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**Human papillomavirus Genotype Distribution and Co-infection
with Sexually Transmitted Pathogens in Reproductive age
Women in Urban Gambia
Bah Camara, H.**

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Human papillomavirus Genotype Distribution and Co-infection with Sexually Transmitted Pathogens in Reproductive age Women in Urban Gambia

Haddy Bah Camara

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Abstract

Cervical cancer is the second most common female cancer in The Gambia, and there have been few studies carried out on the causative agent, human papillomavirus (HPV) in this country. The Gambia introduced the quadrivalent HPV vaccine for girls between the ages of 9-13 years in the urban area in 2014; nationwide immunization will take place in 2019. This study determined the common circulating oncogenic HPV types in the urban region where most cervical cancer cases were reported. Two hundred and thirty-two women between 20 – 49 years of age from the urban region who attended a polyclinic were recruited for this work.

Endocervical and high vaginal swabs were collected, and a socio-demographic questionnaire was administered to capture potential risk characteristics associated with HPV. Nucleic acid amplification techniques and DNA sequencing were carried out to determine the HPV genotype using PGMY09/11 consensus primers. Phylogenetic analysis was carried out on the Gambian HPV sequences to further confirm the identification of The Gambian HPV genotypes and its relatedness to sequences of the same types from other geographical locations. Microbiological and nucleic acid amplification analyses were used to determine the prevalence of other sexually transmitted pathogens in the participants. Blood samples, endocervical swab, and Pap smear were collected from HIV positive participants and these participants were followed up and re-examined every nine months for 24 months, to determine the persistence of cervical HPV infection, HPV antibodies, and cytological cervical changes. Pseudo-typed neutralisation assays were performed to characterise high-risk HPV antibodies in HIV positive women.

Among the sample population studied, HPV prevalence was found to be 12% (28/232). HPV 52 was the most prevalent (17.9%) genotype detected in cervical samples. The Gambian high-risk HPV genotypes, except for a novel putative HPV 35 genotype, were 98 -100% identical to those submitted in the GenBank database. Prolonged (> 5 years) use of hormone contraceptive was the only variable found to associate statistically with HPV infection. Fifty percent (14/28) of participants infected with HPV were co-infected with *Ureaplasma urealyticum/parvum* and 25% (7/28) with HIV. HPV sero-prevalence was found to be 51.7% (15/29) in HIV positive participants. HIV positive women had been exposed to multiple HPV types and HPV 52 antibody was more prevalent 24% (7/29). Other high-risk HPV genotypes were found to cause high-grade cervical lesions and cancer in HIV positive women. Future studies to investigate oncogenic HPV genotypes in cervical cancer specimens will be useful in providing evidence for policies and future evaluation of the quadrivalent HPV vaccine in The Gambia.

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Declaration

I declare that the present work was carried out in accordance with the Guidelines and Regulations of the University of Westminster. The work is original except where indicated by special reference in the text.

Until the outcome of the current application to the University of Westminster is known, the work will not be submitted for any such qualification at another university or similar institution.

Any views expressed in this work are those of the author and in no way, represent those of the University of Westminster.

Signed: Haddy Bah Camara

Date: 15th July 2019

Publications

Journals

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Abbreviations

ART	Antiretroviral therapy
ARV	Antiretroviral
AIDS	Acquired immuno-deficiency syndromes
APC	Antigen presenting cells
ASR	Age standardised rate
AZT	Zidovudine
AOR	Adjusted odds ratio
BLAST	Basic Local Aligement Search Tool
Bp	Base pair
BV	Bacterial vaginosis
COX	Cyclooxygenase
CIN	Cervical intraepithelial neoplasia
CD	Cluster of differentiation
DVI	Direct visual inspection
ECS	Endocervical swab
EDTA	Ethylenediaminetetraacetic acid
EFSTH	Edward Francis Small Teaching Hospital
EFV	Efavirenz
EMA	European Medicine Agency
EPI	Expanded programme on immunization
FP	Family planning
FGM	Female genital mutilation
GBoS	Gambia Bureau of Statistics
GTT	Germ tube test
HPV	human papillomavirus
HR-HPV	High risk human papillomavirus
HIV	human immunodeficiency virus
HIC	High income countries
HVS	High vaginal swab
HSIL	High squamous intraepithelial lesions

HLA	histocompatibility leucocyte antigen
IARC	International Agency for Research on Cancer
IDC	Infectious disease clinic
KMC	Kanifiyng Municipal Council
LR-HPV	Low risk human papillomavirus
LSIL	Low squamous intraepithelial lesions
LPV/r	lopinavir/ritonavir
LBC	Liquid based cytology
MRC	Medical Research Council
NGO	Non-Governmental Organization
PCR	Polymerase chain reaction
pHR-HPV	Probable high-risk human papillomavirus
PsV	Pseudo-typed virus
PGE2	Prostaglandin E2
q-PCR	Quantitative polymerase chain reaction
RT-PCR	Real Time-polymerase chain reaction
Rpm	Revolutions per minute
SD	Standard deviation
STI	Sexually transmitted infection
STM	Specimen transport medium
SOP	Standard Operating procedure
SCJ	Squamous columnar Junction
TZ	Transformation zone
TAE	Tris acetate ethylenediaminetetraacetic acid
3TC	Lamivudine
TDF	Tenofovir
VIA	Visual inspection acetic acid
VILI	Visual inspection Lugol's iodine
VLP	Virus like particles
WCR	West Coast Region
WHO	World Health Organization

Chapter 1: General Introduction

1 Introduction

1.1 Study country profile and population data

The Gambia is a small country in West Africa with a population of less than 2 million (Gambia Bureau of Statistics, 2013). Females between the ages of 15-64 years represent more than 50% of the population. The Gambia has three main ethnic groups namely; Mandinka, Fula, and Wollof. Mandinka is the largest ethnic group representing 42% of the population. The Gambia has a relatively young population and children under the age of 5 years old account for more than 300,000 of the population. Based on the 2015 global population census, the United Nations estimated that by 2017 nearly half a million of the female population will be comprised of 15-49 years old (Fig 1.1).

The Gambia has a total land area of 11,300 km². The river Gambia and a vegetation of open Sudan savannah dominate the geography of the area. The Gambia has an estimated total population of less than 2 million people (GBOS, 2013 census). Subsistence farming, stock rearing, and petty trading are the major occupations particularly, in the middle and eastern hinterlands. The Gambian government is the major health service provider. The public health care system has three tiers, based on the primary health care strategy. Primary health care is delivered through 492 village health posts, dispensaries, and minor health centres. Secondary health care is provided by qualified medical doctors at 38 major health centres. There are five (5) hospitals that provide tertiary care and /or specialist care in the country. The health work force distribution has an urban bias as 66% of personnel are in the urban region (WHO, 2016 country cooperative strategy). The distribution of health workers is hindered by poor infrastructure and insufficient availability of health technology for service delivery in the less well-developed rural regions. The system is complemented by more than 30 private and non-governmental organizations (NGO) clinics mainly in or around the capital city that provide general medical care.

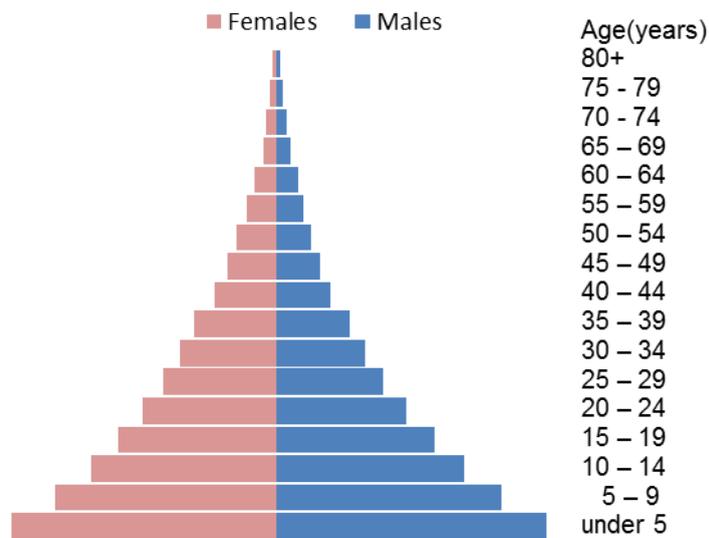


Figure 1.1 Estimated population pyramids for The Gambia, 2017

The Gambia has a relatively young population and was estimated that the under 5 years old accounts for 190,765 and the 15 - 49 years old 429,606 of the total female population (1,001,933) in 2017 (Data source: United Nations, Department of Economic and Social Affairs, Population Division (2017).

World Population Prospects: The 2017 Revision, DVD Edition

(<https://esa.un.org/unpd/wpp/Download/Standard/Population/>) [Accessed on 05/08/2018].

1.2 Viruses

Viruses are small obligate intracellular organisms and their genomes are made up of either RNA or DNA, which is surrounded by a protective coat. The nucleic acid contains the genetic information necessary to program the synthetic machinery of the host cell for viral replication (Gelderblom, 1991). A fully assembled infectious virus is called a virion. The simplest virions consist of two basic components: nucleic acid and a protein shell called the capsid. The functions of the capsid are to protect the viral genome from nucleases and it facilitates the attachment of the virion to specific receptors exposed on the host cell (Lodish *et al.*, 2000). The various virion components are synthesized separately within the cell after which, it is assembled to form progeny particles. This assembly type of replication is unique to viruses and distinguishes them from all other small, obligate, intracellular organisms. Self-assembly of virus capsids follows two basic patterns: helical symmetry, in which the protein subunits and

the nucleic acid are arranged in a helix, and icosahedral symmetry, in which the protein subunits assemble into a symmetric shell that covers the nucleic acid-containing core. Some virus families have an additional covering, called the envelope, which is usually derived in part from modified host cell membranes and can be considered as an additional protective coat (Gelderblom, 1991)

1.2.1 Classification of viruses

Viruses are grouped based on morphology, chemical composition, and structure of the genome, and mode of replication. The genome of a virus may consist of DNA or RNA, which may be single-stranded (ss) or double-stranded (ds), linear or circular (Lodish *et al.*, 2000).

RNA viruses

Seventy percent (70%) of viruses are RNA virus and their genomic structure varies considerably. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or be distributed on two or more nucleic acid segments (multipartite genome). In addition, the strand of single-stranded RNA (ssRNA) may be either a sense strand (plus strand) that serves as a messenger RNA (mRNA) or an antisense strand (minus strand), which is complementary to the sense strand and has no translational function and hence cannot produce viral components (Fields *et al.*, 1996, Gelderblom, 1991). Single-stranded RNA e.g. the retrovirus genome has two identical sense strand ssRNA, which is transcribed into double-stranded, circular proviral DNA. This DNA, facilitated by the viral integrase, becomes covalently bonded into the DNA of the host cell to initiate the subsequent transcription of the sense strands that eventually give rise to retrovirus progeny. On the other hand, the double-stranded RNA (dsRNA) viruses e.g., members of the reovirus family, have several separate segmented genomes and each segment has a complementary sense and antisense strand that is hydrogen bonded into a linear double-stranded molecule. The replication of these viruses is complex; only the sense RNA strands are released from the infecting virion to initiate replication (Gelderblom, 1991).

DNA viruses

Most DNA viruses have either a single genome of linear double-stranded DNA (dsDNA), however, the papovaviruses including the polyomaviruses and papillomaviruses contain

circular dsDNA. The dsDNA act as a template for mRNA and for self-transcription. The papovirus capsid has two or three structural proteins. In addition, there are 5-6 nonstructural proteins, which are encoded that are functional in virus transcription, DNA replication and cell transformation (Lodish *et al.*, 2000).

Single-stranded linear DNA is found with the members of the Parvovirus family, which includes the parvovirus. The virion contains 2–4 structural proteins, which are differently derived from the same gene product, whilst the circular single-stranded DNA of only 1.7 to 2.3 kb is found in members of the Circovirus family which comprise the smallest propagated viruses. The isometric capsid measures 17 nm and is composed of 2 protein species only (Lodish *et al.*, 2000, Gelderblom, 1991).

1.2.2 The human papillomaviruses

The human papillomaviruses (HPV) are small, non-enveloped, double-stranded deoxyribonucleic acid (DNA) icosahedral viruses. HPVs belong to the family *Papillomaviridae* and they infect epithelial cells of the mucosa and skin (deVilliers *et al.*, 2004). HPV has a circular genome with a diameter of approximately 55 nm. The circular double-stranded DNA virus has a genome size of 8.0 kilo-base (kb) attached to cellular histones, which is enclosed in a protein capsid shell (deVilliers *et al.*, 2004; Munoz *et al.*, 2003). The capsid shell is made up of approximately 72 pentameric capsomers containing two late structural proteins, namely; L1 (major capsid) and L2 (minor capsid), which are involved in the packaging of the virus. In addition to coding for the two structural proteins, the HPV genome also encodes the early gene proteins (E1–E7) that direct the replication, transcription and oncogenic transformation of the virus (Table 1). The HPV viral structure also contains a long control region (LCR), which contains the cis-regulatory elements that are important for replication and transcription of the viral DNA (IARC, 2011; Buck *et al.*, 2008). Table 1.1 summaries the functions of the HPV proteins.

Table 1.1 Functions of human papillomavirus early (E) and late gene (L) proteins

HPV proteins	Functions
E1	involves in viral replication, has adenosine triphosphatase (ATPase) helicase activities, which allows it to bind to the viral origin of DNA replication as a hexameric complex
E2	is the main regulator of the viral gene transcription and act as a co-factor to <i>E1</i> in DNA replication
E4	involves in facilitating and supporting viral genome amplification, the regulation of late gene expression, and the assemble of the virion progeny and the mediation of virus release
E5	induces unscheduled cell proliferation by inhibiting apoptosis
E6	involves in viral DNA synthesis, prevents cell differentiation and interacts with <i>p53</i> , a tumour suppressor and cell apoptosis inducer
E7	induces cell proliferation through interaction with tumour suppressor proteins, primarily <i>p105Rb</i>
L1	is the major viral structural protein and assembles in capsomeres and capsids. Together with L2 are involved in the packaging of the new virions. <i>L1</i> also encodes neutralizing epitopes of the virus.
L2	is the minor viral structural protein and is involves in facilitating virion assemble. <i>L1</i> also encodes neutralizing epitopes of the virus

1.2.3 Classification of papillomaviruses (PV)

Papillomavirus classification has been based on genomic cross-hybridisation and restriction digest techniques for over three decades. With the advancement of molecular and phylogenetic analysis, PV taxonomy became much easier as phylogenetic analysis compares either the whole viral genome sequences or sub-genomic fragments. Initially, before the improvement of these newer techniques, PV is grouped together with polyomaviruses under the *Papovaviridae* family based on their non-enveloped capsids and circular double-stranded DNA structure. However, further genomic analysis revealed the difference in genomic size, structural organisation and nucleotide sequences indicating that they are not as similar as previously thought (de Villiers *et al.*, 2004).

Research carried out on the papillomavirus genome has shown that the virus is very stable and sequence changes in the genome can arise due to mutation, however, this event is very rare (de Villiers *et al.*, 2004). In addition, the rate of mutational changes that occur is proportional to those of the infected host DNA genomes. Papillomavirus isolates are classified into types and many have been isolated from animals, birds, and humans. Humans are the only host in which PV has extensively been studied and more than 200 HPV types have been identified based on complete genome analysis (Daudt *et al.*, 2016; Doorbar *et al.*, 2012). It is widely accepted that many HPV types might exist due to the detection of other sub-genomic types. Presently, complete HPV genome sequencing has been carried out on almost all isolated HPV genomes and their sequences are entered into a GenBank, PaVE and EMBL databases. Although the major capsid (*L1*) gene, which is the most conserved region of the HPV genome, has been used to classify HPV types. HPV is only defined as a new type when the cloned DNA sequences of the *L1* gene differ by >10% from the closest type on the database. In addition, sequences that are different by < 2% and 2-10% to the closest known genotype are defined as HPV variants and subtypes, respectively (Bernard *et al.*, 2010; de Villiers *et al.*, 2004).

1.2.4 HPV genotype

More than 100 HPV genotypes have been shown to infect both male and female mucosal tracts. Approximately, half of these have been shown to infect the genital tract and are termed high-risk (HR) or low-risk (LR) genotypes depending on their oncogenic potentials (Parkin and

Bray, 2006; zur Hausen, 2002). Fourteen HR-HPV genotypes are known to cause cervical cancer or are associated with other oropharyngeal and anogenital cancers in humans. These HR-HPV types include HPV 16, 18, 31, 33,35,39,45,51,52,56, 58, 59, 66 and 68. Other HPV genotypes including HPV 26, 53, 67, 70, 73 and 82 have been classified as possible carcinogenic types; hence they are termed probable HR-HPVs (Bouvard *et al.*, 2009; IARC, 2011). HR-HPV genotypes 16 and 18 have been documented to be strongly associated with invasive cervical cancer and other related cancers such as vaginal, anal, oropharyngeal, head, and neck (IARC, 2007; Bulk *et al.*, 2007; Hoots *et al.*, 2007; Smith *et al.*, 2007(a); Khan *et al.*, 2005; Clifford *et al.*, 2005). Globally, HR-HPV 16 and 18 are accountable for most of these cancer cases, however, the distribution of HR-HPV types can vary from one country to another or even within the same country (Bruni *et al.*, 2010; Castellsague *et al.*, 2007).

Infection with the low-risk HPV (LR-HPV) genotypes do not cause cancers but can cause benign or low-grade cervical changes (Lacey *et al.*, 2006). LR-HPV genotypes are associated with skin warts including verruca vulgaris, deep plantar and palmar, myrmecial warts, flats and common warts. These skin warts are normally benign and most of them resolve spontaneously. The genital LR-HPV causes juvenile recurrent respiratory papillomatosis, condyloma acuminata and the most common LR-HPV associated with genital infections are HPV 6 and 11(Jablonska *et al.*, 1997).

1.3 Natural history and epidemiology of genital HPV Infection

HPV is a ubiquitous pathogen and natural infection has mostly been studied in the cervix. However, the natural history of HPV in the cervix may be similar to the natural history of HPV lesions in other anogenital areas (IARC, 2007). HPVs are highly epitheliotropic and they cause productive infections only within stratified epithelial cells (IARC, 2011). The virus initially infects the basal epithelial cells through micro-abrasions in the skin, genital organs and oropharyngeal cavity and causes differentiation of the infected basal epithelial cells, which maintain the active virus life cycle (Schneider *et al.*, 1987). The replication of the virus occurs in the basal differentiating cells and these cells are made up of proliferating cellular components of stratified epithelia. The rapid increase in the number of cell components 20-100 extrachromosomal copies of viral DNA per cell facilitates the establishment of the viral

genome into the nucleus of the infected cell (Fig1.2) (Oguchi *et al.*, 2000; Frattini *et al.*, 1996; Schneider *et al.*, 1987).

The ability of the virus to establish its genome into infected cells is facilitated by the virus early gene proteins (E1 and E2), which are first expressed from the early promoter to regulate the host's cell life cycle and genome replication by forming a complex that binds to sequences at the viral origin of replication (Hubert and Laimins, 2000). Since both E1 and E2 replication factors are also expressed at the early promoter, the viral copy numbers in undifferentiated cells are controlled by E2 expression. The E6 and E7 proteins of the HR-HPV types act as viral oncoproteins, however, for the LR-HPV types, no such functions are associated with these proteins. High-risk E6 binds to the p53 tumor suppressor protein to form a trimetric complex with the cellular ubiquitin ligase E6AP, leading to rapid turnover of p53, whilst E7 binds to the Retinoblastoma (Rb) tumor suppressor as well as other proteins involved in cell cycle regulation (Flores *et al.*, 2000; Thomas *et al.*, 1999). However, these proteins are expressed at a low level during natural infections.

As HPV infected basal cells undergo cell division, viral genomes are divided into daughter cells, whereby one daughter cell loses contact with the basement membrane and migrates into the suprabasal compartment. This causes its withdrawal from the cell cycle to commence a programme of terminal differentiation of the epithelial cells to become squamous cells. As infected cells leave the basal layer, they remain active in the cell cycle due to the action of the E7 protein. The infected cells re-enter the S phase in highly differentiated cells and activate the expression of cellular replication factors required for viral replication. The presence of E7 causes the retention of nucleic throughout all layers of infected epithelia (Fig 1.2). The virus late genes, *L1* and *L2* are expressed late and form icosahedral capsids to encapsidate the viral genome to form progeny virions in the nucleus (Woodman *et al.*, 2007; Schiffman and Kjaer, 2003). The virions mature after epithelial cell differentiation and are released during the natural shedding of senescent cells at the end of the epithelial cell life cycle (Fig1.2) (zur Hausen, 2002; Flores *et al.*, 2000). Although most women will be infected with the virus few will develop precancerous and invasive cervical cancer (section 1.6.1).

Most individuals are infected with genital HPV infection shortly after beginning their first sexual encounter with the majority of incidence seen in women below 25 years of age (Moscicki *et al.*, 2012; Franceschi *et al.*, 2006; Castle *et al.*, 2005; Collins *et al.*, 2002). Thereafter,

prevalence declines and plateaus at 30 - 35 years of age. However, a second HPV prevalence peak has been reported in women 50 years old and older in countries such as Costa Rica and Colombia (Bruni *et al.*, 2010; Woodman *et al.*, 2007; Franceschi *et al.*, 2006). HPV infections in young and adolescent women are known to be transient and can resolve after a few months of natural infection or within 2 years (Woodman *et al.*, 2001; Richardson *et al.*, 2003).

HPV prevalence rates in males are less well defined due to the difficulty of obtaining adequate samples for HPV DNA testing (Smith *et al.*, 2007(b)). HPV infection in males is estimated to be in the range of 15% to 45%, which is similar to the prevalence reported for women (Partridge and Koutsky, 2006; Gerberding, 2004). Infection with HPV is also asymptomatic in men; however, they are also at risk of developing HPV-associated diseases such as genital warts, penile and anal cancers (Nielson *et al.*, 2007; Schiffman and Kjaer, 2003).

1.3.1 Immune response against natural infection with HPV

The innate immune and adaptive cellular and humoral responses control HPV infection and clearance of free virions. However, naturally-acquired HPV infection can evade the innate immune system thereby causing a delay in activating the adaptive immune response (Moscicki, 2005; Ho *et al.*, 2004). The poor immune response to HPV infection is linked to a lack of induced inflammation and apoptosis during HPV replication, no viraemic phase occurs to fully activate the systemic immune response (Bonanni *et al.*, 2009). Secondly, the replication and release of HPV virions occur in the suprabasal compartment instead of the basal epithelium, where the contiguity to the immune system would have generated a stronger response (Frazer, 2007). Although the immune system is poorly activated during HPV infections, both humoral and cellular responses against HPV have been demonstrated in spontaneous regression of genital warts in a mouse model. Infiltration of CD4⁺ and CD8⁺ cells and macrophages were observed in the mouse wart epithelium and stroma with an increase in the level of pro-inflammatory cytokines, which is an indication of a T helper 1 immune response (van der Burg *et al.*, 2007; Stanley, 2006).

Antibodies produced during natural HPV infections are type-specific and are directed against the L1 major capsid protein conformational epitopes of the virus (Carter *et al.*, 2000). However, it has been shown that approximately 60% of individuals will develop IgG antibodies to HPV after natural infection; whilst in others, this may take up to eighteen months

(Carter *et al.*, 2000). Antibodies developed after natural infection are type-specific and usually decline within a year after infection. Although there is little evidence that HPV antibodies provide cross-protection in humans, it has been demonstrated that HPV antibodies have neutralisation effects on sero-positive animals after re-exposure to the same virus. Dogs that were immunised with canine oral papillomavirus (COPV) were found to transfer immunity to other susceptible dogs (Suzich *et al.*, 1995). Studies on the natural history of HPV antibodies have shown that antibody titres are very low following sero-conversion and the acquisition of HPV antibodies is mostly associated with persistent infection (Steele *et al.*, 2008). Furthermore, it has been shown that the responses of antibody IgA correlate with that of IgG and the detection of the same HPV DNA specific type (Sasagawa *et al.*, 1998; Wang *et al.*, 1996).

However, serum IgA antibody conversion is less frequent and occurs later when serum IgG conversion has already taken place. Secondly, serum IgA antibodies can be detected up to approximately fourteen months after sero-conversion, whilst serum IgG may persist for up to two years (Onda *et al.*, 2003; Hagensee *et al.*, 2000).

1.4 HPV transmission and acquisition

1.4.1 HPV transmission by the sexual route

The most common mode of anogenital HPV transmission is through the horizontal route (Kjaer *et al.*, 2001; Rylander *et al.*, 1994). HPV transmission is mostly by sexual contact with an infected cervix, anus, vagina, and penis. Women whose partners were infected with HPV were known to develop HPV-related genital warts after 4 - 6 weeks (Bleeker *et al.*, 2002; Rylander *et al.*, 1994). Many epidemiological studies have confirmed the role of sexual activity in HPV transmission (Castellsagué *et al.*, 2003; Sellors *et al.*, 2003; Bleeker *et al.*, 2002). HPV is easily transmitted because it can penetrate the mucosa or skin through a small abrasion of the epithelium. HPV prevalence reported in lesbians has demonstrated that HPV transmission is not strictly through penetrative intercourse, but it can be transmitted through genital to genital sexual contact (Marrazzo *et al.*, 2001). There is overwhelming evidence that HPV infection can also be transmitted through other sexual practices such as oral sex, peno-anal intercourse as

well as the use of insertive sex toys (Gervaz *et al.*, 2003; Edwards & Carne, 1998; Sonnex *et al.*, 1999).

1.4.2 HPV transmission by non-sexual routes

HPV transmission can occur through the non- sexual routes such as vertical (perinatal) transmission, skin contact, and fomites (Ryndock and Meyers, 2014; Frega *et al.*, 2003; Sonnex *et al.*, 1999). HPV infection in one anogenital site such as the introitus can be transferred to another site through self-infection (Winer *et al.*, 2003). Sonnex *et al.*, (1999) have demonstrated that patients with genital warts were found to harbour HPV on their hands and a similar finding was also reported for university students with HPV genital warts (Winer *et al.*, 2010). These findings show further that HPV infection can be transmitted through digital-genital contact due to individual improper hygiene or that of others through non-sexual physical contact. However, some of the studies on genital HPV transmissions by the non-sexual route have generated controversial results. HPV research carried out on virginal women have shown that HPV transmission through this route is not common (Dillner *et al.*, 1999), while an earlier study has shown approximately 50% of participants infected with the virus were sexually inexperienced (Sun-Kuie *et al.*, 1990). Nonetheless, a study carried out on pre and post virginal women has shed some evidence that HR-HPVs are only transmitted via the sexual route (Kjaer *et al.*, 2001).

HPV infection can also be transmitted perinatally via an infected birth canal during vaginal delivery and the HPV genotypes found in infected newborns are similar to those found in their mothers (Hahn *et al.*, 2013; Freitas *et al.*, 2013; Hong *et al.*, 2013; Park *et al.*, 2012; Smith *et al.*, 2010). Furthermore, nosocomial (hospital-acquired) HPV infection has also been documented (Casalegno *et al.*, 2012). Nosocomial infection is a common route for many viruses to gain access to their host and HPV is no exception. A potential route for hospital-acquired HPV infection is using transvaginal ultrasound probes, which are used to perform endovaginal cavity ultrasounds (Ryndock and Meyers, 2014; Casalegno *et al.*, 2012). Although these probes are disinfected after each use, the virus is known to be very stable and most of the clinical disinfectants cannot neutralise the virus (Ma *et al.*, 2012; Ryndock and Meyers, 2014). Ultrasound probes were found to be positive with HR-HPV types and not only where these probes infected with HPV DNA, but with free virion indicating that HPV can also be

transmitted through fomites (M'Zali *et al.*, 2014; Casalegno *et al.*, 2012; Ma *et al.*, 2012; Kac *et al.*, 2010).

1.5 Risk factors for acquiring HPV infection

The link between the acquisition of oncogenic HPV genotypes and sexual activity has been established through cross-sectional and longitudinal studies. The most consistent predictor for the acquisition of HPV infection is the number of lifetime sexual partners (Cuschieri *et al.*, 2004(a); Karlsson *et al.*, 1995). The risk of acquiring HPV infection is estimated to be directly proportional to the numbers of sexual partners (Moscicki *et al.*, 2001; Ley *et al.*, 1991). Having a new sexual partner, short intervals between new sex partners, concurrent sexual partners and a male partner with multiple sexual partners are all known risk factors for HPV infection (Burchell *et al.*, 2010; Cotton *et al.*, 2007).

Age is an important risk factor, as HPV infection is consistently most common in sexually active women below the ages of 25 years old (Moscicki *et al.*, 2012; Franceschi *et al.*, 2006). The overall association with age may be a result of riskier sexual behaviour such as multiple sexual partners, non-use of condoms and co-infection with multiple STI pathogens (Ley *et al.*, 1991). However, there is also evidence that adolescent and young adults are more susceptible to HPV infection due to the epithelial cells that make up the cervix. Once puberty occurs, the columnar epithelial cells gradually transform into squamous epithelial cells. During this transformation, transitional squamous, glandular and metaplastic cells are formed, which all promote HPV replication (Moscicki *et al.*, 1999). Early sexual debut may accelerate the process of cervical transformation, as adolescents with multiple sexual partners tend to have more mature cervixes than sexually inexperienced adolescents (Curtis *et al.*, 2004; Moscicki *et al.*, 1999). In women older than 45 years old, the strongest risk predictors for HPV persistence are linked to older age, early menopause and the use of hormone replacement therapy (Gonzalez *et al.*, 2010).

1.6 The burden of HPV-related diseases and its role in the development of premalignant, malignant and non-malignant disease

HPV has been associated with cervical, vaginal, vulva, penile and anal precancer and cancers. HPV has also been linked to a subset of head and neck cancers. Approximately 70% of these HPV-related diseases are known to be caused by HR-HPV16 and 18 (Smith *et al.*, 2007; Clifford *et al.*, 2003). Non-malignant HPV-associated diseases such as anogenital warts and juvenile recurrent respiratory papillomavirus are caused by LR-HPV 6 and 11. HPV infections with HR genotypes can persist and progress to cause precancerous lesions, and eventually invasive cancer. The transformation zone of the cervix is the area between the original squamocolumnar junction (SCJ) and the new SCJ where the columnar epithelium has been replaced or is being replaced with the new metaplastic squamous epithelia. The size and shape of the transformation zone depend on age, parity, prior infections and exposure to female hormones (Autier *et al.*, 1996). HPV infection in the cervix is most likely to cause cancer at the transformation zone (TZ) due to its susceptibility to carcinogenic HPV genotypes (IARC, 2011; IARC, 2007).

1.6.1 Pathogenesis of HPV infection

Most HPV infections are spontaneously cleared by the immune system, but persistent infection with oncogenic types may cause low grade squamous intraepithelial lesion (LSIL), high grade squamous epithelial lesion (HSIL) or progress to cervical cancer. The risk of persistence and progression to cervical cancer is related to the type of HPV, infection with multiple types and high viral burden in the cervix (Woodman and Collins, 2002). As explained in section 1.2, HPV virions are released during the natural shedding of senescent cells at the end of the epithelial cell life cycle to initiate a new infection. In the context of HPV infection leading to cervical cancer, persistent infection causes LSIL or cervical intraepithelial neoplasia (CIN), which enhances productive viral replication within the host cells. Viral synthesis occurs in both productive infections and CIN grade 1 (CIN1). Progression to high-grade CIN (CIN 2/3) develops with de-regulated gene expression (IARC, 2007). Unlike the early viral promoter, the late promoter expression is not regulated by E2 protein, and high levels of expression occur upon differentiation, leading to increased viral DNA copy number. The increase in viral DNA

copy number also increases the expression of the replication proteins (Schiffman *et al.*, 2007). In CIN 1/2 infections, the HR-HPV genomes are present as episomes. However, during progression to high-grade CIN 3 and cervical cancer, the episomal viral DNA often becomes integrated into the host genome. This integration normally occurs within the E2 open reading frame (ORF) resulting in loss of E2 repressive action, which leads to high expression of oncoprotein E6 and E7. The oncogenic E6 forms a trimetric complex with p53 and E6AP, resulting in ubiquitination of p53 and subsequent degradation, leading to a reduction in the half-life of *p53*. Furthermore, the function of the *Rb* gene as major regulators of the cell cycle exit during epithelial differentiation is abrogated by the binding of oncogenic E7 protein to *Rb* (Woodman *et al.*, 2007; Jeon *et al.*, 1995). The rapid turnover of p53 and the blocking of the *Rb* gene lead to a cellular state which cannot support the full viral life cycle resulting in cervical carcinoma (Fig. 1.2).

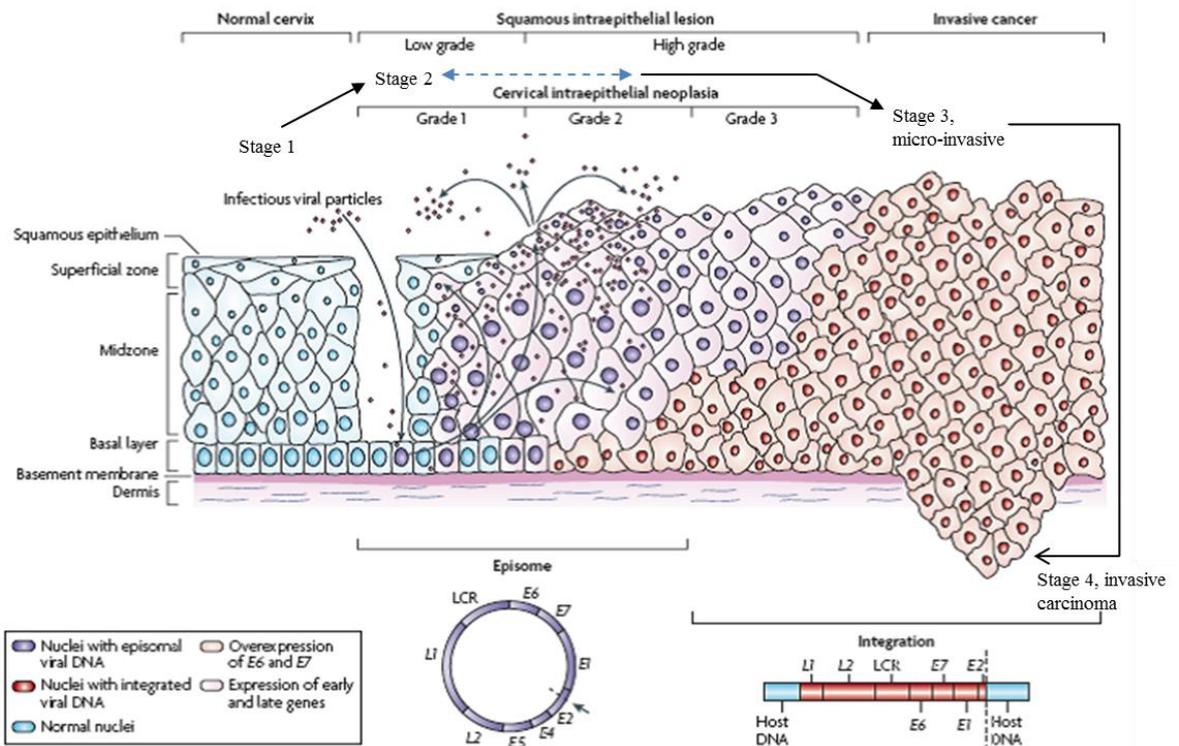


Figure 1.2 Progression of persistent HPV infection to cervical diseases (adapted from Woodman et al., 2007).

Stage 1: Human papillomavirus access the basal cells through micro-abrasions of the cervical epithelium. Once the basal cells are infected, the HPV early genes *E1-E7* are expressed and the viral genome is replicated from the episomal DNA (purple nuclei). **Stage 2:** The viral genome is further amplified in the suprabasal compartment (midzone and superficial zone), and within the compartment both the *L1* and *L2* (late genes) capsids are expressed. The viral genome is then encapsidated by the *L1* and *L2* genes to assembly the progeny virions, which are shed to initiate a new infection. Persistent infection leads to cervical intraepithelial lesions (CIN1/2) that can spontaneously regress. **Stage 3:** Persistent infection with oncogenic HR-HPV genotypes can progress to high grade CIN3, which left untreated progresses to micro-invasive cancer by integration of the viral genome into the host DNA (red nuclei). **Stage 4:** The upregulation of the two high risk oncogenes *E6* and *E7* are responsible for invasive cancer. The *E7* forms a complex with the product of the Retinoblastoma gene (*Rb*) to cause uncontrolled cell proliferation (Chellappan *et al.*, 1992). Binding of the *E6* gene to the *p53* gene protein product degrade the complex and lead to loss of DNA repair, thus preventing the cell to undergo apoptosis (Thomas *et al.*, 1996). This leads to a cellular state that can no longer support the full viral life cycle therefore results in invasive carcinoma.

1.6.2 Epidemiology of cervical cancer

Cervical cancer is the second most common cancer affecting women, worldwide with 530,000 new cases reported each year (de Martel *et al.*, 2017; Ferlay *et al.*, 2013). Nearly all cervical cancer cases reported each year are attributed to HPV infection with approximately half of the cases diagnosed in women below the age of 50 years old (de Martel *et al.*, 2017). An incidence rate of 60,240 cervical cancer burden is observed in Western Europe compared to 120,000, 93,000 and 69,000 cases reported for India, sub-Saharan Africa, and Latin America, respectively (Ferlay *et al.*, 2013) (Table 1.3).

Cervical cancer is a leading cause of mortality in women with more than 250,000 cases reported each year, approximately 80% of which occurs in resource-limited countries (Ferlay *et al.*, 2004). It is expected that 60% of women living in developing countries will succumb to cervical cancer as the 5-year survival rate depends at which stage the disease is presented as well as treatment availability (Sankaranarayanan *et al.*, 1998). The unavailable or inadequate cervical cancer screening programmes together with a lack of effective treatment may have contributed to the low 5-year survival rates reported, in many developing countries. It is estimated that if the current trend continues, cervical cancer mortality will rise from 80% to 90% in developing countries by the year 2020 (Ferlay, 2001).

HPV genotypes 16 and 18 have been reported to be the most types detected in cervical cancer cases, worldwide with HPV 16 more prevalent in squamous cell carcinoma (Clifford *et al.*, 2017). HPV 16 may be found either in an integrated or episomal form in invasive cervical cancers (Sigurdsson *et al.*, 2007; Munoz *et al.*, 2003). The frequency with which HPV 16 is found in integrated forms increases with the severity of the lesion (Cricca *et al.*, 2006). In contrast, HPV 18 is the type most strongly associated with cervical adenocarcinoma and it is most frequently found in integrated forms in high grade cervical intraepithelial lesion (HGICIN) or invasive cancers (Clifford *et al.*, 2003). However, HPV16 prevalence remains high in both cell carcinomas (Clifford *et al.*, 2017; de Sanjose *et al.*, 2007).

Although HPV 16/18 are mostly detected in 70% of cervical cancer specimens, their prevalence is slightly higher (77%) in Europe and North America than in Africa, Asia

and Central and South America, which have a prevalence rate of 70% (Smith *et al.*, 2007; Clifford *et al.*, 2006).

1.6.3 Cervical cancer burden in The Gambia

More than 50% of the reproductive age female population in The Gambia is at risk of being diagnosed with HPV infections. Analysis of The Gambia National Cancer Registry (GNCR) data from 1990 - 2009 showed 7, 991 cases of malignant tumours were diagnosed, 48% of the total cases were in females. Of these malignant tumours, cervical cancer was the most common female cancer accounting for 33% of all female cancers (Table 1.2). Liver cancer is the second most common female cancer (24%), followed by breast cancer (11%) and non-Hodgkins lymphoma (4%) (Bah *et al.*, 2013). Eighty percent (80%) of Gambian women diagnosed with cervical cancer do not attain the 5-year relative survival rate due to late stage diagnosis of the disease. Furthermore, less than 20% of cervical cancer cases are diagnosed histologically with squamous cell carcinoma being the most common type reported in The Gambia (Wall *et al.*, 2005).

Table 1.2 Number of cervical cancer cases, ASR per 100,000 person-years with 95% confidence intervals of cases by period: The Gambia, 1990-2009

Periods	Total female cancer cases n *(%)	**ASR (95% CI)
1990 -1994	199 (31)	13.9 (11.8-16.0)
1995 -1999	361 (34)	20.2 (17.9-22.4)
2000 – 2004	276 (30)	13.7 (11.9-15.4)
2005 – 2009	418 (34)	19.1 (17.2-21.1)
Total (1990 – 2009)	1,254 (33)	16.9 (15.9-17.9)

n *(%) = Number of cervical cancer cases and Percentage of total female cancer cases in period;
 **ASR = Age-standardized incidence rate per 100,000 person-years (Standardized to the World Standard Population) (Bah *et al.*, 2013).

1.6.4 Cervical cancer prevention and control in The Gambia

Currently, The Gambia has not fully established integrated cervical cancer prevention and control services. At present, there is no cervical cancer screening policy as recommended by the World Health Organization and secondly, due to limited resources, the country is unable to embark on a population-based screening. There is an “ad-hoc” cervical cancer screening program in some parts of the urban area of the country using the direct visual inspection with acetic acid (VIA) method. The SOS Mother and Child clinic is a private clinic situated in the Kanifing Municipality Council (KMC) and was the first clinic to offer cervical cancer screening using VIA in the Gambia. The Gambia Ministry of Health and Social Welfare (MOHSW) with assistance from developing partners has recently introduced VIA screening in few government health facilities with foresight to expand VIA services across the country by 2020 (Cervical cancer strategic plan 2016 - 2020).

In 2014, the Gambia embarked on a 2 year HPV vaccination school-based demonstration project in the urban West Coast Region (WCR) of the country, supported by GAVI. A two-dose delivery schedule of the quadrivalent HPV vaccine was administered to girls aged 9 -13 years old as recommended by the WHO. It is envisaged that the quadrivalent vaccine will be scaled-up and integrated into the national immunisation program by 2019.

1.6.5 Global burden of cervical cancer

Approximately 70% of the global cervical cancer burden occurs in developing countries, accounting for almost 12% of all cancers in women. In comparison, cervical cancer accounts for <1% of all cancers in women in developed countries (de Martel *et al.*, 2017). Countries with a cervical cancer age-standardised rate (ASR) greater than 30 per 100,000 per person are found in sub-Saharan Africa (Fig 1.3) (de Martel *et al.*, 2017; Ferlay *et al.*, 2013). In developed countries that have introduced the HPV vaccine and cervical cancer screening with wider coverage, the incidence of cervical cancer and mortality are less than 10 per 100,000 per person (Crosbie *et al.*, 2013). The absence of HPV vaccination and effective cervical cancer screening and treatment in the developing countries may increase the annual global cases from 530,000 to 1 million by 2050, because of a worldwide increase in the number of young women (Ferlay, 2001). Although HPV 16 and 18 accounts for 70% of cervical cancer, the six most

common HR-HPV types found in cervical cancer after HPV 16/18 are: HPV31/33/35/45/52/58. These other HR types and HPV 16/18 together account for approximately 90% of all cervical cancers, globally (Table 1.3). Cervical cancer causes nearly 3 million years of life lost and the burden is observed more in reproductive age women, globally (WHO, 2017). Mortality due to cervical cancer does not only affect families but it also has devastating effects on societies and economies. Reproductive age women that are affected are normally in their mid-adult lives, working and raising a family as well as contributing to the socio-economic development of their societies.

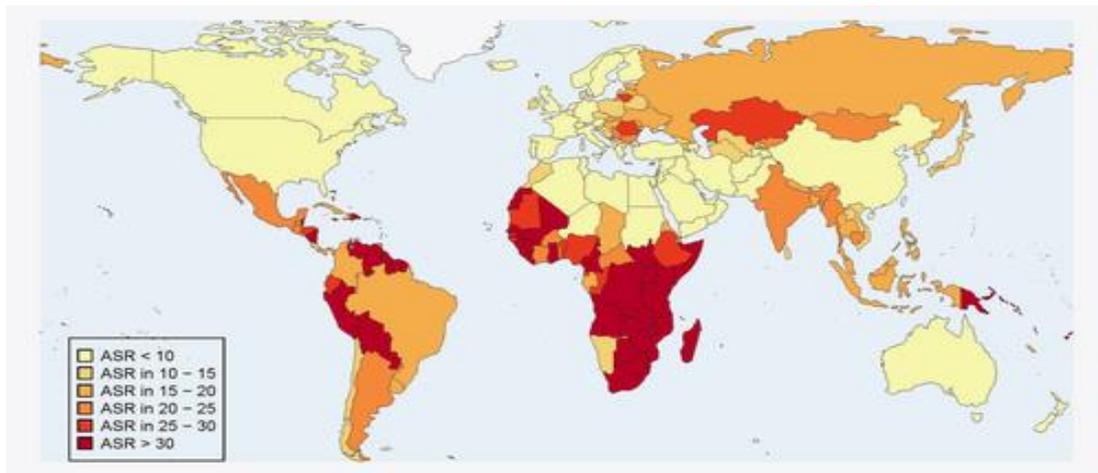


Figure 1.3 Age standardised cervical cancer incidence rates associated to HPV infection, worldwide.

Cervical cancer mortality age standardised rate varies 18-fold between countries ranging from less than 2 per 100,000 per women in developed countries to greater than 30 per 100,000 per in sub-Saharan Africa and some developing countries (dark red coloured areas) (de Martel *et al.*, 2017; Ferlay *et al.*, 2013).

Legend: ASR – Age Standardised Rate

1.7 HPV-associated non-cervical anogenital cancers: vulva and vaginal cancers

Although the global cervical cancer incidence is higher than vulvar and vaginal cancers, global cases of vulvar carcinoma and vaginal cancer have been estimated to be approximately 8,500 and 12,000, respectively (de Martel *et al.*, 2017; Plummer *et al.*, 2016). The highest incidence of these cases was reported in more developed countries, specifically in Europe (Table 1.3). A meta-analysis of data in 14 countries has shown that the age range of diagnosis for both diseases was between 45-82 years old (Bosch *et al.*, 2007).

1.7.1 Anal and penile cancers

Unlike vulvar/vaginal cancers, anal and penile cancers have been reported to account for 35,000 and 13,000 of cancer cases associated with HPV infection, worldwide (Alemany *et al.*, 2016; Serrano *et al.*, 2015). The global distribution of anal cancer is seen more in females

(51%) than in males (48%). However, it is more frequent in men in less developed countries than in developed countries (Table 1.3). The incidence of anal cancer has been reported in populations that have a large proportion of men who have sex with men (MSM) (de Martel *et al.*, 2015; Goldstone *et al.*, 2011; Parkin and Bray, 2006). The anus is susceptible to infection with HPV because it has a transformation zone like those observed in the cervix (Heideman *et al.*, 2007). Associations between anal cancer and HR-HPV infections, especially with HPV 16 in HIV-positive MSM have been documented (de Martel *et al.*, 2015; Goldstone *et al.*, 2011; Hoots *et al.*, 2007). Cancer of the penis is rare and accounts for approximately 0.5% of all cancer cases in men, but 21% of HPV-associated cancers in men, globally (Parkin and Bray, 2006; Ferlay *et al.*, 2013). The highest incidence rates of penile cancer cases have been reported in India (3,200), Europe (2,700) and Latin America (2,000) compared to sub-Saharan Africa (1,000) (Table 1.3).

Male circumcision has been documented to have contributed to HPV clearance and a decreased risk of penile cancer cases seen in countries such as North Africa and some parts of Asia (Lu *et al.*, 2009; Castellsague *et al.*, 2002). However, the role of male circumcision in preventing HPV infections in their female partners is still controversial.

Table 1.3 Number of cancer cases caused globally by human papillomavirus by region, cancer sites and gender

Region	Cervix	Vulvar/Vagina	Anus		Penis	Head and Neck		Attributed to HPV	AF (%)
	F	F	M	F	M	F	M	both sexes	both sexes
Africa									
sub-Saharan Africa	93,000	2,100	1,000	1,200	1,000	150	360	99,000	15.8
Northern Africa/ Western Asia	10,000	650	430	350	70	80	240	12,000	2.2
Asia									
India	120,000	2,800	2,600	1,900	3,200	1,000	5,600	140,000	13.8
Other central Asia	29,000	460	490	410	30	300	760	31,000	6.3
China	62,000	1,600	5,900	3,600	1,300	270	950	75,000	2.5
Japan / Republic of Korean	13,000	460	600	560	250	350	1,500	16,000	1.8
Other Eastern Asia	54,000	1,000	550	530	1,100	280	1,000	59,000	6.2
America									
Latin America	69,000	2,500	1,000	1,900	2,000	280	980	78,000	7.1
Northern America	14,000	3,300	1,800	2,700	1,100	1,900	7,000	32,000	1.8
Europe									
Europe	58,000	5,100	2,700	4,200	2,700	2,800	11,000	87,000	2.5
Oceania									
Australia /New Zealand	940	150	150	190	50	80	290	1,900	1.3
Other Oceania	1,300	30	10	10	10	10	30	1,300	11.1
Less developed countries	370,000	8,300	10,000	7,600	6,800	2,100	8,600	410,000	6.7
More developed countries	160,000	12,000	6,800	10,000	6,100	5,500	22,000	220,000	2.8

* Attributable fraction (AF) for HPV infections is the proportion of cancer cases that would not have occurred if HPV had been totally absent from the population.

(de Martel *et al.*, 2017; Ferlay *et al.*, 2013)

1.7.2 Head and neck cancers

The burden of head and neck cancers varies widely, and this variability is associated with alcohol and tobacco use. Approximately half a million cases and more than 200,000 deaths were reported in 2002, with the greatest burden seen in developing countries (Parkin, 2006). Recently, a report attributed 38,000 cases of head and neck cancers to HPV infection with 72% of cases observed in the developed countries (de Martel *et al.*, 2017; Castellsague *et al.*, 2016; Ferlay *et al.*, 2013). In comparison to the global relative contribution of HPV 16/18 in cervical cancer (70%), HPV 16/18 are responsible for nearly 85% of head and neck cancers (Table 1.4).

Three cancer sites in the head and neck region have been associated with HPV infection; namely: the oropharynx, oral cavity, and the larynx. HPV-related head and neck cancers are mainly represented by oropharyngeal cancer for which consistent evidence has indicated that two main types exist: one that is caused by HPV and the other is associated with tobacco and alcohol intake (Rietbergen *et al.*, 2014; Marur *et al.*, 2010). The incidence of oropharyngeal cancer has been increasing over the past two decades in some high-income countries. This may be due to an increase in HPV infection possibly because of more oral-genital sex and an increase in the number of lifetime sexual partners (Chaturvedi *et al.*, 2011; Gillison *et al.*, 2008).

Table 1.4 Global relative contributions of HPV risk types by invasive cancer at different anatomical sites, 2012

Sites	Number attributed to HPV	Relative contribution of HPV 16/18		Relative contribution of HPV 6/11/16/18/31/ 33/45/52/58	
		Number	Percent	Number	Percent
Cervix	530,000	370,000	70.8	470,000	89.5
Head and neck	38,000	32,000	84.9	34,000	89.7
Anus	35,000	30,000	87.0	33,000	95.9
Penis	13,000	9,100	70.2	11,000	84.6
Vagina	12,000	7,400	63.7	9,900	85.3
Vulva	8,500	6,200	76.2	7,400	87.1
Total HPV sites in women	570,000	410,000	71.4	510,000	89.6
Total HPV sites in Men	60,000	50,000	82.3	55,000	90.4
Global total HPV sites	630,000	460,000	72.4	570,000	89.7

HPV infection accounts for 630,000 cancer cases globally in both men and women with the highest burden seen in women, 2012. The relative contribution of the nonavalent vaccine was nearly 90% compared to the bivalent vaccine (HR-16/18) 72%, therefore making it more suitable against cervical cancer prevention, globally (de Martel *et al.*, 2017).

1.7.3 Anogenital warts

Anogenital warts or condyloma acuminata are commonly found in the cervix, vagina, vulva or perianal and anal in females. In men, the penis, scrotum and perianal and anal regions are the most common sites for condyloma acuminata (Moscicki *et al.*, 2005). The prevalence of genital warts is estimated to be within the range of 0.2-13% depending on the age distribution and the risk of STI in the study population (Patel *et al.*, 2013). Genital wart prevalence also varies by region; however, infection risk is higher in the young and adolescent population (Koshiol *et al.*, 2004). The incidence of genital warts peaks in women aged 15-24 years old, and in 20-29 years old men. A sharp decline in incidence rate is observed in women after 29 years old but

remains high in men until the age of 40 years (Hoy *et al.*, 2009). All anogenital warts are associated with an HPV infection with HPV types 6 and 11 found in 90% of cases, globally. Nearly 50% of women infected with HPV 6 or 11 will develop genital warts after a year of exposure to the virus with an average time of 2 - 3 months (Garland *et al.*, 2009; Kjaer *et al.*, 2007). More than twenty-five percent of HPV positive individuals with genital warts will spontaneously clear the infection; however, recurrence may occur in 30% of cases, irrespective of whether initial clearance occurs spontaneously or with treatment (Wiley *et al.*, 2002). Since genital wart infections may persist or recur, there is a greater risk of an increase in HPV transmission in communities (Giuliano *et al.*, 2011). However, not all individuals infected with HPV 6 or 11 will develop genital warts (Lacey *et al.*, 2006). The primary risk for anogenital wart is linked to sexual activity with an infected partner; a high number of sexual partners, anal sex and infection with an STI (Goldstone *et al.*, 2011; Giuliano *et al.*, 2011). Anal condyloma is a clinical manifestation of HPV 6 or 11 infections in the anal canal and on the perianal skin, but HPV 16 and 18 may also be found in the lesions (Chan *et al.*, 2009). Lesions can be asymptomatic or symptomatic with itching, burning, spontaneous bleeding or bleeding due to anal intercourse (Dunne *et al.*, 2006).

The clinical manifestation of anal condyloma may differ between HIV-positive and HIV-negative individuals. HIV-positive individuals are prone to infection with multiple HPV genotypes due to their immunosuppression status. HPV 16 and 18 have been detected in anal and giant condyloma (Bushke-Löwenstein tumours) of HIV-positive individuals, which exposes them to a greater risk of developing high grade anal intraepithelial neoplasia (AIN) that can progress to invasive anal cancer (Anderson *et al.*, 2004; Byars *et al.*, 2001).

1.8 Prevention of HPV infection

1.8.1 HPV prophylactic vaccines: A primary prevention

The HPV vaccine has the potential to compliment cervical cancer screening in the prevention of HPV infection and the control of cervical cancer morbidity and mortality, worldwide. Currently, three recombinant HPV prophylactic vaccines are commercially available: these are Cervarix[®], a bivalent HPV-16/18 vaccine; Gardasil[®], a quadrivalent HPV-6/11/16/18 vaccine; and Gardasil[®] 9, a nonavalent HPV6/11/16/18/31/33/45/52/58 vaccine. All three vaccines are prepared from

virus-like particles (VLP) using recombinant technology (Zhou *et al.*, 1991; Hagensee *et al.*, 1993). The preparation of these vaccines is based on HPV L1 protein forming virus-like particles (VLP) when expressed alone in a variety of cell types (e.g *Saccharomyces cerevisiae*) that are highly similar to the HPV virus both morphologically and antigenically (Kimbauer *et al.*, 1992). The purified L1 protein does not contain viral genetic material or attenuated live biological products; therefore, it cannot multiply and is not infectious (Table 1.5).

Table 1.5 Characteristics of human papillomavirus prophylactic vaccine

	Bivalent	Quadrivalent	Nonavalent
Manufacturer & trade name	GlaxoSmithKline, Cervirax®	Merck, Gardasil /Silgard®	Merck, Gardasil 9®
Virus-like particle types	16,18	6,11, 16, 18	6, 11, 16, 18, 31, 33, 45, 52, 58
Substrate	Baculovirus expression system	<i>Saccharomyces cerevisiae</i> (Baker's yeast)	<i>Saccharomyces cerevisiae</i> (Baker's yeast)
Adjuvant	Amorphous aluminium hydroxide (500 µg) and 50 µg 3-deacylated monophosphoryl lipid A (GSK ASO4 adjuvant)	Amorphous aluminium hydroxyphosphate sulphate (225 µg) (Merck aluminium adjuvant)	Amorphous aluminium hydroxyphosphate sulphate (500 µg) (Merck aluminium adjuvant)
Schedule*: 3 doses at interval of	1 month between doses 1 and 2; 6 months between doses 1 and 3	2 months between doses 1 and 2; 6 months between doses 1 and 3	2 months between doses 1 and 2; 6 months between doses 1 and 3

*The World Health Organisation Strategic Advisory Group on vaccination made some recommendations of reducing the three-dose schedule to a two-dose after reviewing new research findings. The new research findings indicated that no immunogenicity difference was observed between two doses in adolescent to that of three doses in young adults (Romanowski *et al.*, 2015; Dobson *et al.*, 2013). The recommended dose schedules are as follows: 0 and 6 months for the first dose, and 12 months for the second dose for females 9 -14 years old (WHO, 2017). Countries under the GAVI vaccine alliance have switched their vaccine programmes to the recommended schedule. However, a three-dose schedule over 6 months is recommended for those aged 15 years or older and those that are immunocompromised (Meites *et al.*, 2016).

1.8.2 HPV prophylactic vaccines: mode of action

The mechanisms by which the HPV vaccines prevent HPV infections have not been fully defined. The primary mechanism of action in which the HPV virus-like particle vaccine prevents HPV infections caused by the targeted genotypes is by eliciting the production of serum neutralising anti-HPV IgG antibodies from the capillaries to the basal epithelial mucosa cells. These neutralising antibodies bind to the virus particles and block their entrance into the host cells (Joura *et al.*, 2015; Villa *et al.*, 2005; Harper *et al.*, 2004). Protection from HPV infection can also be mediated by exudation of the serum anti-HPV IgG onto the genital mucosal surface after the formation of micro-abrasions, which activate the innate inflammatory immune responses thereby facilitating the recruitment and migration of the immune cells to the site of inflammation (Stanley *et al.*, 2014).

1.8.3 HPV vaccine protection against infections

Viral vaccines protect against infections that have a bloodstream phase such as hepatitis B, rubella and measles, therefore antibodies developed after exposure to these viruses are sustained and are good markers of infection (Stanley, 2006). In contrast, HPV infection does not have a bloodstream phase as the infection occurs at the mucosa. The antibody response after natural infections does not protect against subsequent infections as the responses are slow to develop and of low titre and avidity (WHO, 2017; Stanley, 2006). A long-term follow-up following administration of the HPV vaccines to pre-sexual adolescent women has shown that antibody levels remained stable up to 6 years in women with no prior infection with HPV (Dasbach *et al.*, 2007, Gall *et al.*, 2007). Similarly, women with prior or ongoing infection with one of the targeted quadrivalent vaccine genotypes were found to have a higher immune response to that genotype they were infected with prior to vaccination (Villa *et al.*, 2006). This may suggest that vaccination enhances the antibody levels generated by natural infection. HPV vaccination with both the bivalent and quadrivalent vaccines have been shown to induce an antibody response that is moderately (60 -78%) capable of neutralising infections caused by closely related HPV genotypes (Kavanagh *et al.*, 2014; Wheeler *et al.*, 2012; Smith *et al.*, 2007). This could be due to cross neutralising epitopes and homology between the related species, for example, HPV16 and HPV31 genotypes (Drolet *et al.*, 2015;

Wheeler *et al.*, 2012). Although detectable neutralising antibodies have been observed 10 years after post-vaccination, no data on antibody persistence beyond 10 years has been reported (Garland *et al.*, 2016). These vaccines cannot eliminate pre-existing cervical lesions since the target antigens (L1) major capsid proteins are not expressed in infected basal epithelial cells (Hildesheim *et al.*, 2016). However, they have been shown to be efficacious in the prevention of precancerous lesions such as CIN1/2 and genital warts by preventing initial infections (Dominiak-Felden *et al.*, 2015; Ali *et al.*, 2013).

1.8.4 Global HPV vaccine implementation

The first HPV prophylactic vaccine was licensed in 2006 and since then two other vaccines (quadrivalent and nonavalent) have been developed. All three vaccines have proven to be safe, highly immunogenic and induce protection against HPV infections and its associated-diseases (Sankaranarayanan *et al.*, 2016; Drolet *et al.*, 2015; Schiller *et al.*, 2012). In 2009, the WHO recommended the inclusion of the HPV vaccine into national immunisation programs to prevent the burden of HPV-related cervical cancer (WHO, 2009). The HPV vaccines have been introduced in more than 85 countries in the past 12 years as a part of their national immunisation program, worldwide. More than 80% (74) of these countries are in high-income (HIC) and upper-middle-income countries (UMIC). In contrast, only 12 countries (13%) in resource-limited countries have a national HPV vaccination program (LaMontagne *et al.*, 2017). Most of the national HPV vaccination in resource-limited countries e.g Rwanda in 2011/2012, Bhutan (2010), Vanuatu (2013) and South and Central America (2017) were facilitated through donor agencies such as pharmaceutical companies, cervical cancer foundations and the Pan American Health Organisation (PAHO) (Gallagher *et al.*, 2018; LaMontagne *et al.*, 2017).

Although the proportion of resource-limited countries with a national HPV vaccination program is 13%, thirty-one countries have carried-out HPV vaccine demonstration or small scale pilot projects, from 2013-2016 (LaMontagne *et al.*, 2017; Brotherton and Bloem, 2015) (Figure 1.4). Sexually inexperienced females, within the ages of 9 -14 years benefitted from these HPV pilot projects. In addition, countries like Uganda, Senegal and The Gambia were funded through the Global Alliance for Vaccine and Immunization (GAVI) projects (GAVI alliance, 2014). Some of these countries are yet to introduce the vaccine in their national

immunisation programs. However, more than ten GAVI eligible funded countries have been granted approval to include the HPV vaccine in their national immunisation programs by 2019.

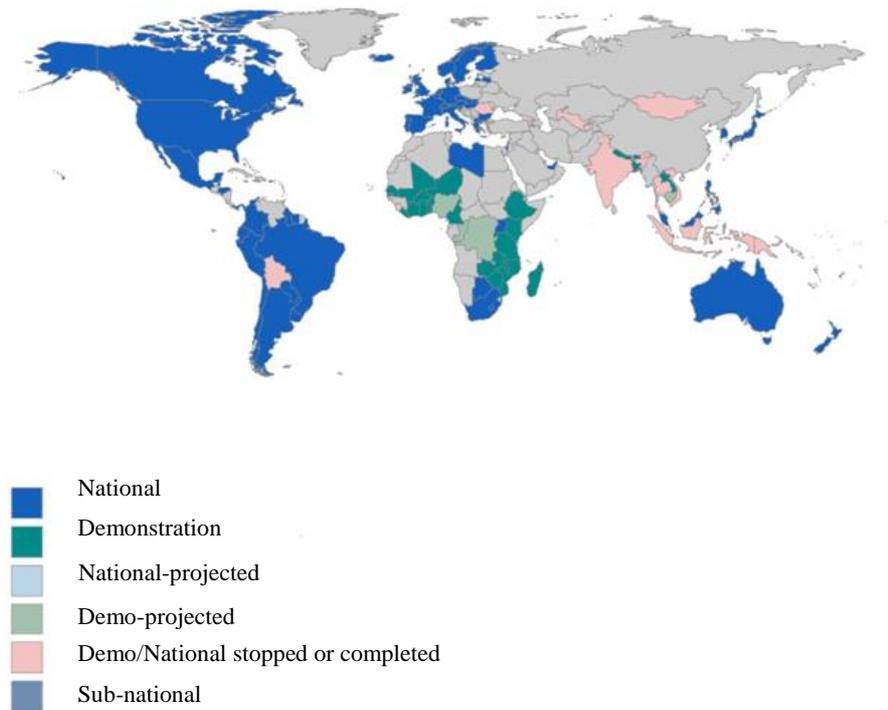


Figure 1.4 Global status of HPV vaccine implementation in 2016

HPV vaccine implementation is higher in high- and upper-income countries compared to sub-Saharan Africa where more than 80% of cancer cases are reported. In some countries, demonstration was stopped due to the ending of the GAVI project funding (pink colour coded) (LaMontagne *et al.*, 2017).

1.8.5 HPV vaccine coverage and effectiveness

High vaccine coverage in a target population is achieved by vaccinating adolescent females prior to their first sexual encounter. Targeting this cohort has shown the greatest impact of these vaccines (WHO, 2016). Some countries have implemented catch-up programs for older females together with the routine school-based 9 -14 years programs to increase overall population coverage (Brisson *et al.*, 2016). A successful national HPV vaccination coverage greater than 80% was achieved in some developed countries with high-income, for example, Scotland and Australia

(Sinka *et al.*, 2014; Brotherton *et al.*, 2011). HPV national vaccination coverage of more than 80% was also achieved by Bhutan and Rwanda (Dorji *et al.*, 2015; Binagwaho *et al.*, 2013). The success of this approach could be the introduction of a catch-up vaccination program (Scotland, Australia and Bhutan) targeting females up to 26 years old, and in the case of Bhutan, 18 years old (Brotherton *et al.*, 2016; Dorji *et al.*, 2015). In contrast to Scotland or Australia, less than 50% vaccine coverage was achieved by Japan as their vaccination program was suspended in 2013 due to negative media reports on vaccine safety (Tanaka *et al.*, 2016; Larson *et al.*, 2014). A similar negative social media report on vaccine safety was also reported in Ireland, which resulted in a decrease in vaccine coverage from 86% (in 2014) to 50% in (2016). The Global Advisory Committee on Vaccine Safety (WHO) and the European Medicines Agency (EMA) maintained a vigilant post-licensure survey and have confirmed the continued safe use of the vaccine (EMA, 2016; WHO, 2015).

The recent development on the administration of the HPV vaccine in boys 12-13 years old in some high-income countries for example in Australia, America, Demark and in England (from next year) is anticipated to reduce the burden of genital HPV infections and increase herd immunity amongst the population (Tabrizi *et al.*, 2014). Vaccination of boys alongside girls would reduce the non-cervical diseases that can have serious morbidity, healthcare, and socio-economic burdens. However, there is much controversy surrounding the cost-effectiveness of HPV vaccination in boys. In America, the burden of non-cervical diseases was estimated to be 8% compared to 92% of cervical diseases, thereby the cost of vaccinating boys was reported as not being cost-effective (Hu and Goldie, 2008). Similarly, In the United Kingdom, it was highlighted that achieving a high HPV vaccine coverage of over 75% in girls and young adults is more cost-effective than vaccinating boys (Cuschieri., 2009). Herd immunity studies in Australia have shown a reduction in genital warts in both vaccinated women and their heterosexual partners (Machalek *et al.*, 2017; Tabrizi *et al.*, 2014). This shows further that vaccinating females may lead to a reduction of HPV-associated diseases in men over time. However, others have argued that if the primary objective of HPV vaccination is to eradicate HPV infections, a gender-neutral vaccination approach should be taken as MSM would not benefit from a female-only vaccination program (Smith and Canfell, 2014). A gender-neutral approach may not only reduce HPV-related diseases in males but will also reduce HPV infection and transmission to females. This will eventually result in a reduction in the pool of circulating vaccine-typed infectious virus in a community (Lee and Garland, 2017). Despite, the controversies surrounding the cost-

effectiveness of vaccinating boys, many high-income countries have started offering the vaccine to boys as it has been demonstrated that the benefit in reducing HPV infections by vaccinating both genders outweigh the cost (Machalek *et al.*, 2017).

In 2014, only 1% of young females ages 10-20 years living in resource-limited countries were reported to have been fully vaccinated (Bruni *et al.*, 2016). Although there may be an increase in HPV vaccine coverage since then, the availability of HPV vaccine in resource-limited countries is still reported to be low. Therefore, it may take several decades for these countries to include boys in their vaccination programs.

1.9 Methods for detecting genital HPV infections by screening

Secondary cervical cancer prevention refers to screening of women at risk of cervical cancer, most of whom are without symptoms, with the aim of detecting and treating precancerous changes, which may, if not treated, lead to cancer (WHO, 2014).

1.9.1 Detection of HPV by nucleic acids

The HPV genomes and its transcripts can be directly detected using hybridisation procedures such as Northern and Southern blots, *in-situ* hybridisation, dot blots, Hybrid Capture™. HPV DNA and RNA can also be detected using PCR techniques and genotypes identified by DNA sequencing.

1.9.2 HPV DNA detection and sequencing

Several screening methods are now available to identify women that are at risk of developing cervical cancer. However, an ideal clinical test should be able to correctly identify all women who have cervical diseases (sensitivity) and those who do not have cervical diseases (specificity). Therefore, ideally, a cervical cancer screening test should have a sensitivity of 100% to detect all true positive cases, however, this is rare. The two most common methodologies widely used for the detection of genital HPV are the Hybrid Capture™ II and PCR with consensus primers. Both assays have equivalent sensitivities (> 90%) and specificities (> 80%) and are suitable for high throughput testing, and for automation processing; therefore, they are both useful for large epidemiological studies and in clinical settings (Söderlund-Strand *et al.*, 2005). However, many of these techniques have their advantages and disadvantages in detecting genital HPV. The

consensus primers target the highly conserved region of the HPV *L1* gene and have the potential to detect more than 30 HPV types. They are widely used in PCR protocols to detect genital HPV and the most common types used are: single consensus primers GP5/6 and its modified version GP5+/6+ that amplifies 150 bp (base pair) fragment of the *L1* gene, multiple primers MY09/11 and its modified version PGMY09/11 (450 bp) and SPF, which amplifies a smaller fragment (65 bp) of the *L1* gene. (Gravitt *et al.*, 2000; Jacobs *et al.*, 1995; de Roda Husman *et al.*, 1995). Although these consensus primers can detect a broad range of HPV types and are used in many epidemiological studies, they may differ considerably in their ability to amplify specific HPV types in a multiple infections (Söderlund-Strand *et al.*, 2009), which contributes to discordant results in HPV prevalence studies.

Currently, there are many commercial PCR-based HPV detection kits available. These include the Amplicor™ which detects 13 HR-HPV, linear array HPV genotyping detects 37 HPV genotypes (13 HR and 24 LR), Inno-LiPA assay detects 32 HPV types, and the Hybrid Capture™2 identifies 13 HR-HPV. However, these test kits are expensive and do not discriminate between all HPV genotypes making them not cost-effective to use in resource-limited countries.

On the other hand, DNA sequencing has the advantage of identifying multiple HPV genotypes present in clinical samples if consensus primers are used for PCR and the amplicons cloned into a plasmid or directly sequenced (Gharizadeh *et al.*, 2003). Although DNA sequencing has the advantage of identifying multiple HPV types, direct sequencing has been shown to be unsuitable in detecting multiple HPV types in a sample as it only shows the overexpressed type (Vernon *et al.*, 2000).

1.9.3 HPV RNA detection

The detection of HPV viral RNA transcripts has also been employed to evaluate the expression of *E6/E7* HPV genes in infected cells by using reverse transcriptase PCR (RT-PCR) and quantitative (q) RT-PCR (Culp and Christensen, 2003). Furthermore, studies have shown that the detection of *E6/E7* transcripts of HR-HPV can show the persistent HPV type and it is more specific than HPV DNA detection in identifying individuals who developed high-grade cervical disease (Cuschieri *et al.*, 2004(a); Molden *et al.*, 2005). In addition, a strong correlation was seen between integrated HR-HPV transcript detection and the development of high-grade cervical lesions using HPV RNA studies (Klaes *et al.*, 1999). HPV RNA testing in biological samples is

hindered by the easy degradation of RNA compared to DNA especially after long storage; therefore, it may not be suitable for epidemiological studies (Habis *et al.*, 2004).

1.9.4 HPV screening by non-molecular techniques

Unlike nucleic acid-based methods, non-molecular techniques used in the detection of HPV infections do not detect the presence of HPV but the clinical sequelae of HPV infection. Cytology and histology testing are widely used in the detection of precancerous and cancer cells in the cervix of females. In the 1970s, cytological and histological investigations associated the presence of koilocytes in cervical smear with HPV infection (Torre *et al.*, 1978). The presence of koilocytes in histological sections may sometimes be difficult to interpret due to either the presence of fixation artefacts or poor dehydration techniques, which both can result in the presence of cells with peri-nuclear halos giving the cells a ‘koilocyte-like’ appearance (Wright *et al.*, 2002).

1.9.5 Papanicolaou (Pap) smear

The cytology-based technique most commonly used especially in resource-limited countries is the Papanicolaou smear (Pap smear). The Pap smear can show the presence or absence of abnormal cervical cells consistent with the histological diagnosis of cervical dysplasia or cancer. Although the Pap smear plays an important role in the initial success in reducing cervical cancer incidence, the clinical performance of the technology is not without limitations. The Pap smear test has been shown to have a false negative rate between 12 - 33% due to an inadequate sampling of the transformation zone and /or poor fixation of cervical cells (Hartmann *et al.*, 2002). The inclusion of inflammatory and necrotic materials or blood with cervical cells during collection has been shown to lead to inaccurate cytopathological diagnosis (Koss, 1993). Studies have also revealed that the Pap smear test has a sensitivity range of 30 - 86% in detecting high-grade cervical lesions and more than 20% of women diagnosed with cancer had a negative Pap smear results (Gibb *et al.*, 2011; Koss, 1989).

1.9.6 Liquid-based cytology

The limitations of the Pap smear technique led to the introduction of the liquid-based cytology (LBC) in 1996, which is now widely used in many developed countries. Unlike the Pap smear

technique, where cervical cells are directly spread on a microscopic slide, the LBC technique employs the use of an FDA-approved cervical collection device and transport medium with preservative. The improvement in sample quality has increased the clinical sensitivity of LBC over Pap smear in the detection of high-grade lesions or cervical cancer from 86% to 93% (Chinaka *et al.*, 2014; Claver *et al.*, 2001). In addition, the automated slide processing of the samples is thought to minimise obscuring artefacts such as blood cells that hinder cytology result interpretation (Chinaka *et al.*, 2014). The LBC technique has been demonstrated to have greater sensitivity in detecting glandular lesions /adenocarcinoma than the Pap smear (Wang *et al.*, 2002; Schorge *et al.*, 2002). However, a meta-analysis carried out on LBC technologies has shown that there was no significant difference between LBC performance over the use of Pap smear (Arbyn *et al.*, 2008; Davey *et al.*, 2006). Despite the benefits shown by LBC, studies on cost-effectiveness have shown the cost of LBC is very expensive compared with the Pap smear, which may hinder its use as a screening method in many resource-limited countries (Chinaka *et al.*, 2014; Denny *et al.*, 2006).

1.9.7 Direct visual inspection

In resource-limited countries, cervical cancer incidence and mortality have not been decreased by cytology screening and early treatment compared to in high-income countries. This may be due to several barriers such as lack of specialised equipment and enough technical staff with the necessary skills, several health-care visits before treatment is achieved, which patients may be loss to follow-up and limited health budget from national budgets. These challenges prompted the WHO to propose screening using the visual cervical inspection method as an alternative to cytology screening in resource-limited countries.

The direct visual inspection (DVI) employs the use of either diluted solution of acetic acid (VIA) or Lugol's iodine (VILI) in the cervix. The VIA method involves washing the cervix with diluted 3 - 5% acetic acid and directly examined for aceto-whitening after 1 minute. The epithelial cells of the cervix will appear aceto-white if infected with mucosal HPV (both HR and LR types). The aceto-whitening is a result of a reversible coagulation that occurs to the intracellular proteins, upon acetic acid application. The intracellular proteins are found in high amounts in neoplasia and produce dense aceto-whitening when 3-5% acetic acid is applied (Sankaranarayanan *et al.*, 2007; Denny *et al.*, 2006). Although inflamed and regenerating cervical epithelial cells can also become aceto-white with acetic acid, however when associated with

CIN is well defined because it is usually densely opaque and localised in the transformation zone (Gravitt *et al.*, 2010). In comparison with the VIA, the VILI stains the abnormal lesions mustard-yellow and the glycogen that is found in cervical epithelial cells is stained darker by the iodine. However, the iodine partially stains areas of immature squamous metaplasia, neoplasia or condyloma or not at all (Sankaranarayanan *et al.*, 2004a). The application of DVI as a screening tool in cervical cancer prevention has been evaluated in several large clinical trials; these have found VIA to have sensitivity values of approximately 70% and most reported low specificities (Bhatla *et al.*, 2007; Cronjé *et al.*, 2003; Denny *et al.*, 2002). In addition, all these studies have reported low negative predictive values, which is very important for national screening programmes (Bhatla *et al.*, 2007; Cronjé *et al.*, 2003; Denny *et al.*, 2002). Comparison studies carried out in India and Africa on VIA with VILI, have found VILI to have a greater sensitivity than VIA, however, both methods have equivalent specificity (Sankaranarayanan *et al.*, 2004 (a); IARC, 2005). Most evaluation trials on the efficacy of VIA in cervical cancer screening have used CIN 2 or 3 and cancer as the outcome measure, which can lead to study bias as only women positive for VIA are referred for colposcopy. In several publications, it has been argued that the use of CIN 2 as a measure for VIA efficacy is inconsistent and unreliable as majority of CIN 2 lesions regress spontaneously, therefore it would be more effective to use CIN 3 or CIN3+ to evaluate efficacy of VIA screening (Mittal *et al.*, 2014; Moscicki *et al.*, 2010).

Most resource-limited countries have adopted the use of VIA for cervical cancer screening due to its low cost, ease of use and the test can be performed by a trained primary health care provider. The test results are obtained immediately making treatment of abnormal cervical lesion possible during the same visit (Gravitt *et al.*, 2010; Sankaranarayanan *et al.*, 2004(b)). However, the VIA screening has also been shown to have some limitations and one of its major limitations is its low positive predictive values (IARC, 2007; Bhatla *et al.*, 2007). In the study of Basu *et al* (2015), 92.8% of women who had a positive result with VIA had a normal cervix or low-grade lesions (CIN 1) on colposcopy/biopsy. A similar finding was reported in HIV positive women with metaplastic changes, flat condyloma, low-grade lesions and even with cervicitis having a false positive result with the VIA test (Denny *et al.*, 2002). Another limitation of the VIA is that there is no standardised quality control in place, which is very important due to the subjective nature of the test. This can cause over referrals for treatment or colposcopy, which is

used to evaluate women with abnormal cytological results. Despite the limitations highlighted above, DVI may be best suited screening option for developing countries.

1.10 Treatment of HPV-associated cervical diseases

Currently, there is no treatment for HPV infection; screening and treatment of pre-invasive cervical lesions are the only available options to prevent progression to cervical cancer (WHO, 2013). Cervical pre-cancerous lesions can be treated using ablation methods, which include destroying abnormal tissues by burning or freezing (cryotherapy) or surgically removing the abnormal tissue (cone biopsy or loop electrosurgical excision procedure [LEEP]). In resource-limited countries, where cervical cancer screening programmes are available, cryotherapy is most commonly used for the treatment of pre-cancerous lesions (WHO, 2011). However, when the lesion is large, LEEP is mostly used to surgically remove the affected tissue.

Treatment of invasive cervical cancer is mainly through radiotherapy, but the combination of chemotherapy with radiotherapy has been shown to be more effective in terms of survival rate than radiotherapy or surgery alone (Ryu, 2002). Chemotherapy with platinum compounds such as cisplatin can be used in conjunction with surgery or radiotherapy or can be used alone as palliative care in advanced or recurrent disease. Depending on the stages of the disease, cone biopsy to simple or radical hysterectomy and pelvic lymphadenectomy may be performed (Shepherd *et al.*, 2001). However, dual therapy such as surgery and radiotherapy are more expensive, and the risk of complication is known to be greater than a single radical treatment (Waggoner *et al.*, 2003).

1.11 Sexually Transmitted Infections (STIs)

Sexually transmitted infections (STIs) are of major public health importance as they predominantly affect adolescents and young adults and have complications which constitute a great socio-economic burden. Complications resulting from failure to diagnose and treat infections include pelvic inflammatory diseases (PID), infertility, ectopic pregnancy, chronic pelvic pain and cervical cancer. The impact on fetuses and newborns can be devastating, as manifested by miscarriages, stillbirths, neonatal deaths, mental retardation, neonatal conjunctivitis and pneumonia (Yohannes *et al.*, 2013; Boyer *et al.*, 2006).

Currently, approximately half a billion STI cases occur each year, worldwide with a million new infections acquired every day (WHO, 2015). Out of the estimated, 357 million STI cases reported globally for each year, four of the most common STI namely; gonorrhoea, syphilis, Chlamydia, and trichomoniasis account for 78 million, 5.6 million, 131 million and 143 million, respectively. The impact of these infections is increased due to their potential to facilitate transmission and acquisition of HIV infection (WHO, 2014; GDHS, 2013). Studies carried out in African countries with a high HIV prevalence have found that HIV infectiousness is increased by more than 5% per sexual contact if either sexual partner are infected with an STI as compared to 0.2%, if neither partner are infected (Johnson *et al.*, 2012; Boily *et al.*, 2009). *Chlamydia* and *gonorrhoea* are major precursors for adverse reproductive health outcomes and untreated chronic infections account for most health care visits with serious health consequences and additional economic and social implications (Ginindza *et al.*, 2017; Basera *et al.*, 2016).

Global estimates have shown *Chlamydia trachomatis* (*C. trachomatis*) prevalence in women to be 4.2% with a region prevalence range of 1.8% - 7.6%. The highest prevalence was reported in the regions of the Americas (7.6%) and Western Pacific (6.2%) compared to 3.8% reported for the African region. In comparison, a higher global estimate prevalence of other STIs such as trichomoniasis (11.5%), gonorrhoea (1.7%) and syphilis (1.8%) was reported for the African region (Newman *et al.*, 2015; WHO, 2015). Possible contributing factors to this finding could be increased condom used, better socio-economic and clinical STI services in developed countries. The 3.8% *C. trachomatis* global estimates reported for the African region could possibly be because most African countries do not screen for *C. trachomatis* and most infections are asymptomatic, which can also confound the reported prevalence. In addition, a lack of laboratory testing may lead to underestimation of *C. trachomatis* prevalence in Africa compared to European countries that screen and report cases annually.

In recent years, evidence-based studies have emerged associating *Ureaplasma*, *Mycoplasma genitalium* (*M. genitalium*) and *Mycoplasma hominis* (*M. hominis*) with sexually transmitted infections. *M. genitalium* is widely accepted as a 'true' STI pathogen compared to *Ureaplasma* species, which are common commensals found in the lower genital tracts in healthy individuals with a colonisation rate of 40 - 80% in sexually active asymptomatic women and a lesser frequency of 20 - 29% in healthy men (Xiao *et al.*, 2010; Kong *et al.*, 2000). Colonisation of the female urogenital tract with *Ureaplasma* is linked to hormonal status and persistence colonisation can lead to ascending infection from the vagina to the upper reproductive tracts with a variety of

clinical outcomes such as infertility, adverse pregnancy outcomes and bronchopulmonary dysplasia in neonates (Yamazaki *et al.*, 2012; Waites *et al.*, 2005).

Infections with *Ureaplasma* in men are normally subclinical but can lead to epididymitis, spermocystitis, prostatitis and urethritis as well as a complication in spermatogenesis, sperm function and motility (Zhang *et al.*, 2014). *Ureaplasma* associated changes in the sperm membrane are known to induce the production of anti-sperm antibodies, which are associated with a higher risk of infertility (Softer *et al.*, 1990). However, the influence of *Ureaplasma* on sperm function is controversial. A reduction in penetration rate in hamster oocytes was observed when the sperm was infected with *Ureaplasma* in an *in-vitro* study (Guillet-Rosso *et al.*, 1987). A similar finding of a reduction in pregnancy rates has also been reported after *in-vitro* fertilisation (IVF), linking IVF failure to *Ureaplasma* infected sperm (Knox *et al.*, 2003). In contrast to these findings, fertility and pregnancy with IVF were found not to be influenced by the colonisation of the sperm with *Ureaplasma* but endometritis associated with *Ureaplasma* (Kanakas *et al.*, 1991). However, significant spontaneous abortion rates have been observed in women, whose male partners were infected with *Ureaplasma* (Stovall *et al.*, 1993). These findings, therefore suggest a pivotal role for male partners in female infections.

The available evidence regarding pathogenicity and clinical importance of *Ureaplasma* is yet inconclusive (Yamazaki *et al.*, 2012; McKechnie *et al.*, 2011), leading to the controversial debate on routine screening and treatment of the infection in reproductive age women. However, the antibiotics of choice for treating *Ureaplasma* infections include those that inhibit bacterial protein synthesis (macrolides and tetracyclines) and DNA synthesis (fluoroquinolones) as *Ureaplasma* are innately resistant to the beta-lactams, sulfamethoxazole, trimethoprim and rifampin antibiotics (Waites and Taylor –Robinson, 2011). On the other hand, *M. genitalium* is linked to causing non-gonococcal urethritis (NGU) in men (Kim *et al.*, 2011). The organism has also been implicated in causing cervicitis and was isolated in approximately 29% of women with cervicitis attending an STI clinic (Donders *et al.*, 2009). Women with salpingitis and endometritis were found to be unresponsive to Cefoxitin and treatment failure was associated with *M. genitalium* infections (Ekiel *et al.*, 2009 (b); Haggerty *et al.*, 2008). There may have been a study bias in Haggerty *et al.*, (2008) study, as it was a follow-up study on women with PID. Recurrent infections can occur in participants that have engaged in risky sexual behaviours during treatment, confounding treatment failure. Although, a study carried out on Peruvian women with spontaneous preterm birth has shown evidence of an association between *M. genitalium* and

preterm birth (Hitti *et al.*, 2010), its role in preterm birth delivery is less defined. Nonetheless, its role as an STI pathogen in men with NGU and reproductive tract infections in women has been documented (Kim *et al.*, 2011; Hilton *et al.*, 2010).

1.11.1 Association of STIs with HPV infection

Several studies regarding the role of STIs in the development of CIN and invasive cervical cancer in HPV-infected women have been carried out. Several previous studies showed that specific STI pathogens including *C. trachomatis*, herpes simplex virus-2 (HSV-2), *Mycoplasma* species, *Ureaplasma* species, and *Trichomonas vaginalis* (*T. vaginalis*) were associated with HPV infection, CIN, and cervical cancer risk. Although a few studies reported no association between *C. trachomatis*, HSV-2 infections with HPV, CIN, or cervical cancer risk (Bhatla *et al.*, 2013; Dahlström *et al.*, 2011).

C. trachomatis is the most frequently investigated pathogen and association with HPV infection, especially high-risk HPV, CIN, and invasive squamous cervical carcinoma (SCC) have been demonstrated (da Silva Barros *et al.*, 2012; Verteramo *et al.*, 2009). However, no association between *C. trachomatis* with glandular lesions including *in-situ* adenocarcinoma, invasive adenocarcinoma, or adeno-squamous was found; therefore, the role of *C. trachomatis* in cervical carcinogenesis may be restricted to SCC (Quint *et al.*, 2009). According to a meta-analysis on invasive cervical cancer by IARC, HSV-2 co-infection with HPV may increase the risk of invasive cervical carcinoma including both squamous and adenocarcinoma (Smith *et al.*, 2004). In addition, *T. vaginalis*, *Mycoplasma*, and *Ureaplasma* species have been associated with HPV infection and abnormal cervical cytology (Lazenby *et al.*, 2014; Donders *et al.*, 2013; Biernat-Sudolska *et al.*, 2011).

Although the risk association between STI pathogens with HPV is not well understood, it is perhaps a result of an inflammatory reaction at the cervix leading to microabrasion of the epithelial cells. The damage of the epithelial cells allows HPV to have direct access to the basal epithelial cells, where the virus initiates its life cycle. *C. trachomatis* is well known in causing cervicitis and HSV-2 causes periodic ulcer sores, therefore co-infection with these pathogens may induce a more profound inflammatory reaction (Rasmussen *et al.*, 1997). A body of literature has suggested that cervical carcinogenesis may be associated with chronic inflammation (Castle and Giuliano, 2003; Chan, 2002). Furthermore, cyclooxygenase-2 (COX-

2), a prostaglandin G/H synthetase enzyme that is induced in inflammatory cells such as macrophages upon stimulation by cytokines and endotoxin (Oshima *et al.*, 1996; Xie *et al.*, 1992) was found to be expressed at an increased level in human cervical cancer specimens (Kulkarni *et al.*, 2001), this further suggests that inflammation may be linked to cervical carcinogenesis. Prostaglandin E₂ (PGE₂), which is mediated by COX-2 during inflammatory reactions, is a potent inducer of interleukin (IL)-10. Both PGE₂ and IL-10 are known to suppress the cell-mediated T_H1 responses by inhibiting the production of IL-12 by antigen presenting cells (APC) (Harizi *et al.*, 2002). The suppression of IL-12 production during chronic inflammation causes a shift from T_H1 cell-mediated immune response to T_H2 humoral response favouring HPV persistence (Moscicki, 2005). Furthermore, an association has been documented between cervicitis caused by *Ureaplasma* infection and high-grade lesions in women infected with HR-HPV genotypes (Castle *et al.*, 2001). This association may be due to prolonged co-infection, *C. trachomatis* endotoxin, for example, may also promote HPV persistence through a decrease in functional antigen presenting cells in the genital tract; thereby inhibiting cell-mediated immune response. Similarly, an association between HPV and HIV has been established. The recruitment of CD4⁺T cells and macrophages induces inflammatory cytokine production, enhancing the replication and reverse transcription of the HIV virus thus facilitating HIV progression to Acquired Immuno-deficiency Syndromes (AIDS) (Gage *et al.*, 2000).

1.11.2 Diagnosis and management of STIs

The main aim of STI primary control is to interrupt STI transmission and prevent adverse consequences through adequate diagnostics, and early and effective treatment. It has been demonstrated that control of STIs could contribute considerably to reducing the incidence of HIV infection, therefore the syndromic management approach was introduced in resource-limited countries as a secondary prevention control.

STIs are diagnosed in resource-limited countries using the syndromic management approach in which clinical algorithms for commonly presenting signs and symptoms (e.g. urethral discharge or genital ulcer disease [GUD]) are used in case management. The algorithm provides treatment for the commonest biological causes of the syndrome. For example, for GUD, treatment is provided concomitantly for the two commonest causes, chancroid and syphilis. Other STI

syndromic indicators include vaginal discharge, male urethral discharge, lower abdominal pain, scrotal swelling and ophthalmia neonatorum (WHO, 2013).

These indicators may be important but are not enough to determine the extent and characteristics of the changing trend in new and re-emerging pathogens and their antibiotics resistance profile. The signs and symptoms of the different STIs are not specific and therefore make accurate clinical diagnosis difficult. Furthermore, the syndromic approach for vaginal discharge is poorly predictive of the presence of cervical chlamydia and/or gonorrhoea. To improve the sensitivity and specificity of the syndromic management algorithm, risk assessment (for example, age < 21 years, more than one sexual partner, new partner in the last 3 months) was introduced by the WHO, but this has not been very successful (Newman *et al.*, 2015; Yohannes *et al.*, 2013).

In addition, several limitations hinder wide acceptability of syndromic management and these include, overtreatment of patients and those with mixed infections may be inadequately managed (WHO, 2015). Such patients may have to return for further investigation, suffer prolonged morbidity and then be re-treated at a further cost. Another limitation of this approach is asymptomatic cases are missed. The adaptation of syndromic management in the fight against HIV by resource-limited countries has been effective in reducing the burden of *N. gonorrhoeae* infection more than other STIs (WHO, 2015). Infection with *N. gonorrhoeae* is normally symptomatic in men; therefore, early treatment reduces the transmission rate. However, non-treatment of sexual partners can cause re-infection and prolong exposure to antibiotic treatment, which can lead to antibiotic resistance.

1.12 Study rationale

With recent development of introducing HPV vaccination into The Gambia Expanded Programme on Immunization (EPI) and the addition of cervical cancer as AIDS-defining illness by the WHO, there is a strong need to have current baseline data on HPV infection rates and circulating genotypes for future evaluation of the quadrivalent vaccine in the prevention of HPV infections and related diseases in The Gambia. Moreover, the current investigational tools available for cervical cancer screening in The Gambia are the VIA and Pap smear tests, which have a lower sensitivity in comparison to HPV DNA tests (Cuzick *et al.*, 2006). Secondly, Pap smear screening is not systematic in The Gambia and the quality control guidelines for conventional smears are often not implemented. Additionally, education of the general population regarding the connection

between HPV and cervical cancer, and the necessity to do regular Pap smear for detecting precancerous cervical lesions is an utmost importance. Furthermore, to reduce the incidence of cervical cancer, it is important not only to diagnose and treat CIN through cytological screening but also to identify and eliminate co-factors that are involved in HPV persistence and development into CIN and cancer. Therefore, additional evaluation of STIs will be helpful to appropriately diagnose and treat patients with oncogenic high-risk HPV result and abnormal cervical cytology.

1.12.1 Study Aims

The aims of the study were to establish the circulating HPV genotype distribution, its co-infection with selected STI pathogens, and socio-demographic and behavioural risk factors that may be associated with HPV/STI in The Gambia.

1.12.2 Study Objectives

The following objectives were investigated:

- To determine HPV prevalence and genotype distribution amongst reproductive age women
- To determine the intratype variability of the Gambia high-risk HPV sequences and compare these with previously published high-risk sequences of HPV isolates from other geographical locations
- To determine HPV co-infection with selected genital pathogens
- To determine the prevalence and distribution of *Ureaplasma* and other selected STI pathogens
- To determine socio-demographic and risk factors associated with HPV/ STI
- To establish HPV sero-prevalence and estimate time of HPV sero-conversion in HIV-positive women
- To establish the cytological changes of HPV acquisition and viral persistence in the cervix of HIV- positive women.

1.12.3 Expected study outcomes

Data obtained from this study will contribute to existing knowledge and provide the basis for The Gambian Government and the Ministry of Health and Social Welfare (MOHSW) to:

- Decide on reviewing the current indicator tools for STI management
- Strengthen laboratory evidence base STI diagnosis
- Have baseline data on circulating oncogenic HPV type in urban Gambia
- Introduce HPV vaccination on the population at risk
- Monitor circulating oncogenic HPV genotypes through screening
- Strengthen the information and communication on HPV and STI prevention and control through:
 - education,
 - raising awareness and other local strategies to promote behaviours that reduce the risk of HPV/STI transmission.

Chapter 2: Materials and Methods

2 Sample collection and processing

2.1 Study location

This study was a collaborative work between Edward Francis Small Teaching Hospital (EFSTH), The Gambia and the University of Westminster, London. The EFSTH (formerly the Royal Victoria Teaching Hospital) was built in 1953 and is a 650-bedded tertiary hospital located in the capital city, Banjul.

The EFSTH was chosen for the study location because it is the main referral and the only teaching hospital in the country with various other departments such as Obstetrics and Gynaecology, Internal Medicine, Paediatrics, General Surgery and Accident/Emergency, all devoted to care of adult and paediatric medical cases. The EFSTH also runs a busy polyclinic outpatient department seeing over 100,000 cases a year. The polyclinic is where the sexually transmitted infection (STI) and family planning (FP) clinics are located.

The infectious disease clinic (IDC) is also in EFSTH and deals with the management of patients diagnosed with HIV. These clinics attend to patients from all over the country with more than 80% from the capital city, Banjul, West Coast Region (WCR) and Kanifing Municipal Council (KMC). These three regions are the most populated urban regions of the country and the residents are from multicultural backgrounds.



Figure 2.1 Map of The Gambia showing the capital city, Banjul where the EFSTH

hospital is located (Google map, 2018)

<https://www.google.com/search?ei=PsZW97UKIiIgAb9j7rgAQ&q=The+Gambia+map&oq>.

2.2 Ethics

Ethical considerations for participants' recruitment and sample collection were reviewed and approved by The Gambia Government and Medical Research Council (MRC) Joint Ethics Committee, Gambia (Appendix E1). Ethical approval was further granted by the University of Westminster Research Ethics Committee, London (Appendix E2). Permission was granted for the use of laboratory facilities at the clinics for sample reception and preliminary processing by the Chief Medical Director of EFSTH (Appendix E 3). In addition, approval was also given to use the facilities in the Medical Microbiology laboratory for the processing and storage of clinical samples through-out the sample collection period in The Gambia.

2.2.1 Study design

This study involved two related studies, a case-control and a cohort longitudinal study. A sample size of 346 (173 samples from each study group [case and control], respectively) was estimated for the study based on the HPV prevalence rate reported from a previous study (Wall *et al.*, 2005).

The sample size was calculated using a simple Daniel formula:

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

where n = sample size

Z = Z statistic for a level of confidence of 95%, Z value of 1.96, which is conventional was used

P = expected prevalence (a prevalence of = 0.13 (13%) was used based on the HPV prevalence reported (Wall *et al.*, 2005)

d = precision (d was set at 0.05 (5%), to give the width of confidence interval of 10% (0.1).

Reproductive age women, 20 – 49 years old attending a sexual health clinic / Infectious disease clinic (STI/IDC) with symptoms of STI or diagnosed with HIV were recruited in the case group. HIV-positive participants with CD4⁺ T cell count ≥ 350 cells/mm³ and not on antiretroviral therapy (ART) during the time of recruitment were further recruited into a cohort group for a longitudinal study and follow-up every 9 months for 24 months.

Similarly, reproductive age women, 20 - 49 years old with no clinical symptoms of STI attending the family planning clinic for contraceptive services were recruited into the control group.

Inclusion criteria

Participants recruited into the case group had one or more of the following clinical symptoms:

- Vaginal itching \ discharge with fishy or strong odour
- Pain or burning when urinating
- Bleeding between periods
- Pain during sex
- Sores, skin rashes with rough red or reddish-brown spots on hands or feet
- Genital warts
- Lower abdominal pain
- HIV

Exclusion criteria

Participants were excluded from the study if they were diagnosed with cervical cancer or cervical lesions, lived outside the study area, were pregnant or were on their menstrual cycle at

the time of sampling, had reached menopause or were above 49 years old. None of the participants was diagnosed with cervical cancer or cervical lesions at the time of recruitment. Participation was voluntary, and participants were given a participants' information sheet (PIS, Appendix E 5), which contained detailed information about the project and the reasons for their participation in the study. Recruited participants were asked to sign consent forms and copies of these were kept at the respective clinics (Appendix E6).

2.2.2 Data and sample collection

Recruitment of participants and sample collection was carried out between 2015 and 2017. A total of two hundred and thirty-five women (n = 235) attending the STI/IDC and family planning clinics at the EFSTH polyclinic, Banjul were recruited. A socio-demographic and risk factor questionnaire (Appendix E7) was administered to each consented participant. Each collected sample has a unique identifier number that corresponds with the one on the questionnaire. All questionnaire entries were double-checked for missing information and all entry queries were resolved immediately.

2.2.3 Case-control study

Two paired swab samples, endocervical (ECS) and high vaginal swab (HVS) were collected from each participant. Participants were subjected to pelvic examination by a clinician or qualified healthcare provider. Before the cervix and vagina were sampled, a sterile cotton-tipped end swab was used to clean the cervical and vaginal canal of any discharge. Swabs were inserted and rotated through 360° into the cervical transformation zone and vaginal canal to collect the samples. One endocervical and one high vaginal swab were used for routine microbiological identification of *Streptococcus agalactiae*, *Candida albicans*, *Neisseria gonorrhoeae*, bacterial vaginosis, and *Trichomonas vaginalis*. Initial processing of the samples was carried out at the polyclinic laboratory within 30 minutes of collection and transported to the Department of Medical Microbiology, EFSTH.

The two remaining swabs (endocervical and high vaginal swab) for nucleic acid amplification of HPV, *C. trachomatis*, *N. gonorrhoeae*, *Ureaplasma parvum / urealyticum* and *M. genitalium* were immediately placed in a specimen transport media, M4RT, micro-test (Oxoid, Basingstoke, UK) and stored at -70°C until ready for shipping to the University of Westminster, United Kingdom, London. All participants were sampled once except in participants in the

longitudinal study, who were sampled at an interval of 9 months for 24 months. During this period a total of nine hundred and forty (940) swab samples (two-paired samples, an endocervical and high vaginal swabs from each participant) were collected for the case-control study.

2.2.4 Longitudinal cohort study

Twenty-nine (n=29) of the 235 recruited participants were diagnosed with human immunodeficiency virus (HIV) at the IDC clinic. The HIV status of these participants was confirmed by rapid HIV screening using point of care determine® and first response rapid HIV test kits (Abbott, UK). These participants were grouped into a cohort and were followed up and sampled every 9 months in a longitudinal study.

Papanicolaou smears (Pap smears), blood and endocervical swab samples were collected and sexual risk behaviour captured for each visit. Pap smears were collected from the endocervix and ectocervix using a cytobrush and wooden Ayre's spatula by a clinician. The slides were fixed in 70% (v/v) alcohol and stored at room temperature. The endocervical swab for HPV screening was placed immediately into a specimen transport medium, M4RT, micro-test (Oxoid, Basingstoke, UK) and stored at -70°C until ready for shipping. Venous blood samples for HPV antibodies were collected in a non-anticoagulated tube and centrifuged at 12,000 g for 5 minutes. The serum was aliquoted into cryovials, labelled and stored at -20°C until ready for shipping.

All samples for further processing were shipped to the University of Westminster, United Kingdom, London on dry ice using an approved courier and following International Air Transportation Association (IATA) instructions on packaging and transportation of biological and dangerous goods.

2.3 Materials

Materials used in this study were purchased from selected companies including Qiagen (Crawley, UK), VWR Ltd (East Grinstead, UK), Sigma–Aldrich (Dorset, UK), Fisher Scientific Ltd (Loughborough, UK), National Institute of Biological Standards and Control (NIBSC, Hertfordshire, UK) and Addgene Ltd (USA, American). All PCR and DNA sequencing kits were obtained from Qiagen and Sigma–Aldrich Ltd, UK. Solid media used for isolating bacterial strains were obtained from Oxoid Ltd (Basingstoke, UK) and Fisher Scientific Ltd

(Loughborough, UK). They included Sabouraud dextrose agar (SDA) agar, Chocolate agar (CA), Blood agar (BA) and Luria-Bertani agar (LB).

2.3.1 Preparation of media, buffers and stock solutions

Solid media

The solid media used in this study for isolation of pathogens were blood agar (BA), chocolate agar (CA), LB agar and Sabouraud dextrose agar (SDA). Briefly, the required powdered agar (Oxoid, Basingstoke, United Kingdom) were weighed according to manufacturer's instructions and dissolved in distilled water. The mixture was thoroughly mixed to dissolve by either heating on a hot plate or by gently stirring or swirling until the entire powdered agar was dissolved. The dissolved mixture was sterilised by autoclaving at 121°C for 15 minutes. The autoclaved media was left to cool to about 50°C before supplements such as 7% (v/v) defibrinated sheep blood (SR0051) were added in the respective media. Every batch of media that was prepared was quality controlled for both sterility and the ability to support the growth of target organisms. Bacterial reference strains, *Streptococcus pyogenes* ATCC®19615 was used for the blood agar, *Neisseria gonorrhoeae* ATCC® 49981™ for chocolate agar, and *Candida albicans* ATCC®10231 for Sabouraud dextrose agar. These reference strains were cultured on each of the respective media as media controls. The cultured plates (media quality check) and uninoculated plates (sterility check) were incubated for 48hrs at 35 - 37°C under aerobic conditions and in a moist atmosphere enriched with CO₂ (5%). All batches that passed quality controls were labelled and stored at 2 - 8°C for no more than a week.

Preparation of buffers and liquid media

All buffers and liquid medium used in this study were prepared according to the manufacturer's guidelines and where appropriate, sterilised by autoclaving at 121°C for 15 minutes. The buffers used were sterile 0.85% physiological saline, phosphate buffer saline (PBS), 50X concentration of Tris-acetate- ethylene – diamine- tetra-acetic acid (TAE) buffer (stock), 1X TAE buffer (v/v) from stock, 0.5mg /L ethidium bromide, 3% (v/v) acid alcohol, 10% (w/v) KOH, 2.5% (w/v) Safranin, 95% (v/v) ethanol, Crystal violet and Grams iodine. The liquid medium used in this study was LB broth.

2.4 Questionnaire

A socio demographic and risk factor validated questionnaire consisting of 39 questions (appendix E 7) per questionnaire was administered to participants and entered into a database. Electronic data capture was done with a database created using Epi™ info version 7 (<https://www.cdc.gov/epiinfo>). A total of 235 questionnaires were administered to participants who consented and entered into the database. Before statistical analysis was carried out, the database was cleaned, validated and verified by double-entry data management method.

2.5 Methods for microbiological identification

2.5.1 Bacterial vaginosis and *Trichomonas vaginalis*

Detection of bacterial vaginosis and *T. vaginalis* was carried out at the clinic's site laboratory. The high vaginal swabs were used for direct wet mount microscopy, detection of fishy amine odour ('Whiff' test) when mixed with 10% (w/v) potassium hydroxide (KOH) preparation and vaginal pH determination (range 4.0 – 7.0). The wet preparation was viewed microscopically for the presence of 'clue cells', yeast cells, and motile *T. vaginalis*. Bacterial vaginosis was diagnosed using Amsel's clinical criteria, which included the presence of any three of the following: 1) homogeneous white vaginal discharge; 2) a vaginal pH \geq 4.6; 3) the release of fishy amine odour when 10% (w/v) of KOH was added to a vaginal fluid sample; 4) the presence of clue cells on Gram stained microscopic examination representing about 20% of vaginal epithelial cells (Amsel *et al.*, 1983).

2.5.2 Isolation of *Neisseria gonorrhoeae*, *Streptococcus agalactiae* and *Candida albicans*

Endocervical swabs were cultured directly on BA, CA, SDA agar plates at the clinic's site laboratory and transported immediately to the Department of Medical Microbiology laboratory, EFSTH. Both BA and CA plates were incubated overnight at 35°C - 37°C under aerobic and in a moist atmosphere enriched with CO₂ (5%) conditions for up to 24 - 72 hours for identification of *Streptococcus agalactiae* and *Neisseria gonorrhoeae*. SDA plates were incubated micro-aerophilically at 25°C - 36°C for 24 - 48hrs to isolate *Candida albicans* or *Candida* species. Colonies of interest were subcultured and incubated overnight for pure

isolates. Gram staining was performed on all isolated organisms. Pure colonies from overnight cultures were selected for biochemical identification. All tests were carried out according to the manufacturers' instructions.

2.6 Biochemical tests for identification of isolated organisms

2.6.1 *Streptococcus agalactiae*

A catalase test was performed on all pure β -haemolytic Gram-positive cocci colonies from overnight cultures using 3% (v/v) hydrogen peroxide (Reiner, 2010). The catalase test was used to differentiate bacteria that produce the enzyme catalase, such as *Staphylococcus* organisms from non-catalase producing organisms such as *Streptococcus*. All β -haemolytic catalase negative isolates were further selected for biochemical and Lancefield group identification using commercial latex serological test, Streptex™ rapid latex agglutination test (Thermo Fisher Scientific, Loughborough, United Kingdom).

2.6.2 *Candida albicans*

A Gram stain was carried out on all suspected *Candida* colonies for the identification of yeast cells and in addition, the germ tube test (GTT) was also performed. In brief, two to three colonies of suspected *Candida* colonies were emulsified in a 0.5 ml of human serum in a plain tube. The mixture was incubated at 37°C in a water bath for 3 – 4 hours. A wet preparation was prepared and observed microscopically for the presence of budding cells and/or pseudo-hyphae. Isolates that have budding cells and/or pseudo-hyphae were classified as *Candida albicans* and those without budding cells and/or pseudo-hyphae were classified as *Candida* species.

2.6.3 *Neisseria gonorrhoeae*

Gram staining was performed on *N. gonorrhoeae* suspected colonies for the presence of Gram-negative diplococci. A catalase test was also carried on suspected *N. gonorrhoeae* isolates with 30% (v/v) hydrogen peroxide (Reiner, 2010). *N. gonorrhoeae* isolates produced a positive reaction with a strong bubbling within 1-2 seconds. An oxidase test was also carried out on Gram-negative diplococci colonies using oxidase reagent strips (Sigma- Aldrich, Dorset, UK). These strips are impregnated with oxidase reagent N-N, Dimethyl-p-phenylenediamine and alpha-naphthol solution. Briefly, the paper strip was placed on a clean microscopic slide and a

suspected *N. gonorrhoeae* colony was picked using a wooden applicator stick. The colony was gently rubbed onto the test area of the strip. Similarly, *Pseudomonas aeruginosa*, a known oxidase positive and *Escherichia coli*, oxidase negative organisms (obtained from the Department of Medical Microbiology laboratory stock, Gambia) were added to the appropriate areas of the strip to act as controls. Suspected oxidase positive organisms oxidise the phenylenediamine in the reagent and a purple colour was observed within 10 seconds. All presumptive *N. gonorrhoeae* isolates were further confirmed using analytical profile index (API NH; Biomerieux, UK) following the manufacturer's instructions.

2.7 Antibiotic susceptibility determination of *Streptococcus agalactiae* and *Neisseria gonorrhoeae* isolates

All *S. agalactiae* and *N. gonorrhoeae* isolates were tested for their antibiotic susceptibility profile according to the European Committee on Antibiotics Susceptibility Testing (EUCAST, 2015) and British Society for Antimicrobial Chemotherapy (BSAC, 2015) guidelines. Antibiotic susceptibility testing (AST) was carried out using the disc diffusion method on BA plate for *S. agalactiae* and CA plate for *N. gonorrhoeae* (Oxoid, Basingstoke, UK), respectively. Two to three colonies were picked depending on the size of the colony using a sterile disposable plastic bacteriological loop and emulsified in 0.85% (v/v) sterile physiological saline. The turbidity of the suspension was compared with 0.5 McFarland turbidity control and adjusted by either adding colonies or adding sterile saline. A 1:100 dilution was prepared from the suspension and evenly streaked onto a BA (*S. agalactiae*) or CA (*N. gonorrhoeae*) plate using a sterile cotton swab to produce a confluent growth.

2.7.1 Disc Diffusion test on isolates

All *S. agalactiae* isolates were tested against the following selection of antibiotic (Oxoid, Basingstoke UK): Ampicillin (Amp, 10 µg), Cefuroxime (CRX, 30 µg), Clindamycin (2 µg), Erythromycin (ERY, 15 µg), Tetracycline (TE, 30 µg), and Penicillin (P, 10 units). *Neisseria gonorrhoeae* isolates were tested against the following selection of antibiotics: Ciprofloxacin (CIP, 5 µg), Cefotaxime (CTX, 30 µg), Tetracycline (TE, 30 µg), Penicillin (P, 10 units), Ceftriaxone (30 µg). An antibiotic disc dispenser was used to place the antibiotic discs onto the inoculated plates for accuracy of the distance between the discs. The plates were incubated aerobically at 35°C - 37°C for 18 – 24 hours. The zones of inhibition observed after incubation

were measured using a calibrated calliper and test results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015) and British Society for Antimicrobial Chemotherapy (BSAC, 2015) guidelines.

2.8 Nucleic acid analysis

2.8.1 Preparation of swab samples for DNA extraction:

Swab samples were transported in a specimen transport medium, Micro TestTMM4[®] (Fisher Scientific, UK) for viruses, *Chlamydia*, *Mycoplasma* and *Ureaplasma*. The transport medium contains the following antibiotics Amphotericin B, Vancomycin and Colistin to inhibit the growth of competing bacteria and yeast. The samples were homogeneously mixed, and the swab sticks discarded. An aliquot of 1.5 ml of the suspension was placed in an Eppendorf tube and centrifuged at 13,000 *g* for 15 minutes and the supernatant was discarded. The cell pellets were suspended in a 1 ml sterile phosphate buffered saline (PBS) and centrifuged at 13,000 *g* for 10 minutes. The supernatant was discarded, and the pellets were used for DNA extraction.

2.8.2 DNA Extraction:

DNA was extracted from the clinical specimens using QIAamp DNA mini extraction kit (Qiagen, Crawley, UK). 200 μ l of the prepared sample was aliquoted into a 1.5 ml microfuge tube and 20 μ l of 20 mg/ml proteinase K, and 200 μ l of AL buffer were added. The mixture was mixed homogeneously by pulse vortexing for 15 seconds and incubated at 56°C in a heat block for 10 minutes. The mixture was briefly centrifuged in an Eppendorf 5415 D benchtop centrifuge to remove drops from the inside of the tube lid. 200 μ l of ethanol (96-100%, (v/v)) was added to the mixture and mixed by vortexing for 15 seconds before briefly centrifuged. The mixture was carefully applied to a spin column in a 2 ml collection tube and centrifuged at 8000 *g* for 1 minute. The filtrate was discarded and 500 μ l of buffer AW1 was added to the column and centrifuged as above. The filtrate was discarded and 500 μ l of buffer AW2 was added to the column and centrifuged at 13,000 *g* for 3 minutes.

The filtrate was discarded, and the spin column was centrifuged again at 14000 *g* for 1 minute to remove excess ethanol. The filtrate and the collection tube were discarded, and the spin column was air dried at room temperature for 10 minutes. The spin column was placed in a new collection tube and 200 μ l of buffer AE was added and incubated at room temperature

for 5 minutes. The tube was then centrifuged at 8000 g for 1 minute. The spin column was discarded and the extracted DNA in the collection tube retained. The extraction process was quality controlled by including sterile distilled water in the extraction process to serve as template extraction (TEX) blank control in each batch of samples extracted.

2.8.3 Quantification of DNA using Nanodrop

The concentration of the extracted DNA was determined using Nanodrop 2000 spectrophotometer (Thermo Scientific, Loughborough, UK). The optical density of 1 µl of the DNA sample was measured at 260/280 (nm). The DNA concentration was calculated by the Nanodrop 2000 software. The extracted DNA was aliquoted in 50 µl aliquots in sterile Eppendorf tubes and stored at -20°C until ready for use.

2.8.4 Primers, positive controls, and PCR.

All primers used in this study were used in previous published studies (Yi *et al.*, 2005, Jensen *et al.*, 2004, Jensen *et al.*, 2003, W.H.O. 2009, Gravitt *et al.*, 2000, Kimmitt *et al.*, 2010). A Basic Local Alignment Search Tool (BLAST) was carried out on all primers to check for specificity and binding regions of the target genes. All primers and probes used in this study were synthesised by Eurofins MWG, Operon, Germany.

Unless otherwise stated, all PCR amplifications were performed with the Peltier thermal cycler, DNA Engine®, BIO-RAD in a 25 µL volume containing 5 µM of each primer, 1x Taq PCR master mix containing 2.5 units of Taq DNA polymerase, 0.2 mM deoxynucleotide triphosphates, and 1.5 mM MgCl₂ (Qiagen, Crawley, UK), 50 ng – 200 ng of DNA template, extraction control and negative control (sterile water). Positive controls used in the study were purchased from Genekam Biotechnology, Duisburg, Germany, unless otherwise stated.

2.8.5 Gel electrophoresis

All PCR amplified products were resolved in 2% (w/v) agarose gel in a 1X Tris-acetic EDTA (TAE) buffer. Agarose gels were prepared from crystalline powder purchased from Sigma Aldrich, United Kingdom. To correctly assign the PCR products the correct band size, DNA size markers 100 base pair DNA ladder (Sigma-Aldrich, United Kingdom) were included. The gel electrophoresis was run at 100 volts for 45 - 60 minutes and stained in ethidium bromide

for 10 – 15 minutes. The amplified PCR products were visualized under ultra violet (UV) light using a transilluminator.

2.9 Histocompatibility leucocyte antigen (HLA) and β -actin PCR:

To confirm the adequacy of the extracted cellular DNA, histocompatibility leucocyte antigen (HLA) and β -actin PCR were used. *HLA* and *β -actin* are house-keeping genes responsible for the maintenance of basic cellular functions and is expressed in all cells of organisms. These house-keeping genes are constantly expressed in all conditions; therefore, they are used as controls in an assay to facilitate the exposure of the underlying cellular infrastructure (Zhu *et al.*, 2008). The thermal cycling consisted of 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 60 seconds, elongation at 72°C for 2 minutes and a final extension step at 72°C for 5 minutes. Samples that were negative for HLA were retested using β -actin Primers (Table 1). PCR analysis was carried out on samples that showed an amplicon size of 230 and 300 (bp) for *HLA* and *β -actin* indicating that adequate cellular DNA has been extracted and inhibitors were not preventing amplification.

Table 2.1 HLA and β -actin primers and PCR amplicon size

Primer Name	Sequence (5- 3)	Amplicon size (bp)
HLA dQ-F	GTGGTGTAAACTTGTACCA	230
HLA dQ-R	GGTAGCAGCGGTAGAGTT	
β -actin- F	TCACCCACACTGTGCCCATCT	300
β -actin- R	CAGCGGAACCGCTCATGCCCA	

2.10 Determination of *Ureaplasma parvum* / *urealyticum* by PCR

Ureaplasma species are frequently isolated in the genital tracts and were grouped under the name *Ureaplasma urealyticum*. The introduction of nucleic acid amplification techniques has changed the classification and knowledge of the distribution of disease by allowing separation of *Ureaplasma urealyticum* species into two groups, *Ureaplasma urealyticum* (T960) and

Ureaplasma parvum (parvo). The technique and common primers UU-1402F and UU-1779R (GenBank accession numbers AF085724 and AF085730) have previously been described and it has been shown that the common primers amplified both *Ureaplasma parvum* and *urealyticum* (Yi *et al*, 2005). The primers (Table 2) amplified 378 base-pair of the *urease* gene. The amplification reaction was carried out as previously described by Yi *et al*, (2005) with slight modifications. In this study, Taq PCR mastermix (Qiagen, Crawley, UK) was used and the thermal cycling consisted of an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, elongation at 72°C for 30 seconds and a final extension step at 72°C for 5 minutes.

2.11 Determination of *Mycoplasma genitalium* by 16S rRNA PCR:

The *M. genitalium* 16S rRNA gene was amplified using the MG16-45 F and MG16-447 R primers (Table 2), which are in the V1 and V3 hypervariable regions respectively. These primers previously described by Jensen *et al.*, (2003) have high specificity binding to most sequences conserved in most eubacteria. The amplification reaction was carried out as follows and it consisted of 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 60 seconds, elongation at 72°C for 30 seconds and a final extension step at 72°C for 5 minutes

Table 2.2 *Ureaplasma* and *Mycoplasma genitalium* primers and PCR amplicon size

	Primer Name	Sequence (5- 3)	Amplicon size (bp)
	UU-1402F	TGC TGG TGG TAC AGG TAT GAA	378
	UU- 1779R	GGA GCA TGT CCW* CCA CCA	
	MG16-45F	TAC ATG CAA GTC GAT CGG AAG TAG C	427
	MG16-447R	AAA CTC CAG CCA TTG CCT GCT AG	

W*: A or T

2.12 Amplification of human papillomavirus late (*L1*) gene

The HPV L1 consensus PGMY09 /11 primer pool consisting of 5 upstream oligonucleotides PGMY11 primer pool and 13 downstream oligonucleotides, PGMY09 primer pool (W.H.O., 2009; Gravitt *et al.*, 2000) was used to amplify HPV from cervical samples (Table 4). These primers amplified 450-bp of the L1 region of the virus. The L1 region is the major capsid protein of the HPV and is the most conserved among all HPV types. It is seldom lost during malignancies making it the targeted region for most frequently used consensus PCRs. HPV L1 PCR was performed twice on the cervical samples, once using 1.5 mM MgCl₂ and then with 3.0 mM MgCl₂ concentration on samples that were negative with the 1.5 mM MgCl₂ concentration. The use of two different MgCl₂ concentrations optimises the analytical sensitivity of detection of the various HPV types and their detection by gel electrophoresis. W.H.O. International standard HPV control was purchased from National Institute for Biological Standards and Control, United Kingdom (NIBSC, Hertfordshire, UK) to serve as a positive and assay sensitivity control.

Briefly, the standard was reconstituted in 0.5 ml nuclease free water and the DNA concentration measured using the Nanodrop[®] system. A ten-fold serial dilution was performed on the positive control and a PCR was carried out to determine the dilution factor that gave clearer product bands when resolved in a 2% (w/v) agarose gel. To determine assay sensitivity, a 1:10, 1:100, 1:1000 dilution of the HPV positive control was included in the PCR amplification. The thermal cycling was carried as described in Table 4. Similarly, 3.0 mM MgCl₂ PCR reaction was carried out as described above with the addition of 1.5 µl of 25 mM MgCl₂ (stock) to the Taq PCR Master mix giving a final concentration of 3.0 mM MgCl₂.

Table 2.3 HPV PGMY09/11 pool primers and PCR thermal cycling reaction

Primer Name	Sequence (5' -3')	Programme
PGMY11-A	GCACAGGGACATAACAATGG	Initial denaturation at 95°C for 9 minutes, followed by 45 cycles of 94 °C for 30 seconds, annealing at 55 °C for 60 seconds, elongation at 72°C for 2 minutes.
PGMY11-B	GCGCAGGGCCACAATAATGG	
PGMY11-C	GCACAGGGACATAATAATGG	
PGMY11-D	GCCCAGGGCCACAACAATGG	
PGMY11-E	GCTCAGGGTTTAAACAATGG	
PGMY09-F	CGTCCCAAAGGAAACTGATC	
PGMY09-G	CGACCTAAAGGAAACTGATC	
PGMY09-H	CGTCCAAAAGGAAACTGATC	
PGMY09-I	GCCAAGGGGAAACTGATC	
PGMY09-J	CGTCCCAAAGGATACTGATC	
PGMY09-K	CGTCCAAGGGGATACTGATC	
PGMY09-L	CGACCTAAAGGGAATTGATC	
PGMY09-M	CGACCTAGTGGAAATTGATC	
PGMY09-N	CGACCAAGGGGATATTGATC	
PGMY09-P	GCCCAACGGAAACTGATC	
PGMY09-Q	CGACCCAAGGGAAACTGGTC	
PGMY09-R	CGTCCTAAAGGAAACTGGTC	
HMB01	GCGACCCAATGCAAATTGG	

“11 primers” are the Forward primers, “09 primers” are Reverse primers, and HMB01 primers amplifies HPV genotype 51

Programme: Initial denaturation at 95°C for 9 minutes, followed by 45 cycles of 94 °C for 30 seconds, annealing at 55 °C for 60 seconds, elongation at 72°C for 2 minutes.

2.13 Real time-PCR (q-PCR)

All real time-PCR reactions were performed with the Rotorgene-Q (Qiagen, Crawley, UK) in a 20 µl reaction volume containing Rotorgene probe mastermix kit (Qiagen, Crawley, UK), 1 µM of each primers (unless otherwise stated), 50 ng -100 ng of DNA template, extraction control and negative control (sterile water) and positive controls (Genekam Biotechnology, Duisburg, Germany) All samples were tested in duplicate within one run and were considered positive if the cycle threshold (Ct) value was ≤ 35 .

2.13.1 Detection of *Ureaplasma parvum* / *urealyticum* by duplex q-PCR

Real time-PCR was carried out to distinguish between the two species, *U. parvum* (parvo) and *U. urealyticum* (T960) in both endocervical and high vaginal swabs. A common primer for the two species UU-1613F and UU-1524R and two species-specific probes (Yi *et al.*, 2005) (Table 3) were used in this reaction. The reaction was carried out in one tube, and the primers and species-specific probes amplified and typed *Ureaplasma* at the same time. The reaction mixture also contained 0.25 µM of each probe. Thermal cycling and detection of fluorescent signals on amplifying the specific target sequence were performed as previously described by Yi *et al.*, (2005) with an addition of a final hold for endpoint detection of fluorescence. It consisted of two holdings of 56°C for 2 minutes, 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, and a final hold of 60°C for 10 minutes. Two channel wavelengths of FAM (absorbance: 470nm; emission 515nm) and JOE (absorbance: 530 nm, emission 557 nm) were used to identify the specific species, *U. parvum* and *U. urealyticum* respectively. Black hole quencher (BHQ) 1 (quenching range: 480 – 580nm) was used for both probes. The fluorescence data were analysed with the allelic discrimination software of the Rotorgene-Q instrument.

2.13.2 Detection of *Mycoplasma genitalium* MgPa gene by Real time-PCR:

Both endocervical and high vaginal samples were tested for the presence of *M. genitalium*. A set of primers targeting the conserved region of the *M. genitalium* adhesion *MgPa* gene was used for the real time-PCR amplification. These primers and probes (Table 3) are specific for

the conserved region of the *M. genitalium* *MgPa* gene, which is interspersed with hypervariable sequences that are correlated with the *MgPa* repeated regions (Jensen *et al.*, 2004). The q-PCR reaction mixture also contained 75 nM FAM fluorophore – labelled *MgPa* probe. The cycling was carried out as follows: two holds of 50°C for 1 second, 95°C for 10 minutes and 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, and a final hold of 60°C for 10 minutes using FAM channel 470 –515(nm) wavelength.

Table 2.4 Real Time (q)- PCR primers and probes for *Ureaplasma* and *M. genitalium*

Primer and probe name	Sequence	Amplicon size (bp)
UU -1613F	AAG GTC AAG GTA TGG AAG ATC CAA	90
UU - 1524R	TTC CTG TTG CCC CTC AGT CT	
UU – T960	JOE – ACC ACA AGC ACC TGC TAC GAT TTG TTC- BHQ1	
UU – parvo	FAM – TCC ACA AGC TCC AGC AGC AAT TTG – BHQ1	
<i>MgPa</i> – 355F	GAG AAA TAC CTT GAT GGT CAG CAA	78
<i>MgPa</i> – 432R	GTT AAT ATC ATA TAA AGC TCT ACC GTT ATC	
<i>MgPa</i> – 380	FAM – ACT TTG CAA TCA GAA GGT – BHQ1	

2.13.3 Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* cryptic plasmid gene by duplex quantitative real time-PCR

A duplex real time-PCR was carried out for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoea* (NG), using dual-labelled probes as previously described (Kimmit *et al.*, 2010). Primers targeting the 144 bp region of the NG cryptic plasmid and 111bp region of the CT cryptic plasmid were used (Table 5). In addition to the reaction mixture (section 2.13), 10 µmol/L of each primer and 10 µmol/L of each probe were added. Both thermal cycling and detection of fluorescent signals were performed as follows: 95°C for 15 minutes and 50 cycles for 94°C for 15 seconds and 60°C for 60 seconds acquiring at FAM 470 – 515 (nm) and JOE 530 – 557 (nm). Black hole quencher (BHQ) 1 (quenching range: 480 – 580 nm) was used for both probes.

Samples that were positive for *N. gonorrhoeae* but negative for *C. trachomatis* were retested for *C. trachomatis* alone to confirm the result. Confirmation of the expected product sizes was carried out on positive samples by gel electrophoresis. Positive samples were purified and sent for confirmation by DNA sequencing.

Table 2.5 Real Time (q) - PCR primers and probes for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Primer and probe name	Sequence (5' – 3')	Amplicon size (bp)
NG primer F	AAC TGC CGG GCG TTT ATA TCG	144
NG primer R	GAC CTT CGA GCAGAC ATC ACG	
NG probe	JOE – ACC GAA GCC GCC AGC ATA GAG CAA CA-BHQ1	
CT primer F	GCC AAT CTT CTT TGA AGC GTT GTG	111
CT primer R	CTA GGG TGC TCA GAC TCC GAC	
CT probe	FAM – TGA AGG TCA CAG GAC ACG CAA CCC CA – BHQ1	

2.14 Purification of amplified PCR products and DNA sequencing:

2.14.1 Purification of PCR product

Following PCR amplification, all PCR amplicons that showed single discrete bands upon gel electrophoresis were purified using a PCR purification kit (Sigma Aldrich, Haverhill, UK) following the manufacturer's instructions. In this assay, the initial buffer, column binding buffer or QC (solubilising and binding buffer) was added to the PCR amplicons. This provides the optimal salt concentration, low pH and enhances the negatively charge DNA to adsorb to the positively charged silica-gel membrane in the QIAquick spin column. Contaminants present in the solution were then eluted by high speed centrifugation at 13,000 g through the column. Wash buffer (PE) was added and centrifuged as above to remove remaining contaminants from the DNA-bound membrane. The final step of this assay was the elution of DNA using a low concentration Tris buffer (EB). To maximise DNA recovery, 40 µL of EB

buffer was added to the centre of the membrane and the column was centrifuged after 1 minute of room temperature incubation.

2.14.2 DNA sequencing by LIGHTRUN™

Purified DNA samples were sent for sequencing to GATC Biotech (Konstanz, Germany) using prepaid barcodes. DNA sequencing by GATC Biotech (Konstanz, Germany) was performed by the LIGHTRUN™ sequencing technique. In this method, 5 µL of the purified DNA sample and 5 µL appropriate primer of concentration 5 µM were premixed and sent for sequencing.

2.15 HPV and *Ureaplasma* genotyping

PCR was performed on samples that were HPV positive for DNA sequencing using the L1 consensus primers, which amplified a 450 bp region of the virus encoding highly conserved amino acids that is suitable for DNA sequencing. Samples that were also *Ureaplasma* positive were sent for DNA sequencing. These samples were purified and commercially sequenced by GATC Biotech (Konstanz, Germany) as described in section 2.14.1 - 2.14.2. The nucleotide sequence data produced from sequencing were then searched in NCBI BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>), Basic Local Alignment Search Tool (BLAST). BLAST is software that finds regions of similarity between sequences by comparing nucleotides or protein input sequences to sequences in a database and calculates the statistical significance of the matches. The likelihood of the match occurring by chance is normally indicated by the Expect (E) value. The lower the E- value was, the more significant the genotype match was. Genotype search was carried out on each of the consensus strands (Forward and Reverse) produced by sequencing. HPV genotype was assigned to the closest known HPV type deposited in the GenBank database when the sequence is 90 – 100% identical to the *L1* gene of that genotype.

2.16 Phylogenetic analysis of high-risk HPV sequences

The high-risk HPV DNA sequences generated were initially identified by BLASTn analyses at NCBI (<http://www.ncbi.nlm.nih.gov/>) against the GenBank sequence database as described in section 2.15. Subsequent analyses on the HR/pHR-HPV sequences were carried out by Dr. Polly Hayes (section 2.16.1 - 2.16.2). These DNA sequences were aligned and edited using

Bioedit (Hall, 1999). Phylogenetic trees for the HR-HPV genotypes were constructed using the edited *L1* variant (PYMG09/11, 412bp) sequences obtained from the study.

The generated HR/pHR-HPV sequences were aligned with representatives of similar published HR/pHR-HPV sequences retrieved from the GenBank database, using MUSCLE (<https://www.ebi.ac.uk/Tools/msa/muscle/>). Phylogenetic analysis was carried out using two methods: Neighbour-Joining (NJ) and Maximum likelihood (ML). All phylogenetic analysis was undertaken using Molecular, Evolutionary Genetics Analysis version 7 (MEGA v7) (Kumar *et al.*, 2016). Neighbour-joining (NJ) trees were constructed using Kimura two-parameter correction methods. The ML analysis was generated in MEGA 7 under the conditions of the Tamura 3-parameter (T92+G) model as determined using the model test function also in MEGA 7. The T92+G model is amongst other models such as T93+1 that have lowest Bayesian information criterion (BIC) scores and they are considered to describe the evolutionary substitution model best (Tamara *et al.*, 2011; Nei and Kumar, 2000).

2.16.1 *p*-distance analysis

As a measure of inter- and intra-divergence of the Gambian HPV-types, uncorrected pairwise genetic distance (*p*-distance) between sequences was calculated using Mega v7. This was carried out using the Gambian HR/pHR-HPV sequences.

2.16.2 Regression analysis

To determine the inter-relationships and movement of the different HPV genotypes, and possibly determine the origin of The Gambia sequences, geographical distance (km) was calculated between different geographical locations “as the crow flies” using the online distance calculator woframalpha.com and plotted against *p*-distance (nucleotide) using linear regression. This was carried out using the Gambian HR/pHR-HPV sequences and other representatives of the different HPV genotypes from different geographical localities obtained from the GenBank database. HPV sequences that formed clades with a bootstrap support of $\geq 90\%$ with the Gambian HR/pHR-HPV sequences were used in this analysis.

2.17 Pseudo-typed virus neutralization assay (PsV):

This arm of the study was a longitudinal cohort study on recruited HIV positive women (n = 29) as described in section 2.1.4.2. Serum samples collected from this cohort were screened against the following high-risk HPV types: HPV 16, 18, 35, 51, 52 and 58.

2.17.1 Amplification of HPV packaging plasmids

Plasmid bacteria stabs p35 SheLL-HPV35 L1L2 a gift from Simon Beddows (Addgene plasmid # 40626) and a pVITRO-HPV 51 L1L2 a gift from Richard Roden (Addgene plasmid # 52583) were cultured on LB agar plates with Ampicillin (100 µg/ml) and Kanamycin (50 µg/ml) antibiotics, respectively. The plates were incubated at 37°C overnight. A single bacterial colony from the different plates were transferred into two different tubes each containing 5 ml LB broth with Ampicillin (100 µg /ml) and Kanamycin (50 µg / ml), respectively and incubated at 37°C on a multi-shaker at 250 (rpm), overnight.

Plasmid extraction was carried out from the overnight LB broth culture using the QIAGEN plasmid miniprep kit (Qiagen, Crawley, UK) following the manufacturer's instructions. The concentration of the extracted HPV plasmid DNA was measured using a Nanodrop ND2000 as described in section 2.8.3. To confirm the identity of the plasmid, a diagnostic restriction enzyme digestion was carried out on the extracted plasmids as follows:

1400 -1500 ng of HPV plasmid DNA was incubated with 1µl (20 U/ µl) of NotI-HF, 1 µl of (20 U/ µl) of NheI-HF (NEB Biolabs, United Kingdom) and 3 µl of 1 x CutSmart buffer in a final volume of 30 µl for 1 hr at 37°C. The products were run on a 1% (w/v) agarose in TBE buffer gel and visualised using a Syngene UV Transilluminator (Syngene).

2.17.2 Production of HPV pseudo-typed viruses

HPV pseudo-types for types 16, 18, 52 and 58 and the bovine papillomavirus (BPV) were already available at the NIBSC (Bissett *et al.*, 2014), where I'd carried-out this part of the research. Pseudo-types for HPV 35 and HPV 51 were created by transfection of HPV plasmids into human embryonic kidney cells HEK 293TT. 7.5 million 293TT cells were seeded in a 75cm² tissue culture flask to a 20 ml final volume of Dulbecco's modified eagle's medium with hygromycin (cDMEM) (Invitrogen, 11140-035) supplemented with

1% (v/v) Penicillin /Streptomycin and 10% (v/v) foetal calf serum (FCS). The flask was incubated at 37°C with 5% CO₂ for 16 - 24 hrs.

19 µg of HPV packaging plasmid and 19 µg pGL4.51 reporter plasmid (Promega, UK) were mixed together and add to 2 ml of Opti-MEM™-1 reduced serum media (Invitrogen 11058-021). 80 µl of lipofectamine™ 2000 (Invitrogen 11668-019) was added to 2 ml of Opti-MEM™ -1 and after 10 minutes at room temperature, the plasmids were added to the lipofectamine and further incubated for 20 minutes at room temperature. The mixture (Opti-MEM/ lipofectamine/HPV plasmid/pGL4.51 reporter plasmid) was transferred to the HEK 293TT cells seeded the day before.

The cells were incubated at 37°C with 5% CO₂ for 4 - 6hrs. The supernatant was gently removed at the end of the incubation period and 20 ml cDMEM was added. The cells were further incubated for 48 hrs.

2.18 HPV pseudo-typed virus maturation and purification

2.18.1 Post-transfection

The cells were collected by trypsinisation with trypsin-EDTA (Gibco, Life Technologies, UK), washed with phenol red free DMEM (prfDMEM) and centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded, and the cell pellet resuspended in 0.5 ml Dulbecco's PBS (Invitrogen, UK), which was supplemented with 9.5 mM MgCl₂ and 1% (v/v) Penicillin /Streptomycin (DPBS-Mg). The suspension was transferred into a 2 ml Eppendorf protein Lo-bind tube and the original tube was rinsed with another 0.5 ml DPBS-Mg and combined with the suspended cells. The tube was centrifuged for 10 minutes at 5000 rpm and the supernatant was discarded. The cell pellet was resuspended in 1.5 volumes of DPBS-Mg. Cells were lysed by adding 1/25 cell volume of 10 % (v/v) Brij-58 detergent and RNA removed using 1/1000 cell volume of RNase mix (Ambion, UK). The cell lysate was incubated at 37°C with 5% CO₂ for 24 hrs.

HPV pseudo-typed viruses were purified on a continuous density gradient of Optiprep® medium (Sigma Aldrich, Haverhill, UK), which is a trade name for 60% (w/v) iodixanol solution. The gradient was prepared in Beckman ultra-centrifuge tubes by layering Optiprep three concentration of Optiprep: 39%, 33%, and 27% in PBS-Mg from the bottom to the top and leave to diffuse for 1- 2 hrs at room temperature. The cell lysate from the previous day was cooled on ice for 10 minutes and clarified by centrifuged at 10,000 rpm at 4°C for 10

minutes. The supernatant was gently loaded on the diffused Optiprep gradient and centrifuged at 50,000 rpm (SW-55ti rotor) at 16°C for 3.5hrs in an Optima X-100 centrifuge (Beckman Coulter). Following centrifugation, 200 µl fractions of the virus from the top of the gradient was transferred into a sterile 96-well plate. Virus fractions in wells 16 – 21 were pooled together, giving a total volume of 1.2 ml of pseudo-typed virus. An equal volume of 27% Optiprep gradient was added to the virus pool to increase the volume of the pseudo-typed virus. The pseudo-typed virus was aliquoted into Eppendorf protein Lo-bind tubes and stored at -80°C until ready for titration.

2.19 Pseudo-typed virus titration by infectivity assay

Tissue culture infective dose (TCID₅₀) for each stock of the PsV was determined by titration. 10,000 HEK 293TT cells in 100 µl of cDMEM per well were seeded in inner wells of a 96-well tissue culture flat bottom sterile plate (Fisher Scientific, UK). The external wells (row A –H and column 1-12) were filled with 200 µl of sterile PBS to avoid evaporation. The plates were incubated at 37°C with 5% CO₂, overnight. The PsV stock was titrated by 5-fold serial dilution with a starting 1/25 dilution and 100 µl of the diluted PsV was added to the HEK 293TT cells seeded the day before. The plates were incubated at 37°C with 5% CO₂ for 72 hrs.

The supernatant from the plates was discarded without disturbing the monolayer of the cells. A 1:1 mixture of prfDMEM and Steady-Glo® Luciferase substrate (Promega, UK) was prepared and 100 µl was added to the wells. The plates were incubated at room temperature for 5 minutes. At the end of the incubation period, the mixture was thoroughly mixed and 90 µl was transferred into a white opaque 96-well plate in the same format and read on a luminometer (Fluostar Omega, BMG Lab Tech) using the Omega software.

The TCID₅₀ for each virus was calculated using the Spearman Karber equation (Appendix C6.1). The pseudo-typed virus infectivity (titration) assay for each virus was tested three times in triplicate as described above and the average TCID₅₀ of each virus was used to calculate the TCID₅₀/ml for each virus stock.

2.20 Neutralisation assay

The 293TT cells were seeded similarly to the infectivity assays (section 2.19). The cells were incubated overnight at 37°C with 5% CO₂. Each HPV pseudo-typed virus (HPV-16, 18, 35, 51, 52, 58 and BPV) was used at a concentration of 300 TCID₅₀ per well in 100 µl and dispensed in a 96-well U-bottom plate. To each well 25 µl of sera diluted 1:8 in prfDMEM were added to the 100 µl PsV to give a final 1:40 dilution of the sera. Virus only control received 25 µl of prfDMEM. Pseudo-typed viruses and sera were incubated at room temperature for 1 hr. 100 µl of the mixture was added to the 293TT cells seeded the day before. 125 µl of prfDMEM was added to the cells only column. The plates were incubated at 37°C with 5% CO₂ for 72hrs. The neutralisation assay was read following the same procedure as described in section 2.19.

2.20.1 Calculation of HPV antibody neutralisation

The raw data was imported and the antibody neutralisation for each sample was calculated by dividing the relative light unit (RLU) values of each antibody dilution with the average “PsV only” RLU values. The value was then multiplied by 100 to give the percentage of cells that were infected, which was then subtracted from 100 to give the percentage of neutralisation. Antibody neutralisation assay was carried out for each of the HR-HPV serotypes listed above and each serum was tested in triplicate for each of the HPV serotypes including the bovine papillomavirus, which served as a negative control.

2.21 Cytology

This part of the work was carried out at the Viapath Bedford hospital, UK. The cytology slides were stained using the Papanicolaou stain, coverslipped and were initially reported by two biomedical science (BMS) cytologists. All abnormal samples were confirmed by a senior BMS cytologist and a final diagnosis was reported by a Pathologist / Advanced Practitioner.

2.22 Data entry and Statistical analysis

Results obtained from the analysed samples were entered into the Epi™ info version 7 (<https://www.cdc.gov/epiinfo>) database according to the unique study number assigned to each participant that corresponded with the questionnaire. The database was checked for duplicate recording, missing variables, and some variables were re-coded for statistical analysis. Statistical analysis was carried out using Epi info version 7 statistical packages. Socio-demographic, behavioural risk factors and clinical outcomes of participants were analysed using standard descriptive statistics such as frequencies and percentages for categorical variables, standard deviation and means were applied for continuous variables. The strength of risk factors associated with STI as an outcome was measured using the Odds Ratio (OR). The ORs of the risk characteristics were interpreted as increase odds (>1.0) or decrease odds (<1.0) rather than a relative risk of the outcome being analysed when the p -value is not significant.

Chi-square-corrected (Yates) and Fisher's exact if any cell of the contingency table contained fewer than five, and Mantel Haenszel (MH) p -values were used to examine associations between categorical variables such as isolation of STI/genital pathogens and selected risk characteristic variables. A confidence interval of 95% (CI) and p -value of ≤ 0.05 were used to determine statistical significance. The student 't' test and pair-wise analyses were also used in a comparative analysis between the study population. A multivariate analysis such as logistic regression was used to determine the association between each risk characteristics and diagnosis of STI in a stepwise model by starting with a single risk exposure variable and building it up by adding more variables. Subsequently, variables that were not significant to STI infection at ($p \leq 0.05$) were removed from the model one at a time. adjusted for age and ethnic groups. The Wald p -test was used to assess the statistical significance of individual predictor variables on the outcome.

Chapter 3: Human papillomavirus genotype distribution and risk factor analysis amongst reproductive age Gambian women

3 Introduction

HPV infection is the most common sexually transmitted infection in reproductive age women (Bruni *et al.*, 2010; De Vuyst *et al.*, 2003). More than 75% of sexually active males and females will be infected with the virus at some stage in their lives, which in most cases will resolve without treatment (Youssef *et al.*, 2016; Dartell *et al.*, 2012). The peak time for acquiring HPV infection is shortly after sexual initiation, however, penetrative sex is not required for transmission. Skin to skin genital contact is a well-recognised mode of HPV transmission (Gervaz *et al.*, 2003; Marrazzo *et al.*, 2001).

Globally, more than 560,000 and 60,000 cancer cases in women and men each year are attributable to HPV, respectively (de Martel *et al.*, 2017; Plummer *et al.*, 2016; Ferlay *et al.*, 2015). This corresponds to 8.6% and 0.8% of all HPV-associated cancers occurring worldwide. In women, the percentage of HPV attributable cancers ranges from approximately 2% in Australia, New Zealand and the US to more than 25% in sub-Saharan Africa (de Martel *et al.*, 2017). The relative contribution of the bivalent vaccine HPV genotypes 16 and 18 and of the nonavalent (9-valent) vaccine types (6/11/16/18/31/33/45/52/58) are 460,000 and 570,000 cancer cases, respectively. This corresponds to 72% and 90% of all HPV-attributable cancer cases in both genders (Alemany *et al.*, 2016; Castellsague *et al.*, 2016).

Although HPV infection is vaccine-preventable, the widespread introduction of the vaccine in resource-limited countries is still in its infancy. Both HPV vaccination and HPV-based screening have been demonstrated to be cost-effective for a comprehensive prevention strategy in developed countries as well as in some developing countries (Jit *et al.*, 2014). The unavailability of the HPV vaccine and the limited use of cervical cancer screening in the developing countries often cause challenges in cervical cancer prevention (Bruni *et al.*, 2016; Santesso *et al.*, 2016).

In The Gambia, the age standardized rate (ASR) of cervical cancer incidence during the period 1990 - 2009 was reported as 16.9 per 100,000 per year (Bah *et al.*, 2013, Sighoko *et al.*, 2010). Furthermore, according to The Gambia Health Management Information system (HMIS), 237 females were diagnosed with cervical cancer in 2016 and 96% of these cases were from the urban regions of the country (Banjul, Kanifing Municipal Council and West Coast Region). Vaccine coverage of more than 70% was achieved with the quadrivalent HPV vaccine demonstration in the West Coast Region of the country. However, the major circulating HR-HPV genotypes are currently unknown in this population. Although the

quadrivalent vaccine targets HPV 6, 11, 16 and 18, the vaccine can only protect against 70% of cancers caused by HPV 16 and 18 genotypes. Other oncogenic types have been documented to cause cancers in the sub-Saharan Africa regions, which are not protected by the quadrivalent vaccine (WHO, 2017). It is envisaged that HPV vaccination will be rolled-out nationwide by 2019. Therefore, there is the need to have a baseline data on HPV infection rates and genotype distribution in this population.

3.1 Aims and objectives

The aims of the work presented in this chapter were to evaluate the potential value of introducing the quadrivalent vaccine in urban Gambia by investigating the major circulating HR-HPV genotypes and the behavioural risk factors associated with HPV infections.

The objectives were to:

- determine HPV prevalence distribution
- characterise the major circulating HR-HPV genotypes
- determine the intratype variability of the Gambia HR-HPV sequences and compare these with previously published HR-HPV sequences isolates from other geographical locations
- determine the socio-demographic and risk characteristics of participants infected with HPV
- determine the prevalence of HPV co-infection with selected STI / genital pathogens

3.2 Determination of adequate cellular DNA in samples

Of the 235 recruited women, 3 (1.3%) had samples from which inadequate cellular DNA had been collected so were excluded from this study. To determine HPV prevalence and genotype distribution, 232 endocervical swabs (case, n = 115, control, n = 117) with adequate cellular DNA were screened for HPV as described in Chapter 2, section 2.12.

3.2.1 HPV prevalence and genotype distribution amongst participants

Of the 232 participants screened for HPV, 28 were positive for HPV DNA, 15 (13.0%) from the case group and 13(11.1%) from the control group. HPV DNA sequencing identified 7 different HR genotypes, 2 different pHR and 7 different LR genotypes. Of the 13% (15/115) HPV positive participants from the case group, 53% (8/15) were infected with HR-HPV, none with pHR-HPV and 47% (7/15) with LR-HPV genotypes. In comparison with the control group, of the 11% (13/117) HPV positive participants, 46% (6/13) were infected with HR-HPV, 15% (2/13) with pHR-HPV and 39% (5/13) with LR-HPV genotypes (Table 3.1). Table 3.2 shows the distribution of the different HPV genotypes identified in both the case and control groups. No statistically significant difference was observed in the distribution of HPV in the two study groups ($p > 0.05$) by student 't'-test. HPV 52 was the most prevalent high-risk genotype identified in this study. Figure 3.1 shows the overall HPV genotype distribution.

Table 3.1 Prevalence of HPV risk types in case and control groups (number and percentage)

	Case group	Control group
HPV risk types	n (%)	n (%)
High-risk	8 (53)	6 (46)
probable high-risk	0 (0)	2 (15)
Low-risk	7 (47)	5 (39)
Total HPV positive	15(13)	13 (11)

Table 3.2 Distribution of HPV genotypes in case and control groups, identified by DNA sequencing and nucleotide Blast analysis

	Case group	Control group
HPV genotypes	(HPV-positive, n = 15)	(HPV-positive, n = 13)
6	1	1
16	0	1
35	0	1
42	1	0
51	2	0
52	2	3
53*	0	1
54	1	1
56	1	0
58	2	0
61	3	1
62	1	0
66	1	1
73*	0	1
83	0	1
89	0	1

High risk-HPV (HR-HPV) oncogenic genotypes (bold) are associated with causing cancers;

* probable high risk-HPV (pHR-HPV) genotypes are associated with intermediate potential risk of cancers. Low risk-HPV (LR-HPV) genotypes (not bold) are associated with causing genital warts or benign epithelial lesions. HPV 18 was not detected in any of the cervical samples.

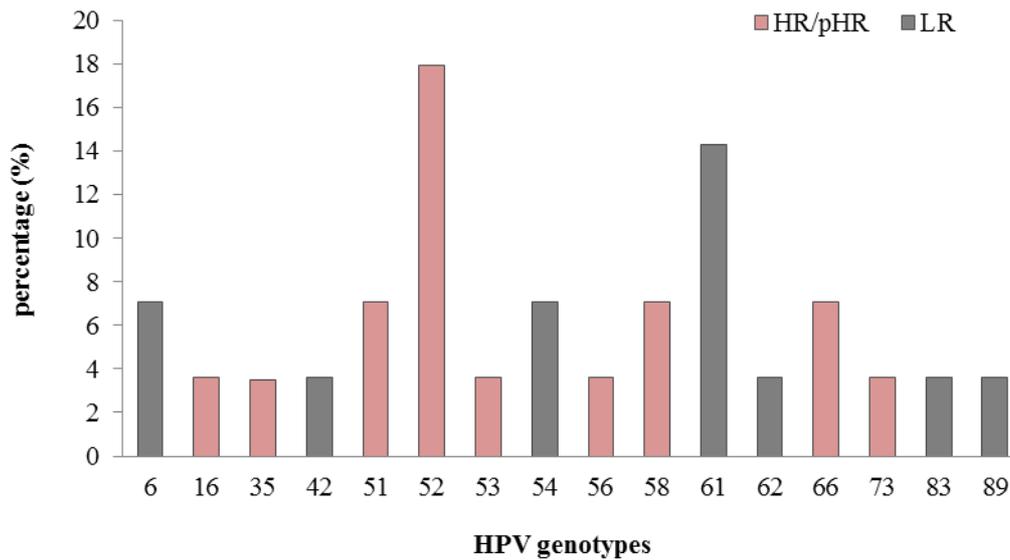


Figure 3.1 Distribution of HPV genotypes in reproductive age women attending a polyclinic, EFSTH, Gambia.

HPV 52 was the most predominate circulating genotype found in this study (17.9%) followed by HPV 61 (14.3%), HPV 6, 51, 54, 58, 66 (each at 7.1%), 16, 35, 42, 53, 56, 62, 73, 83, and 89 (each at 3.6%) of all genotypes identified by DNA sequencing and nucleotide Blast analysis.

Legend: dark grey colour: LR (low-risk genotype), Light pink colour: HR/pHR (high risk/probable high-risk)

3.3 HPV prevalence amongst the different age groups

The HPV age-specific prevalence curve of participants from the case group showed a U-shaped curve in the ages between 21 and 35 years, which was followed by a sharp prevalence increase (21%) in the 36 - 40 age group and a further prevalence decline in the 41 – 49 age group (Fig 3.2). In contrast to the case group, HPV age-specific prevalence in the control group showed a higher peak in the 21 - 25 age group (19%), followed by a steady decline in the ages between 26 and 35 years. A second prevalence peak was observed in the 36 – 40 age group, which was followed by a sharp decline in the 41 – 49 age group. In addition, HPV was observed in 9% of participants between the ages of 41 – 49 years in the case group compared to 0% from the control group (Fig.3.2). The overall HPV age-specific prevalence distribution of participants was investigated and an initial prevalence peak in the 21 - 25 age group (32%, 9/28) was observed, which was followed by a steady decline in the ages between 26 and 40 years, and a sharp prevalence decline in the 41 - 49 age group (Fig 3.3).

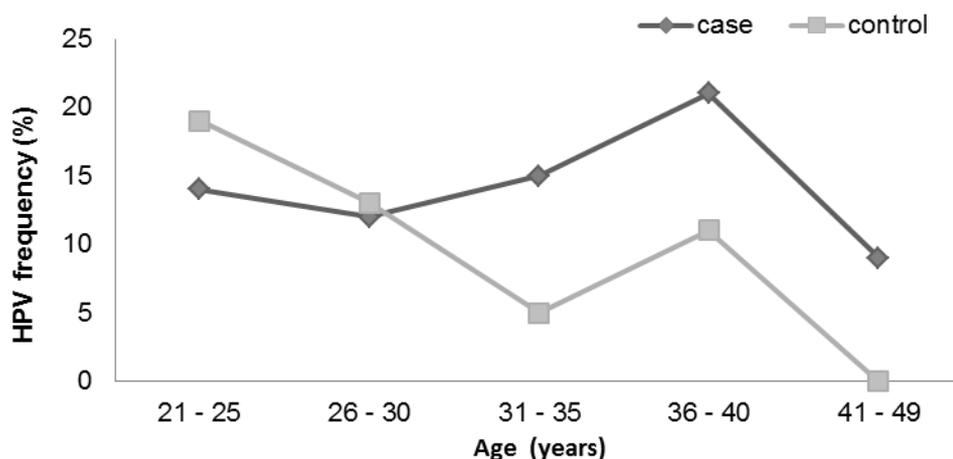


Figure 3.2 HPV age-specific prevalence curve of both case and control groups.

A U-shaped curve of HPV age-specific prevalence was observed in the ages between 21 and 35 years in the case group. HPV age-specific prevalence increase was observed in the 36 – 40 years age group in both study groups, which is followed by a sharp prevalence decrease in the 41 - 49 years age group. In contrast to the HPV age-specific prevalence decrease (0%) observed in the 41 - 49 years age group in the control group, HPV age-specific prevalence of 9% was observed in the 41 – 49 years age group in the case group.

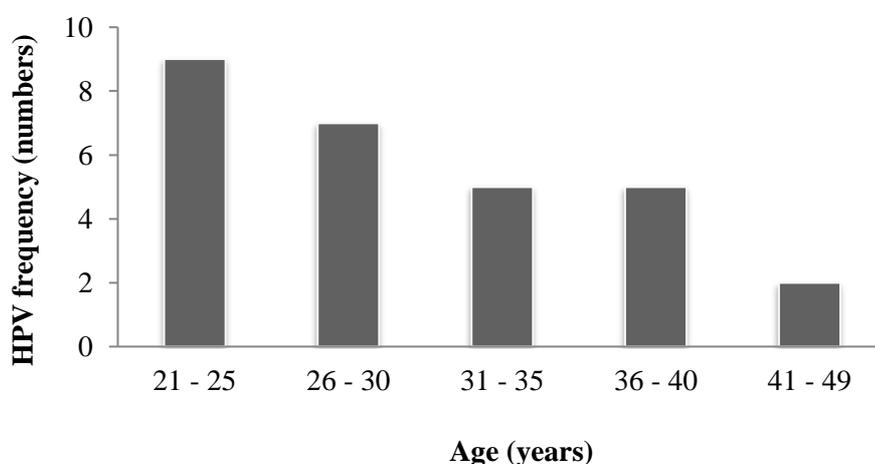


Figure 3.3 HPV age-specific prevalence curve in reproductive age women attending a polyclinic, EFSTH, Gambia.

Overall HPV age-specific prevalence peak 32% (9/28) was observed in the 21 - 25 years age group, followed by a steady prevalence decline in the ages between 26 and 30 years. A prevalence plateau was observed between the ages 31 – 40 years of age, which was followed by a sharp prevalence decline in the older ages (41 – 49 years age group).

3.4 High-risk HPV genotypes prevalence by age and ethnic groups

The prevalence of HR-HPV was found in the different age groups as follows: 29% (4/14) in 21 – 25 age group, 43% (6/14) in 26 - 30, 21% (3/14) in 31 – 35 and 7% (1/14) in 36- 40 age group, respectively. Though, the HPV age-specific curves have shown a second peak in the 36 – 40 years age group in both the case and control groups (Fig. 3.2), it is important to note that only one participant was infected with an HR-HPV genotype. Probable high risk (pHR-HPV) genotypes, HPV53 and HPV73 were only identified in the 21 - 25 years age group. HPV infection was not detected in the 20 years old. No significant association was observed between age group with HPV infection ($p > 0.05$)

Ethnicity-associated differences were also noted, HR/ pHR-HPV prevalence was 31% (5/16) in the Fulas compared to the 19% (3/16) and 12.5% (2/16) observed in the Mandinkas and Wollofs, respectively. However, LR-HPV genotypes were identified mostly in the Mandinka ethnic group, accounting for more than 40% (5/12) of the total LR-HPV types. Prevalence of both HR/pHR and LR-HPV genotypes was lower (12.5% and 8.3%) in the Wollofs compared to the other two major ethnic groups.

3.5 Classification and distribution of HPV risk type by region

Eighteen percent (18%) of participants from the case group resided in Banjul, the capital city, whilst the remaining 82% resided in the Kanifying Municipal Council and West Coast Region (KMC/WCR). However, the opposite was seen in the control group, 70% of the participants resided in Banjul and 30% in the KMC/ WCR. Figure 3.4 shows the classification of HPV types across the three study regions. HPV prevalence across the three regions were found as follows: 12.5% (13/104) in the capital city, Banjul with 6 infected with HR-HPV, 2 with pHR-HPV and 5 with LR-HPV, 11.9% (8/67) in KMC with 3 infected with HR-HPV and 5 with LR-HPV and 11.5% (7/61) in WCR with 5 participants infected with HR-HPV genotypes (Fig.3.4). No statistically significant difference was observed between residing in the capital city, Banjul or KMC/WCR with HPV infection ($p > 0.05$).

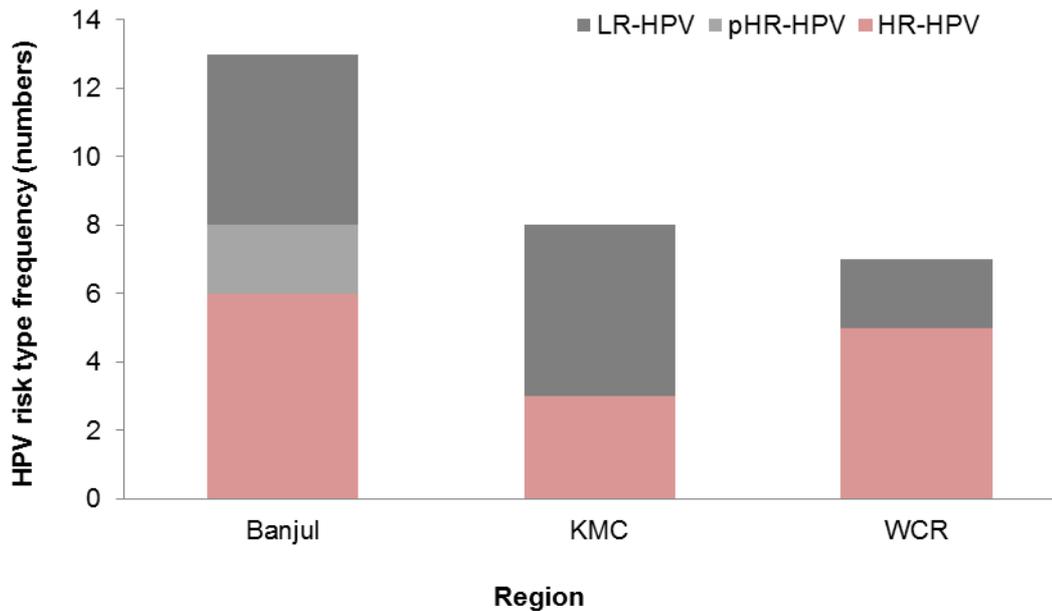


Figure 3.4 Classification of HPV risk type distribution in Banjul City, Kanifying Municipal Council and West Coast Region

A majority 43% (6/14) of participants infected with HR-HPV are residents of the capital city, Banjul, followed by WCR 36% (5/14) and KMC 21% (3/14). No regional variation was observed between those residing in the capital city, Banjul or the KMC/WCR with HPV infection ($p > 0.05$).

Legend and colours:

Pink– High-risk HPV genotypes (HR-HPV)

Dark grey – Low-risk HPV genotypes (LR-HPV)

Light grey– Probable high-risk HPV genotypes (pHR-HPV)

KMC- Kanifying Municipal Council;

WCR – West Coast Region.

3.6 Risk factors associated with human papillomavirus infection amongst reproductive age women.

Statistical analysis of risk characteristics of participants was carried out using bivariate analysis to determine relationships and strength of associations between two variables. In this analysis, the independent variables were participants' risk characteristics and the dependent variable was HPV infection, whilst controlling for age. HPV negative participants were used as a reference group in this analysis. Statistical analysis showed prolonged (> 5 years) use of hormone contraceptive was the only variable that was associated statistically with HPV infection (95% CI=0.9-1.3; $p = 0.03$), in this study (Table 3.3). No association was found between early menarche, having a new sexual partner (s) with HPV infection ($p > 0.05$). Although 82%

(23/28) of married participants were infected with HPV, marital status was not found to be associated statistically with HPV infection ($p > 0.05$) (Table 3.3). Twenty-five participants had reported having sexual intercourse in the preceding 12 months and only 8% (2/25) reported to have used a condom (Table 3.3). In this study, the use of condoms during sexual intercourse was found to have some reduced risk against HPV infection (AOR = 0.7). Although not statistically significant, reporting partners have or had other sexual partners, and had undergone female genital mutilation (FGM) increased the odds of being diagnosed with HPV infection (AOR = 3.5 and 2.1), respectively (Table 3.3). Of the 28 participants infected with HPV, 82% (23/28) had their sexual debut at the age of ≥ 18 years but no statistical association was found between having sexual debut at ≥ 18 years old with HPV infection ($p > 0.05$). Table 3.3 shows the statistical analysis of selected risk characteristics of participants infected with HPV.

Table 3.3 Socio-demographic and risk characteristics associated with HPV Infection, Negative (204), Positive (28) adjusted for age

Characteristics	HPV DNA results		Adjusted OR (95% CI)	P-value
	Negative (204)	Positive (28)		
Ethnic group#				
Fula	40	9	2.1 (1.0, 4.9)	NS
Mandinka	49	8	1.2 (0.4, 2.7)	NS
Wollof	39	3	0.5 (0.1, 1.7)	NS
Age at sexual debut				
≥18 years	131	23	2.2 (0.8, 6.2)	NS
<18 years	70	5		
Marital status				
Married	164	23	0.9 (0.3, 2.5)	NS
Unmarried	40	5		
FGM				
Yes	124	21	2.1 (0.9, 5.7)	NS
No	80	7		
Education				
<12 years	109	11	2.0 (0.9, 4.5)	NS
≥ 12years	95	7		
Lifetime sexual partner(s)				
≥2	77	14	1.8 (0.8, 4.1)	NS
1	124	14		
Partner (s)other sex partners				
Yes	99	13	3.5 (0.4, 28)	NS*
No	27	1		
Hormone contraceptive use				
>5 years	61	13	4.2 (0.9, 1.3)	0.03
<5years	97	10		
Low income				
<D75,000	160	20	1.7 (0.5, 5.5)	NS
>D75,000	8	44		
Condom use last 12 months				
Yes	20	2	0.7 (0.2, 3.3)	NS*
No	166	23		

#Major ethnic group, *Fisher exact due to small sample size numbers, MH- Mantel-Haenszel Odds Ratio, adjusted for age group. NS = not significant, $p > 0.05$

3.7 Human papillomavirus co-infection with STI and other genital pathogens

Two hundred and thirty-two (232) participants screened for HPV were also screened for selected STI/genital pathogens as described in the method section, Chapter 2, section 2.13 - 2.13.3. Of the 28 participants positive for HPV, 15 were from the case group and 13 from the control group. Table 3.4 shows the numbers and percentages of HPV positive participants co-infected with selected STI / genital pathogens. In addition, out of the 14 participants co-infected with *Ureaplasma*, 50% (7/14) were additionally co-infected with one or more of the other screened pathogens.

Table 3.4 HPV co-infection with selected STI / genital pathogens

	Case group	Control group
genital organisms	n (%)	n (%)
<i>Ureaplasma</i>	7 (46.7)	7 (53.8)
<i>Candida albicans</i>	4 (26.7)	1 (7.7)
<i>Streptococcus agalactiae</i>	2 (13.3)	1 (7.7)
<i>T. vaginalis</i>	1 (6.7)	0 (0.0)
<i>Neisseria gonorrhoeae</i>	0 (0.0)	1 (7.7)
HIV	7 (46.7)	0 (0.0)
Bacterial vaginosis*	2 (13.3)	1 (7.7)

*Bacterial vaginosis = polymicrobial syndrome; was diagnosed using Amsel criteria

3.8 BLASTn comparison of The Gambian HR / pHR-HPV DNA sequences to sequences deposited in the GenBank database

All the HR-HPV genotypes identified in this study showed $\geq 98\%$ homology to entries deposited in the GenBank database, except HPV 35 (Table 3.5). Of the three HPV 52 DNA sequences identified from the control group, two were 100% identical to a type isolated in Quebec, Canada and one was 99% identical to a South Korean isolate (Accession numbers: EU077215 and KY077858, respectively). Two of the HPV 52 DNA sequences identified from the case group were 100 and 99% identical to isolates from Quebec, Canada and Croatia (Accession numbers: EU077215 and KF707618, respectively). In addition, one sequence was shown to have an 82% similarity with an HPV 35 isolate from Ecuador (Table 3.5). However, as the difference between this sequence and the reference sequence (Accession number KU050112) *L1* gene differs by more than 10%, it was identified as a novel type. In addition, all other HPV genotypes with DNA homology of 98% with the closest known genotypes were defined as subtype and $> 98\%$ as variant types (Table 3.5). To further confirm the identification and relatedness of the HR/pHR-HPV sequences, a phylogenetic tree was constructed and evolutionary relationships within and between types determined.

Table 3.5 HR/pHR HPV DNA sequences comparison with isolates deposited in the GenBank database

HPV genotype identified	GenBank Accession Number	Isolate Number	Origins	% DNA similarity to Gambian samples
16	KY549284	C484604r11164343NP	Netherlands	98
35	KU050112	ECU-07	Ecuador	82
51	KF707619	R60	Switzerland	98
	KF707618	R72	Switzerland	99
52	KJ676061	CRO 1F6	Croatia	99
	EU077215	23	Canada	100
	EU077215	23	Canada	100
	EU077215	23	Canada	100
53	KY077858	KOR_M10- 4515	South Korean	99
	KU951263	CN10	China	99
56	KU298919	110A.56	Brazil	99
58	HM639676	ww100HK_973	Hong Kong	99
	HQ537776	Rw644	New York	99
66	KU298927	83A.66	Brazil	98
	KU298928	118A.66	Brazil	98
73	KU298936	58c.73	Brazil	99

3.9 Phylogenetic analysis of HR/pHR-HPV sequences

Two phylogenetic trees were constructed using Neighbour-joining and Maximum likelihood methods (Chapter 2, section 2.16) to further confirm the identification of The Gambian HR/pHR-HPV genotypes and compare the sequences to sequences of the same types from other geographical locations. The maximum likelihood tree was used to determine the relationship within and between types and with published sequences retrieved from the GenBank database. Phylogenetic analysis grouped each of The Gambian HR/pHR-HPV genotype sequences within its respective HPV genotype clade. HPV 52 genotypes grouped with comparable sequences from Canada (Accession number: EU077215), Croatia (Accession number: KJ676061), South Korean (Accession numbers: KY077846, KY077858 and KJ676970), China (Accession numbers: KU721792, KT799937 and KT799938), Brazil (Accession numbers: KU298908 and KU298909) with a bootstrap support of 99%. The Gambian HPV 51 grouped with HPV 51 isolates from Switzerland (Accession numbers: KF707618, KF707619, KF707624), Italy (Accession number: KP090001), Japan (Accession number: GQ487711), United Kingdom (Accession number: KP090028, KP090033) and China (Accession numbers: KT725857, JQ902122) with bootstrap support of 96%. The only HPV 16 genotype isolated from this study grouped with HPV 16 isolates from Netherlands (accession number: KY549284), an African-1 variant (AF536180) and a European German isolate (AF536179) with a bootstrap support of 96%. HPV 66 genotype appears to be more divergent compared to the other HPV types (Fig 3.6). Overall, the analysis showed that each of The Gambian isolates grouped within its respective genotype clades and within each genotype. Based on this analysis there was no major distinction between the high risk and probable high-risk genotype sequences here. Overall, no distinct geographical lineages were identified.

3.10 Pair-wise *p*-distance analysis

Uncorrected pair-wise distance (*p*-distance) analyses were carried out on The Gambian HR/pHR- HPV sequences to establish the level of divergence within and between the HR/pHR-HPV sequences. Low nucleotide divergence was observed within all types particularly within HPV 52 genotypes (0.2%). In contrast, *p*-distance divergence was observed between genotypes ranged from 0.181 - 0.368 (18 % - 36 %), however, *p*-distance between HPV 66 and HPV 56 genotype sequences was found to be less divergent (*p*-distance = 0.181) (Table 3.6).

Table 3.5 *p*-distances within (in bold) and between (not bold) HR/pHR-HPV genotypes

	HPV16	HPV51	HPV52	HPV53	HPV56	HPV58	HPV66	HPV73
HPV16	0.008							
HPV51	0.331	0.010						
HPV52	0.250	0.314	0.002					
HPV53	0.321	0.312	0.368	0.008				
HPV56	0.289	0.299	0.325	0.201	0.004			
HPV58	0.210	0.282	0.215	0.309	0.310	0.009		
HPV66	0.305	0.336	0.339	0.232	0.181	0.326	0.017	
HPV73	0.241	0.234	0.234	0.303	0.289	0.214	0.290	0.000

3.11 Regression analysis of *p*-distance versus geographical distance

A regression analysis was carried out as described in Chapter 2, section 2.16.2 to detect any major genetic diversification events between geographical locations (km) to determine inter-relationships and movement of the different geographical isolates and possibly determine the origin of The Gambian sequences. This was carried out using the Gambian HR/pHR-HPV sequences and other representative sequences of the different HR-HPV types from different geographical localities (Europe, America, Asia, and Africa) that formed clades with a bootstrap support of $\geq 90\%$ with the Gambian types. No significant association was observed between the different geographical locations and *p*-distances of the different HPV types ($p \leq 0.05$), except in HPV 66. A significant difference was observed between

HPV 66 *p*-distance (nucleotide) and distance (km) between the different geographical locations ($r^2 = 0.09$; $p = 0.01$) (Fig 3.6 and Table 3.7).

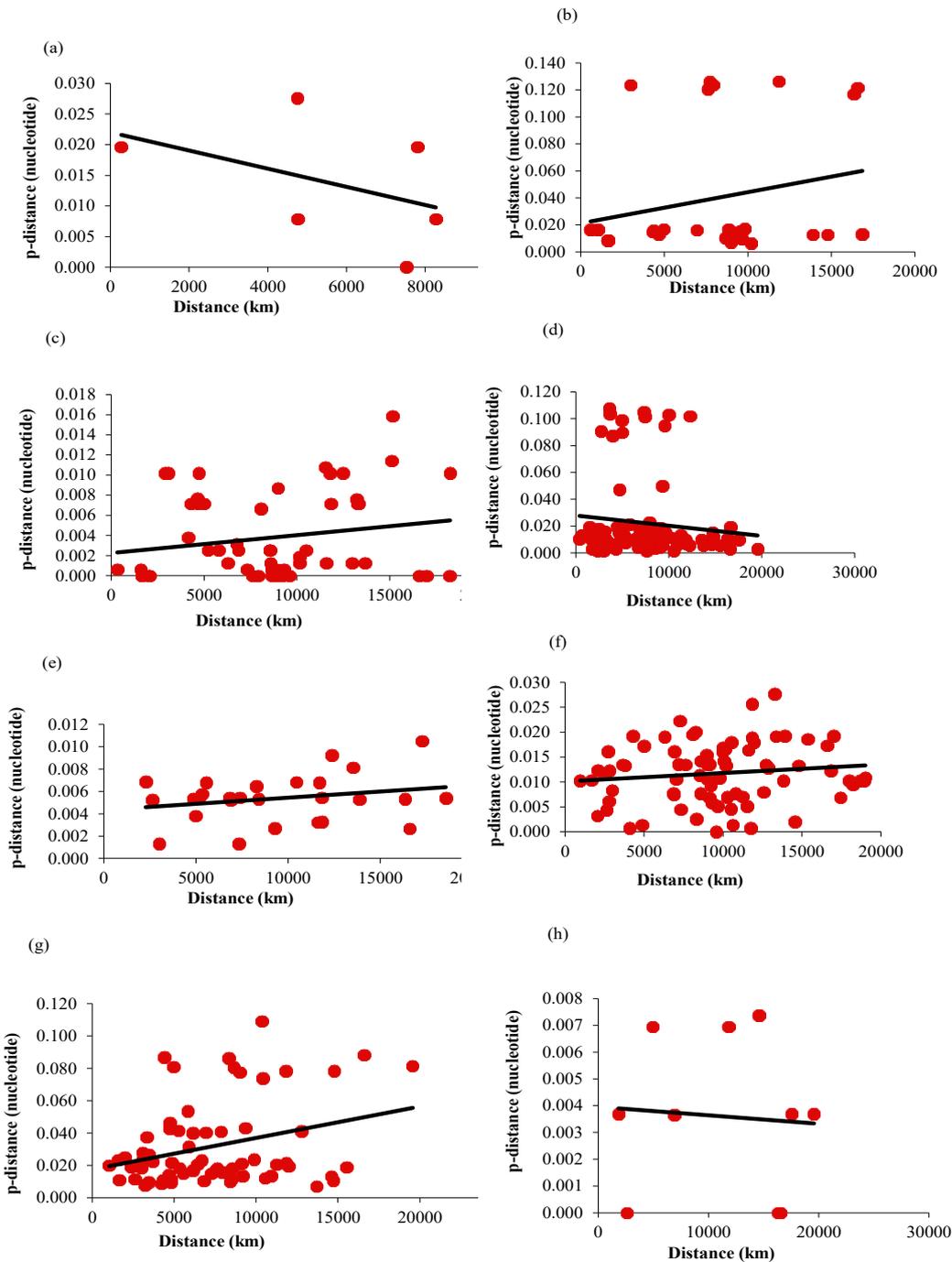


Figure 3.6 Relationship of distance (km) between different geographical locations and *p*-distances of the different HPV genotypes.

Legend: (a) - HPV genotype 16; (b) - HPV 51; (c) - HPV 52; (d) - HPV 53; (e) - HPV 56; (f) – HPV 58; (g) - HPV 66; (h) – HPV 73. Each red dot represents HPV genotype from different geographical locations (Europe, America, Asia and Africa) including the Gambian types.

Table 3.6 Regression analysis between genetic distance of the high risk/probable high-risk HPV sequences and geographical distance (Km)

HPV type	Number of sequences	Range of distances (km)	Slope	Intercept	R-squared	Degrees of freedom	P –value
16	11	279.6 - 8270	-1.48E-6	0.021982	0.19173	4	NS
51	26	599.8 - 16843	2.30E-6	0.02131	0.049467	26	NS
52	25	336.1 -18231	1.78E-7	0.00225	0.035478	53	NS
53	31	1501 -19553	-7.58E-7	0.027843	0.012122	89	NS
56	21	2271 - 18541	1.09E-7	0.004349	0.058124	26	NS
58	33	2036 - 19011	1.68E-7	0.010115	0.016488	76	NS
66	33	1036 - 19553	1.94E-6	0.017575	0.094088	64	0.01
73	9	2641 -19553	-3.20E-8	0.003961	0.005342	8	NS

No significant association was observed between the distances between different geographical locations and *p*-distances of the different HPV types ($p > 0.05$), except in HPV 66 genotype ($r^2 = 0.09$; $p = 0.01$). NS =Not significant ($p > 0.05$).

3.12 Discussion

HPV is the most common sexually transmitted infection in sexually active women; more than 75% of sexually active women will be infected with the virus at some stage in their lives (Dartell *et al.*, 2012). Females between the ages of 15 - 65 years represent 52% of the Gambian population and based on the incidence of 32.4 per 100,000 are at risk of being diagnosed with cervical cancer. Persistent infection with high-risk HPV genotypes can cause HPV progression to cervical diseases. The introduction of any HPV vaccine prevention strategy requires consideration of the major circulating HR-HPV genotypes in a population and cervical diseases. HPV genotyping is very important in primary cervical cancer screening as persistent infections with high-risk genotypes can progress to cervical cancer, especially in females between the ages of 30 years and older. The quadrivalent vaccine has been introduced in the urban region of the Gambia and this is the first research of HPV genotype distribution in the area where most cervical cancer cases are reported.

HPV prevalence was found in the three study regions as follows: Banjul 12.5%, Kanifing Municipal Council 11.9%, and West Coast Region 11.5%. Forty-three percent (6/14) of the HR-HPV genotype distribution was observed in the capital city Banjul, followed by 36% (5/14) in the WCR, and 21% (3/14) in the KMC (Fig 3.4). However, no regional variation was observed in the distribution of HPV genotypes between participants residing in the capital city, Banjul or in the KMC/WCR ($p > 0.05$).

Overall HPV prevalence was found to be 12.1%, which is similar to the 13% prevalence reported for rural Gambia, but less than the 18% reported in nearby Dakar, Senegal and 40.8% in Egypt (Youssef *et al.*, 2016; Xi *et al.*, 2003; Wall *et al.*, 2005). Of the 28 HPV positive samples in this study, 14 (50%) were HR-HPV genotypes and 2 (7.1%) were pHR-HPV genotypes. This is somewhat greater than the HR-HPV prevalence reported for Dakar, Senegal (17.4%) and for south-western Nigeria (19.6%), (Ezechi *et al.*, 2014; Mbaye *et al.*, 2014), where both studies focused on women within the ages of 18 – 80 years of age. The differences in prevalence seen in these studies could be attributed to the different age groups or, perhaps, more importantly could be due to the variability of HPV genotypes in different geographical locations. The 50% HR-HPV prevalence seen in this study could also be reflecting selection bias since cervical samples were collected from individuals who chose to attend a sexual health clinic.

HPV 52 was the most common high-risk genotype identified, accounting for 31.3% of the total HR/ pHR-HPV genotypes and 17.9% of all genotypes. HPV 61 was the most frequent LR genotype identified with an overall prevalence of 14.3% and accounting for 33.3% of all LR-HPV (Fig.3.1). In contrast to earlier work in rural Gambia, where HR-HPV 16 and LR-HPV 42 were the most common genotypes identified (Wall *et al.*, 2005), this study showed that 89% of HPV genotypes identified did not match those included in the quadrivalent vaccine (Fig. 3.1). The different prevalence of HR-genotype between the two studies might be due to sensitivity of the methods used in detecting HPV DNA. The current study employed a pool of primers (modified PGMY09/11), which is more sensitive in detecting many HPV specific genotypes than the single consensus GP5+/GP6+ primers that amplify a 140 bp of the *L1* gene. The sensitivity and specificity of the various HPV detection methods available vary greatly and can cause either an underestimation or over-representation of HPV specific types especially in multiple infections (Vernon *et al.*, 2000). Furthermore, the limitation in HPV detection methods has been a concern in many epidemiological studies, however, the past decades have seen a great improvement in the sensitivity and specificity of these methods due to better quality and stability of reagents and primers (Gravitt *et al.*, 2001).

Similarly, work carried out in an urban region of Senegal, the only country to neighbour The Gambia also found that HPV 52 was the most common genotype, in the Dakar region (Mbaye *et al.*, 2014). The same observation was also seen in studies carried out in Kenya and Tanzania (Dartell *et al.*, 2012; Rahman *et al.*, 2011; De Vuyst *et al.*, 2003). This augments the findings of Bruni *et al.*, (2010) that HPV 52 is a major genotype in Africa. HPV 16 and 18 are the predominant circulating genotypes found in Southern Africa, Europe and America. However, HPV 16 was found to be the fifth most common HR genotype with a prevalence of 7.1% and the eighth most identified genotype with an overall prevalence of 3.6%. HR-HPV 18 was not detected in any of the cervical samples. The reason for this finding is not known, it could possibly be due to chance, which need addressing with a larger sample size. Although cervical cancer is more prevalent in Africa compared to Europe and America, HPV 16 and 18 seems to lose its predominance as the major circulating genotype in some parts of Africa. Studies in Africa have shown that other HR genotypes such as HPV 31, 35 and 58 are the major circulating genotypes (Akarolo-Anthony *et al.*, 2014; Gage *et al.*, 2012; Okonofua, 2007; Thomas *et al.*, 2004), indicating that the HPV bivalent and quadrivalent vaccine may not be as effective in Africa as previously thought (Luquea *et al.*, 2010; Blossom *et al.*, 2007; Clifford *et al.*, 2003).

Considering the high burden of cervical cancer cases and the lower prevalence of HPV16 and 18 in Africa, it could be that other HR-HPV genotypes may be responsible for the high burden of the disease. In addition, a study in Asia found that HR-HPV 52 and 58 genotypes (3.8% and 5.6%, respectively) were associated with a number of cases of invasive cervical cancer and high squamous intraepithelial lesions (HSIL) (Smith *et al.*, 2007(a)). This finding further asserts the importance of determining the major HPV genotypes in cervical diseases and cancers before introduction of the vaccine and is an important step in effective HPV infection prevention strategies. Furthermore, it has been demonstrated here that 10.7% of participants were infected with HPV genotypes targeted by the quadrivalent vaccine and 35.7% were positive for HPV genotypes included in the Gardasil 9 vaccine.

This data is an important public health implication as the HPV quadrivalent vaccine may be of limited value for The Gambia if the circulating non-HPV 16, 18 HR- genotypes are responsible for cervical cytological abnormalities and progression to cervical cancer. The LR-HPV genotypes are associated with causing cutaneous lesion, respiratory papillomatosis and condyloma acuminata, which HPV 6 and 11 are responsible for almost 90% of these cases, globally (Poljak, 2011). Although LR-HPV-types associated conditions rarely result in death, they may cause significant occurrence of diseases such as genital warts, which is very common and highly infectious. Of the 12 LR genotypes detected, HPV 61 was the most identified genotype, representing 14.3% and 33.3% of the total HPV and LR genotypes, respectively. In contrast, HPV 6 is the most common type detected in America and Europe including some other parts of Africa (Youssef, *et al.*, 2016; Mbaye *et al.*, 2014; Munoz, *et al.*, 2003). However, it was the second most common LR genotype identified (16.7%) in this study.

DNA sequence analysis has shown that none of the HR/pHR-HPV genotypes detected were identical to isolates from Africa found in the GenBank database, but rather isolates from America, Asia and Europe. In addition, The Gambian HPV 35 isolate might be a novel type as the *L1* gene sequence differs by more than 10% to the closest known HPV 35 genotype isolated in Ecuador.

Phylogenetic analysis also showed that all 15 HR/pHR-HPV sequences formed clades with most isolates detected in other geographical locations such as Europe, America and Asia except HPV genotype 16, which also formed clades with isolates that belong to the African branch (African-1 and African-2).

Based on previous phylogenetic studies, the geographical locations of HPV 16 genotypes were classified as European, African-1, African-2 and Asian-American (Chen *et al.*, 2005; Wheeler *et al.*,

1997). The Gambian HPV 16 was also found to group with isolates from these three geographical locations (Fig.3.5). The finding of The Gambian HPV 16 as the only HR-HPV type forming clades with isolates from the African-1 and 2 geographical locations was not surprising as HPV 16 has been reported to potentially have evolved separately from Africa and Europe and transferred to the rest of the continent through colonial migrations (Chan *et al.*, 1992). Studies on HPV 16 oncogenicity have shown that infection with the non-European variant is strongly associated with the development of severe cervical lesions and cervical cancer in Europe and Mexico (Tornesello *et al.*, 2004; Berumen *et al.*, 2001).

Furthermore, the HPV 52 variants detected in The Gambia were found to form clades with isolates from Europe and Asia with strong bootstrap support (Fig 3.5). The *p*-distance between HPV 66 sequences and HPV 56 sequences were found to be more alike to each other (*p*-distance = 0.181). This could be due to both genotypes belonging to HPV species 6, evolved from the same common ancestor and are closely related (de Villiers *et al.*, 2004). Furthermore, HPV genotype 66 was the only type in this study that appears divergent compared to the other HR-HPV types. This could be a result of relatively recent divergence events reflecting migration patterns of *Homo sapiens* in different geographical regions. Based on the current results it could be that these HR/pHR-HPV genotypes may have been imported into The Gambia or, more likely, could be recent movement of HPV from The Gambia mediated by the historical movement of people. This highlights a key difference with an earlier study in rural Gambia, which found that most of the HR-HPV sequences were homologous to isolates from Africa (Wall *et al.*, 2005). Contributing factors may be linked to the fact that the urban area is a popular tourist destination, therefore, the lifestyle and sexual behaviour of the participants may be different.

Infection with HPV is common in young females; however, most of these infections are transient and resolve within 12 months, with only a small percentage developing persistent infection (Youssef *et al.*, 2016; Giuliani *et al.*, 2006). The 32% HPV prevalence peak seen in the 21 - 25 years age group (Fig.3.3) follows population norms of sexual initiation as 77.8% of the participants had their sexual debut at the age of ≥ 18 years. Although not statistically significant, sexual debut at the age of 18 years or older was found to increase the odds of being infected with HPV by two-fold (AOR =2.2; *p* > 0.05) (Table 3.3). A second HPV prevalence peak was observed in the 36 – 40 years age group in both study groups (Fig 3.2), which may be caused by several factors including the inability to clear the infection due to poor immune response, reactivation of latent infection due to hormonal factors or

co-infection with other genital STI pathogens. In addition, most of these women live in a polygamous relationship, which could also lead to the acquisition of new HPV infections.

In contrast to a review by Clifford *et al.*, (2005), which indicated that HPV prevalence increases with age in Gambian women, this study found a sharp decline in prevalence in those greater than 40 years old, which is consistent with viral transience. A similar finding was also observed in Abuja, Nigeria (Akarolo-Anthony *et al.*, 2014). Studies carried out in Africa and Asia have reported a biphasic or a flat shaped HPV age-specific curve in older ages (Xi *et al.*, 2003; Franceschi *et al.*, 2006). However, 41.7% of the HR-HPV genotypes were found in the 26 - 30 years age group, which highlights the importance of early and regular HPV and cervical cancer screening. The data also revealed that 93% of participants never had cervical cancer screening (chapter 5, Table 5.1). This may be due to either lack of awareness about cervical cancer or accessibility to screening, or both. The Gambia Histopathology Laboratory is situated at the EFSTH, Banjul and it is currently the only laboratory offering cytology in the country. In addition, there is no decentralised national cervical cancer screening programme; this makes access to screening a significant problem. Cervical cancer screening programmes are very limited in the developing countries and it has been documented that only 5% of women from these countries are screened for cervical cancer compared to 75% of women from developed countries (WHO, 2017). The high burden of HPV infections and low coverage of cervical cancer screening observed in the developing countries may be attributed to a lack of resources to implement efficient vaccination and screening programmes. Currently, The Gambia uses the visual inspection acetic acid (VIA) test for cervical cancer screening and it is being carried out in only a few major health facilities in the urban region of the country. Cervical cancer screening using HPV DNA has been shown to be better in identifying women at higher risk of developing cervical dysplasia than the non-genotyping screening and it has a higher negative predictive value than Pap smear or VIA (Cuschieri and Cubie, 2005). HPV DNA is particularly important for monitoring of the high-risk genotypes that are associated with cancers (Venturoli *et al.*, 2008; Brink *et al.*, 2006). The minimum recall time for women who screened negative for HR-HPV DNA is 5 years compared to recall-time of 3 years with the VIA screening. However, it is recommended to combine HPV DNA with VIA screening in developing countries to detect the stages of the infection to avoid over or under treating of individuals (WHO, 2013).

Whilst HPV infection plays a vital role in cervical cancer development, other socio and risk co-factors appear to contribute to the increased risk of disease progression. The use of hormone contraceptive for more than 5 years was found to be statistically associated with HPV infection ($p = 0.03$) (Table

3.3). A similar finding has also shown that women, who used hormone contraceptives for more than 5 years are at increased risk for developing cervical cancer (Bosch *et al.*, 2006). However, association studies of HPV positive women and long-term use of hormone contraceptives have failed to reach consensus (Smith *et al.*, 2003). There is a potential association between HPV infection and prolonged use of hormone contraceptives in the development of cervical cancer, which needs addressing with a larger study population.

In this study, partners having other sexual partners was found to increase the odds of being infected with HPV by more than three-fold (AOR = 3.5) (Table 3.3). This interaction may be linked to polygamy, which is a common practice in The Gambia and Africa and has implications of increased frequency of sexual activity with more than one partner (chapter 5). Furthermore, 91.7% (22/24) of participants who were HPV-infected reported they did not use a condom during sexual intercourse in the preceding 12 months. Eighty percent of the respondents were married and are less likely to report using a condom during sexual intercourse than unmarried women. Consistent use of condoms during sexual intercourse can reduce the risk of acquiring HPV infection. However, it has been shown that protection is only partial as condoms cannot prevent skin to skin HPV transmission in the genital areas not covered by the condom or during non-penetrative sex (Trottier and Franco, 2006; Winer *et al.*, 2006).

HPV prevalence was found to be 32% in the Fula ethnic group and this ethnic group were at increased odds of being diagnosed with HPV by two-fold (AOR = 2.1) than the other two major ethnic groups (Table 3.3). This observation was also highlighted by Wall *et al.*, (2005) HPV study in rural Gambia. Similarly, Sighoko *et al* (2010) also noted an ethnicity variation in their study on cervical cancer in The Gambia. They found the Fula ethnic group was more at risk of being diagnosed with cervical cancer compared to the other ethnic groups. The differences seen in the prevalence of HPV infection in the different ethnic groups may be linked to possible genetic factors as previously reported (Sighoko *et al.*, 2010; Wall *et al.*, 2005; Allsopp *et al.*, 1992), or FGM being a predisposing factor. FGM is a common cultural practice amongst certain ethnic groups of the Gambia (Chapter 5). The data showed that all the Fula (9/9) women who were infected with HPV underwent FGM. In The Gambia, Wolof women are least likely to have had FGM and possibly to be at reduced risk for HPV infection (AOR= 0.5) (Table 3.3). This study further showed that participants that have undergone FGM were twice likely to be diagnosed with HPV infection (AOR = 2.1). Similarly, studies of Senegalese and Malian women also found FGM to be a risk factor for HPV infection (Osterman *et al.*, 2018; Bayo *et al.*, 2002).

HPV-related cervical cancer can take up to 20 years to develop depending on HPV type and viral load (Verteramo *et al.*, 2009). However, co-infection with other genital STI pathogens can facilitate the progression and integration of HPV in the host genome. Twenty-five percent of women infected with HPV were found to be co-infected with HIV. The incidence of HIV has been shown to exacerbate HPV acquisition, persistent infection with multiple genotypes and increases the carcinogenicity of high-risk genotypes (Cameron *et al.*, 2016). In addition, a worldwide meta-analysis on HPV and HIV has shown that HR-HPV 16 and 18 accounted for most of the invasive cervical cancer compared to normal cytology in HIV infected women.

However, other HR-HPV genotypes also accounted for a significant proportion of high-grade lesions and invasive cervical cancer in African women infected with HIV (Clifford *et al.*, 2017; Cameron *et al.*, 2016). It has also been shown here that 50% of the participants infected with HPV were co-infected with *Ureaplasma parvum/urealyticum*. Other authors have also found high *Ureaplasma* prevalence in females with HSIL compared to those with normal cytology (Ekiel *et al.*, 2009 (a); Lukic *et al.*, 2006). Although *Ureaplasma parvum/urealyticum* infections are known to be sexually transmitted, they are often not diagnosed and treated. These microorganisms can cause chronic pelvic inflammatory diseases and infertility if left untreated (Further discussion in Chapter 4). In addition, *Ureaplasma* can damage the vaginal epithelium and causes cervical mucus degradation thus potentially facilitating the progression of HPV infection to cervical cancer (Yong, 2017). This work adds to the body of evidence that *Ureaplasma* infection may be an important co-factor in HPV infection. This highlights the importance of probably screening for *Ureaplasma parvum/urealyticum* and providing appropriate treatment to HR-HPV infected women, especially those with abnormal cytological results.

In conclusion, there is a difference between the major circulating HR-HPV genotypes in this study and the rural one; however, both studies underscore the need for a multivalent vaccine that targets all major HR-HPV genotypes in the general population. Although the quadrivalent vaccine has been piloted in The Gambia, this study raises important public health issues with HPV vaccination programmes in developing countries. The introduction of accessible HPV DNA testing and cytology screening would be beneficial to Gambian women in cervical cancer prevention. Furthermore, HPV DNA testing would be an effective screening method in the monitoring and evaluating the impact of the quadrivalent vaccine in the population. This will provide an informed policy decision as to whether HPV vaccine types have disappeared from the population and how the prevalence of genotypes not included in the vaccine is affected. Future studies to investigate HPV genotype

distribution in cervical cancer specimens would be necessary for better planning and cervical cancer intervention strategies in The Gambia.

Chapter 4: Distribution of *Ureaplasma parvum* and *urealyticum* in symptomatic and asymptomatic Gambian women seeking primary health care

4 Introduction

Ureaplasma species belong to the class *Mollicutes* and more than 200 species are found in humans, arthropods, plants and vertebrate animals (Waites *et al.*, 2005). Seventeen of the *Mollicutes* species are found predominantly residing in the respiratory and urogenital tracts of humans and five are known to be pathogenic. Of the five pathogens, four are urogenital pathogens, namely: *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, and *Ureaplasma urealyticum* whilst *Mycoplasma pneumoniae* is a respiratory pathogen (Paralanov *et al.*, 2012).

Mycoplasma genitalium (MG) has been identified as a ‘true’ sexually transmitted pathogen and is linked to causing non- gonococcal urethritis (NGU) in men and has been isolated in >10% of men with symptomatic NGU compared to < 2% in asymptomatic individuals (Kim *et al.*, 2011; Ross *et al.*, 2009). The organism has also been implicated in causing cervicitis and an increased risk of pelvic inflammatory disease (PID), endometritis and infertility in women (Umeno and Jensen., 2017). However, *Ureaplasma* and *Mycoplasma hominis* are frequently found in the human urogenital tract in both healthy individuals and symptomatic patients. Although colonization with *Ureaplasma* is common, the organism can be sexually transmitted and evidence of association with a variety of clinical outcomes such as non-gonococcal urethritis and non-*Chlamydial* infections in men, pelvic inflammatory diseases (PID), cervicitis, infertility and chorioamnionitis have been documented (Yamazaki *et al.*, 2012; Waites *et al.*, 2005). Initially, *Ureaplasma urealyticum* was thought to be the only species from this genus to infect humans until recently when genomic characterization showed that the two biovars were distinctly different in size and they have differential growth responses to manganese (Echahidi *et al.*, 2000). Genomic characterization shows *Ureaplasma parvum* has a smaller genome size of 0.75 - 0.76 mega base-pairs (Mbp), whereas *Ureaplasma urealyticum* has a larger genomic size of 0.88 - 1.2 Mbp. The two biovars were later classified into two species, *Ureaplasma parvum* (*U. parvum*) biovar 1 or parvo and *Ureaplasma urealyticum* (*U. urealyticum*) biovar 2 or T960.

Fourteen *Ureaplasma* serotypes have been identified to infect the human mucosal tracts, which includes *U. parvum* serotypes 1,3, 6 and 14 and *U. urealyticum*, which includes the serotypes (2, 4, 7 -13) (Kong *et al.*, 2000). Some earlier studies have limited the pathogenicity and virulence of *Ureaplasma* to one or few of the 14 identified serotypes; serotypes 3, 4, 6 and 8 were initially associated with pathological outcomes (Polvsen *et al.*, 2002). Recently, it has been demonstrated that individual serotypes are not likely to be associated with differential pathogenicity as the host factors

and antigen variability are also important determinants for *Ureaplasma* infection (Xiao *et al.*, 2011). The development of nucleic acid amplification methods and the appropriate species differentiation of *U. urealyticum* and *U. parvum* have enhanced the understanding of the pathological role of these organisms in reproductive age women (Yamazaki *et al.*, 2012; McKechnie *et al.*, 2011).

There is limited laboratory-based evidence of STI prevalence in the Gambia as suspected cases are treated using the syndromic management approach. The syndromic treatment was designed by the World Health Organisation (WHO) in the early 1990's to prevent and reduce the risk of HIV transmission in Africa. The current STI syndromic management, which is based on symptoms alone may be important but is not enough as most of these clinical symptoms overlap. In The Gambia, the standard regime treatment guidelines for non-gonococcal urethritis, cervicitis and other PIDs include Ciprofloxacin (500 mg) stat dose, Metronidazole (flagyl) (2 g) and Doxycycline (100 mg) as first-line primary treatment as part of the syndromic management approach. Erythromycin (500 mg) is given as a second-line treatment option, where *N. gonorrhoeae* treatment with Ciprofloxacin had failed. Furthermore, the first national STI laboratory-based survey in The Gambia was carried out in 1992, using antenatal women. The prevalence of STI was found to be 29% at that time and since then no other laboratory-based evidence has been carried out on STI, except on HIV, which was reported as 2% (Sentinel surveillance, 2016).

Since studies have identified pathological conditions that are associated with *Ureaplasma* infection in reproductive age women (Yamazaki *et al.*, 2012), the characterisation of *Ureaplasma* species in Gambian women may help investigate the significance of this organism in clinical pathology. Furthermore, there is no current data on the incidence of *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis* in The Gambia, except *Ureaplasma*, which was reported as 0% (Demba *et al.*, 2005). Therefore, there is a strong need to have current laboratory-based evidence data on the incidence of STI to strengthen the information, education and communication (IEC) on STIs prevention and control in The Gambia.

4.1 Aim and Objectives

The aims of the work presented in this chapter were to study the epidemiological pattern and microbial burden of *Ureaplasma* and other STI/genital pathogen infections in symptomatic and asymptomatic reproductive age women.

The following objectives were considered:

- To determine the burden of urogenital *Ureaplasma* in reproductive age women
- To characterise urogenital *Ureaplasma* serotypes amongst women with (case group) or without (control groups) symptoms of STI
- To determine the socio-demographic and risk characteristics of participants infected with urogenital *Ureaplasma*
- To determine the prevalence of selected STI pathogens
- To determine the antibiotic susceptibility pattern of selected isolated pathogens.

4.2 Prevalence of *Ureaplasma parvum* and *urealyticum* amongst participants

Two-paired samples of endocervical swabs (ECS) and high vaginal swabs (HVS) from each participant was screened for urogenital *Ureaplasma* using duplex real time-PCR (q-PCR) with conserved primers that detect both *Ureaplasma parvum* and *urealyticum* in a single reaction tube as described in chapter 2, section 2.13.1. Conventional PCR was carried out on samples that were positive with q-PCR as described in section 2.10. PCR amplicons were purified and sent for DNA sequencing as described in chapter 2, section 2.14 and 2.15.

Urogenital *Ureaplasma* was detected in 46% (107/232) participants, 41% (47/115) were from participants that reported one or more symptoms of sexually transmitted infections (case group) and 51% (60/117) were from asymptomatic participants (control group). Of the 47 participants (case group) positive for *Ureaplasma*, 89% (42/47) had vaginitis /cervicitis and vaginal discharge, 63% (30/47) had lower abdominal pain, 53% (25/47) had dysuria (painful and burning urination) and 51% (24/47) reported dyspareunia (pain during sexual intercourse). *Ureaplasma parvum* was identified in 79 participants and 48% (38 /79) were from the case group and 52% (41/79) from the control group (Table 4.1). *Ureaplasma urealyticum* was detected in 25 participants with 68% (17/25) identified from the control group, whilst three participants were infected with both species (Table 4.1). However, there was no statistically significant difference between the incidence of *Ureaplasma* prevalence amongst the case and control groups ($p > 0.05$). Table 4.1 shows the overall prevalence of *Ureaplasma* species in both case and control groups.

Table 4.1 Prevalence of urogenital *Ureaplasma* in the study group (number and percentage of positive cases)

Pathogens	Case (n = 115)		Control (n = 117)		Overall Distribution (n = 232)	
	n	%	n	%	n	%
<i>U. parvum</i>	38	33.0	41	35.0	79	34.1
<i>U. urealyticum</i>	8	7.0	17	14.5	25	10.8
<i>U. parvum</i> + <i>U. urealyticum</i>	1	0.9	2	1.7	3	1.3
Total	47	40.9	60	51.2	107	46.2

Fourty-one percent (41%) and (51%) participants from the case and control groups were infected with *Ureaplasma*, respectively. No statistically significant difference of *Ureaplasma* prevalence was observed in the two groups ($p > 0.05$)

4.3 Prevalence of *Ureaplasma parvum* and *urealyticum* amongst different age groups

Of the recruited age groups, *Ureaplasma* prevalence (56%) was found in the 31 - 35 years age group compared with 45% distribution in the 26 - 30 years age group (Table 4.2).

Table 4.2 *Ureaplasma* prevalence amongst the different age groups

Age group (Years)	Total number recruited	Prevalence n (%)
20	7	3 (42.9)
21 – 25	53	22 (41.5)
26 – 30	57	26 (45.6)
31 – 35	46	26 (56.5)
36 – 40	33	14 (42.4)
41 – 49	36	16 (44.4)

A pair-wise statistical comparison was carried out amongst the age groups, firstly, *Ureaplasma* distribution in the 20 years old was compared with the other age groups (comparing to: 21 - 25 years, $p = 0.9$, 26 - 30 years, $p = 0.8$, 31 - 35 years, $p = 0.7$, 36 - 40 years, $p = 0.7$, 41- 49 years, $p = 0.8$ and the total $p = 0.5$), no statistically significant difference was observed. In addition, all other pair-wise comparison shows no statistically significant differences amongst the age groups ($p > 0.05$) with *Ureaplasma*. However, the total *Ureaplasma* distribution (107/232) amongst the different age group showed *Ureaplasma* prevalence decreases in the older ages, 36 - 40 years and 41 - 49 years age group (13%, 14/107 and 14%, 16/107), respectively.

The distribution of *U. parvum* amongst the different age group showed the incidence was higher in the age range of 26 - 35 years of age in both study groups (Table 4.3). However, *Ureaplasma urealyticum* was more prevalent (35%) in the 21 – 25 years old in the control group when compared with the case group, which shows a prevalence of 25% in the 21 - 25 years and older age group (Table 4.3).

Table 4.3 Prevalence of *Ureaplasma parvum* and *urealyticum* in the different age groups of symptomatic (case group) and asymptomatic (control group) participants

<i>Ureaplasma</i> species	Case group	Control group
	n (%)	n (%)
<i>U. parvum</i> (n = 79)		
Total positives	38 (48.1)	41(51.9)
Age group: 20 (years)	1 (2.6)	0 (0.0)
21 – 25	6 (15.8)	7 (17.1)
26 – 30	10 (26.3)	12 (29.3)
31 – 35	10 (26.3)	9 (21.9)
36 – 40	2 (5.3)	8 (19.5)
41 – 49	9 (23.7)	5 (12.2)
<i>U. urealyticum</i> (n = 25)		
Total positives	8 (32.0)	17(68.0)
Age group: 20 (years)	1 (12.5)	1 (5.9)
21 - 25	2 (25.0)	6 (35.3)
26 – 30	1 (12.5)	3 (17.6)
31 – 35	2 (25.0)	4 (23.5)
36 – 40	2 (25.0)	2 (11.8)
41 – 49	0 (0.0)	1 (5.9)

Ureaplasma DNA was identified in all age groups (20 - 49 years old) with 74% (79/107) incidence observed with *U. parvum*. However, none of the participants in the 41- 49 years age group from the case group were infected with *U. urealyticum*. Both *Ureaplasma parvum* and *urealyticum* DNA were detected in three participants, 1 from the case group within the age group 41- 49 years and 2 from the control group within the age groups of 21 - 25 and 31 - 35 years.

Furthermore, the overall *Ureaplasma parvum/urealyticum* prevalence amongst the different age groups was also considered, to determine whether there would be any differences in the distribution. No significant difference in the total distribution of *U. urealyticum* was observed in the different age groups. *U. urealyticum* was still predominantly found in the 21 – 25 age group 32% (8/25). However, a slight increase in the total *U. parvum* distribution in the 26 – 30 years age group was observed 28%

(22/79). Figure 4.1 shows the total *Ureaplasma* species distribution in the participants by age group. No statistically significant difference was observed in the distribution of either *U. parvum* or *urealyticum* with age group ($p > 0.05$).

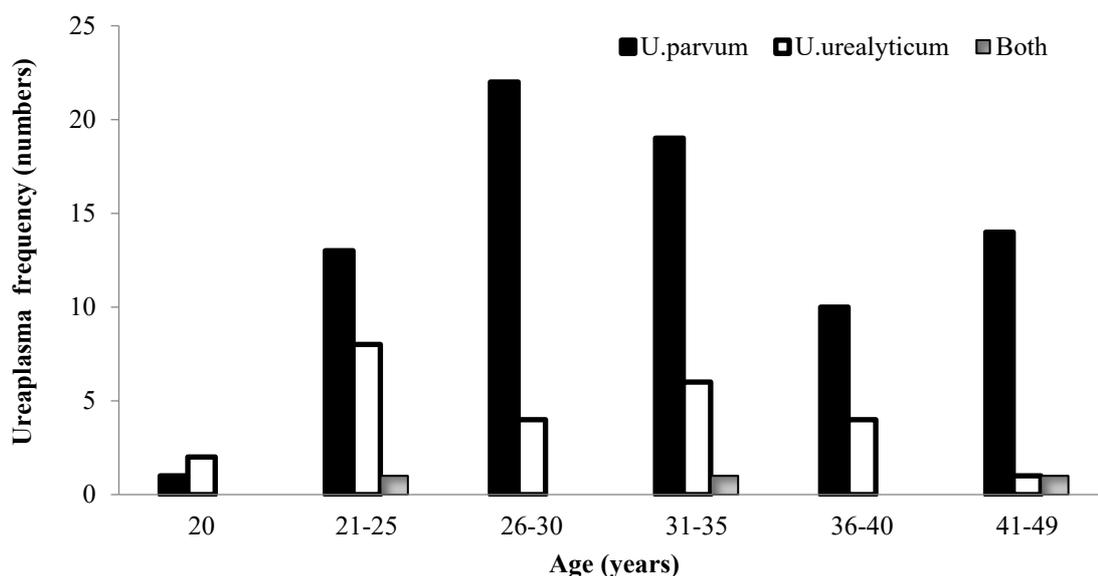


Figure 4.1 Total distribution of *Ureaplasma parvum* and *urealyticum* amongst the different age groups of reproductive age women seeking primary healthcare, polyclinic, EFSTH, Gambia

Ureaplasma urealyticum was more prevalent in the 21 - 25 years, age group (8/25) whilst *U. parvum* was detected more frequently in the 26 - 30 years age groups (22/79). *U. parvum* was more prevalent than *U. urealyticum* across all the age groups except in the 20 years old, where *U. urealyticum* distribution was 8% (2/25). Both *Ureaplasma parvum* and *urealyticum* DNA was detected in three participants. Legend: Both = *U. parvum* and *U. urealyticum*

4.4 Socio-demographic and risk characteristics of participants positive for *Ureaplasma*

Socio-demographic and risk factors data have shown that 85% (91/107) of participants positive for *Ureaplasma* were married and 17% were married more than once. A number of participants (38%) had more than 5 pregnancies with 3% declared having more than ten pregnancies. However, the

numbers of miscarriages, still-births or spontaneous abortions were not captured during data collection as participants were only asked for the total number of pregnancies they had. Amongst the participants infected with *Ureaplasma*, approximately 72% (77/107) used hormone contraceptives. Eighty seven percent (14/16) from the case group and 13% (2/16) from the control group reported having their menstrual period stopped due to either contraceptives or breastfeeding. However, none of the participants had had a natural menopause. Since the overall data on *Ureaplasma* incidence shows a higher distribution between the ages of 26 - 35 years (26 - 30 years age group = 45%; 26/57; 31 - 35 years age group = 56%; 26/46), selected risk variables were identified amongst participants between the ages of 26 - 35 years of age (Table 4.4).

Table 4.4 Selected risk variables in 26 – 35 years old participants infected with *Ureaplasma* (n = 52)

Variables	<i>Ureaplasma</i> n (%)	95% CI	p-value
Hormone contraceptive used	39 (75)	1.5(0.8, 2.8)	NS
≥ 5 years pregnancies	17 (32)	1.3(0.5,3.0)	NS
Sexual debut (< 18 years old)	26 (50)	0.5(0.3, 0.9)	0.05
New sexual partner (last 12 months)	9 (17)	0.3 (0.1,1.0)	0.05
Condom used (last 12 months)	7 (13)	1.3(0.5, 3.3)	NS
FGM	37 (71)	0.8(0.5, 1.4)	NS

P- value is based on chi-square-corrected (Yates).

2 – tailed-*p* was used in this bivariate analysis and adjusted for age group

OR = Odds Ratio, 95% CI = Confidence Interval. NS = Not significant, *p*-value > 0.05

A bivariate analysis on potential risk characteristics that may be associated with *Ureaplasma* infection was carried out and only early sexual debut (< 18 years) and new sexual partner (last 12 months) were found to be statistically associated with *Ureaplasma* infection ($p \leq 0.05$) (Table 4.4).

4.5 *Ureaplasma* co-infection with genital STI pathogens

Women of reproductive age often harbour urogenital infections, therefore, the incidence of other selected genital STI pathogens with *Ureaplasma* was investigated. Bacterial vaginosis was the most

common infection identified in participants with *Ureaplasma*, accounting for approximately 22% (23/107), followed by Gram-negative bacteria (GNB) 15.9% (17/107) and infection with *Candida albicans* 14% (15/107). GNB was isolated more frequently from the older age groups (> 40 years old) compared to the younger age groups. There was no statistically significant difference observed between being infected with *Ureaplasma* and with other genital STI pathogens (Table 4.5).

Table 4.5 Co-infection of urogenital *Ureaplasma* species with selected genital pathogens (number and percentage of positive cases)

Genital pathogens	<i>Ureaplasma</i> species			p-value
	<i>U. parvum</i> (n =79)	<i>U. urealyticum</i> (n = 25)	Both (n = 3)	
<i>C. albicans</i>	14 (17.7)	1 (4.0)	0 (0.0)	NS
HPV	9 (11.4)	5 (20.0)	0 (0.0)	NS
<i>T. vaginalis</i>	5 (6.3)	4 (16.0)	0 (0.0)	NS
<i>S. agalactiae</i>	10 (12.7)	2 (8.0)	0 (0.0)	NS
<i>N. gonorrhoeae</i>	5 (6.3)	2 (8.0)	1 (33.0)	NS
Bacterial vaginosis*	17 (21.5)	5(20.0)	1 (33.0)	NS
Gram (-) bacteria	8 (10.1)	8 (32.0)	1(33.0)	NS

None of the organisms co-infecting with *Ureaplasma* were statistically associated with the infection. NS = not significant ($p > 0.05$). Bacterial vaginosis* = polymicrobial syndrome

4.6 Distribution and prevalence of *Ureaplasma* serotypes

A total of four hundred and sixty-four (464) samples (paired samples, ECS = 232, HVS = 232) were screened for *Ureaplasma*. HVS specimens were collected because most reproductive age women are colonized by this organism in the vagina. Of the 464 samples screened, 33.6% (78/232) of the ECS, 42.2% (98/232) HVS and approximately 14.4% (67/464) ECS and HVS samples were positive. *U. parvum* was detected in ECS, HVS, and both (ECS and HVS) as follows: 61%, 79% and 51%, respectively. The prevalence of *U. parvum* in these paired samples was found to be 30%. In

comparison, *U. urealyticum* was detected in ECS, HVS and both (ECS and HVS) as follows: 19%, 22% and 16%, respectively. *U. urealyticum* accounts for approximately 8.8% of the total *Ureaplasma* infection.

Subtyping of positive samples was carried out by DNA sequencing as described in chapter 2, section 2.14.2 and 2.15. An NCBI BLAST ([http:// www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) search was performed for each sequenced product to allocate the *Ureaplasma* genotype (for ease of referencing, serotype will be used henceforth). Subtyping shows that *U. parvum* serotype 3 was the most prevalent type in both samples, followed by serotype 1. In addition, different serotypes of *U. parvum* were detected within the same sample. In contrast, all the samples positive for *U. urealyticum* were identified as serotype 10 (Figure 4.2). Furthermore, 61% (65/107) of participants were identified to harbour *Ureaplasma* species in both the vagina and cervix, 28% (30/107) had vaginal colonisation and 11% (12/107) had cervical infection with *Ureaplasma*. Table 4.6 shows the total number of participants infected or colonised with different *Ureaplasma* serotypes in the cervix, vagina or both.

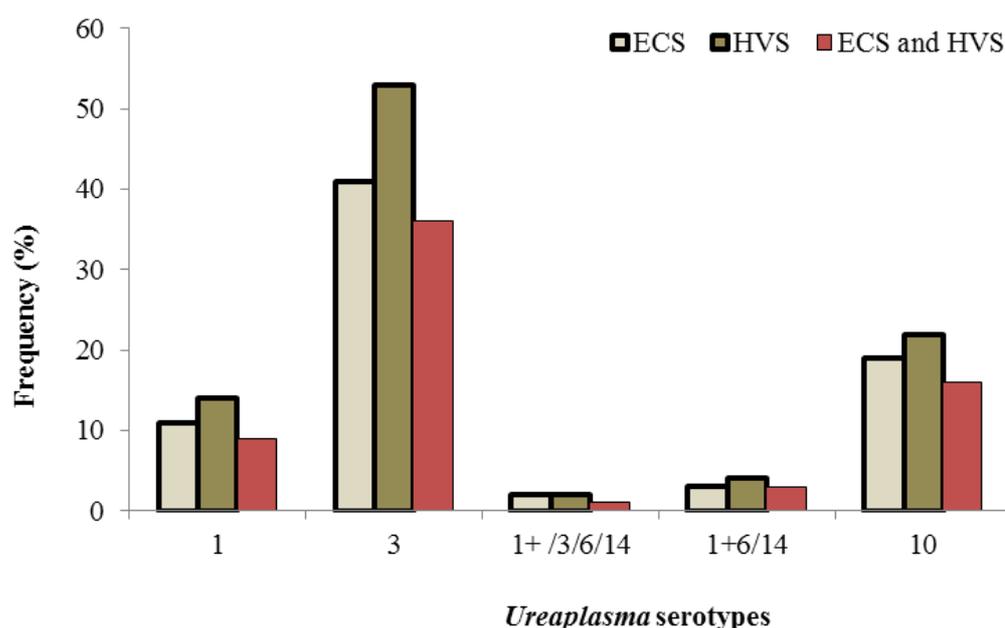


Figure 4.2 *Ureaplasma* serotype distribution in endocervical and high vaginal swab samples of reproductive age women seeking primary health care at a polyclinic, EFSTH, Gambia

A difference in the distribution of *Ureaplasma urealyticum* serotype 10, *Ureaplasma parvum* serotype 1 and serotype 3 in the cervix and vagina was observed. *Ureaplasma* was identified mostly from the high vaginal swab (HVS) samples. Legend: ECS = endocervical swab, HVS = high vaginal swab, ECS and HVS = endocervical swab and high vaginal swab.

Table 4.6 Total number of participants infected or colonised with *Ureaplasma* either in the cervix, vagina or both

Anatomical sites	Serotypes				
	1	3	1+ /3/6/14	1+6/14	10
Cervix	2	5	1	1	3
Vagina	5	17	1	1	6
Both*	9	36	1	3	16
Total participants	16	58	3	5	25

Three participants were identified to harbour both serotype 3 and 10 in the cervix and vagina. Twenty-four participants were colonised with *U. parvum*. Multiple colonisations with different *U. parvum* serotypes were also observed in eight participants. Six participants were colonised with *U. urealyticum*, serotype 10. Both* = cervix and vagina. *U. parvum* serotypes detected were serotype 1, 3, 1+3/6/14 and 1+6/14.

4.7 Prevalence of other sexually transmitted/genital pathogens amongst participants

Distribution of selected STI/genital pathogens and bacterial vaginosis in participants was determined using either real time-PCR (q-PCR) for *C. trachomatis*, *N. gonorrhoeae* and *M. genitalium* or microbiological and biochemical analysis for *C. albicans*, *N. gonorrhoeae*, *T. vaginalis*, and *S. agalactiae* as described in chapter 2, section 2.5.1 – 2.13.3. STI/genital pathogens were identified more frequently in the case group 41% (48/115) as expected than the control group 38% (45/117). 16% (18/115) symptomatic patients (case group) were infected with *C. albicans*, 13% (15/115) with *T. vaginalis*, 7% (8/115) with *S. agalactiae* and 6% (7/115) with *N. gonorrhoeae* (Fig 4.3). 30% (34/115) of symptomatic participants were diagnosed with bacterial vaginosis.

Approximately 39% (45/117) of asymptomatic participants (control group) were positive for one or more of the screened organisms. *Streptococcus agalactiae* was the most prevalent organism isolated in this group of participants, 11.1% (13/117). Figure 4.3 and 4.4 shows the prevalence of genital pathogens in both the case and control groups, respectively.

Cervical samples that were culture positive for *N. gonorrhoeae* were also positive by q-PCR for the cryptic plasmid gene. However, two *N. gonorrhoeae* culture negative samples had a positive real time-PCR result. The two samples were rescreened twice by both real time and conventional PCR and they remained positive. The positive PCR amplicons were purified and sent for confirmation by DNA sequencing as described in chapter 2, section 2.15. Sequenced product search by an NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) identified the organism as *N. gonorrhoeae*. *Chlamydia trachomatis* was not detected in any of the samples; however, a monoplex real time-PCR for *C. trachomatis* was carried out on all the cervical samples that were positive for *N. gonorrhoeae*. This was necessary as *N. gonorrhoeae* can compete with *C. trachomatis* for a binding site in a duplex real time-PCR. All the samples tested negative for the *C. trachomatis* cryptic plasmid gene.

Endocervical and high vaginal swabs (n = 400) samples were screened for *M. genitalium* using primers as described in chapter 2, section 2.11 targeting the *16S rRNA* gene, located in the V1 and V3 hypervariable region of the gene. All 400 samples were negative for this gene. Samples were rescreened using real time-PCR as described in chapter 2, section 2.13.2 targeting the *M. genitalium MgPa* gene using specific primers and probes for the conserved region of the gene. All the samples were also negative for the *MgPa* gene. Although none of the samples were positive for the targeted genes for *C. trachomatis* and *M. genitalium*, nonetheless, the targeted genes were identified in all the positive controls.

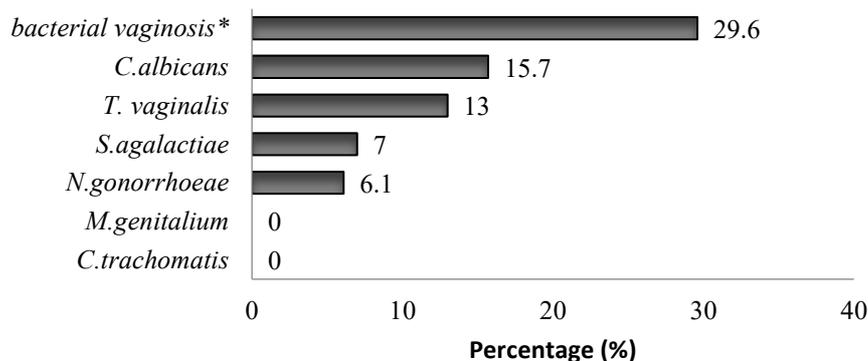


Figure 4.3 Percentage distributions of selected genital pathogens in symptomatic (case group) women attending a polyclinic at EFSTH, Gambia.

Bacterial vaginosis was most prevalent in the case group accounting 30% (34/115). None of the participants were positive for *C. trachomatis* and *M. genitalium*. *bacterial vaginosis was diagnosed using Amsel's criteria (Amsel *et al.*, 1983)

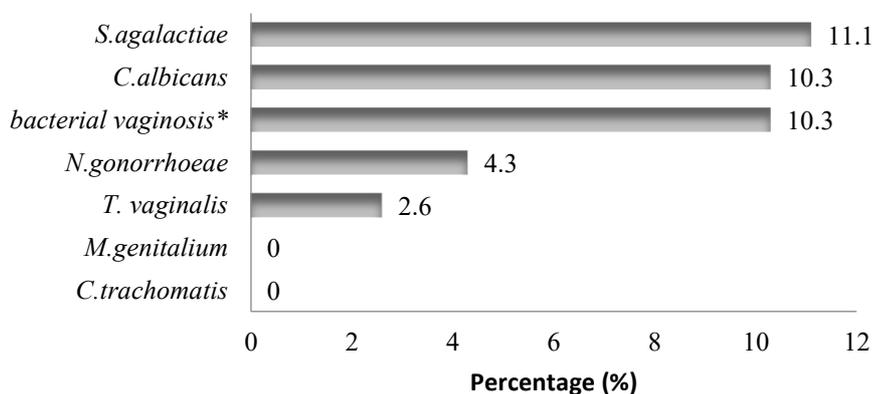


Figure 4.4 Percentage distributions of selected genital pathogens in asymptomatic (control group) women attending a polyclinic at EFSTH, Gambia

S. agalactiae (11%) was the most prevalent organism followed by *C. albicans* and bacterial vaginosis (each of 10%) and a small percentage, 2.6 % (3/117) women were positive for *T. vaginalis*. However, none of the samples were positive for *C. trachomatis* and *M. genitalium*. *bacterial vaginosis was diagnosed using Amsel's criteria (Amsel *et al.*, 1983).

4.8 Antibiotic susceptibility determination of isolates

The antibiotic susceptibility testing was interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2015) and British Society for Antimicrobial Chemotherapy (BSAC, 2015) guidelines and it showed that all *S. agalactiae* isolates (20/20) were resistant to the antibiotic tetracycline, followed by clindamycin and penicillin, each at 45% (9/20). Seventy-five percent (15/20) of *S. agalactiae* isolates were sensitive to erythromycin. Ciprofloxacin resistance was observed in 44% (4/9) of *N. gonorrhoeae* isolates. Seventy-eight percent (7/9) of *N. gonorrhoeae* isolates were sensitive to Ceftriaxone and Cefotaxime. However, two isolates of *N. gonorrhoeae* were found to be resistant to both Ceftriaxone and Cefotaxime, 22% (2/9).

4.9 Discussion

Ureaplasma species are found in the lower urogenital tracts of reproductive age women and can cause infections in the upper urogenital tracts. The increased use of nucleic acid amplification techniques has enhanced knowledge and the classification of these microorganisms.

The aim of this chapter was to study the epidemiology and distribution of *Ureaplasma* species and its clinical significance in symptomatic (case group) and asymptomatic (control group) reproductive age women seeking primary health care at the EFSTH, polyclinic, Gambia. *Ureaplasma* was isolated more in the control group (51%) than in the case group (41%). However, no statistically significant difference was observed between the distribution of *Ureaplasma* in the symptomatic (case group) and asymptomatic (control group) ($p > 0.05$). A similar trend was observed in other studies, Domingues *et al.*, (2002) reported a 43% prevalence rate in asymptomatic women in Guinea Bissau, and 52% was reported by Kim *et al.*, (2014) in asymptomatic Korean women. The 46% *Ureaplasma* prevalence observed in this study is inconsistent with the 0% prevalence reported by Demba *et al.*, (2005) Gambian study. The differences seen between their study and the current study can be attributed to the diagnostic methods used. In Demba *et al.*, (2005), culture methods were used whilst this current study employed nucleic acid amplification techniques (NAAT). It had been well documented that NAAT have greater sensitivity in detecting *Ureaplasma* than culture (Stellrecht *et al.*, 2004). Culture has an advantage of differentiating colonisation from infection, a concentration of $>10^4$ colony forming units per millilitre (CFU/ml) is considered as an infection (Dhawan *et al.*, 2012; Demba *et al.*, 2005). However, it has longer incubation periods, which does not favour the isolation of *Ureaplasma* due to its sensitive nature to the metabolic by-products generated during long incubation periods (Kacerovský and Boudys, 2008; Demba *et al.*, 2005).

Ureaplasma species was detected in 107 participants, *U. parvum* accounting for 74% (79/107), *U. urealyticum* 23% (25/107) and 2.8% (3/107) participants were infected with both *Ureaplasma* species (Table 4.1). This finding is consistent with other studies carried out in reproductive age women in both developed and developing countries (Choi *et al.*, 2012; Beeton *et al.*, 2009; Domingues *et al.*, 2002). *Ureaplasma* age-standardised prevalence was found to be 51% within the ages 26 - 35 years and a prevalence decrease was observed in the 36 years old and above. A similar prevalence distribution was also reported by Govender *et al.*, (2009). However, *U. parvum*, which was the most frequently isolated species in this study, was found to be most

prevalent in the 26 - 30 years age group 28% (22/79) whilst *U. urealyticum* was more prevalent in the 21 - 25 years age group 32% (8/25). In contrast to this finding, *U. parvum* has been reported to be more prevalent in the 21 - 25 years age group and *U. urealyticum* in the over 25 age group (Domingues *et al.*, 2002; De Francesco *et al.*, 2009).

The differences noted could be attributed to the fact that Gambian women may be more sexually active between the ages of 26 - 35 years of age compared to other countries, where sexual activity is reported more frequently in the under 26 years old (GDHS, 2013; Navarro *et al.*, 2002). Furthermore, frequency of sexual activities has been reported to be associated with *Ureaplasma* colonisation (Waites *et al.*, 2005). The 32% prevalence of *U. urealyticum* in the 21 – 25 years age group may be a concern as this organism has been documented to be associated with adverse reproductive outcomes such as infertility, habitual abortion and ectopic pregnancy (Hunjak *et al.*, 2014).

In the current study, 81% (38/47) of participants from the case group infected with *U. parvum* had cervicitis/vaginitis, indicating that *U. parvum* may also cause infections and if left untreated may cause endometritis and salpingitis (Holmes *et al.*, 2008). *Ureaplasma parvum* serotype 3 was the most prevalent serotype in both the endocervical and HVS samples (Figure 4.2). This finding is consistent with the literature that *U. parvum* serotype 3 is the predominant type found in reproductive age women (Kotrotsiou *et al.*, 2013; Polvsen *et al.*, 2001). Serotype 3 has been associated with spontaneous abortion in reproductive age women with cervical colonisation (Donders *et al.*, 2000). The ascending infection seen with *Ureaplasma* from vaginal colonisation to cervical infection could be due to the organisms' ability in producing protease, which breaks down IgA and thus facilitates the organism to evade the immune system response (Marovt *et al.*, 2014).

The 51% of *Ureaplasma* prevalence observed in the asymptomatic participants further support the hypothesis that reproductive age women can be carriers of *Ureaplasma* in their vaginal normal flora and they may be at risk of developing ascending infections. Vaginal colonisation by *Ureaplasma* was found to be 28% (30/107) in the participants and *U. parvum* represented 80% (24/30) of the total *Ureaplasma* vaginal distribution (Table 4.6). *Ureaplasma* colonisation in the female urogenital tract has been linked to a younger age, low socio-economic background, multiple sexual partners, black racial ethnicity and the use of hormone contraceptives (Marovt *et al.*, 2014; Kotrotsiou *et al.*, 2013; Tibaldi *et al.*, 2009). Approximately 60% *Ureaplasma* prevalence has been reported in black West /Central African and in black African–American

women compared to other racial ethnicities (Tibaldi *et al.*, 2009; Newton *et al.*, 2001). This may explain the 46% *Ureaplasma* prevalence also observed in this study. Colonisation of the female urogenital tract with *Ureaplasma* is thought to be associated with hormonal status. The influence of hormones on *Ureaplasma* vaginal colonisation has been demonstrated in oestradiol-treated mice (Furr and Taylor-Robinson, 1989) and in human urogenital tracts (Waites *et al.*, 2012).

In this study, 72% (77/107) of women infected with *Ureaplasma* were identified to be using hormone contraceptives. Although not statistically significant, the use of hormone contraceptive (> 5 years) in the study, was found to increase the odds of being infected with *Ureaplasma* (OR = 1.5, $p > 0.05$). Moreover, 51% of the younger age group (21 – 35 years old) reported using contraceptives, which may increase the vaginal epithelial glycogen content due to the high oestrogen level. This can enhance lactic acid production and reduces vaginal pH, therefore promoting *Ureaplasma* vaginal colonisation (Gupta *et al.*, 2000). A decrease in the prevalence of *Ureaplasma* between the ages of 36 – 49 years of age was observed. This could be due to either reduced sexual activity in the older age groups and/or an increased in the vaginal pH.

Women of reproductive age frequently suffer from urogenital infections; therefore, the incidence of other concomitant organisms with *Ureaplasma* was investigated. Participants infected with *Ureaplasma* were mostly found co-infected with bacterial vaginosis (Table 4.5). However, there was no significant association between bacterial vaginosis and *Ureaplasma* infection ($p > 0.05$). The predominant detection of Gram-negative bacteria (GNB) 15.9% (17/107) in the older age groups infected with *Ureaplasma* can probably be attributed to either poor hygiene or an atrophic vaginal mucosa and /or a neural vaginal pH, providing a conducive environment for enteric GNB to colonise the lower urogenital tracts (Kokotos, 2006). In addition, 4% of asymptomatic (control group) participants were diagnosed with gonorrhoea (Fig 4.4), which could have gone untreated and possibly caused future adverse reproductive outcomes. This further evidenced that asymptomatic STI poses a significant public health challenge.

C. trachomatis and *M. genitalium* were not detected in any of the screened samples (Fig.4.3 & 4.4). This finding is inconsistent with Demba *et al.*, (2005) study, which found chlamydial infection to be 15% (34/227) in symptomatic Gambian women. In a Kenyan study, a *C. trachomatis* prevalence of 6% (18/300) was reported in asymptomatic reproductive age women (Kohli *et al.*, 2013). The chlamydial cryptic plasmid gene was also targeted in Demba *et al.*, (2005) study; therefore, the inconsistency seen in the two studies could possibly due to the frequent exposure of participants to dual antibiotic treatments or past infections, resulting in

acquired immunity. Comparing *C. trachomatis* prevalence in The Gambia and Kenya with England, where there is a national screening programme, a prevalence rate of 46% was reported in 2015 amongst asymptomatic 15-24 years old (<https://www.fpa.org.uk/factsheets/sexually-transmitted-infections>). The disparities observed in these studies could be attributed to differences in sexual behaviour as well as the targeted age groups. Reports have shown that the 15 – 24 years age groups are more susceptible to chlamydial infection due to persistence columnar epithelial cells in their cervixes, which favours the growth of *C. trachomatis* (Johnson, 1989).

Antibiotic resistance with Erythromycin and Ciprofloxacin against some strains of *N. gonorrhoeae* and *S. agalactiae* were observed. Ciprofloxacin and Erythromycin resistance observed in this study may be a result of selective pressure as in The Gambia all STIs suspected cases are treated with these first-and second-line antibiotics. Similarly, increased reports on treatment failure and reduced susceptibility to Cephalosporins, the last line of treatment option for *N. gonorrhoeae* have been reported (WHO, 2013, Tzelepi *et al.*, 2008). The same pattern was also observed in this study as *N. gonorrhoeae* resistance to Cefotaxime and Ceftriaxone have been documented. Resistance to Tetracycline by all isolates was observed, however, this finding is not surprising as Tetracycline has been extensively used as a broad-spectrum antibiotic for more than three decades, in The Gambia.

In summary, the 51% asymptomatic *Ureaplasma* prevalence and the no statistically significant difference in the distribution of *Ureaplasma* between the two study groups make it difficult to associate significant clinical outcomes with *Ureaplasma* infection. This finding adds to the literature that *Ureaplasma* may be mere co-factors associated with urogenital infections. Approximately in half of the women treated for STI, there was no microbiological aetiology of the symptoms. Therefore, a presumptive diagnosis of genital infections based on symptoms alone may be erroneous and can increase the burden of antibiotic resistance. The study also highlights the need to undertake a national laboratory-based STI and antibiogram surveillance for effective policy decision making in the prevention and treatment of STIs in The Gambia.

Chapter 5: Socio-demographic and risk factors associated with sexually transmitted infections in The Gambia

5 Introduction

Sexually transmitted infection (STI) is a term used to refer to various clinical syndromes that can be acquired or transmitted through sexual activity. STIs can be caused by bacteria, viruses, fungi, protozoa and ecto-parasites. Sexually transmitted infections are a major global public health concern with 86% of the burden found in resource-limited countries (Newman *et al.*, 2015). It is estimated that for those aged 15–44 years, STIs excluding HIV are the second most important cause of healthy life lost in women after maternal mortality and morbidity (WHO, 2015). Despite this burden, STIs have been accorded low priority in many developing countries and most countries lack an effective STI control programme.

Several epidemiological studies have characterised risk factors associated with STI transmission and acquisition in adolescents and adults. These factors include urban residency, a young age at sexual debut, being in a multiple, sequential or concurrent sexual relationship, partners' sexual behaviour, and inconsistent use of condoms (Niyazi *et al.*, 2016; Cordova *et al.*, 2014; Chadambuka *et al.*, 2011). However, physical and sexual partner violence have been reported in resource-limited countries to be a contributing factor for STI transmission and acquisition (Rahman *et al.*, 2014; Dhakal *et al.*, 2014). Gender-based violence lies in the patriarchal society in many African countries and is used to control women, which can easily lead to risky sexual behaviours (Rahman *et al.*, 2014; Garcia-Moreno and Watts, 2000). Similarly, a systematic review carried out in sub-Saharan Africa has evidenced that taking illicit drugs, alcohol and gender-based violence increases the risk of STIs and are associated with STI (Jewkes *et al.*, 2010; Kongnyuy and Wiysonge, 2007; Kalichman *et al.*, 2007; Garcia-Moreno and Watts, 2000).

The risk of acquiring STI was found to be two-fold greater in those that take illicit drugs and alcohol compared to those that do not (Yohannes *et al.*, 2013; Diclemente *et al.*, 2002). Alcohol and drugs have been shown to have a disinhibiting effect that may lead to risky sexual behaviours and disease transmission (Kalichman *et al.*, 2007). Although there is an increased risk of acquiring and transmitting STIs through commercial sex workers, Yohannes *et al.*, (2013) found that many individuals who have sex with commercial sex workers do so mostly due to peer pressure, socio-economic dependency, cultural and environmental factors.

Considerable efforts are being made to identify interventions that could reduce sexual risk behaviours, however, change in risk behaviours itself remains a complex issue. Therefore, it is

important to characterise risk factors that are associated with STIs at a national level for improved and more effective intervention programmes.

Prevention and early treatment of STIs is a high public health priority in The Gambia, however, there is limited information on the current prevalence and behavioural risk factors associated with STIs for an effective development of successful prevention, diagnosis and management strategies.

5.1 Aim and objectives:

The aim of the work presented in this chapter was to determine STI prevalence and characterise the socio-demographic and behavioural risk factors associated with STI in reproductive age woman.

The objectives were to:

- Identify socio-demographic and risk factors that are associated with individual STI /genital pathogens
- Determine the prevalence of STIs in symptomatic (case) and asymptomatic (control) groups
- Identify emergent risk factors that are associated with STIs in The Gambia.

5.2 Statistical analysis of socio-demographic and risk characteristics of study participants

Socio-demographic, behavioural risk factors and clinical outcomes of participants were analysed as described in Chapter 2, section 2.22

5.3 Socio-demographic characteristics of participants

Of the 232 subjects with adequate cellular DNA samples (case, n =115, control, n=117), the mean age for both study participants was 31.5 (± 7.4 SD) with an age range of 20 – 49 years. The data showed that 55% (63/115) of the older women within the age group 31 - 49 years of age visited the STI clinic more than the younger age group between the ages of 20 – 30 years, 45% (52/115). The opposite was observed in the family planning clinic, where women between the ages of 21 - 30 years, 56% (65/117) visited the family planning clinic for contraceptive services more than the older age groups (Table 5.1). The three major ethnic groups of The Gambia were represented in this study, Mandinka (24.6%, 57/232), Fula (21%, 49/232) and Wolof (18%, 42/232). The Mandinka ethnic group accounted for 32% (37/115) of the total ethnic group that attended the STI clinic and the Wolof were the least represented ethnic group 11% (13/115) to report to the STI clinic. However, the Wolof accounted for the greatest number 25% (29/117) of visits to the family planning clinic. Seventy percent of participants (83/117) residing in the capital city, Banjul visited the family planning clinic more than the STI clinic. In comparison, 81% (94/115) of participants that are residents of KMC/WCR visited the STI clinic more than the family planning clinic (Table 5.1).

More than 70% (82/115) of participants from the case group were married, 17% (20/115) single and 11% (13/115) were divorced, whilst approximately 89% (105/117) from the control group were married. Approximately, 43% (99/232) of participants had completed a secondary education and 4% from the case and 8% from the control group had a college education (Table 5.1). The greatest annual household income reported by both groups was between D10, 000 – D35, 000 (Gambian Dalasi) equivalent to £153 - £537 (GBP). 46% (109/232) of participants lived in a household between 2 - 6 occupants. However, the data also showed that 10% (24/232) of participants lived in a household of more than 18 occupants. Thirty-three percent (case group) and 28% (control group) of participants were petty traders. Approximately 23% (26/115) and

30% (35/117) of participants from the case and control group gave housewife as their occupation and only 10% (24/232) reported being employed in the civil service.

The acceptability of using self-sampling for STI screening was also assessed and 18% (43/232) preferred self-sampling. Cervical cancer screening programs have been introduced in some parts of the urban region of the country, but, this study has shown that more than 90% of participants had never undergone cervical cancer screening. Table 5.1 shows the socio-demographic characteristics of the participants.

Table 5.1 Socio-demographic characteristics of participants

Variables	Case n (%) (n =115)	Control n (%) (n = 117)
Ethnic group		
Fula*	22 (19.13)	27 (23.08)
Jola	12 (10.43)	8 (6.84)
Mandinka*	37 (32.17)	20 (17.09)
Sarahule	11 (9.57)	2 (1.71)
Serere	10 (8.70)	20 (17.09)
Wollof*	13 (11.30)	29 (24.79)
Others	10 (8.70)	11 (9.40)
Age group (Years)		
20	5 (4.35)	2 (1.71)
21 – 25	22 (19.13)	31 (26.50)
26 – 30	25 (21.74)	32 (27.35)
31 – 35	26 (22.61)	20 (17.09)
36 – 40	14 (12.17)	19 (16.24)
41 – 49	23 (20.00)	13 (11.11)
Level of education		
None	14 (12.17)	23 (19.66)
Primary	23 (20.00)	15 (12.82)
Secondary	45 (39.13)	54 (46.15)
College	5 (4.35)	8 (6.84)
Islamic studies	28 (24.35)	17 (14.53)
Marital status		
Married	82 (71.30)	105 (89.74)
Divorced	13 (11.30)	5 (4.27)
Single	20 (17.39)	7 (5.98)
Residency		
Banjul	21 (18.26)	83 (70.94)
KMC/WCR**	94 (81.74)	34 (29.06)
Occupation		
Civil servant	12 (10.43)	12 (10.26)
Business woman	22 (19.13)	15(12.82)
House wife	26 (22.61)	35 (29.91)
Petty trader	38(33.04)	33 (28.21)
Others	17 (14.78)	22 (18.80)

Table 5.1 (continued)

Number of people in household

2 -6	46 (40.02)	63 (53.83)
7 -11	42 (36.52)	38 (32.48)
12- 17	13 (11.31)	6 (5.12)
18+	14 (1.18)	10 (8.53)

Total household income last 12 months

<D10,000 (< £153.00)	6 (5.22)	9 (7.69)
D10,000 - 35,000	40 (34.78)	15 (12.82)
D35,000 - 50,000	11 (9.57)	23(19.66)
D51,000 - 65,000	11 (9.57)	18 (15.38)
D66,000 - 75,000	14 (12.17)	13 (11.11)
D76,000 - 85,000	6 (5.22)	7 (5.98)
>D85,000	27 (23.48)	32 (27.35)

Acceptance of self-sampling

Yes	11(9.57)	32 (27.35)
No	103 (89.57)	81 (69.23)
Don't mind either way	1 (90.87)	4 (3.42)

Past screening for cervical cancer

Yes	7(6.09)	7 (5.98)
No	108 (93.91)	110 (94.02)

Family member diagnosed with cervical cancer

Yes	2 (1.74)	3 (2.56)
No	113 (98.26)	114 (97.44)

*The three major ethnic groups of the Gambia. The Mandinka ethnic group were under-represented (42% of the eligible population vs 25% of those with adequate cellular DNA), the Wollof and Fula ethnic groups were slightly over-represented (16%, 18% of the eligible population vs 18%, 21% of those with adequate cellular DNA).

Key: KMC/WCR** = Residents of Kanifing Municipal Council and West Coast Region.

5.4 Behavioural characteristics of participants

Participants reported risk factors that were likely to increase their chances of acquiring and transmitting STIs (Table 5.2). Although a small number of participants 10 (8.7%) and 6 (5.1%) from both the case and control groups had sexual intercourse at the age of less than 14 years due to early marriages, most participants 61% (case group) and 72% (control group) had their sexual debut at the age of 18 years or older. One participant from the case group reported sexual debut at the age of 9 years because of sexual abuse. Approximately, 45% (case group) and 33% (control group) of participants reported having more than 2-lifetime sexual partners. Of the women with one-lifetime sexual partner, 60% stated that their partners have other sexual partners. Approximately 29% (24/82) of participants from the case group and 14% (15/105) from the control group were married more than once. Three participants had symptoms of STI but reported never having had sexual intercourse. Approximately 84% (95/112) and 99% (116/117) of participants from the case and control groups reported having had sexual intercourse in the last 12 months (Table 5.2). Of the 95 participants from the case group that had one or more symptoms of STI, 22% (21/95) declared to have had one or more new sexual partners in the last 12 months compared to 15% (18/117) from the control group. Participants' attitudes toward safer sex practices were assessed and the data showed that more than 80% (189/229) of participants' partners had never used a condom during sexual intercourse in the last 12 months. Female genital mutilation (FGM), which is a cultural practice in the Gambia and other parts of Africa, had been performed on 73% (84/115) of women from the case group and 52% (61/117) from the control group, respectively (Table 5.2).

Table 5.2 Behavioural characteristics of participants

Variables	Case n (%)	Control n (%)
Age at sexual debut**		
< 18	43 (38.39)	32(27.35)
≥18	69 (61.61)	85 (72.65)
Number of lifetime sexual partner (s)		
1	60 (53.57)	78 (66.67)
≥2	52 (46.43)	39 (33.33)
Partner (s) had sex with others *		
Yes	36 (26.67)	48 (60.00)
No	8 (13.33)	20 (25.00)
Don't know / not sure	16 (33.33)	12 (15.00)
Sexual intercourse in the last 12 months**		
Yes	95 (84.81)	116 (99.15)
No	17(15.18)	1 (0.85)
New sexual partner (s) in the last 12 months		
Yes	21 (22.10)	18 (15.65)
No	74 (77.89)	97 (84.35)
Condom use in the last 12 months		
Yes	15 (15.79)	7 (6.03)
No	80 (84.21)	109 (93.97)
Female genital mutilation		
Yes	84 (73.04)	61 (52.14)
No	31 (26.96)	56 (47.86)
Smoking		
Yes/Quit	3 (2.61)	
No	112 (97.39)	117 (100.00)
Hormone contraceptive use		
Yes	53 (46.09)	117 (100.00)
No	47 (40.87)	
Yes, but stopped using them	15 (13.04)	
History of pregnancy		
Yes	93 (80.87)	114 (97.44)
No	22 (19.13)	3 (2.56)

* n = varies as only women with one lifetime sexual partner were asked this question

** 3 Participants reported to never have had sexual intercourse

5.5 Socio-demographic and risk characteristics of participants infected with HIV (n = 29)

Of the 232 women with adequate cellular DNA in the sample collected, 29 had been diagnosed with human immunodeficiency virus (HIV). These participants were followed up as a cohort group every 9 months for a period of 24 months, to determine HPV sero- prevalence and sero-conversion as described in Chapter 6.

The socio-demographic and risk characteristics of HIV infected participants are shown in Table 5.3. Of these participants, 96% (28/29) were residents of KMC/WCR and only 3% (1/29) lived in the capital city. Over fifty percent (17/29) of these women belonged to the two major ethnic groups of the Gambia; Mandinka 38% (11/29) and Fula 17.2% (6/29), with the Wollof, the third major ethnic group accounting for 3% (1/29) and the minor ethnic groups of the country constituting the remaining 41%. Sixty-two percent (18/29) reported being married and the remaining were either never been married 10% (3/29), divorced 17% (5/29) or widowed 10% (3/29). Of the 62% married women, 50% (31/62) had remarried more than once. Eighty-six percent (25/29) were engaged in income generating activities with petty trading, 38% (11/29) and business 24% (7/29) were the most common.

Approximately 52% (15/29) had two or more lifetime sexual partners with 2 women reported to have had more than 6 sexual lifetime partners. Of the 48% (14/29) who declared to have had one lifetime sexual partner, 71% (10/14) of their partners have other sexual partners with 60% (6/10) declared having 2 or more co-wives. Furthermore, 62% (18/29) reported to have sexual intercourse in the preceding 12 months and only 22% (4/18) had used a condom. Three women reported having had sexual intercourse with a new partner and only 1 out of the 3 reported to have used a condom.

Table 5.3 Selected socio-demographic and behavioural characteristics of HIV positive women (n = 29)

Variables	Number	Percentage
Residency		
Banjul	1	3.4
KMC/WCR	28	96.6
Ethnic group*		
Mandinka	11	37.9
Fula	5	17.2
Wollof	1	3.4
Age group (years)		
21 – 25	4	13.8
26 – 30	7	24.1
31 – 35	7	24.1
36 – 40	5	17.2
41 – 49	6	20.7
Marital status		
Married	18	62.1
Unmarried	11	37.9
Occupation		
Business	7	24.1
Petty trading	11	38.0
Housewife	4	13.8
Others	7	24.1
Level of education		
<12 years	20	69.0
≥12 years	9	31.0
Total lifetime sexual partners		
1	14	48.3
≥2	15	51.7
Sexual intercourse last 12 months		
Yes	18	62.1
No	11	37.9
Condom use last 12 months		
Yes	4	22.2
No	14	77.8
New sexual partner(s) last 12 months		
Yes	3	16.7
No	15	83.3

Table 5.3 (continued)

Partners had other sexual partners		
Yes	10	71.4
No	4	28.6
Number of partners' other sexual partners		
1	4	40.0
≥2	6	60.0
FGM		
Yes	23	79.3
No	6	20.7
Ever screened for cervical cancer		
Yes	1	3.4
No	28	96.6

*The three major ethnic groups

5.6 Risk characteristics of participants diagnosed with bacterial vaginosis and trichomoniasis

Bacterial vaginosis was diagnosed in 46 participants out of the 232 screened giving a prevalence of 19.8% (Table 5.4). Approximately, 30% (34 /115) and 10% (12/117) of participants were positive from the case and control groups, respectively. All 30% of participants positive for bacterial vaginosis from the case group had symptoms of offensive vaginal discharge and itching. Bacterial vaginosis distribution was 22 % (10/46) in the Mandinka, compared to 17% (8/46) in the Fula, and 15% (7/46) in the Wollof, major ethnic groups. The data showed that bacterial vaginosis prevalence increases from 17% in women who reported one-lifetime sexual partner, to 25% in women with two-lifetime sexual partners and to 36% in women with ≥ 3 lifetime sexual partners. 31% of participants positive for bacterial vaginosis were married more than once. Although not statistically significant, remarriage was found to increase the odds of being diagnosed with bacterial vaginosis by two-fold (AOR 2.2, $p > 0.05$) (Table 5.4).

The prevalence of trichomoniasis was 16 % (3/18) in the 21 - 25, 11% (2/18) in the 26 – 30 age group and a prevalence increase was noted in the over 30 years age groups, with a prevalence range of 17% - 28%. Of the 18 participants infected with *T. vaginalis*, the Mandinka ethnic group accounts for 27% (5/18) of the distribution, followed by the Fula 22% (4/18) and the Wollof, 5%

(1/18). Participants with trichomoniasis had a vaginal pH range of 5.0 – 6.0. 91% (11/18) had symptoms of vaginal discharge and itching. Less than 12 years of education (AOR 2.5) and FGM (AOR 2.2) increased the odds of infection with *T. vaginalis* by two-fold (Table 5.4).

Table 5.4 Bivariate analysis on selected socio-demographic and behavioural risk characteristic of participants diagnosed for bacterial vaginosis and trichomoniasis

Risk variables	Bacterial vaginosis		<i>T. vaginalis</i>	
	AOR (95% CI)	p-value	AOR (95% CI)	p-value
New sexual partner (last 12 months)	1.3 (0.5, 3.4)	NS	1.0 (0.1, 3.9)	NS*
Age at sexual debut (< 18 years old)	0.7 (0.4, 1.5)	NS	1.7 (0.6, 4.5)	NS
Not using condom (last 12 months)	1.8 (0.5, 9.9)	NS*	1.3 (0.2, 5.8)	NS
Low level of education (< 12 years)	1.0 (0.5, 2.0)	NS	2.5 (0.8, 7.4)	NS
FGM	1.2 (0.6, 2.3)	NS	2.2 (0.7, 6.9)	NS
Remarriage	2.2 (1.0, 5.0)	NS	1.3 (0.2, 5.2)	NS*
Petty trading	1.2 (0.6,2.3)	NS	1.8 (0.6, 4.8)	NS
Low income	0.9 (0.4, 2.0)	NS	1.4 (0.4, 4.3)	NS*

P-value is based on chi-square-corrected (Yates) except * where Fisher exact was used.

2 – tailed-*p* was used in this bivariate analysis and adjusted for age group

AOR = Adjusted Odds Ratio, 95% CI = Confidence Interval, NS = not significant, *p*-value > 0.05

5.7 Characteristics of participants infected with *C. albicans*, *N. gonorrhoeae* and *S. agalactiae*.

Candida albicans was isolated from 30 participants with 60% (18/30) from the case group. Twelve participants from the control group were also infected 40% (12/30). *C. albicans* was most prevalence in the 26 - 30 age group 40% (12/30), followed by 23% (7/30) in the 21 - 25 age group. Sixty-three percent (63%) of the participants diagnosed with candidiasis lived in a household of more than 7 occupants with three participants living in a household of twenty occupants. Twenty percent (6/30) of participants infected with *C. albicans* were co-infected with HIV. *C. albicans* was detected in 70% (21/30) of participants who had their sexual debut at ≥ 18 years old, 73% (14/19) of them reported taking contraceptives and 52% (11/21) had 3 - 4 pregnancies.

Similarly, *N. gonorrhoeae* was isolated most frequently in the 21 – 25 years age group, accounting for 45% (5/11) of the total infections. Furthermore, statistical analysis showed that all the age groups were at risk of infection with *N. gonorrhoeae* with a risk three-fold greater in the 21 - 25 years age group (OR = 3.7). *N. gonorrhoeae* distribution was 54% (6/11) in participants that had their sexual debut at the age of ≥ 18 years, but this was not associated statistically with the infection ($p > 0.05$).

Twenty participants were infected with *S. agalactiae* with two co-infected with *C. albicans*. The Fula ethnic group was found to be associated statistically with *S. agalactiae* infection ($p = 0.02$). Fig.5.1 shows the percentage distribution of STI in the different major ethnic groups.

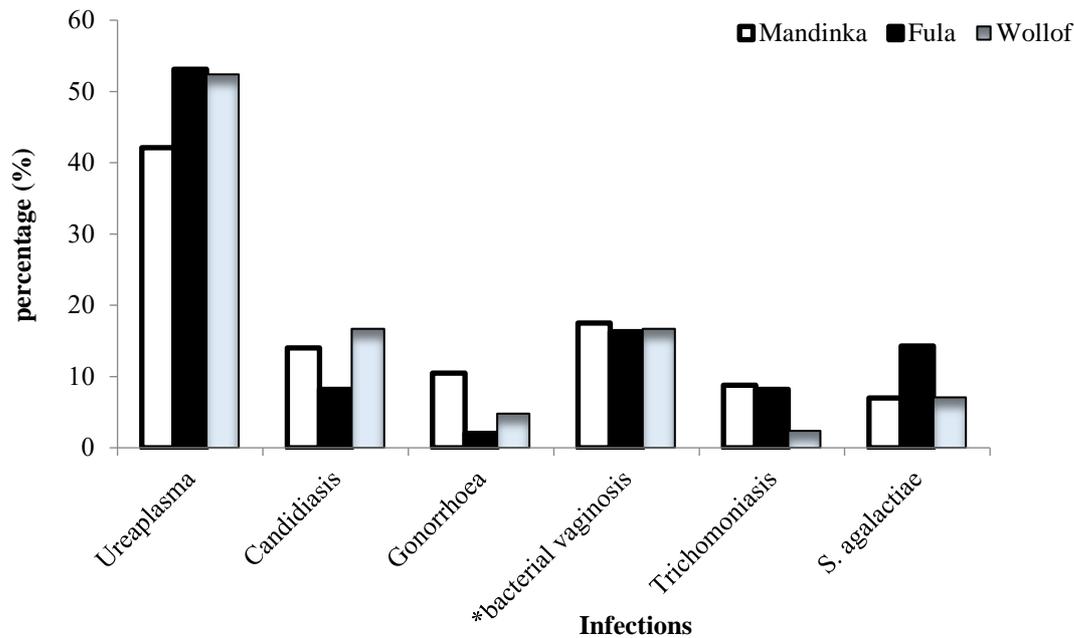


Figure 5.1 Percentage distributions of sexually transmitted infections in The Gambia major ethnic groups, attending a polyclinic, EFSTH

All the different major ethnic groups were infected with STI pathogens with the Fulas accounting for 53% of the total *Ureaplasma* and *S. agalactiae* (14%) infections. The Mandinkas accounted for the highest distribution of gonorrhoeae (10%), bacterial vaginosis* (17%) and trichomoniasis (8%), whilst candidiasis (*C. albicans*), 17% was isolated more from the Wollof ethnic group. (Total number recruited; Mandinka = 57, Fula = 49, Wollof = 42). *bacterial vaginosis diagnosed using Amsel's criteria (Amsel *et al.*, 1983).

5.8 Prevalence of STI / genital pathogens amongst participants

Of the 115 participants (case group) that reported one or two symptoms of STIs, a total of 69% (79/115) were infected with at least one of the screened pathogens (*T. vaginalis*, *Ureaplasma*, *N. gonorrhoeae*, *C. albicans*, *S. agalactiae*). Surprisingly, 67% (78/117) of participants from the control group were infected with one or more pathogens. Of the 232 participants screened, 157 were positive giving an overall prevalence of 67.7%. *Ureaplasma* was the most prevalent organism representing 68% of the total isolates, however of the infections that are symptomatic, bacterial vaginosis was most common, followed by candidiasis, and trichomoniasis (Table 5.5). Approximately 57% (90/157) of participants were infected with one STI / genital pathogen, 31% (49/157) with two, whilst 6% (10/157) with ≥ 3 STI / genital pathogens. Statistical analysis showed that *T. vaginalis* and bacterial vaginosis were associated significantly with STI ($p = 0.01$).

Table 5.5 Prevalence of sexually transmitted infections (STIs) in reproductive age women, 20 - 49 years old attending a polyclinic, EFSTH, Gambia

Pathogen	Positive (n)	Prevalence (%)		AOR (95%CI)	p- value
		n = 232†	n =157**		
<i>Ureaplasma</i>	107	46.2	68.2	0.6 (0.3,1.0)	NS
Bacterial vaginosis**	46	19.8	29.3	3.8 (1.8,8.1)	0.01
<i>C.albicans</i>	30	12.9	19.1	1.8 (0.8,4.1)	NS
<i>S.agalactiae</i>	20	8.6	12.7	1.0 (0.4,2.7)	NS
<i>T.vaginalis</i>	18	7.8	11.4	5.3 (1.4,19.2)	0.01
<i>N.gonorrhoeae</i>	11	4.7	7.0	1.8 (0.5,6.8)	NS
HPV	28	12.1	17.8	1.3 (0.6,3.1)	NS
<i>C. trachomatis</i>	0	0	0	0	0.0
<i>M.genitalium</i>	0	0	0	0	0.0

† Number of participants recruited

**Number of participants infected with STI / genital pathogens

*bacterial vaginosis was diagnosed using Amsel's criteria.

NS = not significant, p-value > 0.05, AOR- Adjusted Mantel - Haenszel (MH) odds ratio, age group was controlled

5.9 Statistical analysis of risk factors associated with STI

Clinical outcomes of participants have shown that 157 participants were infected with one or more STI pathogens. Collinearity was observed between being married and had sexual intercourse in the preceding 12 months during stratification analysis. Since collinearity was observed between the two variables, it was not entered in the final analysis. A bivariate analysis was carried out on risk factors that may potentially associate with STIs in The Gambia. As more than 80% of study participants were married, marital status was looked at as a risk for STI and it was found to be associated statistically with STI ($p = 0.001$) (Table 5.6). Having ≥ 2 lifetime sexual partners, not using condoms, contraceptive used > 5 years and FGM were found significantly associated with STI ($p < 0.05$) (Table 5.6).

Multivariate logistic regression analysis was carried out on risk factor variables that were only associated with STI at the p -value ≤ 0.05 levels from the bivariate analysis. To control for confounding effects in the analysis; age group and ethnic group were adjusted in the model. Ethnic group was controlled in this final analysis because there were differences in the distribution of ethnic groups between the sample and the eligible population (Table 5.1), and disease prevalence between ethnic groups (Fig 5.1). The model was created in a step-wise procedure by starting with a single exposure variable and building it up by adding more variables. Subsequently, variables that were not significant to STI infection at ($p \leq 0.05$) were removed from the model one at a time. Marital status, and not using a condom during sexual intercourse were the only variables associated statistically with STI ($p \leq 0.05$) when age and ethnic groups were adjusted (Table 5.6).

Table 5.6 Bivariate and multivariate logistic regression analyses showing risk factors associated with sexually transmitted infections

Risk variables	Bivariate		Multivariate	
	OR (95% CI)	p-value*	AOR (95%CI)	p-value**
Married	0.2 (0.1, 0.5)	0.001	0.4 (0.1,0.8)	0.02
≥2 lifetime sexual partners	0.5 (0.3, 1.0)	0.05	1.0 (0.5,2.2)	NS
sexual debut (< 18 years old)	1.7 (0.9, 2.8)	NS		
Not using condom (last 12 months)	2.9 (1.1, 7.4)	0.03	0.3 (0.1,0.9)	0.03
education (< 12 years)	1.5 (0.9, 2.5)	NS		
FGM	2.5 (1.4, 4.3)	0.002	1.8 (0.8, 3.9)	NS
Partner(s) have other sexual partners	2.2 (0.9, 5.3)	NS		
Petty trading	1.4 (0.8, 2.4)	NS		
Contraceptive used (>5 years)	0.5 (0.2,1.0)	0.05	0.6 (0.2,1.4)	NS
Multiparity	2.8 (0.2, 3.0)	NS		

**p* - value is based on chi-square-corrected (Yates). 2 – tailed-*p* was used for the bivariate analysis **Based on Wald test, Adjusted for both age group and ethnic group.

OR/AOR = Odds Ratio /Adjusted Odds Ratio. NS – not significant (*p* > 0.05).

5.10 Discussion

In developing countries, the estimation of most of the STIs burden are based on either syndromic management or history of self-reported STI. In this study, samples were collected from reproductive age women attending a polyclinic for either STI / HIV management or family planning contraceptive services. These clinics attend to more than 80% of patients from the capital city, Banjul, the West Coast Region and the Kanifing Municipal Council as they are the only tertiary referral clinics. The data has shown that more than half of the women regardless of whether they had symptoms of STIs or not were infected with one or more STI/genital pathogens. The overall prevalence for at least one STI was found to be 68% with *Ureaplasma* being the most prevalent organism (46%). The STI prevalence observed in this study is consistent with other African studies. Oliveira *et al.*, (2007) found an overall STI prevalence of 45% in Brazilian women and Siawaya, (2014) found a prevalence of 65% in Gabonese women infected with at least one STI pathogens. The high prevalence seen is also consistent with The Gambia national STI surveillance data, which reported STI prevalence of 29% (Gambia Rapid Assessment Survey, 1992) confirming that STIs are important public health issues in The Gambia. However, the overall STI prevalence reported in this study is greater (68%) than the national prevalence, which could be attributed to the increased number of pathogens screened in this study. Another contributing factor is the national STI survey was conducted on antenatal women more than three decades ago, whilst this study focused on non-pregnant women attending a sexual health clinic. Secondly, the epidemiological, detection and statistical methods used have changed over time, therefore this could also add to the prevalence differences.

The prevalence of each of the pathogens isolated from the participants was more than a 10% except for *N. gonorrhoeae* (7.0%), (Table 5.5), which is greater when compared with the global estimates (0.3 -1.2%). In the global estimates, *T. vaginalis* prevalence amongst reproductive age women is reported as 1.9% to 7.8% (Newman *et al.*, 2015), which is in line with the 7.7 % prevalence reported for *T. vaginalis* in the current study. Though adolescents and young adults (15 – 24 years old) are consistently identified as having the highest rates of STI (WHO, 2014; Ohene *et al.*, 2008), this research showed STI prevalence was 38% (88/232) between the ages of 26 - 35 years of age, in The Gambia.

STI prevalence decrease was also observed with increasing age in this setting. This finding is consistent with The Gambia demographic and health survey (GDHS, 2013), where STI self-

reported was found to be 2.7% (45/1653) between the ages of 25 - 29 years and prevalence decrease was also noted in the older ages. The prevalence increase observed between the 26 - 35 years of age may be due to risky sexual behaviours such as unprotected sex, a high number of sexual partners or partners' sexual behaviour as 75% of participants in the 31 - 35 age group reported their partners had other sexual partners. STI prevalence decrease in the older ages have also been noted in several studies carried out in reproductive age women indicating either less sexual activity and/or stronger immune responses in these women (Ginindza *et al.*, 2017; Oliveira *et al.*, 2007; Kongnyuy and Wiysonge, 2007).

Having a higher education (>12 years) has a major effect on individual's sexual behaviour and it is thought to contribute to one's attitude towards risk factors that may be associated with STI (Gaspar *et al.*, 2015; Ojiyi *et al.*, 2013). However, less than 12 years of education appears to be a potential risk but was not statistically associated with STI (OR 1.5, $p > 0.05$). This finding agrees with Ginindza *et al.*, (2017) study, which found that women who have <12 years of education are likely to be infected with an STI. Although the GDHS, (2013) found self-reported STI increases with education, this study found STI to be more prevalent in women with none or less than 12 years educational backgrounds, which is consistent with other findings (Kakaire *et al.*, 2015; Arbabi *et al.*, 2014). It has been demonstrated that women with none or below 6 years educational background tends to lack formal employment and may be financially dependent on male sexual partners, therefore unable to negotiate for safe sex (Ncube *et al.*, 2012). Most of the participants (68%) with symptoms of STIs were low-income earners (< D76, 000 per annum; equivalent to < £1166 GBP) and 31% (71/232) were petty traders. Among occupation variables, those with secured means of livelihood such as housewives were less at risk of STI compared to petty trading (OR =1.4). Women, whose occupation was given as petty trading were found to have no formal education or had only 6 years of education (primary education). The underlying factors might be multiple sexual partners as those who engage in sexual relations with multiple partners most likely do it for economic reasons (Francis *et al.*, 2014; Okesola *et al.*, 2000). In addition, it has been demonstrated that low-income earners with a less than 6 years educational background are less likely to access health care information on STIs prevention (Arbabi *et al.*, 2014; GDHS, 2013) than the high-income earners with a more than 12 years educational background. Furthermore, Tenkorang, (2012) has shown that highly educated women are more likely to practice safer sex by using a condom with their partners compared

with the less educated women. This may be linked to the fact that individuals with higher education can access information on STIs that helps to protect themselves and their partners. In contrast to these findings, frequent sexual activity with different partners has been reported in highly educated employed women compared to unemployed or employed women with none or a lower educational background (Siawaya, 2014; Kohli *et al.*, 2013; Berhan and Berhan, 2012). These findings could be linked to women acceding to liberation bridging all sexual taboos and experimenting with their sexuality. Furthermore, Annang *et al.*, (2010) have also reported that higher education has a differential impact on racial/ethnic groups as some racial/ethnic groups with higher education were at significant risk of STIs. Another possible risk factor observed was with multiparity, it increased twice the odds of acquiring STI (OR 2.8) (Table 5.6). This could probably be linked to an early sexual exposure such as early marriage, early sexual debut, age at first pregnancy, and multiple sexual partners through remarriages as they are all closely related with synergistic effects (Wand *et al.*, 2011). Long-term exposure through unprotected sex in trying to get pregnant can also increase the chances of acquiring sexually transmitted diseases.

Female genital mutilation (FGM) is a practice that is performed on young females and it involves the removal of either part or all of the external female genitalia or injury to the female genital organs (WHO, 1997). FGM is a common cultural practice amongst certain ethnic groups of The Gambia and more than 50% of females have undergone FGM before the age of 5 years. However, 75% of females between the ages of 15 - 49 years old had undergone FGM in The Gambia with slightly a higher level of 79% seen in the rural area compared to 72% in the urban area. In this study, 73% (171/232) of participants diagnosed with STI had undergone FGM. This cultural practice is least practised by the Wollof ethnic group as shown in this study. Eighty-nine percent of the Mandinkas, 79% of the Fulas compared to 9% of the Wollof ethnic group had undergone FGM. The finding is consistent with The Gambia demographic health survey (GDHS, 2013), which also found the Wollof to be the ethnic group least likely to practise FGM in the general population. Multivariate analysis showed FGM was not significantly associated with STI ($p > 0.05$) but having undergone FGM, increased the odds of STIs by approximately two-folds (AOR = 1.8) (Table 5.6). The observation between FGM and STI could be a result of genital tissue damage leading to chronic inflammation and more susceptibility to infections (Osterman *et al.*, 2018). Furthermore, since most circumcised females are susceptible to recurrent genital infections, this can result in an impaired immune response and an inability to clear the infection.

In addition, FGM was found to increase the odds of being diagnosed with trichomoniasis by two-fold (AOR 2.2), but no association with the infection was observed ($p > 0.05$), (Table 5.4).

Demba *et al.*, (2005) also reported a similar finding between FGM and bacterial vaginosis in symptomatic women reporting to a sexual health clinic in The Gambia. A similar study conducted in The Gambia has also shown FGM to be a significant risk factor of acquiring bacterial vaginosis and herpes simplex virus type 2 (Morrison *et al.*, 2001). Although in this current study *Chlamydia trachomatis* was not detected in any of the samples, Morrison *et al.*, (2001) reported an association of FGM with chlamydial infection. FGM was also found to be a risk factor for STI in studies conducted in Tanzania and Nigeria (Klouman *et al.*, 2005; Okonofua *et al.*, 2002).

The role of male circumcision in STI acquisition and transmission has been controversial. Many cross-sectional studies carried out on the impact of male circumcision on STIs have found it to be a protective factor for their female sexual partners (Auvert *et al.*, 2009; Castellsagué *et al.*, 2002). Contrary to these findings, some longitudinal studies found no protective effect of male circumcision with STIs (Turner *et al.*, 2008; Gray *et al.*, 2000). It is widely accepted that the foreskin of the penis provides a favourable environment for the growth of pathogenic organisms such as *C. trachomatis*, *T. vaginalis*, and *N. gonorrhoeae*, which causes urethral infections (Holmes *et al.*, 1999). Therefore, the removal of the penis foreskin reduces the burden of these infections in their sexual partners compared to the female sexual partners of uncircumcised men. In The Gambia, more than 90% of men underwent pre-pubertal circumcision and Gambian women are more likely to be married to circumcised men. These findings may explain the low burden of STIs observed in the Wollof ethnic group as they are least likely to have undergone FGM. This finding adds to the controversial debate on FGM association with STI and whether circumcised women with circumcised male sexual partners are at greatest risk of acquiring an STI compared to uncircumcised women with circumcised male sexual partners.

Being married was identified to be statistically associated with STI ($p = 0.02$) (Table 5.6), however, controversial findings on being married with STIs have been demonstrated. Investigations have identified being single to associate significantly with STI (Francis *et al.*, 2014, de Lima *et al.*, 2014; Nyarko *et al.*, 2014), whilst others found an association with being married. The inconsistency in these findings may be a result of study bias as several studies that did not demonstrate a significant relationship between being married and STI had samples that were collected from predominantly unmarried participants. Secondly, some studies may be affected by a diagnostic bias. For example, if investigators are more likely to screen for and report cases

of STI in single young adults or adolescents, these cases may be systematically over-represented.

The interaction observed between being married with STI in this study may be due to polygamy. Polygamy is a common practice in The Gambia and in some parts of Africa and has implications of increase frequency of sexual activity with more than one partner. Approximately, 39% of married Gambian women live in polygamous union with one or more co-wives (GDHS, 2013), which increases the risk of acquiring and transmitting STI. In many African countries including The Gambia, not all sexual partners of women diagnosed with an STI are treated; therefore, this may increase the risk of recurrent STIs and treatment failure.

Inconsistent use of or not using a condom during sexual intercourse has been shown to be a risk factor for STI (Boyer *et al.*, 2006; Shlay *et al.*, 2004). Not using a condom was found to be statistically associated with STI ($p \leq 0.05$) (Table 5.6). The use of a condom in the last 12 months by both case and control respondents was comparatively lower than that reported by other studies (Karim *et al.*, 2003; Zellner *et al.*, 2003). This could be attributed to many of the respondents being married and are less likely to report using a condom during sexual intercourse than unmarried women. Another contributing factor could also be a poor negotiating power with their partners on condom use during sexual intercourse especially those in a polygamous relationship. In addition, studies carried out in many developing countries have shown STIs increases with wealth as men with high socio-economic status engage more in sexual risk behaviours, therefore predisposing their sexual partners to STIs (Berhan and Berhan, 2013; Awusabo-Asare and Annim, 2008).

Advocacy of safe sex practises and the use of condoms have been the driving message on STIs prevention, worldwide. In most developing countries the lack of access to free condoms is a contributing factor for unprotected sex. Ohene *et al.*, (2008) reported that approximately 60% of sexually active Ghanaian females do not know where to obtain condoms. A similar finding was also reported in The Gambia demographic health survey, (GDHS, 2013) that more than 70% of young female adults do not know where to obtain a condom compared to 22% of young male adults.

Several risk factors have also been identified to be associated with sexually transmitted infections. Early age at sexual debut had been demonstrated to be associated statistically with STI in single factor analysis (Boyer *et al.*, 2006; Navarro *et al.*, 2002). However, none of these investigations demonstrated any statistically significant associations with multivariate analysis due to small

sample sizes, the same was also observed in this study. Sixty-six percent of participants had their sexual debut at the age of ≥ 18 years old. This indicates that most Gambian women have their sexual debut at the time of their first marriage, as 80% were married at ≥ 18 years and reported having their sexual debut at the same time.

Age at sexual debut may be causally related to STI through changes in vaginal flora, mucus production, and persistence of columnar epithelium on the cervix, which supports the growth of pathogens such as *C. trachomatis* (Berman and Hein, 1999). Early sexual debut may also be an indicator of other aspects of sexual activity, which may directly increase risk. These include multiple sex partnerships that have an increased likelihood risk of having sex with an infected partner, and sexual intercourse with non-regular partners, which also have increased risk due to a reduced familiarity between partners (Evans *et al.*, 1997; Kost and Forrest, 1992).

In many developing countries, contraceptive use is associated with frequent sexual activity especially in young unmarried adults and could be a risk factor for STI (Tweedie *et al.*, 2000; Ohene *et al.*, 2008). It had been shown that most women associate contraceptive use with preventing unwanted pregnancies; therefore, its association with STIs through risky behaviours is normally not considered (Ohene *et al.*, 2008). Women who use oral contraceptives or intrauterine device (IUD), are less likely to use barrier contraceptive (condom) and this cohort of women tend to have a higher STI prevalence than non-users (Navarro *et al.*, 2002). However, results obtained on contraceptive studies have shown some disparities, for example; Mosure *et al.*, (1997) found that contraceptive users have decreased risk of being infected with STI than non-users. Similarly, the use of IUD was found to be a protective factor against chlamydial infection (Evans *et al.*, 1997). The investigators hypothesized that the IUD may enhance local immune response and accelerate the development of cervical squamous epithelial cells, which prevents Chlamydial infection. However, no significant difference was found for IUD use compared with the use of other methods by these investigators. Although, this current study did not differentiate the use of different contraceptive methods with STI, it has found the use of contraceptive for more than 5 years to be associated statistically with HPV and STI ($p < 0.05$) in a bivariate analysis (Tables 3.1 and 5.6), however when age and ethnic groups were controlled in a multivariate analysis, no significant association was found with STI ($p > 0.05$) (Table 5.6). The overall prevalence of 30% of bacterial vaginosis reported for the symptomatic (case group) participants is high when compared with the prevalence reported from other African countries. A prevalence of 20% was reported in Burkina Faso and in Gabon, 23% in symptomatic women

in Malawi, and 24 % of women seeking primary health care in Morocco (Oliveira *et al.*, 2007; Costello *et al.*, 1998; Ryan *et al.*, 1998; Ledru *et al.*, 1996). The finding in this study is consistent with Demba *et al.*, (2005), which reported bacterial vaginosis to be present in 47% in symptomatic Gambian women. The risk of being diagnosed with bacterial vaginosis clearly increased with the reported number of lifetime sexual partners. The same finding was also observed in a population-based study in rural Uganda (Sewankambo *et al.*, 1997). Bacterial vaginosis and trichomoniasis were found to be more prevalent in the Mandinka ethnic group compared to the other two major ethnic groups. However, *Ureaplasma*, *Streptococcus agalactiae*, and HPV (chapter 3) prevalence were observed more in the Fula ethnic groups. The difference seen in pathogen prevalence in the ethnic groups could be due to differences in vaginal flora or genetic variation in the different ethnic groups (Allsopp *et al.*, 1992).

Although some researchers have cited bacterial vaginosis as a syndrome rather than an STI, it is one of the most common conditions reported in sexual health clinics, worldwide (Demba *et al.*, 2005). Bacterial vaginosis and trichomoniasis were diagnosed more in the older age group (> 30 years old) as observed in other studies (Ginindza *et al.*, 2017; Oliveira *et al.*, 2007). The high incidence seen in older women could be due to physiological changes such as high vaginal pH as bacterial vaginosis is a poly-microbial syndrome caused by a shift in the vaginal flora. The vaginal flora, which is predominantly made up of lactobacilli is gradually or totally replaced with anaerobic bacteria such as *Gardnerella vaginalis*, *Bacteroides*, *Atopobium vaginae* and other Gram-negative bacteria in older women resulting in a higher vaginal pH (Hill, 1993).

Recent systematic reviews have indicated that the use of self-sampling for STI screening was highly acceptable in many developing countries (Huynh *et al.*, 2010; Stewart *et al.*, 2006). The acceptability of self-sampling for STI screening was assessed in this study and only 18% preferred self-sampling, citing embarrassment as a reason. Comparing the low rate of acceptability of self-sampling in this study with reports from other developing countries for example: in India, Uganda and Nicaragua, self-sampling acceptance were: 95%, 64% and 50%, respectively (Bansil *et al.*, 2014). Data on self-sampling has indicated that most women preferred self-sampling because it is less embarrassing, less invasive and more private than the conventional speculum collection (Quincy *et al.*, 2012; Dzuba *et al.*, 2002). A good correlation between self-sampling and health care provider sampling has been reported, therefore it could be a potential future collection option for cervical cancer screening programmes in developing countries (Gravitt *et al.*, 2011; Safaeian *et al.*, 2007).

In conclusion, the results indicate that there is a strong need to implement STI screening and treatment in women of reproductive age. Since many risk factor analyses were performed in this study (chapters 3 and 4), those factors that were statistically significant with only one organism may well have arisen by chance except being married and not using condoms, which were the only variables found to be statistically associated with STI in a multivariate analysis. Therefore, the use of a condom during sexual intercourse should be advocated for, to reduce the risk of STI acquisition and transmission. Embarrassment and social stigma could be a contributing factor for most women not reporting symptoms of STIs. However, 72% of participants preferred a health care provider sampling. Therefore, it was not a contributing factor for not reporting symptoms of STIs in this study. Nonetheless, women should be educated on the potential health and social behavioural risks of asymptomatic STIs. Furthermore, results obtained on the acceptability of self-sampling may not be representative of Gambian women as more data is needed to validate this finding. Although this study has highlighted some important public health issues, there is a need for STI programs to strengthen intervention strategies to increase awareness on risk factors associated with STIs in Gambian women, especially in women between the ages of 26 - 35 years of age. There is also a strong need to encourage sexual partner(s) treatment to reduce or prevent recurrent infections, which can lead to significant reproductive health complications in women and their newborn babies.

Chapter 6: Human papillomavirus sero-prevalence and estimation of sero-conversion in a cohort of HIV-positive women

6 Introduction

The association between HPV and genital diseases is well established. HPV infection is usually a transient infection in immune-competent individuals with an average duration of 8 months (Ho *et al.*, 1998). However, HPV infections in HIV-positive individuals are burdensome and the progression of cervical diseases is aggressive as these individuals are less likely to clear the infection due to reduced cell-mediated immune (CMI) responses, which increases their risk of developing persistent infections, cervical dysplasia and cancer (Ezechi *et al.*, 2014). It has been documented that even after cancer treatment in these individuals, the chances of recurrence after 5-years are between 40 - 60%, which can be linked to the level of immunosuppression, social and behavioural risk factors (Rein, 2000 (b), Cuthill *et al.*, 1995). This association is such that in 1993, invasive cervical cancer was added to the list of AIDS-defining illnesses.

A meta-analysis carried out mostly in developed countries has shown an association between infection with multiple HR-HPV genotypes and HIV-positivity (Clifford *et al.*, 2017; Blossom *et al.*, 2007). Furthermore, HIV-positive women tend to be more susceptible to co-infection with HR-HPV types other than HPV 16 and 18; however, this can vary geographically (Luque *et al.*, 2006; Levi *et al.*, 2004). This could be due to increased HIV prevalence in developing countries, where other oncogenic HR- HPV genotypes are more common (McKenzie *et al.*, 2010; Clifford *et al.*, 2017). For example, in Africa, HPV genotype 51, 52, 53, 56 and 58 have been reported to be more prevalent in HIV-positive women with cervical dysplasia than HPV 16 (Sahasrabudde *et al.*, 2007; Blossom *et al.*, 2007).

The introduction and increased accessibility of antiretroviral therapy (ART) in resource-limited countries in the last decade has contributed in the improvement of clinical outcomes and life expectancy in HIV-positive individuals. The Gambia introduced ARVs in the early 2000s and started using the 2013 adopted HIV treatment guidelines recommended by the WHO, in 2015. The WHO recommended first-line and second-line treatment regimens for HIV-positive adults and adolescents are as follows: The first-line regimen includes a combination of nucleotide reverse-transcriptase inhibitor (NtRTI), Tenofovir disoproxil fumarate (TDF), and a nucleoside reverse-transcriptase inhibitors (NRTIs) e.g. either lamivudine (3TC) or Emtricitabine (FTC), and an integrase strand transfer inhibitor (INSTI), Dolutegravir (DTG). A combination of TDF, 3TC or FTC, and non-nucleoside reverse-transcriptase inhibitors (NNRTI), e.g. Efavirenz

(EFV) is given as an alternative treatment. Zidovudine (AZT) can also be given in combination with EFV and 3TC or FTC as an alternative treatment.

The preferred second-line treatment regimens include a combination of AZT, 3TC and a protease inhibitors (PIs) e.g. Lopinavir/ritonavir (LPV/r) or Atazanavir/ritonavir (ATV/r) if TDF was used in the first-line therapy or TDF, 3TC and LPV/r or ATV/r, if AZT was used in first-line therapy. In HIV-2 or dual infection (HIV-1 and HIV 2) treatment, the preferred regimen is a combination of TDF, 3TC and LPV/r or an alternative regime with AZT, 3TC and LPV/r.

HIV-positive individuals who are prescribed on ARV treatment have been found to be at a reduced risk of being infected with oncogenic HR-HPV than those who are not (Ezechi *et al.*, 2014; Schuman *et al.*, 2003). The consistent use of ARV drugs has been reported to restore and enhance the immune response of HIV-positive individuals, thus preventing infections caused by other STI pathogens (Broker *et al.*, 2001). Furthermore, HIV-positive women with low CD4 count and who are not prescribed on ARVs may be at greater risk of latent HPV reactivation (De Vuyst *et al.*, 2008). This can occur because of severe immunodeficiency and can lead to cervical diseases. Therefore, the use of ARV drugs may reduce persistent infection with HPV and facilitate cervical lesion regression in HIV-positive women (Heard *et al.*, 2004).

Humoral immune responses following natural HPV infection of the anogenital tract are not always observed. If sero-conversion does occur, antibodies against HPV capsids may persist for years and are therefore reflective not only of recent, but lifetime HPV exposure (Stanley *et al.*, 2006). HPV seropositivity has been associated with both virus-related (e.g persistence of infection, viral load, and viral type) and host-related factors (e.g increasing age, HIV-induced immunosuppression and sexual risk behaviour (Hildesheim *et al.*, 2007).

Since the introduction of ARVs in The Gambia, there is little or no information on the prevalence, distribution of HPV and cervical cancer in women who are HIV-positive. The incidence of HPV infection in African women is known to range from 30 to 60% with the most prevalence seen in women co-infected with HIV (Baay *et al.*, 2004; Wright *et al.*, 2000). Sero-conversion following HPV infections in immune-competent individuals is estimated to be between 8 - 24 months, however, longitudinal studies analysing HPV sero-conversion following HPV infections in HIV-positive individuals are scarce. More insight into the associations between HIV infection and HPV sero-response is crucial to interpret results from sero-surveillance, which can, for example, be used to monitor HPV prevention strategies. As reproductive age women, especially HIV-positive, are at increased risk of HPV infection and HPV-related diseases,

understanding these issues among this group is important. Furthermore, immunity to HPV virus-like particle vaccines is type-specific, it is therefore, important to also characterize the distribution of HPV and cervical diseases in this group to develop effective cervical cancer prevention strategies.

6.1 Aim and objectives

The aims of the work presented in this chapter were to characterise the distribution of HPV and cervical diseases, determine sero-prevalence, and estimate the duration of HPV sero-conversion in a cohort of HIV-positive women.

Objectives:

- To determine HPV sero-prevalence and type-specific distribution in cervical and serum samples
- To estimate the time of sero-conversion over a period of 24 months
- To determine cervical cytological changes over a period of 18 months
- To determine and estimate cervical lesion regression, persistent infection and duration of immune response.

6.2 Baseline data on recruited HIV-positive women

Twenty-nine women diagnosed with HIV were recruited in this arm of the study, 72% (21/29) were HIV-1, 14% (4/29) HIV-2 and 14% (4/29) were infected with both HIV-1 and 2 (Table 6.1). Of the 29 women, 9 had CD4 cell count between 350 – 499 (cells/mm³) and 55% had a CD4 count of ≥ 500 cells/mm³. Eighty-six percent (86%) of these women were not prescribed ART at the time of recruitment. It is noteworthy that at the beginning of this study, the recommended CD4 cut off for HIV treatment by the W.H.O was < 350 cells/mm³. Table 6.1 shows the baseline laboratory and treatment characteristics of participants.

Table 6.1 Baseline laboratory and treatment characteristics of recruited HIV positive women (n = 29)

Variables	Number and (percentage)
HIV status	
HIV-1	21 (72)
HIV-2	4 (14)
HIV-1 and 2	4 (14)
CD4 cell count (cells/mm³)	
<350	4 (14)
350 – 499	9 (31)
≥ 500	16 (55)
Antiretroviral (ARVs) drug use	
On ARVs	4 (14)
Not on ARVs	25 (86)

6.3 HPV sero-prevalence and cervical diseases amongst HIV-positive women

6.3.1 HPV sero-prevalence and serotype distribution

Serum samples from HIV-positive women were each mixed with one of the pseudo-typed viruses and a reduction of the infectivity was measured. Serum samples that did not reduce the infectivity to less than half at a dilution of 1:40 were defined as negative for neutralization as described in chapter 2, section 2.20. Fifteen samples were found to neutralize pseudo-typed viruses of one or more of the following HPV types: HPV 16, 18, 35, 51, 52 and 58.

HPV sero-prevalence was found to be 51.7% (15/29) and antibodies against HPV52 were the most common at 24% (7/29). Of the 15 women whose samples tested positive, 8 had HPV antibodies to a single serotype with HPV 51 accounting for 37.5% (3/8) of the single serotypes detected, followed by HPV 52, 25% (2/8) and HPV16, 18, and 58 each at 12.5% (1/8). Seven (7) women were sero-positive with multiple HPV serotypes with neutralising antibodies against two HPV types detected in 3 women whilst 4 women had neutralising antibodies against three HPV types (Table 6.2).

Table 6.2 Number of women sero-positive for HR-HPV neutralising antibodies against one or multiple HPV types at baseline

HPV serotypes	Number of women
16	1
18	1
35	0
51	3
52	2
58	1
16/35/52	2
16/58	1
18/35/52	1
18/52/58	1
35/51	1
35/52	1

Participants were screened against selected HR-HPV genotypes in this arm of the study. HPV-52 was the most common genotype detected in multiple infections and HPV-51 was the most prevalent type in single infections amongst the study participants.

6.3.2 Cervical diseases, HPV DNA and neutralising antibodies at baseline

Baseline cytology showed that of the 5 women diagnosed with severe high-grade cervical lesions, one had neutralising antibodies against HPV 16, two had antibodies against HPV 51 and the remaining two were sero-negative. One of the women with neutralising antibodies against HPV 51 had severe HSIL which suggested invasive cervical cancer. HPV DNA genotype 51 was also detected in a cervical swab sample from this patient (Table 6.3).

Three participants were diagnosed with low-grade cervical lesions with one woman having neutralising antibodies against HPV 16, 35 and 52. Of the two remaining women with LSIL, 1 had antibodies against HPV 58 and 1 tested sero-negative. Nine women had normal cytology

results and HPV neutralising antibodies were detected in their sera. In addition, HPV DNA (1 high-risk and 3 low-risk types) was detected in four of the women with normal cytology (Table 6.3). Only six women had normal cytology results, the absence of HPV DNA and were also sero-negative against the screened HPV serotypes at baseline.

Table 6.3 Number of women with CIN lesions, with and without neutralizing antibody and HPV DNA

Cytology grade	Presence of HPV DNA (type)	HPV neutralising antibodies
	HSIL	Positive (51)
	Negative	51
	Negative	16*
	Negative	sero-negative**
	Negative	sero-negative
LSIL	Negative	sero-negative
	Positive (58)	16,35, 52
	positive (56)	58
Normal	positive (52)	51, 52
	positive (42)	35, 52
	Positive (6)	51
	positive (61)	sero-negative

patients were unable to give a second sample at the 9 months follow-up visit as both patients died. ‡ was diagnosed with cervical cancer (HPV51‡). ** Patient sero-converted to HPV 16 at the 9 months follow-up visit.

Key: HSIL – High-grade squamous intraepithelial lesion; LSIL – Low-grade squamous intraepithelial lesion

6.4 Presence of HPV DNA, neutralising antibodies and cervical lesions in HIV-positive women.

6.4.1 Follow-up at 9 months

Of the 29 women recruited, only 18 gave a follow-up second sample at 9 months. Of the 15 women that were sero-positive at baseline, 10 gave a second sample, two had died and three were lost to follow-up. Out of these women, three were found to produce additional HPV antibodies at 9 months. Of the 14 women that were sero-negative at baseline, 8 gave a second sample and two women sero-converted to HPV16 and HPV 35, respectively. However, none had HPV DNA detected in the cervix. The remaining 6 women remained sero-negative. A participant with normal

cytology and a presence of HPV 52 DNA at baseline (Table.6.3) was diagnosed with HSIL and was still found to be persistently infected with HPV52 DNA at the 9 months follow-up visit. In addition, the presence of HPV DNA (HPV 69) was detected in a woman who had HSIL and neutralising HPV 51 antibodies at baseline. Lesion regression was observed in one participant who was diagnosed with LSIL at baseline.

6.4.2 Follow-up at 18 months

At the 18 months follow-up visit, only 12 women provided a third sample and they were all on ARVs treatment at the time of the visit. Of these women, the 6 remaining sero-negative participants were found to be still sero-negative at the 18 months follow-up visit. However, of the 6 sero-positive women, five remained sero-positive with the same neutralising HPV antibodies and one became sero-negative at the 18 months follow-up. The presence of HPV DNA (HPV 66) was found in only 1 woman who in three consecutive samplings had neutralising antibodies against HPV 18.

In addition, the data also showed that three women who were sero-positive to multiple HPV serotypes at either baseline or at the 9 months visit became persistently sero-positive with only one serotype and lost neutralising activity against the other types at the 18 months visit. Cytology results of the 12 women who provided serum samples showed that only two of the women who were initially diagnosed of HSIL at baseline and at 9 months had lesion regression to LSIL at the 18 months follow-up visit. The remaining 10 women had normal cytology. Table 6.4 summaries the follow-up cytology results.

6.4.3 Follow-up at 24 months

Table 6.4 Number of samples with normal and abnormal cytology results from baseline to 18 months.

	Normal	LSIL	HSIL
Collection interval (number of samples)			
Baseline (n = 23)	15	3	5
9 months (n = 18)	14	0	4
18 months (n = 12)	10	2	0

Among those that gave a second sample at 9 months, cervical lesion regression was observed in one participant who had LSIL at baseline. In addition, one participant who had a normal cytology result at baseline was found to have a HSIL at 9 months. Cervical lesion regression from HSIL to LSIL was observed in two participants at the 18 months follow-up visit.

At the end of the follow-up visit, only 11 women provided a fourth sample at 24 months. Of these women, 7 were sero-positive at baseline and sometime during follow-up. Five women remained sero-positive until the end of the 24 months follow-up period (Table 6.5) and two women lost their neutralising antibodies by the 24 months follow-up visit. Only 4 out of the remaining 6 sero-negative participants gave a fourth sample and one sero-converted to HPV-58. Only 3 women who gave four consecutive samples remained sero-negative for neutralising HPV antibodies, had no presence of HPV DNA and had three consecutive normal cytology results (Pap smear was not collected at the 24 months follow-up visit due to the timeframe as this part of the work was carried-out externally). Cervical swabs that were collected at the time of serum collection were all negative for the presence of HPV DNA.

Table 6.5 Number of women persistently sero-positive for HR-HPV neutralising antibodies at the 24 months follow-up visit

HPV serotypes	Number of women
18	1
52	1
35/52	1
52/58	1
18/35/52	1

Five women with four consecutive samples were found to persistently have the same HPV neutralising antibodies. HPV52 was the most neutralising antibodies detected in multiple infections.

6.5 Discussion

HPV is known to have the ability to evade the host's immune response as the virus does not cause an inflammatory reaction, which enhances the full activation of the cell mediated immune response (Bonanni *et al.*, 2009). However, more than 50% of infections are naturally cleared or become undetectable within 12- 24 months of infection (Hildesheim *et al.*, 2007; Trottier and Franco, 2007). Both the innate and adaptive (cellular and humoral) immune systems play a role in the immune response at the site of HPV infection in the genital tract. The cell-mediated immune (CMI) response, which is central against the early HPV proteins, is decreased in HIV-positive individuals (Clifford *et al.*, 2017; Stanley, 2006; Passmore *et al.*, 2006). Therefore, HIV-positive individuals may be unable to mount an effective CMI response against HPV infection, which could lead to persistent infection with the virus.

In this arm of the study, serum samples from HIV-positive women were screened for neutralising antibodies against six high-risk HPV genotypes namely: HPV 16, 18, 35, 51, 52 and 58. Fifteen serum samples (51.7%) were positive for neutralising antibodies against one or more screened HPV types (Table 6.2). Nearly half of these participants (7/15) were sero-positive with more than one HPV type at baseline, which could reflect cross-reactivity or infection with multiple types (Stanley *et al.*, 2006). However, subsequent follow-up data showed persistent infection with multiple types; therefore, it seems likely that these HIV-positive women were infected with multiple HPV types. Furthermore, it has been demonstrated that natural infection with HPV induces highly type-specific neutralising antibodies, which discriminate between even closely related species (Clifford *et al.*, 2006). The high prevalence of multiple neutralising HPV antibodies observed can result from HPV-induced upregulation and persistence infection of HPV (Moscicki *et al.*, 2004). Importantly, it can also result from continued sexual exposure to novel HPV types during periods of severe immunosuppression, which may be an important factor in this study as more than 52% of participants reported having two or more lifetime sexual partners and of those with one-lifetime sexual partner, 60% of their partners have two or more co-wives. Furthermore, most of these women's partners do not use condoms during sexual intercourse, therefore predisposing them to new infections (Chapter 5).

The detection of HPV type-specific antibodies in this study did not strictly correlate with the HPV DNA results indicating that the antibody response may most likely be due to previous exposure to the virus. In addition, it has been observed that persistent, rather than transient HPV infection is associated with sero-conversion (Petter *et al.*, 2000).

HPV sero-conversion to HPV 16 and 35 were observed in two women at the 9 months follow-up visit and one participant sero-converted to HPV 58 at 24 months. This finding is consistent with the literature where the average time for sero-conversion is estimated to be between 8 -12 months. However, sero-conversion after 18 months to HPV16, 18 and 6 had been reported in 55-70% of women infected with HPV (Ho *et al.*, 2004; Carter *et al.*, 2000). The presence of HPV DNA was not detected in the cervical samples of the participants who sero-converted at 9 and 24 months, respectively. The HPV immune response generated could possibly be due to vulval or vaginal infections that were not detected in the cervical samples. Sero-conversion was found to be transient and that sero-positivity could be intermittent as most participants that were sero-positive with multiple neutralising antibodies lost neutralising activity to some other types and two women lost their neutralising antibodies by the 24 months follow-up visit. This may reflect antibody titre, which can fluctuate around the antibody detection threshold as type-specific HPV antibody peaks and decline within 12 months of infection (Steele *et al.*, 2008, Carter *et al.*, 2000).

Although HPV infection can lead to sero-conversion, not all infected individuals will generate an immune response to the HPV L1 capsid protein. Approximately, 60% of individuals will develop serum antibodies after natural infections (Viscidi *et al.*, 2004; Carter *et al.*, 2000). This may explain why three participants with four consecutive samples neither sero-converted nor had HPV DNA detected up to the end of the 24 months follow-up period. Secondly, the participants that provided third follow-up samples were prescribed on ARVs by the 18-month visit and this could have partially restored their cell-mediated immunity.

Of the participants diagnosed with HSIL at baseline, two died by the 9 month follow-up visit, and one had HPV 16 neutralising antibody, but no HPV DNA was detected. The failure to detect HPV DNA in this patient could result from loss of the *L1* open reading frame during viral integration into the host genome as integration often occurs in the L1 region (Depuydt *et al.*, 2007).

The other participant was diagnosed with invasive cervical cancer and HPV-51 DNA and anti-HPV 51 was detected in both cervical and serum samples. This evidenced that oncogenic HPV types other than HPV 16/18 may cause cervical cancer in The Gambia. Cervical lesion

regression was observed at the 18 months follow-up visit in women who were diagnosed with either LSIL or HSIL at baseline or at 9 months, respectively.

Furthermore, partial restoration of the immune function by ARVs may have induced the cell-mediated immune response, which would have induced the cervical lesion regression observed in these patients. In addition, only 5 women were found to persistently have the same neutralising antibodies (Table 6.5) up to the end of the 24 months follow-up. Antibodies generated in response to a persistent infection may prevent sequential auto-infection within the genital tract. However, antibody levels after natural infections may not be adequate for long-term immunity in these patients (Mckenzie *et al.*, 2010).

In conclusion, the availability and accessibility to antiretroviral therapy are such that HPV-associated cervical diseases and cancers may become a major problem in HIV- positive women in future due to increased life expectancy. The concurrent infection with multiple high-risk HPV types in HIV-positive women increases the risk of cervical diseases. Although 31% (9/29) of participants had normal cytology at baseline, multiple neutralising antibodies were found in 66% (6/9) of those with HPV antibodies indicating that these women were exposed to multiple HR-HPV types. It is therefore important to routinely screen these women for early detection and management of precancerous lesions. The relatively low prevalence of vaccine-type HPV16 and 18 reported in this study corroborate other findings reported in the African sub-regions (Gage *et al.*, 2012; Sahasrabuddhe *et al.*, 2007; De Sanjosé *et al.*, 2007). This suggests that the current vaccine available for the Gambia will benefit only a minority of HIV-positive women as other HR-HPV other than HPV 16 and 18 were found to constitute the majority of circulating types both in cervical and serum samples of this cohort. Studies on the safety and efficacy of HPV vaccines among HIV-positive women have demonstrated promising results, therefore this group would benefit from vaccination to boost their antibody levels and duration of protection against HPV infection.

Chapter 7: General conclusion, study limitations, future work and summary

7 General conclusions and recommendations

Infection with oncogenic human papillomavirus genotypes is a major public health concern, globally. Persistent infection with these types can lead to cancers of the cervix, vulva, vagina, anus, penis, and head and neck (IARC, 2007; Bulk *et al.*, 2007; Hoots *et al.*, 2009). Amongst the HPV-associated cancers, cervical cancer is the most common cancer affecting women below the age of 50 years old (de Martel *et al.*, 2017). Approximately 70% of the global cervical cancer incidence is reported in developing countries, where access to adequate and regular cervical cancer screening and treatment is not readily available. It is estimated that if this trend continues cervical cancer mortality will increase by 10% each year in these countries (Ferlay *et al.*, 2001). Regular cervical cancer screening and HPV vaccination programmes have been demonstrated to have reduced the burden of cervical cancer and anogenital warts in developed countries (Machalek *et al.*, 2017). In contrast, only 13% of countries in developing countries have HPV vaccination programmes (LaMontagne *et al.*, 2017). Lack of proper vaccination and screening programmes as well as low socio-economic development, environmental risk factors and co-infection with sexually transmitted infections, contributed to the highest mortality rate seen each year, in developing countries (Ekiel *et al.*, 2009; Ferlay *et al.*, 2004).

The work presented here studied the distribution and prevalence of human papillomavirus genotypes, as well as co-infection with selected sexually transmitted pathogens in reproductive age women attending a primary health clinic, at Edwards Francis Small Teaching Hospital, Banjul, The Gambia. In addition, the ability of the human papillomavirus to persist and cause cervical changes in the cervix of a cohort in women infected with HIV was also studied.

The overall prevalence of HPV infection reported (12%) in this study, the first urban Gambian study is similar to the Gambian rural study, which reported a prevalence of 13% (Wall *et al.*, 2005). However, there was a difference in the most prevalence circulating genotypes distribution between the two studies. This present study observed HPV 52 genotype to be the most common circulating genotypes and HPV 16 the eighth genotype identified. In contrast, Wall *et al.*, (2005) found HPV 16 to be the most prevalent type, in their rural study. HPV genotype variability in different geographical regions and within the same country has also been reported elsewhere (Bruni *et al.*, 2010; Castellsague *et al.*, 2007).

Although The Gambia introduced the quadrivalent HPV vaccine in 2014, it has been demonstrated here that less than 11% of participants were infected with HPV genotypes

targeted by the quadrivalent vaccine. In contrast, 35.7% were positive for HPV genotypes included in the nonavalent vaccine. This finding will be further investigated using cervical cancer biopsy samples. In addition, the prevalence of HPV antibody was found to be 51% in a cohort of 29 women infected with HIV. HPV 52 was also the most common type found (Table 6.2). Although antibodies to HPV 52 genotype was the most commonly found, it was predominantly found in multiple infections in HIV-positive women. On the other hand, HPV 51 antibody was the most common type found in patients with a single genotype infection (Table 6.2). An HIV-infected participant was diagnosed with cervical cancer and both HPV 51 DNA and serum antibody were detected in the samples provided. This shows further that other oncogenic HPV types other than HPV 16 and 18 might also be responsible for cervical cancer in The Gambia. Furthermore, this work also observed that nearly 42% of women between 26 and 30 years old were infected with HR-HPV genotypes, which can resolve or progress to cervical diseases. Therefore, the present study supports the idea that women above 30 years old and those that are infected with HIV should be screened regularly for the presence of HPV oncogenic genotypes and cytological cervical changes, for early diagnosis, treatment and follow-up. In addition, the “save my mother” campaign on cervical cancer screening initiated by the SOS Mother and Child private clinic have reported an increase in women accessing this service. Therefore, the Gambian Ministry of Health should encourage the use of this strategy across the major government health clinics that are offering VIA screening.

The 32% prevalence of HPV infection in the 21 – 25 years old reported in this study is in line with the global trend of the infection as HPV infection begins shortly after sexual initiation (Moscicki *et al.*, 2012; Franceschi *et al.*, 2006; Castle *et al.*, 2005). However, most of these infections are transient and will resolve spontaneously. A novel putative fragment of HPV 35 may have been found in this study as the *L1* gene sequences of The Gambian type and the reference type differ by more than 10%. This finding will be further investigated.

The phylogenetic analysis carried out on the isolated HR/pHR-HPV genotypes was important in demonstrating the evolutionary similarities as either divergent or convergent with published isolates from other geographical locations (Fig 3.6). The Gambian HR/pHR-HPV isolates were found to group within its respective genotype clades and within each genotype. No major distinction between the high-risk and probable high-risk genotype sequences were found. Although the prevalence of the most circulating HR-HPV genotypes in both the urban and rural studies is different, both studies advocate for a multivalent vaccine that would also target other circulating oncogenic genotypes in The Gambia. The nonavalent vaccine (9-V) may be the best vaccine candidate for The Gambia if the current cost of the quadrivalent vaccine is

comparable to the cost of two doses of the nonavalent vaccine. Currently, two Chinese companies (Innovax and Walvax) are evaluating the efficacy of two other new HPV vaccines targeting HPV genotypes 16 and 18 (Wu *et al.*, 2015; Schiller *et al.*, 2015). The possibilities of introducing these two new vaccines by 2020, worldwide and the technology transfer of existing vaccines to manufacturers in middle income countries such as Brazil, India and Argentina (Baker *et al.*, 2015; Leonardo *et al.*, 2011; Padmanabhan *et al.*, 2010) may increase market competition thereby reducing vaccine prices.

A single dose for HPV prevention has been reported to provide enough protection to significantly reduce the burden of cervical diseases (Kreimer *et al.*, 2015). However, findings on the efficacy and duration of protection of a single dose vaccination have been of concern as the numbers of incident and persistent infections in these studies were either too small due to the sample size and/or limited follow-ups (Basu *et al.*, 2016). Delivery of a single dose of HPV vaccine with high coverage would be much easier for developing countries that face issues with logistics, financial and programmatic challenges. Although a single dose of HPV vaccine may be promising, more evidence is needed on the cross-protection effect and duration of protection as the peak incidence of HPV infections occurs in 20-25year olds. Therefore, protection with a single dose of HPV vaccine in young girls 9-14 years old must be demonstrated to last for at least 10 years (Wang *et al.*, 2015) especially in countries like The Gambia, where the present study reported a high prevalence of oncogenic HR-HPV in women above the age of 25 years.

The Gambia will be implementing a national HPV vaccination programme in 2019; it is also important to have a national cervical cancer screening programme. Although the World Health Organisation (WHO) recommended a single visit algorithm for HPV-associated cervical diseases in developing countries, the most cost-effective methods to triage and treat HPV-positive women in these countries are yet to be determined (Ogilvie *et al.*, 2017). Presently, apart from small scale projects, HPV testing as a screening method has not been nationally instituted in developing countries. This could be due to cost as HPV testing is expensive to establish and maintain as a primary screening technique (Basu *et al.*, 2017).

The Gambia is best suited for the single visit algorithm; that is screening with VIA and treating at the same visit as there is only one cytology laboratory. However, including HPV DNA screening in the future may be beneficial to reduce unnecessary colposcopy referrals, provide more robust triage of women with HR-HPV genotypes, and monitor the replacement of the quadrivalent HPV vaccine types in The Gambia, for an effective prevention strategy.

Other findings such as HPV co-infection with other STI pathogens have also been documented. Of the 28 women infected with HPV, 27 (96%) were found to be co-infected with at least one STI/genital pathogen; 50% of these women were found to be co-infected with *Ureaplasma*; 79% (11/14) of women co-infected with *Ureaplasma* had the organism detected in both endocervical and high vaginal swab samples. This finding is not novel but adds to the body of literature that women who are above 30 years old and co-infected with HPV may be treated for *Ureaplasma* infection, to investigate whether treatment will help prevent cervical lesions that may progress to high-grade cervical diseases and cancer (Ekiel *et al.*, 2009a). However, treating *Ureaplasma* is still a controversial debate and more data is needed to determine treatment benefit.

Although in The Gambia, a majority of suspected STIs are treated using the syndromic management approach, this study underscored the need to strengthen laboratory-based evidence in the diagnosis of STI to limit overuse of antibiotics. The finding of asymptomatic gonorrhoea in the control group (4.3%) and the isolation of ciprofloxacin, ceftriaxone and cefotaxime resistant *N. gonorrhoeae* strains is a cause for concern not only for The Gambia but globally as reduced susceptibility to the cephalosporins has been documented (WHO, 2013, Tzelepi *et al.*, 2008). This work shows further that microbiological analysis of clinically significant isolates and their antibiogram should be carried out to confirm a diagnosis and to advise clinicians on the appropriate choice of antibiotic regimen, to reduce therapeutic failure.

A vital public health issue found in this study was the high STI prevalence seen in asymptomatic (control group) participants. Approximately, 66% (78/117) were found to harbour at least one or more STI/genital pathogens. Secondly, these STI infections; Chlamydia, gonorrhoea, and trichomoniasis are asymptomatic in women and can remain untreated, therefore resulting in longer infection periods (Holmes, 2008). This evidenced further that asymptomatic STI can pose significant public health challenges in reproductive age women. Furthermore, recurrent STI infection is very common, particularly with *T. vaginalis* following the current recommended single dose treatment, as male partners are normally not treated, which can facilitate progression of HPV infection to cervical diseases because of inflammatory reactions. The global trend of antimicrobial resistance, especially with *N. gonorrhoeae* makes it important to improve the monitoring and evaluation of changes in STI incidence and resistance profile, through robust national STI surveillance.

In chapter 4 of this study, the distribution of *Ureaplasma* in reproductive age Gambian women was also investigated. A 46% *Ureaplasma* prevalence was seen in this study and 41% of

women that reported symptoms of STI had *Ureaplasma* infection, however, its clinical significance in this study population cannot be ascertained as 51% of asymptomatic participants were colonised with the organism. The substantial *Chlamydia* prevalence difference between Demba *et al.*, 2005 (15%) and the present study (0%) despite targeting the same cryptic gene needs further investigation.

Potential risk factors that may be associated with HPV/STI infections in The Gambia were also investigated in this study. More than 80% of women indicated that their sexual partners did not use condoms and this finding was also observed in HIV-positive women. This may be since most of the participants were married. The interaction between HPV and HIV is enhanced by the same mode of sexual transmission; therefore, this study proposes that clinicians should place emphasis on HPV prevention through counselling and regular cervical cancer screening for all HIV-positive women, especially in women who do not use condoms. Although the effectiveness of condom uses in preventing HPV infection remains controversial, health care providers who counsel HIV-positive women, especially younger women, should emphasize the importance of preventing HPV infection and other STIs through consistent condom use with all male sexual partners. The present work has shown that older women in a polygamous relationship are at greater risk of being infected with HPV/STI, in The Gambia. Therefore, there is a need to strengthen HPV/STI awareness in this group as well as the younger population using the local media, cultural drama and during clinic health sensitisation.

The acceptability of self-sampling for HPV/STI screening was assessed in this study and only 18% of participants preferred self-sampling. This number is comparatively lower than other findings in other developing countries. Self-collected samples have been shown to have the potential to significantly increase HPV screening coverage (Ogilvie *et al.*, 2017). Therefore, self-sampling could be an option for The Gambia, for STI laboratory base screening to facilitate effective treatment of patients. In future, self-sampling could be used for HPV DNA testing, when a national cervical cancer screening programme is in place to increase screening coverage.

7.1 Study limitations and future work

7.1.1 Study limitations.

The present study has some important limitations, first, the sample size was small (235) as the initial sample size calculated was 346. Though plans were made to recruit the actual

sample size, this was not possible due to the 2016 political impasse in the country. Since the study was time limited the desirable sample size could not be achieved. Furthermore, since reproductive age women (20 - 49 years) were targeted, this study was unable to determine whether HPV prevalence in the urban population would have continued to increase, remained stable or decrease. Secondly, the differences between the HPV results of the present study and previous rural study may be explained as, this research studied an urban population of women attending a primary health care for either STI/HIV management or contraceptive services, whilst the other study was a community survey in which participants 15 -54 years old were visited and invited to participate. Secondly, the information on sexual behaviour was self-reported and could be subject to recall bias.

Due to the sensitive nature of the topic on sexual behaviour, social desirability bias cannot be excluded. Although confidentiality and anonymity were maintained throughout the study, women may have had the tendency to give more “desirable” answers such as a lower number of lifetime sexual partners or older age at sexual debut.

Another limitation of the study was observed in the cohort follow-up during participant recruitment. Some of the participants gave an incorrect or changed their telephone numbers after recruitment, which led to participants lost to follow-up in each scheduled visit. Though follow-up studies to establish an epidemiology pattern of an infection or disease in Africa may be important, these are difficult to carry out in an African setting.

7.1.2 Future work

Characterising oncogenic HPV genotypes in cervical cancer cases before the introduction of any vaccine type is necessary for an effective cervical cancer prevention strategy. Therefore, future studies to investigate epidemiological correlates of oncogenic HPV genotypes in cervical cancer specimens will be useful in providing evidence for policies and future evaluation of the quadrivalent vaccine, in The Gambia. The use of HPV DNA testing and cytology in high-risk groups will be advocated for monitoring replacement of HPV vaccine types with other HPV genotypes that may possibly cause cervical cancer in The Gambia.

The 46% prevalence rate of *Ureaplasma* species reported in this study should be investigated further targeting women who reported gynaecological complications such as infertility, spontaneous abortions and preterm pre-labour rupture of membranes (pPROM) to ascertain its clinical importance in The Gambia. Furthermore, future work will be carried out on the characterisation of *Chlamydia trachomatis* in the collected samples targeting a different gene.

7.2 Summary of findings

In summary, HPV 52 was the most common circulating HPV genotype found in this study. HPV 51 genotype was found in a patient who died of cervical cancer. Prolong use of hormone contraceptives (> 5 years) was the only risk variable found to be statistically associated with HPV infection. Gambian women between the ages of 26 - 35 years were found to be mostly infected with STIs. Marital status and partners not using condoms during sexual intercourse were the only variables found to be significantly associated with STIs. Differences between the three Gambian major ethnic groups were noted in this study. The Wolof were the tribe least to be infected with HPV/STI and to practise FGM, whilst the Fula and the Mandinka ethnic groups tend to be infected more with HPV/ STI and FGM was a common practice. Multiple HPV neutralizing antibodies were found in women infected with HIV and seropositivity was intermittent. Sero-conversion was observed during the 9 and 24 months follow-up period. However, detectable HPV antibodies types did not strictly correlate with the HPV DNA genotypes results.

8 References

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9 Appendices

Appendix A: Preparation of reagents and solutions

Preparation of 50X Tris base, acetic acid and EDTA (TAE) buffer

A 1.1 Ethylene diamine tetra acetic acid (EDTA)

Compound	Amount
EDTA	73g
Deionised water	350 ml

A magnetic stirrer was used to mix the mixture and 10M NaCl was added to adjust the pH to 8.0. The volume was made up to 500 ml with deionised water.

Table A 1. 2 50X TAE buffer (1 Litre of solution in deionised water)

Compound	Amount
Tris base (Mw -121 g/mol)	242g
Glacial acetic acid	57.1 ml
0.5 M EDTA	100 ml

Note: 0.5 M EDTA was prepared at pH 8.0. The volume was made up to 500 ml with deionised water.

To prepare a 1x TAE working buffer, the 50X TAE buffer was diluted 50X in deionised water before use.

Table A1.3 10X phosphate buffered saline (PBS) in 1 Litre of solution

Compound	Amount (g)
NaCl	58.44
KCl	74.55
Na ₂ HPO ₄	141.96
KH ₂ PO ₄	136.09

Note: Add deionised water to bring to 1 Litre and autoclave to sterilise.

To prepare a 1x PBS working buffer, the 10X PBS was diluted 10X in deionised water before use.

Table A 1.4 6X gel electrophoresis loading dye

Compound	Amount
Bromophenol blue	2.5g
Glycerol	3ml

Deionised water was added to bring the volume to 10 ml. The loading dye was diluted to 1X by mixing in a ratio of 1 part of loading dye to 5 parts of DNA sample before samples were loaded into agarose gels.

Table A1.5 Preparation of Ethidium bromide (10 mg/ml) in solution

Compound	Amount
Ethidium bromide	1g
Deionised water	500 ml

The mixture was dissolved with care and deionised water was added to bring the volume to 1 litre (1000 ml).

General precaution:

Wear gloves and lab coat always when handling ethidium bromide or ethidium bromide contaminated solutions, glassware, pipette tips, etc.

Appendix B: Preparation of media and antibiotics

B 1.1 Preparation of Luria-Bertani (LB) agar and broth.

LB agar and LB broth were purchased from Fisher Scientific, Loughborough in a powdered form. LB agar and LB broth were prepared following the manufacturer's instructions.

Table B 1.1 Luria-Bertani (LB) agar in 1litre solution

Compound	Amount
LB agar base	40g
Deionised water	800 ml

The LB agar was dissolved, and the volume was made up to 1litre (1000 ml) and autoclave to sterilise

Table B 1.2 Luria–Bertani (LB) broth in 1 litre solution

Compound	Amount
LB broth	25g
Deionised water	800 ml

The LB broth powder was dissolved, and the volume was made up to 1 litre (1000 ml) and autoclave to sterilise

B 1.3 Ampicillin

Ampicillin was obtained from (Sigma, Gillingham, UK) and a stock of 100 mg /ml was prepared by dissolving

Table 1.3 Ampicillin preparation (100 mg/ml)

Compound	Amount
Ampicillin powder	1g
Deionised water	10 ml

The solution was mixed to dissolve and filter sterilised with a 0.2 µm syringe filters (Millipore, Hertfordshire, UK). The solution was stored at -20°C in 500 µl aliquots.

B 1.4 100X Penicillin-Streptomycin solution

100X penicillin-Streptomycin was purchased from (Sigma, Gillingham, UK) and stored at -20°C in 1 ml aliquots

Appendix C: Preparation of embryonic kidney 293TT cells, cell counting, pseudo-typed virus titration and neutralisation assays.

C1: Calculation of human embryonic kidney 293TT cells for transfection

C1.1 Cell counting

Under sterile conditions, human embryonic kidney cells (293TT) that reached 50% confluent growth were trypsinised and 10 µl of cells was added to 10 µl of trypan blue dye (1:1 ratio). 10 µl of the mixture was charged into a disposable counting chamber and counted using the automated countess II machine (Life Technologies, UK).

The concentration of viable cells was calculated by multiply the number of viable cells by the dilution factor.

The number of cells needed for transfection was calculated as follows:

To seed 7.5×10^6 293TT cells in a final volume of 20 ml, 7.5×10^6 was divided by the number of viable cells =X (ml) of cells, therefore in a 20 ml final volume, X (ml) was subtracted from 20 ml to give the amount of cDMEM medium needed to add to the cells.

C1. 2: Preparation of HPV plasmid DNA for Transfection:

To transfect 19 µg of HPV plasmids, 19 µg was divided by the virus concentration to give the amount needed for the transfection. Below is the concentration of each of the HPV plasmids with the required volume used for transfection:

Table C1.2.1 Concentration of HPV plasmid DNA and required volume for transfection

HPV genotype	DNA concentration	Volume
35	0.773 µg/µl	24.6 µl
51	0.708 µg/µl	26.8 µl
52	3.7 µg/µl	5.1µl
Reporter plasmid PGL4.51	1.9 µg/µl	10 µl

C1. 3: Calculation of 10,000 human embryonic kidney (293TT) cells in100µl media / well for infectivity (Titration) and neutralization assays

To seed 10,000 cells /well for 1 plate (60 wells) for 100 µl /well, the cells were counted as described in C1.1. The following calculation was employed to determine the number of cells required for the assay using the following formula:

$$C_1V_1 = C_2V_2$$

C_1 = cell count

$V_1 = X$

$C_2 = 1.0 \times 10^6$ (10,000 x 100)

$V_2 = 10$ ml (6 ml + extra 4 ml, for ease of calculation)

Therefore (X ml) cells = C_2V_2/C_1 . The X value was subtracted from the final volume to have the amount of medium needed to add to the cells for a 10 ml volume.

C_1 - initial concentration, V_1 - initial volume

C_2 - final concentration, V_2 - final volume

C1. 4: Preparation of Optiprep® step gradient solution; Optiprep 46% stock:

The 46% Optiprep® stock was prepared as follows:

5.2 ml of 5 M NaCl and 4 ml of Phosphate buffer saline $p^H7.2$ was added to 30.7 ml of 60% Optiprep® (Iodixanol solution) (w/v).

From the 46% Optiprep® stock, 27%, 33%, and 39% gradient solutions were prepared for a 10 ml volume as shown below:

Table C1.4.1 Preparation of Optiprep gradient solutions

Gradient (%)	46% Optiprep (ml)	0.8M NaCl/PBS (ml)
27	5.9	4.1
33	7.2	2.8
39	8.5	1.5

C1.5 Plating/seeding of 293TT cells

10,000 HEK 293TT cells in 100 µl of cDMEM per well was seeded in an inner wells of a 96-well tissue culture flat bottom sterile plate (Fisher Scientific, UK). The external wells (row A –H and column 1-12) were filled with 200 µl of sterile PBS to avoid evaporation

C1.5: Layout of 96-well U-bottom plate of human embryonic kidney 293TT cells for infectivity (titration) and neutralisation assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS											PBS
B		293TT									293TT	
C												
D												
E												
F												
G		293TT									293TT	
H	PBS											PBS

PBS – Phosphate buffer saline (200µl) pH7.2,

Inner plate wells were seeded with 10,000 293TTcells/well /100 µl / well.

C1.6 Template for infectivity (titration) plate to determine pseudo-typed virus Tissue Culture Infective Dose (TCID₅₀)

1	2	3	4	5	6	7	8	9	10	11	12
PBS											PBS
	293TT + PsV							293TT + PsV	293TT +	293TT PsV	
	293TT + PsV							293TT + PsV	293TT +PsV	293TT	
PBS											PBS

Key:

PsV = pseudo-typed virus

PBS = Phosphate buffered saline

Column 11 – human embryonic kidney cells (293TT) only

C6.1 Calculation of TCID₅₀

All pseudo-typed viruses were tested in triplicate and the TCID₅₀ for each virus was calculated using the Spearman-Karber equation.

TCID₅₀ was calculated using the Spearman-Karber equation as follows:

Spearman-Karber formula: $M = x_k + d [0.5 - (1/n) (r)]$

x_k = dose of the highest dilution.

r = sum of the number of “negative” responses.

d = spacing between dilutions.

n = wells per dilution.

To calculate TCID₅₀/ml of virus stock, the original dilution was corrected by multiplying by 5 (dilution factor)

Each virus was used at a concentration of 300TCID₅₀ per well in 100 µl for neutralisation assay.

C1.7 Neutralization assay

Pseudo-typed viruses were diluted in 10 ml of phenol red free (prf) DMEM) supplemented with 10% foetal calf serum and 1X penicillin-streptomycin to give a concentration of 300TCID₅₀ per well in 100 µl. 100 µl of pseudo-typed virus was dispensed in a 96 plate well

Serum samples were diluted in 1: 8 dilutions (10 µl of serum +70µl of prf DMEM. The mixture was thoroughly mixed and 25 µl was transferred to 100 µl of pseudo-typed virus (giving a final dilution of 1:40) and incubated for 1 hr at room temperature.

After 1 hr incubation, 100 µl of the mixture was transferred to the cells seeded a day before as described in C1.5 and incubated for 72 hrs at 37°C with a humidified atmosphere of 5% CO₂ in air. (Note: final volume = 200 µl). The plate lay out is shown on Table C1.7.1

The presence of neutralising antibodies for each sample was determined as 50% inhibition of the HPV pseudo-typed infectivity.

C 1.7.1 Layout of 96-well U-bottom plate for neutralisation assay

1 2 3 4 5 6 7 8 9 10 11 12

PBS											Cells only	PBS
	293TT+ samples							293TT+ samples	293TT+PsV	293TT		
	293TT+ samples							293TT+ samples	293TT+PsV	293TT		
PBS												PBS

Inner wells from 2-9 highlighted in pink all had 293TT cells with samples, row 10 had the virus and the 293TT cells, row 11 had only 293TT cells and row 12 had only phosphate buffered saline (PBS).

Appendix D: Sequence data of high and low risk HPV genotypes identified by DNA sequencing and nucleotide BLAST search (*L1* gene, 450 bp)

HR- High risk genotype; LR- Low risk genotype

HPV 16(HR)

ATaatnatCTTCtaGTGTGCCTCCTGgaggAGGTTGTAAACCAAATTCCAGTCCTCCAA
AATAGTGGAATTCATAGAATGTATGTATGTCATAACGTCTGCAGTTAAGGTGATT
TTGCACAGTTGAAAAATAAACTGTAAATCATATTCCTCCCCATGTCGTAGGTACT
CTTTAAAGTTAGTATTTTTATATGTAGGTTCTGAAGTAGATATGGCAGCACATAAT
GACATATTTGTACTGCGTGTAGTATCAACAACAGTAACAAATAGTTGGTTACCCC
AACAAATGCCATTATTgTGGCCCTGCGcaaaa

HPV 35 HR

GGGACCTCTAACCAGATACACCCTTTAATGACACGCCCCGGCTCTAACCGCAGA
TCCACAATAGACGGTCCCACGAGCTTCATTTAGAGATTCACGTCTATGATCACAC
TAAAAGACAGAAAAATAGGAGTAATTATCTGTTGTTTACGGTAGATGAAAGAAG
GCGTAGAATAAATATGTTTGTGTGTTATGTTGAGTATTGTAGTGTCCGTATATATA
AAAAAGATAATTGTAAGGAATATTTAAGGCATGGAGAAGAATATGATGTACAGT
TTATTTTTCAGTTATGTAAAAGAAGAGTAACAGAAGAGGATAAGACATATATTGA
TAGTAGGAAGGCGTGAGATTGGAGAGGATTGGAATTTTGGCCTTGACGCAGGGC
GTTGGGGGAGATAAGAGGAAAGATATGGTTGTGTGAGGGGGGTGTGGAGGGGG
ACAAAAAAGGGGAATTTTCTATAGGGGGGTGTGCAGAGGGAACAGAGGGGGG
GATGTTTTTCTGGGTTTGTGCGCCGGCGCCGGC

HPV 51 HR

AGTACAAATTTAACTATTAGTACTGCCACTGnnnnmntTTCCCCAACATTTACTCCAA
GTAACTTTAAGCAATATATTAGGCATGGGGAAGAGTATGAATTGCAATTTATTTT
TCAGTTATGTAAAATTACTTTAACTACAGAGGTAATGGCTTATTTACACACAATG
GACCCTACCATTCTTGAACAGTGGAATTTTGGATTAACATTACCTCCGTCTGCTAG
TTTGGAGGATGCATATAgGTTTgTtCgAAATGCAGCCACCAgCTGTCAAAGGACAC
CCCTCCACAGGCTAAGCCAGATCCTTTGGCCAAATATAaattTtGGGATGtTGATTTA
AAGGAACGGTTTTTCgtTAgATTTAGACcAatTTGCATTGgGtCGCA

HPV 51 HR

CGgaggtAatGttaatCcAAAATTccactgTTCAagAaTGGTtaggATCCAttgngtgtAAAtangCcaTtA
CCtctgtagTtAAagtaaTTTTGCATAactgAAAAaTAAATTgCAATTCatactcTtCCcAtgcctAA
TATATTgCTtAAagTtacttgaggtAAAtGTTGGGGAAACCGCAGCagtggcagTGCTAATagTt

AAATTTGTA CTtGgTAGTATCAACACAGGTAATAAAAAGCTGATTGTTCCAGCA
AATGCCATTATTGTGGCCcTGCGCAgtGCa

HPV 52 HR

AcntcccaanTATAGTCCTTtaaGGATCTTCCTTTTCCTTTaGGTGGTGTGTTTTTTTGACA
TGTtATAGCAGTAGAAGTGACAAATCTGTATGTGTCCTCCAAAGATGCAGACGGT
GGTGGGgTAAGGCCAAATTGCCAGTCCTCTAAAATAGTGGCATCCATCTTATGAA
TGTATGTCATAACATCAGCTGTTAATGTAATTTTGCACAATTGAAAAATAAATTG
TAAATCAAATTCCTCGCCATGACGAAGGTATTCCTTAAAATTTTCATTtttatATGTG
CTTTCCTTTTTAACCTCAGCACATAAAGTCATGTTAGTGCTACGAGTGGTATCCAC
AACTGTGACAAACA ACTGATTGCCCAACATATGCCATTATTGTGGCCCTGCGc

HPV 52 HR

atccaCcTCCcAanCnTATAGTCCTTtaaGGATCTTCCTTTTCCTTTaggTGGTGTgTTTTTTT
GACAAGTTATAGCAGTAGAAGTGACAAATCTGTATGTGTCCTCCAAAGATGCAG
ACGGTGGTGGGGTAAGGCCAAATTGCCAGTCCTctAAAATAGTGGCATCCATCTTA
TGAATATATGTCATAACATCAGCTGTTAATGTAATTTTGCACAATTGAAAAATAA
ATTGTAAATCAAATTCCTCGCCATGACGAAGGTATTCCTTAAAATTTTCATTTTTA
TATGTGCTTTCCTTTTTAACCTCAGCACATAAAGTCATGTTAGTGCTACGAGTGGT
ATCCACA ACTGTGACAAACA ACTGATTGCCCAACATATGCCATTATTGTggCCcT
GCGc

HPV 52 HR

cngTTTTTTTGACAAGTTATAGCAGTAGAAGTGACAAATCTGTATGTGTCCTCCAA
AGATGCAGACGGTGGTGGGGTAAGGCCAAATTGCCAGTCCTCTAAAATAGTGGC
ATCCATCTTATGAATATATGTCATAACATCAGCTGTTAATGTAATTTTGCACAATT
GAAAAATAAATTGTAAATCAAATTCCTCGCCATGACGAAGGTATTCCTTAAAATT
TTCATTTTTATATGTGCTTTCCTTTTTAACCTCAGCACATAAAGTCATGTTAGTGCT
ACGAGTGGTATCCACA ACTGTGACAAACA ACTGATTGCCCAACATATGCCATTA
TTGTGGCCCTGCGcAa

HPV 52 HR

TTTtnGAcAAGttATAGCagtagaagtGACAAAtctGTATGTGTCCTCCAAAGATGCagACG
GtggTGGGgtAAGGCCAAATTGCCAGTCCTCTAAAATagtGGCATCCATCttATGAATG
TATGTCATAACATCAGcTGTTAATGTAATTTTGCACAATTGAAAAATAAATTGTAA
ATCAAATTcctCGCCATGACGAAGgTATTCCTTAAAATTTTCATTTTTATATGTGCTT
TCCTTTTTAACCTCAGCACATAAAGTCATGTTAGTGCTACGAGTGGTATCCACAA
CTgtGACAAACA ACTGATTGCCCAACATATGCCATTATTGTGGCCCTGCGc

HPV 52 HR

gtCcngTTGTGGaTnACcaCTCGTagcACtaaCATGACTTTATGTGCTGAGGttAAAAaGGA
AAGCACATATAAAAATGAAAATTTTAAGGAATACCTTCGTCATGGCGAGGAATTT
GATTTACAATTTATTTTTCAATTGTGCAAATTACATTAACAGCTGATGTTATGAC
ATATATTCATAAGATGGATGCCACTATTTTAGAGGACTGGCAATTTGGCCTTACC
CCACCACCGTCTGCATCTTTGGAGGACACATACAGATTTGTCACTTCTACTGCTAT
AACTTGTCAAAAAAACACACCACCTAAAGGAAAGGAAGATCCTTTAAAGGACTA
TATGTTTTGGGAGGTGGATTTAAAAGAAAAGTTTTCTGCAGATTTAGATCAGTTTc
cTTTAGGTTCGa

HPV 56 HR

tgtagtaganncTACTAGAAGTAcTAACATGACTATTAGTACTGCTACAGAACaGTTAAGt
AAATATGATGCACGAAAAATTAATCAGTACCTTAGACATGTGGAGGAATATGAA
TTACAATTTGTTTTTCAATTATGCAAATTACTTTGTCTGCAGAGGTTATGGCATA
TTTACATAATATGAATGCTAACCTACTGGAGGACTGGAATATTGGGTTATCCCCG
CCAGTGGCCACCAGCCTAGAAGATAAATATAGATATGTTAGAAGCACAGCTATA
ACATGTCAACGGGAACAGCCACCAACAGAAAAACAGGACCCATTAGCTAAATAT
AAATTTTGGGATGTTAACTTACAGGACAGTTTTTCTACAGACCTGGATCAATTTCC
ACTAGGTcg

HPV 58 HR

aTaccaCTCgtagcACtAaTATGACAttATGCACTGAAgtaactAAAGAagATACatataAAAAAT
aatAaTTtAAGGAATATgtAcGtCatgTtGAAGAATATGACTtaCagTTtGTTTTTCAGCTTT
GCAAATTACTAAActgCAGAGgtAATGACATATATACATACTATGAATTCAGATA
TTTTGGAGGaCTGgcAATTTGGTTTAAACACCTCcTCCgtCTGCCaGTTTACAGGACAC
ATATAGATTTGTTACCTCCCAGGCTATTACTTGCCAAAAAACAGCACCCCCTAAA
GAAAAGGAAGATCCATTAATAAATACTTTTTGGGAGGTTAACTTAAAGGAA
AAGTTTTCTGCAGATCTGGATCAGttccTTtnGGGACg

HPV 58 HR

gtanttaCTCCAAagTATATTtATTtAaTGGATCTTCCTTTTCTTTaGGGGGTGCtgTTTTTtG
GCAAgTAAATAGCCTGGGaggTAACAAATCTATATGTGTCTgtAAACTGGCAGACGG
AGGaGGTGTtAAACCAAATTGCCAGTCCTCCAAAATATCTGAATTCATAGTATGTA
TATATGTCATTACCTCTGCAGTTAGTGTAATTTTGCAAAGCTGAAAAACAACTG
TAAGTCATATTCTTCAACATGACGTACATATTCCTTAAAATTATCATTTTTATATG
TACCTTCCTTATTTACTTCAGTGCATAATGTCATATTAGTGCTACGAGTGGTATCA
ACCACGGTAACAAATAATTGATTGCCCCAGCAAATGCCATTGTTATgTCCCTGTGc

HPV 66HR

CtnCCcAaacTtataTTTAGccaggnatCCTGCTTTTCTGCaGGGGgcngetnCCCTctGacaTgtaat
aGCtgtgCTTtaataTACcTataTTTATCCtctAAGctaGTtGCAActggtGGGgATAAGCCAATA
TTCCAAtCGtCTAATAAAGtATTattCATATTATGCAAATATGCCatAaCTTCTGCAGTT
AAGGTTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGC
GAAGGTATTGATTGATTTACGGGCATCATATTTAGTTAATGTGCTTTTAGCTGCA
TTAATAGTCATGTTGGTGCTTCTGGTAGTATCCACAACAGtAACAAATACCTGATT
ACCCAGCATATGCCATTATTATgtCCCTGTGcnca

HPV 66 HR

aancTtATATTTaGccaGgggatCCTGCTTTTCTGCAGGGGGctgnCCentCTGACATgtaaTAG
CTGTGCTTttAaTATACCTATaTttaTCCTCtAAGcTaGTTGCAACTggtGGGGATAATCC
AatATTCCAATCGtCTAATAAAGTATTATTCATATTATGCAAATATGCCATAACTTC
TGCAGTtAaGGTTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCA
CATGGCGAAGGTATTGATTGATTTACGGGCATCATATTTAGTTAATGTGCTTTTA
GCTGCATTAATAGTCATGTTGGTGCTTCTGGTAGTATCCACAACAGTAACAAATA
CCTGATTACCCAGCATATGCCATtAtnaTGTCCCTGTGCa

HPV 53 pHR

cAAAnTtnaaTttaGATAGTGGGTCcnnCTTTTcaGGaGGggActgcaTCcTTTTGACAGGTTA
TAGCTGCACTTTTTACATATCTGTATTTGTCCTCTAAGCTAGTGGCAACAGGAGGC
GACAAACCTATATTCCAGTCTTCCAGTAAGGTAGAATTCATAGTATGTAAATAGG
CCATAACCTCAGCAGACAGGGATATTTTACATAGTTGAAACACAAATTGTAATTC
ATATTCCTCTGCATGCCTAACATACTGTTTAATTTGCTTTGAATTATATGTGGACA
TagACTGTGTGGTTGCAGAAAGAGTCATGTTTGTATTCCCTGGTGGTATCCACAACA
GTTACAAATAACTGATTGTTCCAACAGATGCCATTATTATGTCCCTGTGCA

HPV 73 pHR

gtttGATTTACaGtTTGTTTTTCAGTTATGTAAAATTAGTTTAACTACTGAGGTAATGA
CATATATACATTCTATGAATTCTACTATATTGGAAGAGTGGAATTTTGGTCTTACC
CCACCACCGTCAGGTACTTTAGAGGAAACATATAGATATGTAACATCACAGGCTA
TTAGTTGCCAACGTCCTCAACCTCCTAAAGAAACAGATGACCCATATGCCAAGCT
ATCCTTTTGGGATGTAGATCTTAaagaAaAGTTTTCTGCAGaAATTAGACCAgTTTCCC
TTGgGTCg

HPV 6 LR

ccTTTtCAggAntGggCTTTTGACaGgtaatGgccTGTGACTGcACATACCTATAGGTATCTT
CTAATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGA
GGGATTCATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACAT
AATTGAAAAATAAATTGTAAATCATACTCTTCCACATGACGCATGTAATGCTTTAT
AATCAGAATTGGTGTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTT
GGTACTGCGTGTGGTATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATA
CCATTGTTATGTCCCTGTGcaaa

HPV 6 LR

ACCTCcccAAaaaCtaaGgTTCTTATAGGGATCTGGCTTTTCCTTTTcaGGAGTGGGCTT
TTGACAGGTAATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCA
TTTGGGGGAGGCGATAACCCAAAGTTCCAGtCTTCCAAAACAGAGGGATTCATTgT
GTGAATATAGGCCATTACTTCAGCAGACAATGTAATGcTACATAATTGAAAAATA
AATTGTAAATCATACTCTTCCACATGACGCATGTAATGCTTTATAATCAGAATTGGT
GTATGTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTgTg
GTATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATgtCC
CTGTGCatGc

HPV 42 LR

AtntacataCCTATAACTATcTtCTAAAGTTcctGAAGGTGgTGGTGC AACACCAACATTC
CaCTCCTcTaatATGttaGGAttCATATTGtgtATATATGACATTACTTCAACagtnAatgTtAT
CTTACACAATTGaaaTATAAATTgcACATCataTTCTTCAGCAtgtcTTAAATATTCCTTA
AAATTATCAGCTGTatagTATCACCAGATGTTgCAgtgncACACAAAGTCatgTtAGTAC
TacGgataCTATCnnnncanttAAAAaTAGctgaTTtCccaaca

HPV 54 LR

ATTTTTTTGTTGCCCTCCACACCCCCCACTATACAAACCTATTTTTTTCTTCCCTA
CTACTTCTCCTCGGGGGTTATACCATATTTCTTTCTCTACAATAGTGCCATTC
ATTCCATGAATATATGCCTTATCATCTGCTGTAAGGGTTATGGTACATAAATC

HPV 54 LR

tnagntCacagTCCAAAaGTAAaTTtaCTGTAAGGATCCTCCTTTTCCTTTGCAGGGGcann
nTtCTTTTGACATGCAATGGCCtgtACTGTACAAACCTATATGTGTCTCCAAACTA
CTTgtAGCTGGGGGGTTATACCAAAGTTCCAGTCCTCTAGAATAGTGGGATTCAT
TCCATGAATATAGGCCATAACATCTGCTGTAAGGGTTATGGTACATAAATGAAAT
ATAAACTGTAAATCATATTCCTCCACATGTCTAATACTCCCTAAAGTCAGAATT
ATTAAAGCTATCCTGCGTGGATGCTGTAGCACACAATGTTAGGTTAGTACTACGG

GTGGTATCTACAACCTGTTAAAAACAATTGATTGCCCAACAAATACCATTGTTGT
GGCCCTGGGc

HPV 61 LR

tacaccTctggactgCAAAAACCTAtatgtgtCTTctaGACTGGTAGAGGGTGGAGGTACCACA
CCAAAGTTCCAGTCATCCAACAAGGCTTTATTCATATTATGTAGGTAGGCCATAA
TTTCAGGGGTAAATGTATTTTACATAACTGAAAAATGAATTGCAAATCAAATC
TTCTGTATGGCGCAAATATCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGG
GGGGATGTAGCAGtACAaatGGTTACATtagtacTGcgnnTGGTatggacAacGGTtacAaacant

HPV 61 LR

tacaccTctggactgCAAAAACCTAtatgtgtCTTctaGACTGGTAGAGGGTGGAGGTACCACA
CCAAAGTTCCAGTCATCCAACAAGGCTTTATTCATATTATGTAGGTAGGCCATAA
TTTCAGGGGTAAATGTATTTTACATAACTGAAAAATGAATTGCAAATCAAATC
TTCTGTATGGCGCAAATATCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGG
GGGGATGTAGCAGtACAaatGGTTACATtagtacTGcgnnTGGTatggacAacGGTtacAaacant

HPV 61 LR

tGnActGcanAAACCTATaTGTGTCTTcTaaaCtgntanAGGGTGGAGGTACCACACCAAAG
TTCCAGTCATCCaACAAGGCCTTATTCATATTATGTAGGTAGGCCATAATTCAGG
GGTTAAATGTATTTTACATAACTGAAAAATAAATTGCAAATCAAATCCTTCTGTA
TGGcGCAAATATCCCTAAAGCTTgtGGCTTTATATTCAGATACAGGGGGGGATGC
AGCAGTACAAATGgnTaCATTAgTACTGCGGGTGGTATCCACAACGGTTACAAacaAt
TCATTAACCAACAAATACCATTGTTGTGgcCcTGg

HPV 62 LR

GttctgtggTGgnTncTACTagAaGTAATAATTTTACTATTTGTACCGCTCCacTGCTGCA
GCAGAATACAAGGCTACCAACTTTaGGGAATTTTGGCGACACACGGAAGAATtG
ATTTGCAATTTATATTTCAATTGTGCAAAATACAGTTAACCCCCGAAATCATGGC
CTACCTGCATAATATGAACAAGGACCTTTtGGATGACTGGAACCTTTGGGGTTTTAC
CTCCCCCTTCCACTAGTTTAGATGAGACATATCGCTATTTGCAGTCTCGGGCTATT
ACATGTCAAAGGGGGCTGCTTCCCCgtCCCCAAGGTGGACCCGTATGCGCAA
TGACATTTTGGACTGTGGATCTTAAGGACAAGTTGTCTACTGATTTGGACCAGTTT
CccTTGGgtc

HPV 83 LR

GAtccTtatnaGGGgCaGGGgCGGAagnCCcTTTTGgcaggtAatagCACGGgactGCagaTAGCG
ATaGgTATCATCAAGGctGGtGgAaGgAGGtnntAACACGCCAAAATTCACCTCATCCA
ATAAATGTTCAATTCATACTATGTAGGTATGCCATAATTTTCAGGGGTAAAGATGTAT

TTTGCAAAGTTGCAATATAACCTGTAAGTCATATTCCTCGGTGTGGCGGaGGTATT
CCTTAAAGTTAGAGGCTGTGTATTCATTAGCCTGTGTAGCAGCAGCTGAAATAGT
AATATTGGTACTGCGGGTAGTATCCACAACCTGTAACAAATAACTCATTAACCAA
CAAATGCCATtAttaTGTCCCTGTGc

HPV 89 (Cp6108) LR

gTTCTAcacGCTTTAaggAaTATTTAAgACACACtgaGgAaTATGACCTACAGTTTATAT
TCCAACCTATGTAAGATACACCTAACGCCTGAGATAATGTCCTATTTACACAATAT
GAATGACACATTGTTAGATGAATGGAACCTTTGGTGTCATTCCCCCTCCCTCCACTA
GTTTGGATGATACCTATCGCTTTCTTACCTCTCGGGCCATTACATGTCAAAGGGC
ACTGCTGCCCCAGAACCTAAAAGGATCCATATGATAAGTTATCCTTTTGGGATG
TGGATCTTAAGGAACGTTTGTCCACTGATCTCGACCAGTTTCCCTTGggTCGa

Appendix E:

E1 Ethics approval from The Gambia government/MRC Joint Ethics Committee

C/o MRC Unit: The Gambia, Fajara
P.O. Box 273, Banjul
The Gambia, West Africa
Fax: +220 – 4495919 or 4496513
Tel: +220 – 4495442-6 Ext. 2308
Email: ethics@mrc.gm

The Gambia Government/MRC Joint
ETHICS COMMITTEE

03 December 2015

Mrs Haddy Bah Camara
Faculty of Science and Technology
Department of Biomedical Sciences
University of Westminster, London, United Kingdom

Dear Mrs Bah Camara

R015 002v3, Human papilloma virus co-infection with sexually transmitted pathogens amongst women of reproductive age in urban Gambia

Thank you for submitting your response addressing the issues raised by the Gambia Government/MRC Joint Ethics Committee at its meeting held on 31 July 2015.

Your proposed study has now received full Ethics Committee approval and may proceed.

With best wishes

Yours sincerely



Mr Malamin Sonko
Chairman, Gambia Government/MRC Joint Ethics Committee

Documents submitted for review:-

- RePublic approval letter – 28 April 2015
- RePublic reply letter – 15 March 2015
- Revised proposal
- Material and Data Transfer Agreement

The Gambia Government/MRC Joint Ethics Committee:

<i>Mr Malamin Sonko, Chairman</i>	<i>Prof. Umberto D'Alessandro</i>
<i>Professor Ousman Nyan, Scientific Advisor</i>	<i>Dr Momodou L Waggeh</i>
<i>Ms Naffie Jobe, Secretary</i>	<i>Dr Kalfia Bojang</i>
<i>Dr Roddie Cole</i>	<i>Dr Ramatoulie Njie</i>
<i>Dr Ahmadou Lamin Samateh</i>	<i>Dr Jane Achan</i>
<i>Mrs Tulai Jawara Ceasay</i>	<i>Dr Siga Fatima Jagne</i>

**UNIVERSITY OF
FORWARD
THINKING
WESTMINSTER**

23 February 2016

Dear Haddy

Ethics application: VRE1415-0262

Project title: Human papilloma virus co-infection with Sexually transmitted pathogens amongst women of reproductive age in urban Gambia

Applicant: Mrs Haddy Bah Camara

Supervisor: Dr Patrick Kimmitt

Thank you for providing your response to the Conditions set by the Committee.

The Committee considered the response to Conditions and found this satisfactory, including, noting the external approval from the Gambia Government /Medical Research Council Joint Ethics Committee. The proposal was approved.

If your protocol changes significantly in the meantime, please contact me immediately, in case of further ethical requirements.

Yours sincerely

Huzma Kelly

University Research Ethics Committee

I am advised by the Committee to remind you of the following points:

1. Your responsibility to notify the Research Ethics Committee immediately of any information received by you, or of which you become aware, which would cast doubt upon, or alter, any information contained in the original application, or a later amendment, submitted to the Research Ethics Committee and/or which would raise questions about the safety and/or continued conduct of the research.
2. The need to comply with the Data Protection Act 1998.
3. The need to comply, throughout the conduct of the study, with good research practice standards.
4. The need to refer proposed amendments to the protocol to the Research Ethics Committee for further review and to obtain Research Ethics Committee approval thereto prior to implementation (except only in cases of emergency when the welfare of the subject is paramount).
5. You are authorised to present this University of Westminster Ethics Committee letter of approval to outside bodies, e.g. NHS Research Ethics Committees, in support of any application for further research clearance.
6. The requirement to furnish the Research Ethics Committee with details of the conclusion and outcome of the project, and to inform the Research Ethics Committee should the research be discontinued. The Committee would prefer a concise summary of the conclusion and outcome of the project, which would fit no more than one side of A4 paper, please.
7. The desirability of including full details of the consent form in an appendix to your research, and of addressing specifically ethical issues in your methodological discussion.

E 3 Permission letter for sample processing in EFSTH Laboratories

EFSTH
Edward Francis Small
Teaching Hospital



10th December 2014

REF NO: HMB/PF/ 377/(78)

Mrs. Haddy Bah Camara
Chief Scientist/Microbiologist
Edward Francis Small Teaching Hospital
Banjul

Dear Madam,

Reference to your letter dated 10th November 2014. I have the pleasure in granting you permission for you to carry out sample processing, Isolation of Organisms, Microcopy and DNA extraction for the purpose of your study.

The hospital is looking forward in collaborating with your University in other areas of interest.

I wish you well in your endeavours.

Yours faithfully,



.....
Dr Mohammed Ammar al-jaafari
CHIEF MEDICAL DIRECTOR

Cc File
R/file

EFSTH
Edward Francis Small
Teaching Hospital
DEPARTMENT OF OBSTETRICS & GYNAECOLOGY



20th January 2015

Mrs. Haddy Bah Camara
Chief Scientist / Microbiologist
Edward Francis Small Teaching Hospital
Banjul

Dear Mrs. Bah Camara,

I have the pleasure to confirm that women from your study that have abnormal cervical cytology from screening will be offered further management and treatment in our gynaecology clinic for free in line with government policy and the practice in our department. Do not hesitate to contact me if you need further clarification on this.

My colleagues and I at the department are looking forward to collaborating with you on this study.

Yours Sincerely,



Dr. Patrick Idoko MB.BS, FWACS
Consultant Obstetrician & Gynaecologist
Head of Department
patidoko@utg.edu.gm; +2203866852

E 5: PARTICIPANT INFORMATION SHEET

Project Title: Human papillomavirus co-infection with Sexually Transmitted Infection Pathogens amongst Reproductive age Women in Urban Gambia

You are kindly invited to take part in a research project, designed by Haddy Bah Camara of Edward Francis Small Teaching Hospital (EFSTH), Banjul, The Gambia in collaboration with the University of Westminster (UoW), London, UK. Mrs. Haddy Bah Camara and the study team will provide you with all the information concerning the project and your participation. Do not hesitate to contact the study team if there is anything you do not understand. You can confirm your participation by signing or thumb-printing the consent form below.

What is the purpose of the study?

The purpose of this study is to determine whether women infected with sexually transmitted bacteria will also be infected with human papillomavirus, which can cause cervical cancer. The study involves collaboration with Edward Francis Small Teaching Hospital, Gambia and University of Westminster, UK

The results from the study will help in making health policies on HPV and STI management in Gambia; provide education to the community and serve as foundation for future researches on HPV in Gambia.

What will you be asked to do?

Participation in this study is voluntary. Swab or blood specimens will be collected from you as part of your routine clinic appointment. A sterile cotton swab will be introduced into your cervix (womb) to collect the sample or a sterile syringe and needle will be introduced into your vein to collect a venous blood sample (where applicable). This will be done by an experienced staff of the clinic in the safest way possible. You have every right to withdraw from the exercise if you are uncomfortable or unwell.

Risks and Discomfort

It is very unlikely that there will be any side effects for taking part in the study. You may experience slight discomfort when swab / blood sample are being taken. However, this will be done in the safest way possible.

Why have I been asked to participate?

You have been asked to participate because of your present condition and history of infection /Non existing infection (Family Planning client)

Do I have to take part?

Participation is voluntary. However, if you decide to take part, a copy of this information sheet will be given to you to keep. You are free to withdraw at any time without giving reasons and can request the removal of your sample from the study. Moreover, your decision to withdraw will not affect the health care you receive.

What do I have to do?

To be recruited for the study, you are kindly requested to answer some few questions below. You can then confirm your participation by signing/ thumb printing the consent form below.

Confidentiality

This is a student research project which may be published. During the project and in the event of subsequent publication, your participation and any other personal details will be kept highly confidential. Your sample will be given a specific research number and anonymized. Access to identifiable data will be held in The Gambia only by your respective health provider, who has access to your information. Dr Patrick Kimmitt, Dr Edward Wright and Haddy Bah Camara will only handle anonymized samples with no bearing to your identity.

Expenses and Payments

Participation is entirely voluntary and as such there will be no payment for your participation in the study.

Contact for further information

If you have any problem or query about any aspect of the study at any time, please do not hesitate to contact the researcher or the Hospital Public Relation Officer (PRO) on the contacts given below:

EFSTH PRO

Momodou Lamin Jammeh

PhD student:

Haddy Bah Camara

A copy of this consent document has been provided to the participant.

E 7: QUESTIONNAIRE

Please allow me 20 minutes of your time to answer the following questions. Your genuine answers will help the health care provider to make corrective decisions in providing quality health care. Your answers will be kept confidential and known only to the health care provider. Your identity will not be distributed, published or sold.

1. Health facility

Name..... Type.....

2 Participant Identifier number

3 What is your date of birth? : Age in Years (.....)

4. Region.....District.....

5. What is the purpose of your visit today?

Family Planning visit (go to Ques. 7)

STI visit, (go to Ques. 6)

HIV management visit (go to Ques. 7)

6.Do you have any of the following symptoms today?

Vaginal itching\ discharge with fishy or strong odour (Tick)

Pain or burning when urinating

bleeding between periods

Pain during sex

Sores, skin rashes with rough red or reddish brown spots on hands or feet

Genital warts

Lower abdominal pain

Others (specify)

- If YES to any of the questions, find out how long she has/had the symptoms-----

SECTION B: Socio economic background

7. What is your ethnicity?

Wollof

Mandika

Jola

Serere

Sarahule

Fula

Others (Specify)

8. What is the highest level of education you have completed?

- None
- Primary level
- Secondary level
- College
- University
- Quaranic studies

9. What is your current occupation?

- Student
- Petty trader
- Business woman
- Civil servant
- House wife
- Farmer
- Others (specify)

10. How many people are currently living in your household, including yourself? ____

a. Of these people, how many are children ≤ 18 years old? ____

11. Which of these categories best describes your total combined family income for your household for the past 12 months?

- <D10, 000
- D 10,000 - 35,000
- D35, 000 - 50,000
- D51, 000 – 65,000
- D66, 000 – 75,000
- D 76,000 – 85,000
- D85, 000 - 100,000
- > D100, 000
- Don't Know / Not sure

12. Have you ever smoked cigarettes?

- Yes (How many a day?) Specify_
- No
- Quit

13. Have you ever been screened for cervical cancer?

- Yes
- No
- Don't Know
- Not sure

14. Have any of your family members been diagnosed with cervical cancer?

- Yes
- No
- Don't Know

15. This visit, swab samples will be collected from you by a health care provided, but would you have preferred to collect the samples yourself?

- Yes. (if yes, why?)
 - Embarrassment
 - Others (Specify)
 - Don't mind either way
- No

SECTION C: Reproductive and Hormonal factors

B. Menstrual periods

16. At what age did your menstrual periods begin?

- 10 years
- 11 years
- 12 years
- 13 years
- 14 years
- 15 years
- 16 years
- 17 years or older

17. Have you had a menstrual period within the last 12 months?

- Yes, I still have a menstrual cycle (go to question18)
- Yes, but my menstrual cycle stopped within the last year
- No, my menstrual cycle stopped more than one year ago
- Don't know

18. When was your last menstrual period?

|_|_| / |_|_|_|_|_| OR |_|_|

Month YEAR AGE

Don't know

19. Which of these best describes why your menstrual cycle stopped?

Breastfeeding

Birth control or medications

Natural menopause

Surgery to remove the uterus or ovaries

Other (specify) _____

Don't know

Pregnancy

20. Have you ever been pregnant?

No (go to question 24)

Yes

Don't know (go to question 24)

21. How old were you when you first became pregnant?

Less than 15 years

15-19 years

20-24 years

25-29 years

30-34 years

35-39 years

40-44 years

45 years or older

Don't know

22. How many times have you been pregnant? Please include stillbirths, miscarriages, abortions, tubal or ectopic pregnancies, and live births.

1

2

3-4

5-9

>10

SECTION D. Contraceptive use

23. Did you ever take birth control pills for birth control or to regulate menstrual periods?

No, never (go to question 28)

Yes

Yes, but stopped taking them now (go to question 25 &26)

24. How old were you when you first started taking birth control pills?

less than 15 years

15-19 years

20-29 years

30-39 years

40-49 years

25. How old were you when you last took birth control pills?

Still taking birth control pills

Less than 15 years

15-19 years

20-29 years

30-39 years

40-49 years

50 years or older

26. How many total years of birth control pills have you used?

less than 5 years

5-9 years

10-14 years

15-19 years

20-24 years

25-29 years

30-34 years

35 years or more

*Could repeat 14-17 for injectable contraceptive or shot, contraceptive hormonal patch, vaginal ring, intrauterine device, other (specify)

SECTION E. WOMAN’S SEXUAL HISTORY

The next questions are about your sexual history. I do realise that, this is a personal subject, but it is very important to the study. Please take the time to recall the information as accurately as possible. I want to remind you that this is a private interview and that the information you give me will not be linked to your name.

27. Are you married?

- Married*, (Is this your first married)?
 - Yes (go to question 28)
 - No (How many times have you been married?), Specify__
- Single
- Divorced
- Separated
- Widow

28. Have you been circumcised?

- Yes
- No
- Don’t Know

29. How old were you when you first had sexual intercourse with a man?

- |AGE
- NEVER HAD INTERCOURSE

30. Throughout your life, with how many different men have you had sexual intercourse with?

-
- No. of Men
- Don’t know

BOX E1
IF NUMBER OF MEN = 1CONTINUE
IF NUMBER OF MEN = GREATER THAN 1 Q.34
IF DON’T KNOW Q.35

31. Did your partner have any other sexual partners besides yourself, either before he met you or during the time you were together?

Yes

No

Don't Know

32. Besides yourself, how many sexual partners would you say he had?

|_|_|

NO. OF PARTNERS

Don't Know

33. Would you say it was . . . (READ)

2 or 3,

Between 4 and 6,

Between 7 and 10

More than 10

DON'T KNOW

34. Within the last year, have you had sexual intercourse?

Yes (go to 34b)

34b. Does your partner (s) use condoms during sexual intercourse?

---Yes

----No

No

35. During the last year, what is the total number of men with whom you have had sexual intercourse?

|_|_| (go to question 38)

NO. OF MEN

DON'T KNOW

36. Would you say it was...? (READ)

1

2

between 3 and 5

>5

37. Within the last year, were any of these partners new partners, that is, partners with whom you had sexual intercourse for the first time?

Yes

No

38. With how many new partners did you have sexual intercourse in the last year?

NO. OF NEW PARTNERS

DON'T KNOW

39. Would you say it was..... (READ)

1

2

between 3 and 5

>5

Thank you for your participation.