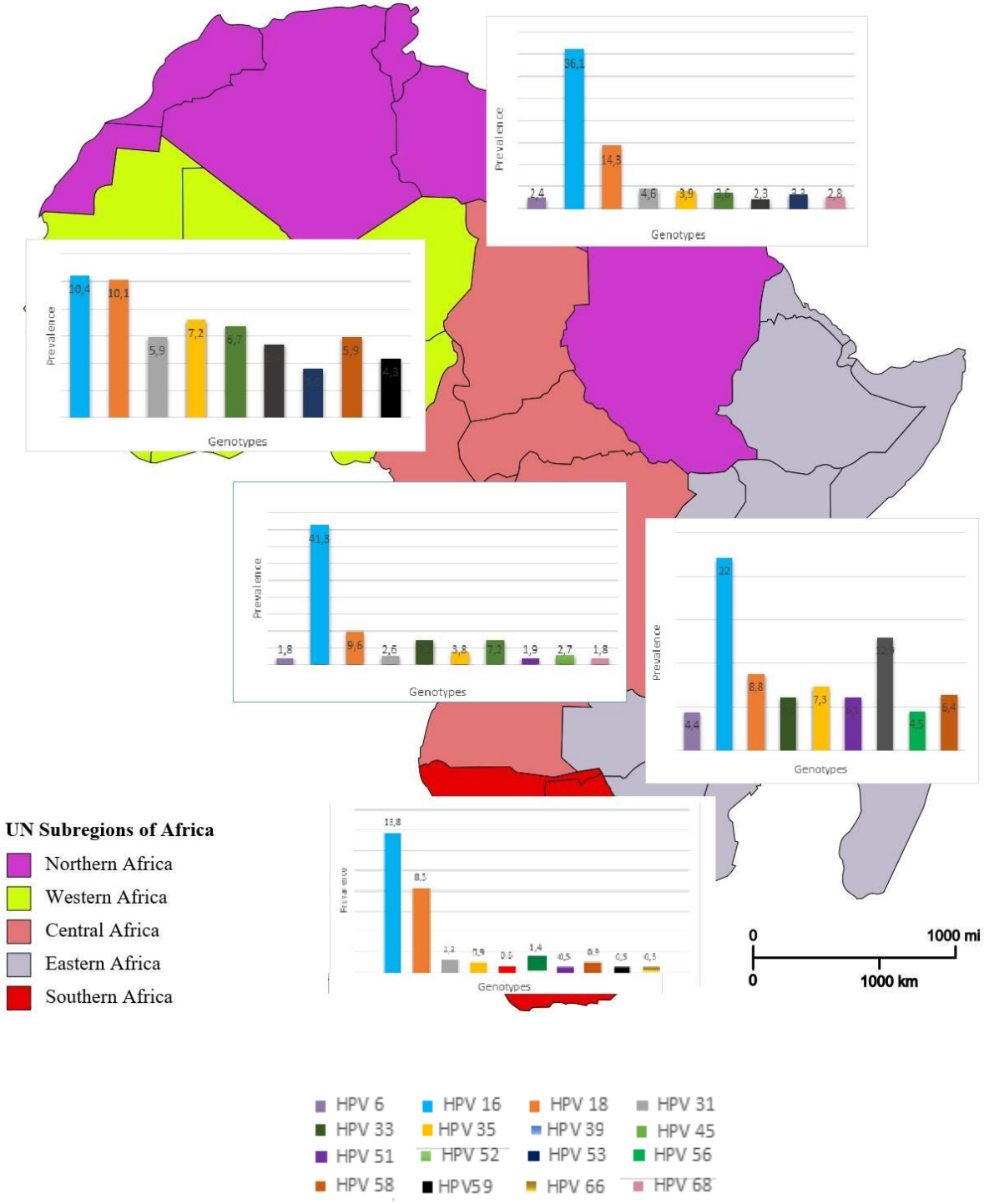

Figure 10 - Prevalence of HPV genotypes in Africa by regions.

5. DISCUSSION

HPV is the most common sexually transmitted virus that has a strong causal relationship with cervical cancer globally (Awolude *et al.,* 2013).

There is evidence that vaccination may be the most effective strategy to prevent cervical and other genital cancers worldwide and currently vaccination against the two most common HPV genotypes is available. But an individual protected against a particular HPV type may still be at risk of infection with other oncogenic types not included in the vaccine. The presence and distribution of multiple HPV genotypes should be taken into consideration when designing a vaccine. Thus, the vaccine should be based on the different HPV genotypes circulating in that particular region (Abate *et al.,* 2013).

Population-based data for HPV type distribution is prerequisite to development of new HPV-screening tests and to assessment of the effect of future vaccination on HPV infections of differing severity, but these data are limited or missing for many world regions (Clifford *et al.,* 2005).

In this regard, the study aimed to determine the most prevalent HPV genotypes in a small population of Angolan women to understand if the current vaccines, since the HPV vaccination program started this year, will be effective against HPV and if not which genotypes should be included in new vaccines trials.

In the first objective of the work the HPV positivity and genotypes was tested for the sample of a population of Angolan women origin, using PCR RFLP. For this we used the typing method described by Nobre, Almeda and Martins, 2008 that allows the discrimination off all known mucosal HPV genotypes (49 HPV types and 2 subtypes), exceeding the genotypes characterized by currently available commercial assays based on RFLP.

The most prevalent genotype was the low-risk HPV-61(35.7%) followed by the oncogenic HPV-16(14.3%) which was similar to Ali-Risasi et al., 2015 that also did not find HPV- 16 as the most prevalent.

The overall HPV prevalence in the Angolan women population was 20.9% which is very similar to HPV prevalence of 21.7% in Central Africa (Cameroon, Democratic Republic of Congo, Equatorial Guinea, Gabon, Republic of Congo, Central African Republic, Chad and São Tomé and Principe) where Angola is also inserted.

Comparing our results from this Angolan women population to other African countries that used Hybrid Capture II (HC2) we observed that HPV prevalence is similar to the study in Tanzania from Dartell et al., 2012 which presented a prevalence of 20.1%. On the other hand, in Democratic Republic of Congo from Mahmud *et al.,* 2012 (11%) that share borders with Angola the presented prevalence tested with HC2 are lower than the one observed in our study from the Angolan population. However, in the studies of Pimentel, *et al.,* 2013 and Schnatz *et al*., 2008 from Nigeria and Leyh-Bannurah *et al.,* 2014 from Ethiopia and Serwadda *et al*., 1999 the HPV prevalence is a slightly lower 16%, 16.6%, 17.5% and 16.3%, respectively.

Nevertheless, when the most prevalent HPV genotypes are compared the differences are many mainly the fact that the most prevalent genotypes are different. The only similarities are the fact that in Dartell et al., 2012 study, HPV-16 also appears as the second most prevalent with 18.8% and the same happens in our study with HPV- 16 representing 14.3% of the infections and in the Leyh-Bannurah *et al.,* 2014 study HPV-56 represent 10.5% of the infection which was very close to our results of 7.1% from HPV-56 prevalence.

Considering the second objective, the studies from Northern regions, showed higher HPV prevalence estimates than Southern regions. HPV prevalence ranged from 21.7% to 35.2%. Although these most frequent types happened to be those most often tested for, HPV-16 was not only the most prevalent type but also had a high relative contribution compared with other types. Among HPV-positive women, HPV-16 accounted for 20.2% of infections. This contribution correlated inversely with the overall HPV prevalence, with the result that the regions with higher HPV prevalence had the lowest relative contributions of HPV-16. This pattern is explained by a higher prevalence of other HPV types in areas where HPV is extremely

common and the increase is not explained by the contribution of any other single type (Bruni *et al.*, 2010).

In our analysis, heterogeneity regarding methods of HPV detection (Table 10), the selection and representativeness of the populations were the most influential variables. To limit heterogeneity related to HPV detection methods, were only included studies using PCR-based methods or HC2. Another source of variability is the differential sensitivity of PCR primers sets to specific HPV types, especially with the less frequent types. The type-specific performance of the assays depends not only on the technique but also on the laboratory and the processing of the specimen (Bruni *et al.*, 2010).

Table 10 - HPV method of detection

	PCR-RFLP	**HC2**
Amplification technique	Target	Signal
Method	*Consensus primer polymerase chain reaction* (*PCR*)	*Liquid-phase signal amplification techniques (HC2)*
Detection	Colorimetric reaction or chemiluminescence	Chemiluminescence
Target area	L1	Entire genome
Analytical sensitivity	Less sensitive than hybridization methods	Less sensitive PCR methods

In the PCR method a broad spectrum of HPV types were amplified by consensus primers (MY09/11), followed by detection with type-specific probes. Detection of the hybridized PCR product is done by chemiluminescence or colorimetric reaction.

RFLP implies the digestion of consensus PCR products with restriction endonucleases and comparison of the digestion pattern with those of known types of HPV. It is useful if unknown types of HPV are present in the specimens, but they have drawbacks as compared with hybridization methods, as RFLP is not suitable for the detection of infections with multiple HPV types, once will usually give an uninterpretable mix-up of sequence patterns. RFLP is less sensitive than hybridization methods because more PCR product is needed to generate a positive signal.

In the case of Hybrid Capture 2 (HC2) method, before the test, the clinical samples are need to be heat-alkaline-denatured. It uses a mixture of RNA probes representing hrHPV types -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68. Detection of the hybrid is done by chemiluminescence and is sensitivity is lower than that of most target-amplification methods, because a mixture of probes is used and HC2 is at present not suitable for high-resolution typing (Brink, Snijders and Meijer, 2007) once the result is qualitative, its given the presence or absence of an HRHPV, nevertheless is very fast (Eide and Debaque, 2012). Non-oncogenic HPVs are not included in the probes (Brink, Snijders and Meijer, 2007) however, another panel of probes is available to demonstrate infection by HPV low risk (Eide and Debaque, 2012).

As described by Clifford *et al.*, 2005, HPV35, -45, and -58 were also more common in Africa. The consistency of these patterns suggests that the prevalence of HPV16 relative to other HPV types truly varies by region.

In our study, the prevalence of HPV -56, -61, -70, -72 and -84 were not corroborated as other common types in Africa, once in some regions they do not appear as the 9 most prevalent at all. First, this might be attributed to the low share of African studies in worldwide reviews and thus limited data from African regions. Second, HPV results from various populations often differ by age ranges, settings and HPV assays used, which makes comparisons difficult (Leyh-Bannurah *et al.*, 2014).

6. CONCLUSIONS

Once Angola like other developing countries has very poor cervical cancer screening and limited resources vaccination may represent a very powerful strategy to fight HPV and cervical cancer burden. In our study, high-risk HPV genotypes which are not targeted by 9v HPV vaccines were identified. However, HPV vaccination remains a promising approach in Angola once its known that the available vaccines may provide cross-protection against HPV genotypes other than the targeted ones and that many oncogenic HPV infections are transient.

Our data on Angolan women population HPV prevalence and genotype distribution would represent a real impact on the choice of immunization policy of the Angola as current vaccines against HPV do not provide reliable or lasting protection against other types. This data can help us to study the genotypes distribution and strengthening the development of new vaccines to be applied in specific regions. Our results indicate that within African geographic regions we may expect different rates of efficacy resulting from a putative vaccination of populations using the currently existing vaccines.

The observed different data between our study and the studies used to compare the results might reflect true differences in the distribution of HPV types in different populations or might be because of differences in the sensitivity of the methods used.

7. REFERENCES

Abate, E., Aseffa, A., El-Tayeb, M., El-Hassan, I., Yamuah, L., Mihret, W., & El-Hassan, A. (2013). Genotyping of human papillomavirus in paraffin embedded cervical tissue samples from women in Ethiopia and the Sudan.Journal of medical virology, 85 (2), p. 282-287.

Ali-Risasi, C., Verdonck, K., Padalko, E., Broeck, D., & Praet, M. (2015). Prevalence and risk factors for cancer of the uterine cervix among women living in Kinshasa, the Democratic Republic of the Congo: a cross-sectional study. Infectious Agents and Cancer, 10 (1), 1.

Angola Geography. (n.d.). Retrived March, 2016, from http://www.worldatlas.com/webimage/countrys/africa/angola/aoland.htm

Angola. (n.d.). Retrived March, 2016, from http://www.who.int/countries/ago/en

Asiaf, A., Ahmad, S. T., Mohammad, S. O., & 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), p. 206-224.

Awolude, O. A., Morhason-Bello, I. O., Denny, L. A., & Adewole, I. F. (2013). Human papillomavirus infection and related cancers in sub-Saharan Africa: burden and tools for prevention. Vaccine, 31, vii-x.

Bahmanyar, E. R., Paavonen, J., Naud, P., Salmerón, J., Chow, S. N., Apter, D. & Jaisamrarn, U. (2012). Prevalence and risk factors for cervical HPV infection and abnormalities in young adult women at enrolment in the multinational PATRICIA trial. Gynecologic oncology, 127 (3), p. 440-450.

Bernard, H. U., Burk, R. D., Chen, Z., van Doorslaer, K., zur Hausen, H., & de Villiers, E. M. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology, 401 (1), p.70-79.

Boccalini, S., Tiscione, E., Bechini, A., Levi, M., Mencacci, M., Petrucci, F. & Bonanni, P. (2012). Sexual behavior, use of contraceptive methods and risk factors for HPV infections of students living in central Italy: implications for vaccination strategies. Journal of preventive medicine and hygiene, 53 (1).

Brink, A. A., Snijders, P. J., & Meijer, C. J. (2007). HPV detection methods. Disease markers, 23 (4), p. 273-281.

Bruni L, Barrionuevo-Rosas L, Albero G, Aldea M, Serrano B, Valencia S, Brotons M, Mena M, Cosano R, Muñoz J, Bosch FX, de Sanjosé S, Castellsagué X. ICO Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Angola. Summary Report 2015- p.12-23.

Burk, R. D., Harari, A., & Chen, Z. (2013). Human papillomavirus genome variants. Virology, 445 (1), p. 232-243.

Bzhalava, D., Eklund, C., & Dillner, J. (2015). International standardization and classification of human papillomavirus types. Virology, 476, p. 341-344.

Bzhalava, D., Guan, P., Franceschi, S., Dillner, J., & Clifford, G. (2013). A systematic review of the prevalence of mucosal and cutaneous human papillomavirus types. Virology, 445 (1), p. 224-231.

Chen, Z., de Freitas, L. B., & Burk, R. D. (2015). Evolution and classification of oncogenic human papillomavirus types and variants associated with cervical cancer. Cervical Cancer: Methods and Protocols, p.3-26.

Clifford, G. M., Gallus, S., Herrero, R., Munoz, N., Snijders, P. J. F., Vaccarella, S., & 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), p. 991-998.

Dartell, M., Rasch, V., Kahesa, C., Mwaiselage, J., Ngoma, T., Junge, J., & Kjaer, S. K. (2012). Human papillomavirus 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), p. 201-208.

de Sanjosé, S., & Alemany, L. (2015). HPV and Cancer: Epidemiology and Mechanism of Carcinogenesis of the Virus HPV. In Tropical Hemato-Oncology (pp. 143-156). Springer International Publishing.

De Sanjosé, S., Serrano, B., Castellsagué, X., Brotons, M., Muñoz, J., Bruni, L., & 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 (Suppl 4), D1-83.

De Villiers, E. M. (2013). Cross-roads in the classification of papillomaviruses. Virology, 445 (1), p. 2-10.

De Villiers, E. M., Fauquet, C., Broker, T. R., Bernard, H. U., & zur Hausen, H. (2004). Classification of papillomaviruses. Virology, 324 (1), p. 17-27.

Doorbar, J., Egawa, N., Griffin, H., Kranjec, C., & Murakami, I. (2015). Human papillomavirus molecular biology and disease association. Reviews in medical virology, 25 (S1), p. 2-23.

Doorbar, J., Quint, W., Banks, L., Bravo, I. G., Stoler, M., Broker, T. R., & Stanley, M. A. (2012). The biology and life-cycle of human papillomaviruses. Vaccine, 30, F55-F70.

Egawa, N., Egawa, K., Griffin, H., & Doorbar, J. (2015). Human papillomaviruses; epithelial tropisms, and the development of neoplasia.Viruses, 7 7), p. 3863-3890.

Eide, M. L., & Debaque, H. (2012, December). HPV detection methods and genotyping techniques in screening for cervical cancer. In Annales de pathologie (Vol. 32, No. 6, pp. e15-e23). Elsevier Masson.

Gallagher, K. E., Baisley, K., Grosskurth, H., Vallely, A., Kapiga, S., Vandepitte, J. & Watson-Jones, D. (2016). The association between cervical human papillomavirus infection and subsequent HIV acquisition in Tanzanian and Ugandan women: a nested case-control study. Journal of Infectious Diseases, jiw094.

Guettiti, H., Ennaifer, E., Attia, L., Chelly, D., Alaya, N. B., Aissa, R. B., & Boubaker, S. (2014). Pre-vaccination Prevalence and Genotype Distribution of Human Papillomavirus

Infection among Women from Urban Tunis: a Cross-sectional Study. Asian Pacific Journal of Cancer Prevention,15 (21), p. 9361.

Haukioja, A., Asunta, M., Söderling, E., & Syrjänen, S. (2014). Persistent oral human papillomavirus infection is associated with smoking and elevated salivary immunoglobulin G concentration. Journal of Clinical Virology, 61 (1), p. 101-106.

Home – ifad.org. (n.d.). Retrived March, 2016, from http://www.ifad.org/

Horo, A. G., Didi-Kouko Coulibaly, J., Koffi, A., Tchounga, B., & Seni, K. (2015). Cervical Cancer Screening Program by Visual Inspection: Acceptability and Feasibility in Health Insurance Companies. Obstetrics and gynecology international, 2015.

Houlihan, C. F., Larke, N. L., Watson-Jones, D., Smith-McCune, K. K., Shiboski, S., Gravitt, P. E., & Hayes, R. (2012). HPV infection and increased risk of HIV acquisition. A systematic review and meta-analysis. AIDS (London, England), 26 (17).

Hufbauer, M., Cooke, J., van der Horst, G. T., Pfister, H., Storey, A., & Akgül, B. (2015). Human papillomavirus mediated inhibition of DNA damage sensing and repair drives skin carcinogenesis. Molecular cancer, 14 (1), p. 1.

Huh, W. K., Ault, K. A., Chelmow, D., Davey, D. D., Goulart, R. A., Garcia, F. A., & Einstein, M. H. (2015). Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance. Gynecologic oncology, 136 (2), p. 178-182.

Hurst, C. S., Hagensee, M. E., Ahmed, S. A., & Smith, J. S. (2015). Validation of Educational Tools for Use in a Human Papillomavirus Intervention Study. Cancer and Oncology Research, 3 (3), p. 35-43.

Info Please (n.d.). Retrived March, 2016, from http://www.infoplease.com/country/angola.html

Jiang, R. T., Schellenbacher, C., Chackerian, B., & Roden, R. B. (2016). Progress and prospects for L2-based human papillomavirus vaccines. Expert review of vaccines, (just-accepted).

Kaderli, R., Schnüriger, B., & Brügger, L. E. (2014). The impact of smoking on HPV infection and the development of anogenital warts. International journal of colorectal disease, 29 (8), p. 899-908.

Keita, N., Clifford, G. M., Koulibaly, M., Douno, K., Kabba, I., Haba, M., & Franceschi, S. (2009). HPV infection in women with and without cervical cancer in Conakry, Guinea. British journal of cancer, 101 (1), p. 202-208.

La Ruche, G., You, B., Mensah-Ado, I., Bergeron, C., Montcho, C., Ramon, R., & Orth, G. (1998). Human papillomavirus and human immunodeficiency virus infections: relation with cervical dysplasia-neoplasia in African women. International journal of cancer, 76 (4), p. 480-486.

Lavezzo, E., Masi, G., Toppo, S., Franchin, E., Gazzola, V., Sinigaglia, A., & Barzon, L. (2016). Characterization of Intra-Type Variants of Oncogenic Human Papillomaviruses by Next-Generation Deep Sequencing of the E6/E7 Region. Viruses, 8 (3), p. 79.

Leyh-Bannurah, S. R., Prugger, C., de Koning, M. N., Goette, H., & Lellé, R. J. (2014). Cervical human papillomavirus prevalence and genotype distribution among hybrid capture 2 positive women 15 to 64 years of age in the Gurage zone, rural Ethiopia. Infectious agents and cancer, 9 (1), p. 1.

Luckett, R., & Feldman, S. (2015). Impact of 2-, 4-and 9-valent HPV vaccines on morbidity and mortality from cervical cancer. Human vaccines & immunotherapeutics, (just-accepted), 00-00.

Mahmud, S. M., Sangwa-Lugoma, G., Nasr, S. H., Kayembe, P. K., Tozin, R. R., Drouin, P., & Franco, E. L. (2012). Comparison of human papillomavirus testing and cytology for cervical cancer screening in a primary health care setting in the Democratic Republic of the Congo. Gynecologic oncology, 124 (2), p. 286-291.

Marek, E., Berenyi, K., Dergez, T., Kiss, I., & D'Cruz, G. (2016). Influence of risk-taking health behaviours of adolescents on cervical cancer prevention: a Hungarian survey. European journal of cancer care, 25 (1), p. 57-68.

Mihigo, R., Anya, B., Okeibunor, J., Ajibola, S., Boakye-Agyemang, C., Muzenda, L., & Nshimirimana, D. (2015). African vaccination week as a vehicle for integrated health service delivery. BMC health services research,15 (1), p. 358.

Moodley, J. R., Constant, D., Hoffman, M., Salimo, A., Allan, B., Rybicki, E., & 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 (1), p. 275.

Muwonge, R., da Ganda Manuel, M., Filipe, A. P., Dumas, J. B., Frank, M. R., & Sankaranarayanan, R. (2010). Visual screening for early detection of cervical neoplasia in Angola. International Journal of Gynecology & Obstetrics, 111 (1), p. 68-72.

Namujju, P. B., Pajunen, E., Simen-Kapeu, A., Hedman, L., Merikukka, M., Surcel, H. M. & Lehtinen, M. (2014). Impact of smoking on the quantity and quality of antibodies induced by human papillomavirus type 16 and 18 AS04-adjuvanted virus-like-particle vaccine–a pilot study. BMC research notes, 7 (1), p. 445.

Niyazi, M., Husaiyin, S., Han, L., Mamat, H., Husaiyin, K., & 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.

Nobre, R. J., de Almeida, L. P., & Martins, T. C. (2008). Complete genotyping of mucosal human papillomavirus using a restriction fragment length polymorphism analysis and an original typing algorithm. Journal of clinical virology, 42 (1), p. 13-21.

Ntekim, A. (2012). Cervical cancer in sub sahara africa. INTECH Open Access Publisher.

Paixão, A., Mancebo, B., Sánchez, L. M., Walter, A., de Fontes-Pereira, A. M. A., Soca, M., & Nicolau, S. (2014). Tamizaje fitoquímico de extractos metanólicos de *Tephrosia vogelii* Hook, *Chenopodium ambrosoides*, *Cajanus cajan* y *Solanum nigrum L.* de la provincia de Huambo, Angola. Revista de Salud Animal, 36 (3), p. 164-169.

Panatto, D., Amicizia, D., Bragazzi, N. L., Rizzitelli, E., Tramalloni, D., Valle, I., & Gasparini, R. (2015). Chapter Eight-Human Papillomavirus Vaccine: State of the Art and Future Perspectives. Advances in protein chemistry and structural biology, 101, p. 231-322.

People & Culture. (n.d.). Retrived March, 2016, from http://www.our-africa.org/angola/people-culture

Pimentel, V. M., Jiang, X., Mandavilli, S., Nwana, C. U., & 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), p. 203-209.

Piras, F., Piga, M., De Montis, A., Zannou, A. R., Minerba, L., Perra, M. T., & Sirigu, P. (2011). Prevalence of human papillomavirus infection in women in Benin, West Africa. Virol J, 8, p. 514.

Poljak, M., Kocjan, B. J., Oštrbenk, A., & Seme, K. (2015). Commercially available molecular tests for human papillomaviruses (HPV): 2015 update. Journal of Clinical Virology.

Ribeiro, A. A., Costa, M. C., Alves, R. R. F., Villa, L. L., Saddi, V. A., Carneiro, M. A. & Rabelo-Santos, S. H. (2015). HPV infection and cervical neoplasia: associated risk factors. Infectious agents and cancer ,10 (1), p. 16.

Ryser, M. D., McGoff, K., Herzog, D. P., Sivakoff, D. J., & Myers, E. R. (2015). Impact of coverage-dependent marginal costs on optimal HPV vaccination strategies. Epidemics, 11, p. 32-47.

Sambo, M. R., & Ferreira, A. V. (2015). Current status on health sciences research productivity pertaining to Angola up to 2014. Health Research Policy and Systems, 13 (1), p. 32.

Sankaranarayanan, R., Anorlu, R., Sangwa-Lugoma, G., & Denny, L. A. (2013). Infrastructure requirements for human papillomavirus vaccination and cervical cancer screening in sub-Saharan Africa. Vaccine, 31, F47-F52.

Schelar, E., Polis, C. B., Essam, T., Looker, K. J., Bruni, L., Chrisman, C. J., & Manning, J. (2015). Multipurpose prevention technologies for sexual and reproductive health: mapping global needs for introduction of new preventive products. Contraception.

Schnatz, P. F., Markelova, N. V., Holmes, D., Mandavilli, S. R., & O'Sullivan, D. M. (2008). The prevalence of cervical HPV and cytological abnormalities in association with reproductive factors of rural Nigerian women. Journal of Women's Health, 17 (2), p. 279-285.

Serwadda, D., Wawer, M. J., Shah, K. V., Sewankambo, N. K., Daniel, R., Li, C., & Gray, R. H. (1999). Use of a hybrid capture assay of self-collected vaginal swabs in rural Uganda for detection of human papillomavirus. Journal of Infectious Diseases, 180 (4), p. 1316-1319.

Silva, J. V. A. V. (2011). Caracterização molecular de agentes infecciosos associados ao desenvolvimento do cancro do colo do útero em jovens no Norte de Portugal: Estudo do *Papilomavírus Humano* e *Chlamydia trachomatis* (Master's thesis). Universidade Fernando Pessoa, Porto.

Sudenga, S. L., Torres, B. N., Botha, M. H., Zeier, M., Abrahamsen, M. E., Glashoff, R. H., & Giuliano, A. R. (2015). Cervical HPV natural history among young Western Cape, South African women: The randomized control EVRI Trial. Journal of Infection.

Tanton, C., Soldan, K., Beddows, S., Mercer, C. H., Waller, J., Field, N., & Sonnenberg, P. (2015). High-risk human papillomavirus (HPV) infection and cervical cancer prevention in Britain: Evidence of differential uptake of interventions from a probability survey. Cancer Epidemiology Biomarkers & Prevention, 24 (5), p. 842-853.

Tran, L. T. H., Tran, L. T., Bui, T. C., Le, D. T. K., Nyitray, A. G., Markham, C. M., & Hwang, L. Y. (2015). Risk factors for high-risk and multi-type Human Papillomavirus

infections among women in Ho Chi Minh City, Vietnam: a cross-sectional study. BMC women's health, 15 (1), p. 1.

Urso, V., Signorini, M. A., Tonini, M., & Bruschi, P. (2016). Wild medicinal and food plants used by communities living in Mopane woodlands of southern Angola: Results of an ethnobotanical field investigation. Journal of ethnopharmacology, 177, p. 126-139.

Van Damme, P., Olsson, S. E., Block, S., Castellsague, X., Gray, G. E., Herrera, T., & Luxembourg, A. (2015). Immunogenicity and safety of a 9-valent HPV vaccine. Pediatrics, peds-2014.

von Karsa, L., Arbyn, M., De Vuyst, H., Dillner, J., Dillner, L., Franceschi, S., & Anttila, A. (2015). European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. Papillomavirus Research.

Wagner, M., Bennetts, L., Patel, H., Welner, S., de Sanjose, S., & Weiss, T. W. (2015). Global availability of data on HPV genotype-distribution in cervical, vulvar and vaginal disease and genotype-specific prevalence and incidence of HPV infection in females. Infectious agents and cancer, 10 (1), p. 13.

Yang, A., Jeang, J., Cheng, K., Cheng, T., Yang, B., Wu, T. C., & Hung, C. F. (2016). Current state in the development of candidate therapeutic HPV vaccines. Expert review of vaccines, (just-accepted).

Zur Hausen, H. (1996). Papillomavirus infections—a major cause of human cancers. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1288 (2), F55-F78.

Zur Hausen, H. (2002). Papillomaviruses and cancer: from basic studies to clinical application. Nature Reviews Cancer, *2* (5), p. 342-350.

Zur Hausen, H. (2009). Papillomaviruses in the causation of human cancers—a brief historical account. Virology, *384* (2), p. 260-265.