Impact of sex work and behavior on the immunological milieu of young female sex workers in Mombasa, Kenya.

By

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Abstract

HIV disproportionately affects adolescent girls and young women in Sub-saharan Africa and having a diverse microbiome has been associated with an increased risk of HIV acquisition. Among African women studies have shown that most women harbour diverse microbiome groups, and this is coupled by high recurrence rates even after successful treatment of BV. We hypothesize that sexual intercourse and sex work has a negative impact on the immunological and microbial milleu of the female genital tract and is associated with increased sexually transmitted infection risk in adolescent and young girls from Mombasa.

Adolescent girls and young women were recruited from hotspot in Mombasa, Kenya and they self-triaged into 3 distinct groups using a triage questionnaire: Non-sex worker group, transactional group and female sex worker group. Cervicovaginal lavage and urine were collected from the study participants. The CVL sample was processed into a supernatant which was used to measure cytokines and soft cup pellet which was used to for microbiome sequencing and gene expression assays. The urine was used for qPCR of sexually transmitted infections.

In the first sub study we analysed microbiome data for 168 samples and found that molecular-BV was common among the participants, and it was highly correlated with cytokines associated with increased risk of HIV acquisition (chapter 4). We further assessed cytokine receptor expression of the samples above and found that most receptors were downregulated especially those that contained diverse microbiome (chapter 5). In this chapter we further assessed whether there was a correlation between barrier marker E-cadherin and, receptors and epidemiological variables and found that participants in the female sex worker and transactional groups have lower levels of E-cadherin (p=0.015). A negative correlation was also observed between E-cadherin and some of the receptors; IL10RA (p=0.027, R= -0.259*), IL1RAP (p=0.046, R= -0.234*), GMCSFRA (p=0.023, R= -0.267*), IFNYR2 (p=0.013, R= -0.291*).

In this cohort we found an interestingly low prevalence of sexually transmitted infections (Chapter 6) specifically *Neisseria gonorrhea* infections 0.5% (5). The most common STIs were *Mycoplasma hominis* 14.9% (114) and *Ureaplasma urealyticum* 18.9% (183) which are not classified as real STIs. When we further assessed whether having an STI or the combination of an STI and diverse microbiome has an impact on immune markers we found that the latter is associated with decreased expression of some receptors; CXCR1(p=0.012), GMCSFRA(p=0.045), IL2Rg (p=0.013), IFNyR2 (p=0.050) and CXCR3 (p=0.017).

In conclusion, this study shows the importance of assessing behavior in studies that are focused in understanding the immunological and microbiological factors that contribute to the disparities of HIV infection especially among adolescent girls and young women.

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In Kenya's street language sheng we say "degree ni mchango" which translates to "completion of a degree is a compilation of many aspects", it took a village to complete this degree!

Dedication

I dedicate this degree to my loving husband Raphael Mawazo Lwoba, “behind every successful woman there is a man who pushes her up the steep hill and cheers her on”. My parents, siblings, nephews, nieces, and grandmother who was so excited when I joined the program but sadly passed away right before my completion. To every child from the Mijikenda community of Kenya ~ My completion of this degree is an example that you can scale heights and break the stereotypic barriers - you are not lazy you just need the right environment to thrive~.

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List of abbreviations

ADGYW – Adolescent girls and young women
AIDs – Acquired immunodeficiency symptoms
APCs - Antigen presenting cells
ARV – Antiretrovirals
ASC - Apoptosis-associated speck-like protein containing a caspase
ATP - Adenosine triphosphate
BV - Bacterial vaginosis
CARD – Caspase-recruitment domain
CAPRISA – Centre for the AIDS programme research in South Africa
CD4 – Cluster of differentiation 4
CRF – Circulating recombinant forms
CCR5 – Chemokine receptor 5
CT – Cervicotype
CST – cervical state type
CVL – Cervicovaginal lavage
CXCR4 – Chemokine receptor type 4
DAMPs - Damage associated molecular proteins
DNA – Deoxyribonucleic acid
EBV - Ebstein barr virus
ECL2 – Extracellular loop 2
FGT – Female genital tract
FP - Fusion peptide
FRT – Female reproductive tract
GM-CSF – Granulocyte-macrophage colony-stimulating factor
HIV - Human Immunodeficiency Virus
HPV – Human papilloma virus
HSV – Herpes simplex virus
IDOL1 - Indoleamine-2,3-dehydrogenase 1
IFN – Interferon
IL – Interleukin
iNKT - Invariant natural killer T
JAK-STAT – Janus kinase/signal transducer and activator
LD – Lactobacillus dominant
MAIT - Mucosal associated invariant T
MIP - Macrophage inflammatory protein
MMP2- Matrix metalloproteinase 2
mRNA – messenger ribonucleic acid
MSM - Men who have sex with men
MyD88 – Myeloid differentiation primary response 88
Nef - Negative regulating factor
NF-kB – Nuclear factor kappa-light-chain-enhancer of activated B cells
NOD – Nucleotide-binding oligomerization domain-containing protein 1
nLD – non-Lactobacillus dominant
NLR - NOD-like receptors
OCT - Optimum cutting temperature

OR – Odds ratio
PAMPs – Pathogen associated molecular patterns
PBS – Phosphate buffer sulphate
P.I – Post infection
qPCR – Quantitative polymerase chain receptor
Rev - RNA splicing regulator
RNA – Ribonucleic acid
SDS-PAGE – Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SIV - Simian Immunodeficiency Virus
STI – Sexually transmitted infection
Tat - Trans activator protein
TCR – T cell receptor
TFV – Tenofovir
Th1 – Type 1 T helper
TIMP4 - Tissue inhibitor of metalloproteinase 4
TLR – Toll-like receptors
TM – Transmembrane
UNAIDS – United Nations Programme on HIV/AIDS
Vif - Viral infectivity factor
Vpu -Viral protein unique
Vpr -Virus protein R
WHO – World health organization
YcDNA- human Y-chromosome

Contribution of authors

Section 1.5.1: Interaction of sexually transmitted infections and the female genital tract milleu (Section adopted from the paper "Mechanisms of sexually transmitted infection-induced inflammation in women: implications for HIV risk.")

Ruth Mwatelah - Planning the review, Prepared the first draft, figure and corrected the review.

Lyle McKinnon – Planning the review, co-authored the first draft and reviewed the paper.

Cheryl Baxter – Planning and reviewing the paper.

Quarraisha Abdool Karim - Planning and reviewing the paper.

Salim S. Abdool Karim - Planning and reviewing the paper.

Chapter 4: Sex work is associated with increased vaginal microbiome diversity in young women from Mombasa, Kenya.

Aida Sivro- Assisted in running the cytokine assay and contributed to writing the first draft of the paper.

Ruth Mwatelah- Carried out statistical analysis and contributed to writing the first draft of the paper.

Cheli Kamabaran- Carried out bioinformatics analysis and contributed in writing the first draft of the paper.

Henok Gebrebrahan- Assisted in downstream analysis of the processed sequences.

Michael Becker- Assisted with bioinformatics analysis.

Huiting Ma- Advised on statistical analysis of the data.

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Florence Mutua: Trained Ruth in the qPCR protocol and assisted in troubleshooting when technical challenges occurred.

Faisal Nuhu: Assisted in RNA extraction and cleanup.

Aida Sivro: Generated cytokine data.

Cheli Kambaran: Generated and conducted microbiome analysis of samples from chapter 4 above whose results are used in this section too.

Sandra Kiazyk: Collaborator. Participated in planning for the analysis and her lab was used for running the samples.

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Chapter 6: Prevalence and risk factors for sexually transmitted infections in AGYW from Mombasa, Kenya

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Chapter 1. Human Immunodeficiency Virus (HIV)

1.1 History of HIV

HIV is a virus of the genus lentivirus and subfamily *Orthoretrovirinae*, one of the two subfamilies of the family *Retroviridae*. There are two types of human immunodeficiency virus types; HIV-1, which has spread worldwide, and HIV-2, mainly found in West Africa. Isolates of HIV-1 have close association with Simian Immunodeficiency Virus (SIV) isolated from chimpanzees in West Central Africa. In contrast, HIV-2 is associated with SIV isolated from sooty mangabeys from West Africa. In addition, studies have shown that the virus was cross transmitted from non-human primates to humans due to its close genetic relation to the Simian Immunodeficiency Virus (SIV). The cross-transmission is thought to have originated from West Africa through some form of cutaneous or mucosal membrane exposure due to the close interaction of humans with non-human primates through bush meat hunting[1]. HIV is further divided into four distinct groups: M, N, O, and P. Phylogenetic studies found that HIV-1 groups M and N are closely related to SIVcp2PH and SIVcp2Pts lineages isolated from chimpanzees in West Central Africa, whereas group P is closely associated with SIVgor isolated from gorillas[2].

HIV was discovered in 1983 and identified as the causative agent of AIDS when 26 young men who had sex with men from New York presented at the hospital with an unusual infection coupled with Kaposi sarcoma and *Pneumocystis carinii* which is currently known as *Pneumocystis jiroveci* [3]. Further, retrospective studies on samples from 1959 have shown that the virus first appeared in Kinshasa 20 years before it was isolated in the USA[2]. Since the discovery of HIV, HIV-1 has spread worldwide, while HIV-2 has been largely constrained to West Africa. M is the most diverse group with 9 subtypes (A, B, C, D, F, G, H, J, K), and several circulating recombinant forms that result from the recombination of two or more parental strains. HIV diversity is caused by different evolutionary pressures including those applied by the immune system and antiretroviral therapy. Recombination may occur through a process whereby two different subtypes that infect a single individual may combine their genetic material resulting in a new type[4]. During this process some of the newly formed viruses survive and end up infecting other individuals, and thus become classified as distinct genotypes called “circulating recombinant forms”.

Subtype C is widely spread and comprises 48% of all HIV-1 infections followed by subtypes A and B at 12% and 11%, respectively; CRF02_AG at 8%; CRF01_AE at 5%: subtype G at 5%; and subtype D at 2%. Subtypes A, D, and C mainly occur in Sub-Saharan Africa while the latter is also found in India. Subtype B is mainly found in North America, Australia, Western Europe, South America, the Middle East, North Africa, and South-East Asia. BF recombinants have been uniquely recorded in S. America while CRF01_AE and CRF07_BC have been found in China and Thailand[5–8].

1.2 Epidemiology of HIV.

According to World Health Organization (WHO) data from 2021, there are 38.5 million people living with HIV worldwide with the number of deaths at 650,000. The prevalence of HIV varies widely across different regions of the world. Africa bears the highest burden of HIV infections accounting for two-thirds of people living with HIV (WHO, UNAIDS sites). Despite a low prevalence of HIV infection in some counties like Canada and Europe, steady increases in HIV infections has been observed in other countries such as U.S, Russia, Ukraine, Brazil, Argentina, and Mexico. For instance, the incidence of HIV in the US jumped from 48,175 per year in 2010 to 67,000 in 2019; this was attributed to increased infections among men who have sex with men (MSM)[9,10]. While a steady decrease of new HIV infections has been noted in some Sub-Saharan African countries, others have experienced an increase in new infections. This were the results of a study where the prevalence of HIV in Africa was measured by space time estimates between 2000 to 2017. Manica district in Mozambique noted 5.7 percentage decrease in infection, while the Guija district noted a 17.2 percentage increase; this example demonstrates that infection patterns are heterogeneous and non-linear, even within different regions of a country[11].

The prevalence of HIV in Kenya has been decreasing, as reported in different National surveys. In 2007, the Kenya AIDS indicator survey reported a national prevalence of 7.1%; a subsequent survey in 2012 noted a prevalence of 5.6%. Results from the latest national survey Kenya population-based HIV impact assessment (KenPHIA) indicated a prevalence of 4.9%. In this study it was noted that the national prevalence of HIV among women was 6.6%, higher than

the national prevalence, and this rate was much higher than the prevalence in men, which was 3.1%. This trend was consistent among adolescent girls and young women (AGYW) compared to men of the same age group[12,13]. A higher burden of HIV infections among AGYW has also been reported in other countries in Sub-Saharan Africa like South Africa, Uganda and Zimbabwe[14]. Studies on the reasons behind disparity in HIV infection rates among men and women have been inconclusive.

1.2.1 Dynamics of HIV prevalence among female sex workers in Kenya.

Female sex workers are disproportionately burdened by a higher prevalence and incidence of HIV in both the developing and developed countries. A study by Kimani et al found that in 1986 the prevalence of HIV among FSWs at enrollment was at 81% and this prevalence declined to about 50% in 1997 as well as a decrease in the incidence. During the whole study period from 1985 to 2005 the decrease in odds of infection was recorder at 6.4% annually[15]. Similar studies assessed the prevalence of HIV have found a similar trend. A study by Manguro et al showed that there has been a 3% decline in HIV prevalence among female sex workers in Mombasa Kenya in the past 20 years due to targeted services[16]. Another study by Tago et al showed that among FWSs in Nairobi accessing programs there has been an increase in condom use, frequent HIV testing and a decrease in reports of symptoms associated with STIs. The study found a 10 year decrease in the prevalence of HIV among FSWs of about 50-80% from the year 2008 in all age groups[17]. A combination of the availability of tailored services in Kenya has resulted in decrease in the prevalence of HIV among female sex workers in Kenya. Despite the decline in HIV prevalence Kenya still records a higher prevalence among FSWs compared to the general population at 29.3% and 4.8% respectively.

Socio economic and political structure in Sub-Saharan Africa is thought to be a major contributor in the hindrance of efforts put towards elimination of HIV among female sex workers. A study by Shannon et al described a couple of factors that may be a source of these roadblocks; in most Sub-Saharan counties sex work is criminalized, and this has been previously associated with increased risk of HIV. Another social factor associated with increased risk of HIV include migration from rural areas to urban areas which contributes by non-linear effects.

Gender empowerment, literacy and access to community organizations that are focused on this population are associated with decreased HIV incidence[18].

1.3 HIV structure and life cycle.

HIV is roughly spherical, with a diameter of about 100nm. It carries a lipid membrane that contains actin, histocompatibility antigens and ubiquitin, derived from the host's cell. The membrane has two important surface glycoproteins crucial for viral entry into the host cell. The inner surface has a matrix protein and a capsid core particle composed of capsid proteins. At the center of the virus, there are three virally encoded enzymes, protease, reverse transcriptase, and intergrade, and three accessory proteins, *nef, vif*, and *vpr* (Figure 1). The genomic material of HIV contains two single-stranded positive sense RNA that are about 10kb in length. the viral genome consists of a 5' LTR region, which is the promotor region for the transcription of viral genes. Next to this region is *gag* that encodes proteins for the inner layer of the capsid protein and nucleocapsid. The *pol* region is crucial for viral replication and contains the code for the enzyme's reverse transcriptase, protease, RNaseH and intergrase. The *env* region forms the two HIV glycoprotein spikes; gp120, the surface glycoprotein, and gp41, the transmembrane protein and it is crucial for viral entry. In between the *pol* and *env* regions, there are six regulatory proteins. *Tat* (transactivator protein) and *rev* (RNA splicing regulator) initiates replication, while *nef* (negative regulating factor), *vif* (viral infectivity factor), *vpr* (virus protein R) and *vpu* (viral protein unique) a crucial for replication, budding and pathogenesis.

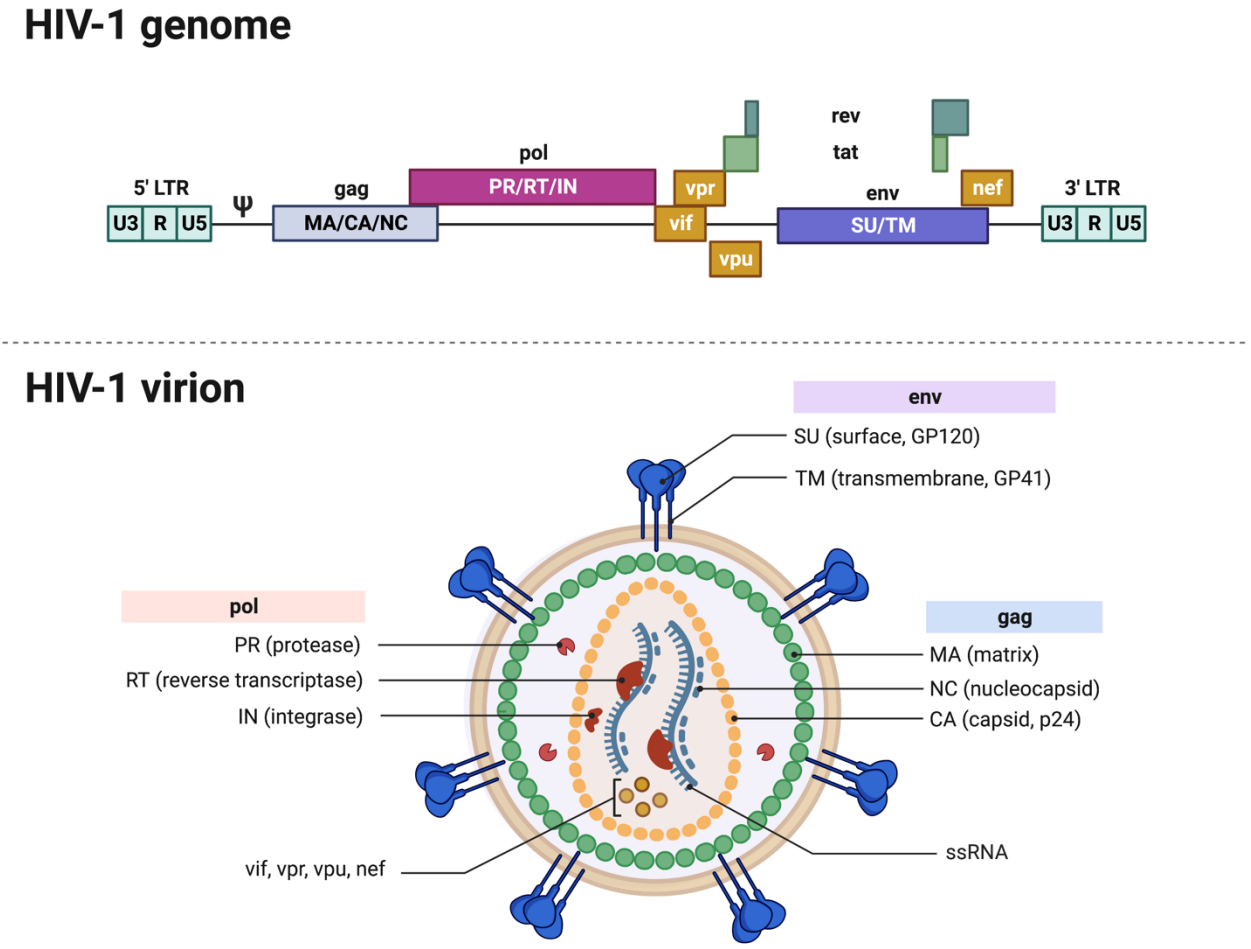


Figure 1 The HIV genome (above) and virion (below).
Created with *BioRender.com*

For HIV to enter the host cell, it requires the expression of a host cell receptor CD4 and a co-receptor, typically CXCR4 or CCR5. Co-receptor usage is important in determining the viral tropism; viruses that mainly use CCR5 are commonly named R5 HIV and are dominant during viral transmission, while the virus that use CXCR4, named R4 HIV, or viruses that use both co-receptors, usually emerge during disease progression[19]. When gp120 binds to the CD4 receptor and its co-receptor initiates refolding of gp41, a process that enhances bonding between the virus and host cell membranes. The interaction of gp120 and CD4 occurs at the interface of the inner and outer domains of gp120. This results in a bridging sheet, one of the major conformational

changes that occurs in the *env* region. Other changes that occur in this region are the flipping of the variable regions V1-V2, exposure of the variable regionV3, and repositioning of the fusion peptide in gp41[20,21]. The crown of the V3 region is then inserted into the deep pocket formed by 7- transmembrane (TM) helices of CCR5, forming a semi-circular grip by extracellular loop (ECL) 2 that wraps around the V3. This prompts the N terminal of CCR5 to form an extended shape with sharp turns which attach to the bridging sheet. These events trigger the glycoprotein complex to translocate and insert itself into the target cell membrane refolding into a hairpin and the virus particles move to the fusion site.

The virus particles are then dispensed into the cytoplasm of the host cell, and the capsid proteins are uncoated to release viral RNA. In the cytoplasm, viral RNA is transformed into proviral DNA by combined action of reverse transcriptase and intergrase. The integration step involves removal of 2 nucleotides from the 3' end of the viral DNA, which then attach to the phosphodiester bonds of the target DNA. The hanging 5' ends are then repaired by cellular enzyme to complete the process. The proviral DNA is then transcribed into messenger RNA, a process that results in synthesis of two important enzymes, Tat and Rev[22,23]. The Tat enzyme initiates the formation of longer RNA strands by binding onto the transactivation response RNA structures (TAR) site. In contrast, the Rev enzyme aids in the transcription of the longer transcripts, expression of structural and enzymatic genes. It also plays a role in inhibiting the production of regulatory, which results in the formation of mature viral particles. Cleaving of the mature viral particles occurs in two stages. In the first stage, the viral RNA associates with the replication enzymes; in the second stage, the core proteins assemble over the complex forming the capsid. Migration of the new viral material to the surface of the host cell occurs then it is cleaved by HIV protease.

1.4 Pathogenesis, Treatment, and Prevention.

1.4.1 The immune response to HIV.

The first line of defense from pathogens in the female genital tract (FGT) is the physical barrier, i.e., the epithelial cells and mucus in the FGT. The migration of HIV into the sub-

mucosae is facilitated by gaps in the epithelial cells, paracellular movement between cells, transcytosis which is mediated by the presence of neonatal Fc receptor and the ability of dendritic cells to infiltrate the lumen and attract HIV through viral receptors TLR-8 and DC-sign[24]. Once an infection is established, there are about 3-4 days post-infection, i.e., the eclipse phase when the viral DNA can be detected. This phase is characterized by rapid local expansion of the virus, followed by the peak period between 10-14 days post-infection, known as the acute phase. The eclipse period presents an opportunity to eliminate the virus before its systemic spread[25,26]. In the absence of antiretrovirals, there is a rapid increase of the virus followed by a decline, after which viral loads normally stabilize at a level known as viral set point which is highly variable between individuals and correlates with disease progression. Finally, the virus disseminates to the tissues via the lymph nodes[27]. HIV evades innate immunity through various mechanisms modulated by the accessory proteins *vpu*, *vif* and *nef*. Both *vpu* and *nef* downregulate the expression of CD4 thus escaping immune surveillance. Other proteins that are downregulated include MHC 1 and CD28 by *nef*; NTB-A and CD1d by *vpu* which protects the virus NKT cell-mediated killing. *Vif* induces degradation of APOBEC3 complex thus preventing their assimilation into the viral particle[28,29].

1.4.2 Treatment.

Reduction of HIV viral load through treatment has been a key factor in reducing transmission of the virus. This concept is known as test and treat at the population level which refers to upscaling HIV testing and offering immediate treatment to individuals who test positive. Results from the HPTN 052 trial showed that early initiation of ART resulted in a 96% reduction in HIV transmission rates. Similarly, a meta-analysis carried out to assess the impact of viral load and transmission showed that the transmission rate among untreated individuals was 5.64 per 100 person-years while that of treated individuals was 0.46 per person-years[30,31]. Through these observations from extensive research the concept of 90-90-90 HIV cascade of care was developed to reduce new infections from HIV. In 2015 the United Nations General Assembly included HIV in its development goals as Millennium Development Goal (MDG) number 6, and jointly with World Health Organization it came up with the HIV cascade of care. In this strategy

the aim is to have 90% of all people living with HIV to be aware of their status, 90% of all people diagnosed with HIV taking antiretroviral (ARV) therapy, and 90% of those on ARVs to be virally suppressed. However, these goals had not been attained by the target year of 2020, as only 81% of the people living with HIV were aware of their status in 2020, leaving a deficit of 3.1 million people. Of those aware of their status only 67% were on ARVs leaving a deficit of 5.4 million, and finally only 59% of those on ARVs were virally suppressed with 5.4 million people yet to attain viral suppression[32]. Poverty, stigma, lack of education, lack of risk perception and unemployment are some of the socio-economic factors that have hampered the efforts to attain these goals in Sub-Saharan countries[33–35].

While treatment was previously based on CD4 levels, the current approach is "test-and-treat" and viral load monitoring is recommended to confirm and detect treatment failure in the first six months after diagnosis, following by annual monitoring. In cases where viral load technology is not accessible, CD4 count monitoring is recommended[36]. Treatment entails combination therapy. The first line of antiretrovirals in most countries consists of two nucleoside reverse transcriptase inhibitors (NRTIs); tenofovir and lamivudine) and a nonnucleoside reverse transcriptase inhibitor (Efavirenz) or an integrase. The drugs work by targeting different stages of the virus life cycle. NRTIs prevent the formation of the 3'-5' phosphodiester bond of incoming 5'- nucleoside triphosphates, therefore, terminating the growth of DNA. NNRTIs change the spatial configuration by forming a hydrophobic pocket at the substrate binding site, reducing polymerase activity[37].

Drug resistance poses a major challenge in the efficiency of ARVs. Many low- and middle-income countries including Kenya lack resources for widespread and regular surveillance of ARV resistance. The pooled prevalence of drug resistance in Africa is estimated at 10.6%, with East Africa recording the highest resistance toward NNRTIs at 57.0%[38]. A recent study in Nairobi, Kenya found that 83.2% of the patients attending viral monitoring clinics had some form of drug resistance; this included triple-class resistance at 15.3% and dual-class resistance at 45.2%[39]. In another study among children and adolescents from South Rift Valley and Kisumu, drug resistance was at 76.0% for NNRTIs, 64.4% for NRTIs and 6.2% for PIs[40]. Drug resistance occurs due to various factors; the HIV replication system does not have a robust proof reading of new genetic material generated during replication, thus resulting in errors that lead to coding and

non-coding mutants that in rare cases may be beneficial to the virus[41]. The viruses' high replication rate especially in untreated individuals coupled with errors results in new variants which may have reduced susceptibility to ART. Other factors include adherence to treatment and poor drug absorption resulting in lower drug concentration[42].

1.4.3 Prevention.

HIV prevention uses a combination of different approaches that range from behavioral to biomedical, all tailored to different demographics according to their risk profile. Intrapersonal behaviors include the number of sexual partners, treatment of sexually transmitted infections, use of condoms, and voluntary medical male circumcision. Biomedical approaches include pre-exposure prophylaxis (PrEP), treating sexually transmitted infections and use of topical microbicides which reduce inflammation thus decreasing the risk of STI and HIV acquisition. The first iteration of oral PrEP consists of two drugs (tenofovir-disoproxil-fumarate and emtricitabine) and was approved for use by the Kenya Pharmacy and Poisons Board in December 2015. A task force was formed to oversee the implementation in 2016 in Kenya and the nationwide roll-out efforts scaled up in May 2017 through community- and facility-based models[43]. PEPFAR initiated the Determined, Resilient, Empowered, AIDS-free, Mentored and Safe (DREAMs) program aiming to scale up PREP uptake among adolescent girls and young women (AGYW) in countries with high HIV burden, including Kenya, in 2014. There was a general, steady (2.5-fold) increase in the uptake of PrEP from 2018 to 2019, although uptake was much higher (62%) among the 20–24-year-old age group compared to AGYW aged 15-19 years (38%). Some challenges faced during the implementation that contribute to a lower prophylaxis-to-need ratio include low demand, lack of adherence and stigmatization[44]. Other studies within Kenya have shown that there is a preference for peer-led PrEP initiatives which may increase the success of PrEP implementation[45,46].

1.5 Sexually transmitted infections: Epidemiology and pathogenesis.

The first cases of STIs in Kenya were recorded in the 1980s where cases of genital ulceration were observed among both female sex workers and men who reported that they had sexual encounters with multiple females including FSWs. During this period HIV cases were quite high in Kenya and many researchers sought to understand the risk factors associated with HIV acquisition. In a study conducted by Greenbalt et al, genital ulceration was associated with HIV among men who had reported multiple sexual partners and contact with FSWs[47]. The association between genital ulceration and HIV was confirmed in a study by Kreiss et al where HIV was isolated from 42% of *Haemophilus ducreyi*-positive women who had genital ulcers[48]. Plummer et al conducted a study to assess the risk factors associated with HIV acquisition. In this study, he found that oral contraceptives, genital ulcers, and *C. trachomatis* infections were the major risk factors of heterosexual HIV acquisition. *C. trachomatis* was highly correlated with HIV among infected women [O.R: 3.7; C.I:1.3-11.2, $p<0.02$] although the significance of the effect disappeared in a multivariable model the group noted that the degree of association remains[49]. In addition to increased risk of HIV acquisition both *C. trachomatis* and *N. gonorrhea* infections were associated postpartum upper genital tract infections (UGTI) that resulted in infertility. In a study among women in labor from Nairobi hospital, *N. gonorrhea* was found to be the single risk factor associated with postpartum UGTI whereas *C. trachomatis* infections were only associated in circumstances where there was co-infection with *N. gonorrhea*[50]. Similarly, Temmerman et al noted that the 2 microorganisms were isolated among women who were reported to have endometritis[51].

Despite the continually high prevalence of sexually transmitted infections (STIs) in Africa, there are limited studies that assess the prevalence of STIs, especially in Sub Saharan countries. In 2020, the World Health Organization estimated that there were 374 million infections attributable to the four common STIs: chlamydia (129 million), syphilis (7.1 million), gonorrhea (82 million), and trichomoniasis (156 million) infections[52]. With the highest incidence seen among key populations which include men and women who sell sex, intravenous drug users and men who have sex with men who are at an increased risk of HIV acquisition. A recent meta-analysis consisting of 18 prevalence studies from Sub-Saharan Africa showed a higher prevalence of STIs among adolescents and young women (AGYW) compared to older women

aged between 25-49 years. In the same study it was observed that in Eastern Africa, the risk was greater among AGYW from key populations[53]. Although few studies focused on AGYW in Kenya, increased risk of HIV has been observed among women aged 18-24 years in high-risk populations. The high prevalence was attributable to behavioral risk factors that increase exposure[54]. Varied prevalence estimates have been reported worldwide for the common STIs, with *Chlamydia trachomatis* infections ranging between 3.1 to 15.0%, *Neisseria gonorrhea* from 1.0 to 1.8%, and *Trichomonas vaginalis* from 5.0 to 13.6%[55–59]. These three are the most studied STIs in Kenya yet very little is known about other microorganisms like *Mycoplasma hominis*, *T. vaginalis* and *Ureaplasma urealyticum.*

C. trachomatis is an intracellular bacteria that parasitizes eukaryotic cells. The infective stage of bacteria, termed the elementary body (EB), is internalized into the host cell through the vacuole and starts its replication cycle by transforming into a reticulate body (RB). The reticulate bodies are metabolically active, replicate approximately 7 times, then transform back into the infective EB that are released from the host cell through lysis[60]. *C. trachomatis* can be classified into 15 serovars based on the differences of the major outer-membrane protein A (ompA). These serovars can further be classified into three different subsets according to where disease is most commonly manifested. Serovars A-C affect the eyes, causing trachomatis, D-K cause urogenital infections, while L1-L3 cause sexually transmitted infections. The most commonly occurring serovars are D to K, with serovar E causing persistent infection especially when exposed to β-lactam based antibiotics, with ~ 15% of *C. trachomatis* post-treatment persistent cases among women up to 42 days[61,62].

N. gonorrhea is a gram-negative diplococcus that causes infection of the female genital mucosa by infiltrating the endocervix. The establishment of infection causes disruption of epithelial junctions, shedding of epithelial cells, and penetration of the bacteria to the sub-epithelia. In response to the infection, neutrophils, dendritic cells, macrophages, and T cells are released to induce a local immune reaction[63]. *N. gonorrhea* can be genetically differentiated from *N. meningitidis* through typing of the *PorB* pseudogene, a highly heterogenous surface loop on the major outer membrane protein classifies the microorganism into porB1a or porB1b alleles, an important tool in assessing transmission network within the community[64,65].

Mycoplasma spp and *Urealyticum spp* belong to the *Mycoplasmataceae* family, a group of small bacteria that lack a cell wall and have the ability to self-replicate. Four microorganisms in this group can cause genital infections - *Ureaplasma urealyticum, Mycoplasma hominis, Mycoplasma genitalium,* and *Ureaplasma parvum*[66,67]. *U. urealyticum* is a major cause of nongonococcal urethritis, and, together with *M. hominis* are responsible for 7.6% of urinary tract infections; *M. hominis* accounts for about 2%-4% of these infections. *M. hominis* has been isolated in approximately 75% of bacterial vaginosis cases, thus making it a contributor of vaginal imbalance in the female genital tract[68].

Trichonomas vaginalis is a protozoa that is the causative agent of a common and curable sexually transmitted infections. The pooled prevalence of *T. vaginalis* infections from a recent meta-analysis was estimated at 16% among female sex workers, with the mean age between 30-36 years bearing the highest burden[69]. In women, this organism is responsible for causing cervicitis and vaginitis. While it is considered as a mild infection it is known to have detrimental effects on the female genital tract by damaging the host epithelia. Upon infection, the ovoid form of *T. vaginalis* transforms into an ameboid form that attaches to the epithelial cells aided by surface adhesins which are upregulated in the presence of iron. The microorganism then attracts more pathogens to the site of attachment forming a mass of ameboid cells. This process results in damage to the host cells and stimulation of an immune response[70].

1.5.1 Interaction of sexually transmitted infections and the female genital tract milleu.

1.5.1.1 Mucosal immune responses to STIs.

STIs result in a large inflammatory response that can, in turn, lead to pathology throughout the genital tract, including pelvic inflammatory disease, ectopic pregnancy and infertility, and degradation of the epithelium. As part of this inflammatory response, an influx of immune cells, including neutrophils, has been associated with the presence of discharge and lesions in the genital tract, resulting in further damage to the epithelial barrier [71]. We and others have shown that this epithelial damage may be due to increased protease expression, which functions to degrade epithelial integrity [72,73].

Although the mechanisms of infection differ, the ability of all STI-causing pathogens to induce an inflammatory response, damage the epithelial barrier, and impair natural innate defenses are believed to increase the risk of HIV acquisition by providing the virus better access to HIV target cells in the sub-mucosa and beyond. Several clinical trials have assessed the impact of STI treatment as a mode to reduce HIV infections. In these studies, contradicting results have been observed for example in a study carried out in Mwanza there was a 40% reduction of new HIV cases in the region when syndromic management of STIs were introduced into government facilities. There was no difference in other regions which were included in this study. What was noted is that this specific region had a budding HIV epidemic whilst the others had mature epidemic which explained the results of the difference. Similar observations recorded in the latter regions were seen in other clinical trials that targeted STI interventions in demographics with mature epidemics. It was postulated that there are several reason that could explain the failures; the design of the intervention was good but not suitable for the population which it was tested on, or the design of the intervention was not good. This is a clear indication that there is no "one fits all" approach to an effective HIV prevention strategy[74,75].

Inflammation caused by STIs may simultaneously increase the number and location of these cells relative to the lumen, or induce phenotypic changes that increase their cellular susceptibility to virus infection[76]. The inflammatory responses induced by STIs are intended to, and in some cases may, play an important role in protecting the host, but in many cases, this response favors the pathogen. This could be due to evasion of the effector mechanisms aimed at pathogen clearance and by causing collateral damage to host tissues[77–79]. For example, in *C. trachomatis* infection, neutrophils are among the first immune cells recruited to the site of infection. Delayed apoptosis is a strategy used by *C. trachomatis* to avoid a complete immune response whereby it reduces the neutrophil sensitivity towards the stimuli from apoptosis, hence contributing toward pathogen persistence[80].

1.5.1.2 Intracellular and extracellular recognition of STI causing pathogens by pattern recognition receptors.

Mucosal epithelial cells are the first barrier against infection, forming an early line of defense against pathogen invasion. Epithelial cells are equipped with receptors that are crucial for pathogen detection, and these cells function to initiate and modulate the inflammatory cascade aimed at inducing pathogen clearance [81,82]. Inflammation leads to a series of reactions which induce adaptive immunity, including effector mechanisms that can clear infection. However, tight regulation of inflammation is required to avoid self-damage [82,83]. In the case of STIs, a combination of immune evasion, potent induction of inflammation, and poor natural immunity represent scenarios in which HIV entry may be increased (Figure 2).

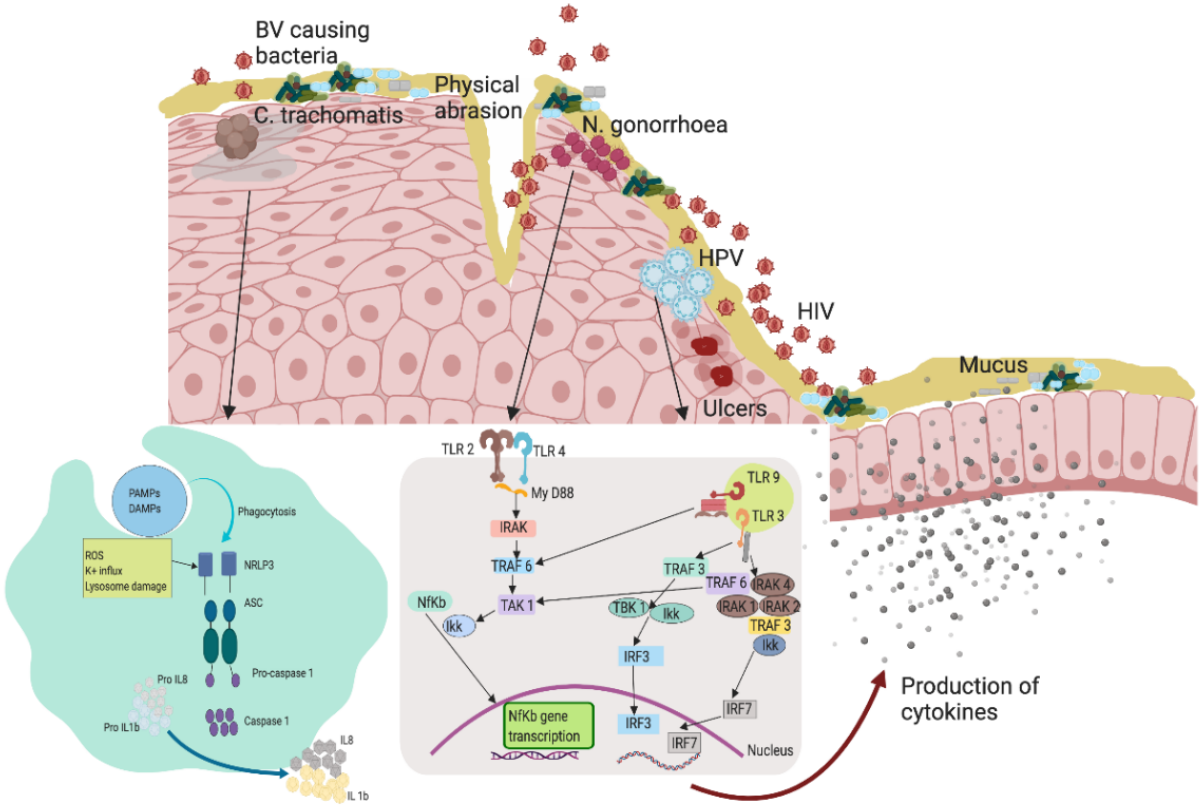


Figure 2. Immune responses to STI in the female genital tract.

Mucosal innate immune responses to STIs in the female genital tract that could potentiate HIV transmission risk. Depicted are several modes through which STIs might increase the risk of HIV acquisition. Infection with STIs results to physical abrasion, ulcer formation and increase pro-inflammatory cytokines resulting in inflammation. Inflammation increases the availability of HIV target cells in the sub-mucosa. During *N. gonorrhea* infection, TLR2 and 4 detect

lipooligosaccharide and induce a NF-KB driven immune response resulting to production of cytokines. Infection with *C. trachomatis* results in death of some cells which in turn produce elementary bodies. *C. trachomatis* infection is detected by inflammasomes resulting to production of IL-1*b* and IL-8 through the NLR3 pathway. TLR 9 detects the CpG island in genomic material of the HPV virus inducing an immune response through the MYD88 pathway. TLR3 detects the viral nucleic acid to induce an immune response through the IRF and IR7 pathways. Created with B*io*R*ender.com*

Toll-like receptors (TLRs) play an important role in detecting pathogens including STIs, and in initiating appropriate innate and adaptive immune responses. TLRs bind to their cognate ligands, resulting in signaling cascades that culminate in the expression of pro-inflammatory cytokines. TLRs can be classified based on their expression and where the location of ligand recognition typically occurs; this can be intracellular (TLR 3,7,8,9,11,12 and 13) or extracellular (TLR1,2,4,5,6 and 10) [84,85]. TLRs recognize pathogen-associated molecular patterns (PAMPs), including bacterial DNA, viral nucleic acid, and viral proteins, with the eventual goal of inducing specific T cell, antibody responses and creating the immediate innate response required for containment of interferons. For example, TLR9 detects the unmethylated CpG sequences in bacterial DNA molecules. Many TLRs signal via MyD88, an important intracellular protein adaptor molecule. MyD88 is responsible for induction of the IL-1 family, a group of 11 cytokines that are mainly inflammatory and regulate innate immune cell function. IL-1R-associated kinase is recruited via MyD88 activation, further activating the NF-kB pathway and culminating in the transcription of pro-inflammatory cytokine genes [86,87].

Several bacterial STIs induce innate inflammatory responses by interacting with extracellular TLRs. A recent study that utilized a 3D model of endocervical cells showed that *M. genitalium* was recognized by TLR2, 4 and 6, a pattern of TLR usage that initiates the NF-kB pathway and is unique to this bacterium [88,89]. In microorganisms such as *N. gonorrhea*, protein elements are detected both intracellularly and extracellularly, both of which can induce an NF-KB-driven inflammatory response. TLR 2 and 4 detect LPS, outer membrane vesicles, porins and other proteins, while additional pattern recognition molecules called nucleotide-binding oligomerization domain-like receptors (NOD) 1 and 2 detect additional biochemical structures of STI-causing pathogens such as gamma glutamyl diaminopimelic acid and muramyl dipeptide, which also results in induction of NF-kB-driven inflammation [63,90].

STIs similarly induce immune responses through inflammasomes, which are multi-protein intracellular structures located in the cytosol. The inflammasome is activated by the signaling of PAMPs, DAMPs (damage associated molecular proteins), changes in the ion concentrations of cytosol and by extracellular adenosine triphosphate (ATP). Once activated, this molecular complex leads to the expression of pro-inflammatory cytokines and can also initiate an inflammatory form of cell death called pyroptosis [91–93]. Activation of the inflammasome often occurs through NOD-like receptors (NLRs, especially NLR3), which interacts with apoptosis-associated speck-like protein containing a CARD (ASC). This protein is located in the nucleus of macrophages and monocytes and is responsible for activating caspase-1, which in turn cleaves and activates IL-1β and IL-8 [93]. *C. trachomatis*, co-cultured with epithelial cells, were found to activate inflammasomes. This resulted in IL-1b and IL-8 production and activation of pyroptosis in the context of the canonical inflammasome. An inactivated form of *C. trachomatis* was tested in the same model and was still found to lead to priming of the inflammasome, but without the resulting inflammatory response, implying that pathogen replication may be critical for cytokine induction [94]. This inflammatory pathway also applies to other STIs; for example, the LPS of *N. gonorrhea* has been shown to harbor hexa-acylated lipid A, which can activate the NRLP3 inflammasome [95].

1.6 Cytokine receptors and their role in immunomodulation the female genital tract.

Cytokine receptors are a group of proteins whose main task is initiating signaling and are associated in modulating several functions which include immune responses, metabolism, blood cell and immune cell development among others. There are 2 classes of cytokine receptors namely class I and II which differ by their structural features and sequence homology whereby class I contains N-terminal extracellular domain and a C-terminal intracellular domain. Whereas class II have an N-terminal extracellular domain that lack WSxWS motif and have a different arrangement in their conserved 4 cysteine residues. The receptors mainly utilize the Janus Kinase (JAK) Tyrosine Kinase to initiate signaling. An imbalance of class I receptors has been associated with inflammatory bowel disease blood cancers, multiple sclerosis, and osteoporosis. Class I signaling through JAK family that is bound to BOX 1 motif occurs through activation of

signaling transducers and activators of transcription (STAT 1-6) while others utilize SRC family kinaces[96,97]. The structure of a receptor contains an extracellular portion that binds to the cytokine and an intracellular portion that initiates the signaling cascade. There are 2 main types of polypeptides present in cytokine receptors that are associated with signaling; components with innate kinase activity or those that depend on signaling kinases. In some cases a third component is required e.g. in the signaling of IL2, receptors Rα, Rβ and Rγ actively play a role in the process with Rβ and Rγ as the main mediators of signaling since they actively associate with JAK1 and JAK3 while Rα is not necessarily required for the signaling process but plays an important role in stabilization of the complex and increasing binding affinity[98].

1.7 Bacterial vaginosis in the female genital tract.

1.7.1 History and classification of bacterial vaginosis.

In 1892 Albert doderlein isolated gram-positive microorganisms from the vagina that were later named as *Lactobacillus* after several naming iterations form the initial name which was his second name. In 1953, Leopold first described small coccobacillary organisms isolated from vaginal samples which were later named as Haemophilus vaginalis by Gardner and Dukes in 1955. Due to the blood requirement for bacteria in the genera *Haemophilus*, the coccobacillus was removed from this genus since they were not able to grow in blood agar. This saw the microorganisms reassigned to the genera *Corynebacterium vaginalis*, but they also did not produce catalase and lacked a cell wall thus they were eventually removed from this genus. At this point, since these bacteria did not resemble any other existing microorganisms, they were given the name *Gardnerella*. Later a group from the University of Washington observed that *Gardnerella* are not the only organisms in existence in this condition that caused a foul smell but there are other anaerobic microorganisms and mycoplasmas. This resulted to the conclusion that vaginitis is caused by multiple microorganisms and their observation of inflammatory cells microscopically led the name change from vaginitis to vaginosis.

1.7.2 Epidemiology of bacterial vaginosis.

The global prevalence of BV which is defined by presence of a diverse microbial population in the female genital tract characterised by abundance of anaerobic bacteria and low populations of lactobacillus species among women of reproductive age has been estimated between 23% to 29% with varying percentages across different continents. Europe and Central Asia had the lowest prevalence at 22.8% while North America recorded a much higher prevalence of 27.4%[99]. Risk factors that have been associated with BV include unprotected sex events, multiple sex partners, female genital mutilation, hormonal contraceptives, oral sex, use of cloth for menstrual hygiene, hormonal changes that occur during the different phases of the menstrual cycle and new sexual partner even though it isn't believed to be sexually transmitted.[100–102]. Genetic factors are also thought to play a contributing role in BV especially among women of African descent but not much is known yet. Cohort studies carried out in mixed populations have found racial disparities which are associated with a higher prevalence of BV in studies carried out in America, where women of African descent were more likely to have a diverse microbial population compared to their white counterparts[103]. Paul et al investigated causes of preterm birth among women and showed that economic risk factors such as low income and stressful life events were associated with BV among African-Americans who experienced high rates of preterm births[104]

In a meta-analysis study, a prevalence of 35.2% was noted in community based population from Southern and East African clinics while a higher prevalence of 49.5% has been recorded among women who engage in sex work (high-risk) in Eastern Africa, with Kenya recording a prevalence of 44% within the same population[53,105]. The prevalence of BV is lower among adolescent girls who are studied prior to sexual debut. Previous studies carried out in Kenya found that a higher proportion of adolescent girls from the community or those that do not have prior sexual experience had *Lactobacillus* dominant microbiome[100,106].

1.7.3 Diagnosis of bacterial vaginosis.

Diagnosis of bacterial vaginosis includes two main methods: Amsel's criteria which is typically used by clinicians and Nugent scoring which is laboratory based. Amsel's criteria were developed in 1983 by Amsel whereby four characteristics were identified as characteristics of abnormal vaginal discharge:

1. A pH higher than 4.5 which is typically indicative of loss of *Lactobacillus spp* thus a shift to a more alkaline state.
2. Fishy odor which is a characteristic of production of amines when 10% potassium hydroxide is added to vaginal discharge samples.
3. Prescence of clue cells which is mainly shedding of *Gardnerella* cells adhered to epithelial cells.
4. Prescence of a white-yellow discharge.

Using these symptoms presence of three of the above characteristics is used by clinicians to diagnose BV and determine whether treatment is required for the patient[107].

Nugent method was developed in 1991 by Robert Nugent as a laboratory diagnostic method which was a modernization of the Speigel criteria. In the Spiegel criteria a gram-stained slide that showed predominance of *Lactobacillus spp* which are gram-positive rods with or without the *Gardnerella spp* (gram variable coccobacilli) was classified as normal. When there was a dominance of mixed species and reduced lactobacillus species the sample was classified as BV[108]. The Nugent scoring system similarly utilises gram staining method to quantify the amount of *Lactobacillus* species, gram negative curved rods which are mostly identified as *Mobiluncus* genus and *Gardnerella* species[109]. A score of the range 0-10 was created to assign to the analysed slides according to the number of observed bacterial groups, *Lactobacillus* was assigned between 0-4, *Gardnerella* between 0-4 and *Mobiluncus* between 0-2 while a score of 7-10 is assigned to slides that are mainly composed of *Gardnerella* and *Mobiluncus* thus diagnosed as BV.

Other advanced methods used in the classification of microbial populations residing in the female genital tract include16s rRNA sequencing is divided into a couple of ways dependent on the method used to isolate the microorganisms and the bioinformatics pipeline used to analyze

the resulting sequences. Some studies have characterized those isolated via 16s rRNA sequencing are characterized into four distinct cervicotypes (CST); CST I which is *L. crispatus* dominant, CST II which is *L. gasseri* dominant, CST III which is mainly composed of *L. iners* and CST IV which is further divided into subgroups A, B and C. CST IVA is mainly composed of BVAB1 and G. vaginalis with modest amounts of Lactobacillus species, CST IVB has a much higher composition of *G. vaginalis*, *Aptobium vaginae* with little population of *Lactobacillus* species. CST IVC is dominated by *Provetella* species and other species include *Anaerococcus*, *Finegoldia* and *Corynebacterium*. A final classification is CST V which is dominated by *L. jensenii*[110]. Other studies by Ravel's research group have further characterized the cervicotypes into 13 distinct groups using a method that compared centroids calculated from a large database of comprehensive taxonomic profile of 13,160 women. The resulting CSTs include CST I-IV that were dominated by *Lactobacillus* spp; *L. crispatus, L. gasseri*, CST *L. iners* and *L. jensenii respectively.* CST IV-A, IV-B, and IV-C which did not have a high relative abundance of *Lactobacillus* species. CST IV-A had a high abundance of *Candidatus Lachnocurva vaginae* and low abundance of *G. vaginalis, CST IV-B* had an inverse abundance of the microorganisms in CST IV-A. CST IV-C which is further divided into 5 subgroups (CST IV-C_1 to CST IV-C_5) had a high abundance of facultative anaerobic bacteria and a low abundance of *Lactobacillus* spp., *G. vaginalis*, *A. vaginae*, and *Ca. L. vaginae*[111].

1.7.4 Treatment and prevention.

Bacterial vaginosis is typically treated using 500mg metronidazole twice a day for 7 days, 0.75% metronidazole gel applied once a day for 5 days or 2% clindamycin cream applied once a day for 7 days. Despite availability of medication BV has a high recurrence rate of about 67% with in 12 months of initial treatment. The cost of treatment has been estimated to be $4.8 billion with recurrent treatment taking up more than half of this cost[99]. BVAB and biofilms are the major modulators of recolonization[112]. The mode of action of metronidazole is penetration of the cell wall of both aerobes and anaerobic microorganisms. This infiltration occurs at a higher rate among the anaerobes, and it is through a process whereby a gradient is created after intake of the parent compound to allow uptake of more. The parent compound is not active thus it undergoes

a 4 to 6 election process to stimulate the reduction of the 5-nitro group to convert it to an active compound which mainly consist of amines and hydroxyl amines. The reduction products interact with macromolecules and disrupt DNA[113]. Metronidazole has been found to be limiting because it also impacts the population of lactobacillus in the female genital tract. Clindamycin which has been found to be more promising in clearing infections from *G. vaginalis*, *Anaerococcus tetradius* and Mobiluncus works by binding to the 50s ribosomal subunit to inhibit protein synthesis by preventing peptide bond formation. In addition to use of antibiotics, there are other treatment options that have been explored for use together with the antibiotic regimen or by themselves. These include treatment of partners to inhibit recolonization this was not found to be successful, use of intravaginal rings which slowly release lactic acid and metronidazole, use of activated charcoal to reduce bad odor and vaginal secretions and lastly the use of probiotics.

Probiotics have been found to increase the amount of lactic acid, immunomodulatory activities and complete with other bacteria to recolonize the female genital tract and displace facultative anerobic bacteria. Some of the commonly used Lactobacillus species include *L. reuteri* RC-14, *L. fermentum*, *L. brevis*, *L. gasseri*, *L. rhamnosus* GR-1, *L. acidophilus*, *L. plantarum* and *L. crispatus*. They are administered orally or as topical applications[112]. Combination of *L. rhamnosus* GR-1, *L. reuteri* RC-14 based probiotics and metronidazole has been found to be 88% successful in treatment of BV patients compared to the use of antibiotic only which was found to have a success rate of 40%. It was also found that bacteriocin from *L. rhamnosus* creates transient pores on G. vaginalis a factor that contributes to its elimination[114]. In a meta-analysis that involved 10 RCTs showed that use of probiotics alone was more efficient for long term treatment of BV compared to the combination of probiotics and antibiotics when the two groups were compared with a placebo group[115]. Microbiome transplant have also been explored as an alternative treatment to BV. This method was adopted from success rates of fecal transplant in patients suffering from Clostridium difficile a study in which the bacterial composition of the patients was repopulated through this method[116]. Results from exploration of vaginal microbiome transplantation (VMT) as a method of treating bacterial vaginosis among 5 patients was marked as success as four of the patients had improved symptoms, recolonization with Lactobacillus species and improvement was also observed when the sample was analyzed microscopically and by the use of Amsel's criteria[117].

1.7.5 Protective role of *Lactobacillus* species and pathogenesis of bacterial vaginosis causing bacteria.

An optimal female genital tract is colonized by *Lactobacillus* species; *L. jensenii*, *L. crispatus* and *L. gasseri* but once this changes to a much more diverse population the condition is called bacterial vaginosis (BV)[118]. *Lactobacillus spp* apart from *L. iners* which has been found to be associated with inflammatory markers are protective in the female genital tract by several mechanisms, including lowering of the pH making the environment acidic an inhabitable to other microorganisms, their adherence to the epithelial cells forming a barrier that inhibits the attachment of other microorganisms through steric hindrance, their production of antimicrobial substances such as bacteriocins and hydrogen peroxide, and their modulation of the immune system at a local level[119–121]. *Lactobacillus spp.* are also able to break down the glycogen to produce lactic acid which is important in the enhancement of growth of *Lactobacillus spp*. Several studies have shown that *Lactobacillus spp.* utilise surface active molecules like peptidoglycan, lipoteichoic acids, biosurfactants and exopolysaccharides to disrupt the activities of BV-associated bacteria. Peptidoglycans are glycan strands that form the cell surface of gram-positive bacteria and are joined by two side chains: N-acetylgucosamine and N-acetylmuramic acid. Peptidoglycans ameliorate the host's immune responses by modulating the immune activity. Peptidoglycan from *L. crispatus* was found to stimulate CD207+ Langerhans cells, thus significantly reducing the expression of HIV receptors. Lipoteichoic acid which is found on the peptidoglycan layer of lactobacillus species modulates the PRRs and immune signaling pathways. *In vitro* studies showed that *L. plantarum* LTA obstructed the formation of *Streptococcus mutans* biofilm on hydroxyapatite discs by decreasing sucrose decomposition. Exopolysaccharides (EPS) play a major role in host cell- bacteria interactions and adhesion. EPS produced by *Lactobacillus spp* have been found to have antiviral, anti-yeast properties and they have the ability to modulate the activities of the immune system[114].

BV is characterised by overgrowth of anaerobes which in turn increase pH levels making the environment conducive for invasion of anaerobic microorganisms including STIs. BV has been associated with increased risk of HIV and STI acquisition by increasing proinflammatory cytokines thus making desirable cells readily available for attachment of HIV[122]. The presence of

anaerobes in the female genital tract is associated with an alkaline pH greater than 4.5, increase in sialidase, depletion of sialic acid, increase in diamines, putrescine and cadaverine[123,124].

G. vaginalis is one of the first organisms to colonise the female genital tract and initiate the shifting of the bacterial population. This bacteria displaces lactobacilli, creating a biofilm that it utilizes to colonize and adhere the epithelium resulting in production of a cytotoxin called vaginolysin. Vaginolysin is a spore forming toxin that binds to CD59 human complement regulatory molecule aiding the attachment of *G. vaginalis*. Formation of the biofilm enhances symbiosis between other microorganisms and *G. vaginalis* by allowing cross talk between microorganism and quorum sensing[125]. In an *in vitro* study by Anton et al., colocalization was observed between bacterial and epithelial cells, a feature only observed in *G. vaginalis* and not by any of the lactobacillus species. Permeability of the ectocervical cells co-cultured with *G. vaginalis* increased in a dose-dependent manner. In this study they also noted that there was blister formation which is indicative of cell death and also increase in lactate dehydrogenase[126]. G. vaginalis induces exfoliation of vaginal epithelial cells in mouse studies where the vaginal wash from mice inoculated with the microorganism. In the same study they showed that *G. vaginalis* was associated with increased production of sialidase which is associated with mucosal barrier degradation, attachment of bacteria and facilitation of bacteria growth by releasing carbon sources[123].

G. vaginalis does not act by itself but benefits from symbiotic relationship with other microorganisms that have been identified in samples of women with bacterial vaginosis. Some of the most identified microorganisms that have been studied include *Provetella spp* specifically *P. bivia* and *P. disiens*, *Aptobium vaginae.* The symbiotic relationship occurs through their metabolites and at very low doses. *Provetella spp* stimulates production of sialidase which subsequently cleaves sialic acid which degrades mucin a component of the mucus which acts as a protective barrier in the female genital tract. Sialidase is also associated with facilitating bacteria growth by releasing carbon sources and also plays a role in bacterial attachment. Another symbiotic relationship between *G. vaginalis* and *P. bivia* revolves around ammonia and amino acids. *G. vaginalis* produces amino acids which are utilised by *P. bivia* to produce ammonia which is used by the latter to enhance its growth[127]. Ilhan et al studied the impact of different species of *Provetella spp* on endometrial epithelial cell models and found that out of 13

different species *P. disiens* had a higher cytotoxicity compared to *P. bivia*. In this model *P. bivia* was the only species that was able to induce inflammation by upregulating IL8, TNF and CCL20 levels compared to other species but it was not significant[128].

A. vaginae has also been identified as a contributor to bacterial vaginosis in collaboration with *G. vaginalis* as the two have always been isolated together. The two have been associated with higher recurrence rates of BV. *A. vaginae* has been associated with distinct characteristics including stimulating a strong immune response which increases production of cytokine and b-defesins factors that have been thought to be contributors of symptoms associated with BV including vaginal odor and adverse pregnancy outcomes. Another notable characteristic of *A. vaginae* is its resistance to metronidazole, which is the drug that is commonly used for treatment of BV[129]. A 3D human epithelial model that evaluated in detail the impact of BV causing bacteria on cells showed that *A. vaginae* was associated with increased MUC10, death receptor Fas and *Sneathia amnii* was associated with increased heat shock protein HSP70 and vascular endothelial growth factor analytes associated with cellular stress. *S. amnii* was also found to alter a very huge number of metabolites mainly amino acids after polymicrobial BVAB community. In Euclidean distance clustering analysis, the polymicrobial, *A. vaginae* and *S. amnii* clustered closely together an indication that they do have a unique metabolic profile[130]. Together these studies show how BV causing bacteria work together to alter the functioning of the physical barriers in the female genital tract and establish an infection; a process that weakens the immune system and sets the precedence for sexually transmitted infections to easily establish their colonies.

1.7.6 Co-infection with sexually transmitted infections, bacterial vaginosis, and HIV

It is increasingly being recognized that STIs are not the only important inflammatory microbes in the genital mucosa. In addition to the mucosal barrier, the composition of the vaginal microbiome can play an important role in providing immune defense at the genital mucosa. Women with certain *Lactobacillus*-dominant communities can produce lactic acid and maintain a low mucosal pH, which inhibits the growth of pathogenic bacteria including STIs. In the absence of *Lactobacillus* spp., except for *L. iners*, a more diverse microbiome population is typical, which is often associated with bacterial vaginosis (BV). BV, defined either by Nugent scoring or

using molecular methods [110], has associated with an increased risk of both STI and HIV acquisition [122,131–137]. Both STIs and BV are associated with increased levels of inflammatory cytokines like IFN-α2, IL-1α, IL-1β, TNF-, IFN-γ, IL-8 [132,138]. Epithelial cells of the genital mucosa produce glycogen, an energy source that allows *Lactobacillus* spp. to flourish [139,140]. Gong et al (2014) suggested that lactic acid is important in protection against *Chlamydia* infection. Lactic acid acts by disrupting the outer bacterial membrane, inactivating the elementary bodies (EB) that mediate the attachment of Chlamydia to the host epithelial cells [141]. Therefore, the presence of BV causing bacteria increases the risk of co-infection with STIs.

Synergism between BV and STIs is in part through the production of metabolites by the BV causing bacteria, which are utilized by STIs as growth factors. An example is seen between BV and *C. trachomatis* infections. Bacterial species that produce tryptophan including have been associated with the increased risk of *C. trachomatis* infection among women whereas Indoleamine-2,3-dehydrogenase 1 (IDOL1) producing species have been associated with decreased risk. IDOL1 inhibits the availability of tryptophan. This shows that molecular BV may play an important role in both first time and recurrent *C. trachomatis* infections [142,143]. Ziklo et al found that Chlamydia infection was associated with reduced IFN-g response. IL-17 was also reduced among infected individuals, and this cytokine is important in boosting host defense and maintaining mucosal barrier. Therefore increased levels of kynurenine, the byproduct of tryptophan breakdown, is associated with increased risk of HIV acquisition [143–145]. Studies have suggested that ethnicity and not metabolic mechanisms may also underlie Chlamydia-HIV co-infection [146–148]. Increased risk of *T. vaginalis* acquisition has been associated with BV in both HIV-positive and negative women. *T. vaginalis* has been associated with decreased levels of *Lactobacillus* spp., especially those that produce H_20_2 [149].

Durable and effective treatment of BV has been a major challenge for the field. Oral or topical metronidazole is effective in the short term, yet recurrence occurs among more that 50% of women. within a 3 to 12-month period [131,150,151]. However, periodic presumptive treatment has proven to be an effective method in reducing STI incidence [152,153]. This both strengthens the case for a causal relationship between BV and STIs and suggests that reducing BV may help to reduce STI incidence.

STI co-infection in HIV+ women, particularly by *N. gonorrhea* or HSV-2, increases inflammatory responses and mucosal HIV shedding [154–157]. In addition to mucosal inflammatory response, STIs such as *N. gonorrhea* have been found to increase plasma viral load and reduce CD4 T cell counts. This is a clear indicator that both STI and HIV act in synergy resulting in detrimental effects to the host. While studies have suggested that STI treatment could result in reduction in HIV shedding and transmission [154], this may be a moot point in the era of effective antiretroviral therapy, which if taken correctly reduces HIV transmission almost completely[158] .

Chapter 2. Rationale

HIV disproportionately affects adolescent girls and young women (AGYW) in Sub Saharan Africa. AGYW are 3 times more likely to be infected by HIV than their age-matched male counter parts, meaning that they acquire the infection roughly 7 years earlier. The increased risk of HIV acquisition among AGYW is likely attributed to a combination of biological and intravaginal behavioral factors. This includes the physiology of the female genital tract which provides the first barrier to prevent infection but can fail to do so when damaged. Co-infection with BV and/or STIs results to increased pro-inflammatory cytokines that are linked to increased acquisition of HIV. Behavioral factors like multiple sexual partners and unprotected sex acts, lack of education on HIV prevention approaches and age-disparate relationships which have been linked to both increased incidence of HIV and increased unprotected sex events[122,159,160]. Viral load is another contributing factor to increased transmission. A study by Hughes et al found that male to female transmission was higher due to high viral load among the males[161]. The *Transitions* study was uniquely designed to capture the epidemiology of the entry point into sex work, including sexual activity with different sex partners. The study allows us an opportunity to measure how diverse vaginal exposures ranging from sexual activity in different contexts for example sex work or not, sex with different partners with and without condoms, vaginal insertions and STIs, define the vaginal milieu.

2.1 Hypothesis and Objectives

The central hypothesis of this is thesis is that sexual intercourse and sex work has a negative impact on the immunological and microbial milleu of the female genital tract and is associated with increased sexually transmitted infection risk in adolescent and young girls from Mombasa.

2.1.1 Sub-hypothesis 1:

Sexual activity and intravaginal behavior are associated with increased inflammatory cytokines and microbial dysbiosis in the female genital mucosa. This is addressed in chapter 4.

2.1.1.1 Objectives

- To assess the impact of multiple sex partners on vaginal dysbiosis and inflammation.
- To determine the impact of condomless sexual intercourse on vaginal dysbiosis and inflammation.

2.1.2 Sub-hypothesis 2:

Expression of cytokine receptors is influenced by vaginal dysbiosis and sexually transmitted infections, and will correlate inversely with cytokine expression in the FRT. This is addressed in chapter 5.

2.1.2.1 Objectives

- To quantify expression of cytokine receptor RNA in the FRT, and correlate expression to the presence of STIs, sexual activity, vaginal microbiota, and age.
- To correlate cytokine receptor expression with expression of cytokines in matched ligand-receptor pairs.
- To determine the effect of high cytokine receptor expression in conjunction with high cytokine expression on HIV-associated biomarkers such as barrier proteins and surrogates of immune cell frequency.

2.1.3 Sub-hypothesis 3:

Sexual activity is associated with increased risk of 'atypical' and typical STIs among adolescent and young women from Mombasa, Kenya. This is addressed in chapter 6.

2.1.3.1 Objectives

- To determine the prevalence of typical and atypical STIs among ADGYW.
- To determine the correlation between sexual behavior and vaginal dysbiosis on typical and atypical STIs.
- To determine the diversity of *C. trachomatis* and *N. gonorrhea* subtypes circulating among adolescent girls and young women from Mombasa Kenya.
- To determine the prevalence of drug resistant mutations associated with *N. gonorrhea*.

2.1.4 Sub-hypothesis 4:

Engaging in sexual activities with different sex partners coupled with intravaginal behaviour associated with sex work are associated with vaginal dysbiosis and inflammation. This is addressed in chapter 7.

2.1.4.1 Objectives

- To assess the impact of multiple sex partners on vaginal dysbiosis and inflammation.
- To determine the impact of semen exposure on vaginal dysbiosis and inflammation.
- To assess the impact of intravaginal behavioral factors on vaginal dysbiosis and inflammation

Chapter 3: Methodology

3.1. Study population

Adolescent girls and young women (AGYW) were recruited from active hotspots in Mombasa Kenya using a two-step approach. In the first step the study team mapped out all the active hotspots in Mombasa registered in the Kenya National bureau of Statistics database which acted as the primary sampling units. Then a multi-stage cluster sampling approach was used to select potential participants from each hotspot as follows:

Stage 1: Creation of a sampling frame was carried out through geographic mapping of adolescent girls and young women that visit hotspot in Mombasa. Active hotspots were selected form the mapped sites and those that were closed were excluded.

Stage 2: Probability proportional sampling approach was used to calculate the number of study participants to be recruited from each of the hotspots.

Stage 3: Community mobilizers for International Centre for Reproductive Health went out to identify possible participants using the inclusion criteria below:

1) Between the age of 14 to 24 years.
2) Has ever had sex.
3) Residing/working within an identified hotspot.

Following informed consent, the study participants self-triaged into 3 grouped based on how they answered an entry level questionnaire. The 3 groups included:

1) Non-sex worker group: AGYW who have experienced their first sex but have not engaged in transactional or commercial sex.
2) Transactional sex worker group: AGYW who have experienced transactional sex. In this case they do expect a gift or award after the sex act, but they do not participate in negotiations before the sex act.
3) Female sex workers: AGYW who have experienced commercial sex and are self-declared sex workers.

In-depth interviews were then administered using a structured questionnaire in English or Kiswahili to capture the socio-demographic, sexual behaviour, STI and HIV testing and treatment data.

3.1.2. Ethics statement

Ethical approval was obtained from the University of Manitoba institutional review board and Kenyatta National Hospital/ University of Nairobi ethics review board. Informed consent was obtained from each study participant before the in-depth interviews and collection of samples. The study was performed according to Helsinki declaration and guidelines.

3.2. General Reagents

PBS was used for diluting the cervico-vaginal supernatant to obtain an adequate concentration which would allow appropriate measurement of ELISA analytes. RNA later was used for preserving cell pellets which were further used for DNA and RNA extraction. Nuclease free water was used to elute RNA samples. Distilled water was used to dilute the wash buffer used to wash off reagents in the imaging assay. Optimum cutting temperature (OCT) embedding solution was used to freeze the tissue sample and to attach the frozen block of tissue samples onto the cutting block of the cryostat.

3.2.1. Bio-Rad reagents and kits

Three different Bio-Rad kits were used to quantify the following cytokines:

- Human Th17 panel (IL17F, GMCSF, IFNγ, IL10, MIP3α [CCL20], IL12P70, IL13, IL15, IL17A, IL22, IL9, IL1β, IL33, IL2, IL21, IL4, IL23, IL5, IL6, IL17E, IL27, IL31, TNFα, TNFβ, IL28α).

- Human cytokine / chemokine panel I (IFNα2, IL12P40, IL1Rα, IL1α, IL7, IL8 [CXCL8], IP10 [CXCL10], MIP1α [CCL3], MIP1β [CCL4], RANTES [CCL5]).
- Human cytokine/chemokine panel III (MIG [CXCL9], ITAC [CXCL11], MIP3α [CCL20], MIP3β [CCL19], IFNλ1).

3.2.2. DNA reagents and sequencing kits

3.2.2.1 Sample homogenization

Cell pellets were homogenized using VWR® Tough Microorganism Lysing Mix tubes (VWR, Pensylvannia-USA).

3.2.2.2 DNA extraction

Extraction of DNA from cell pellets and Urine was carried out using DNeasy Blood& Tissue Kits (Qiagen, Maryland-USA).

3.2.2.3 Sequencing kits and reagents

1. Amplicon PCR was carried out using Platinum™ II Hot-Start PCR Master Mix (Thermofisher Scientific, Massachusetts-USA).
2. PCR product clean-up was carried out using AMPure XP beads (Beckman Coulter, Indianapolis-USA).
3. PCR product quality control was carried out using a D1000 TapeStation kit (Aligent, Santa Clara-USA).
4. Index PCR was carried out using the KAPA HiFi PCR kit (Roche Sequencing and Life Science, Indianapolis-USA).
5. Library quantification was carried out using a Qubit 1X HS dsDNA kit (Lumiprobe Corporation, Maryland-USA).
6. Library denaturation was carried out using the following:

UltraPure™ 1M Tris-HCI (Thermofisher Scientific, Massachusetts-USA).
Sodium Hydroxide [NaOH] (Thermofisher Scientific, Massachusetts-USA).
PhiX Control v3 (Illumina, San Diego- USA).

HT1 Hybridization buffer (Illumina, San Diego- USA).

7. MiSeq cartridge (Illumina, San Diego- USA).
8. Nextera library prep kit (Illumina, San Diego- USA).

3.2.3. RNA reagents and kits

3.2.3.1 RNA extraction

Extraction of RNA from cell pellets was carried out using AllPrep DNA/RNA mini-Kit (Qiagen, Maryland-USA).

3.2.3.2 cDNA synthesis

Reverse transcription of RNA to cDNA was carried out using SuperScript™ IV Reverse Transcriptase (Thermofisher scientific, Massachusetts-USA).

3.2.3.4 Cytokine receptor panel

A 54-analyte open array plate was customized consisting of cytokine receptors of interest measured from data chapter 1. The analytes included:

A. Cytokine receptors of interest (CCR1, CCR2, CCR5, CCR6, IFNαR1, IFNαR2, IFNγR1, IFNγR2, IL10RB, IL10RA, IL1RA, IL6R, IL8RA, IL8RB, IL9R, TNFRSF1α, TNFRSF1β, CXCR3, CCR7, IL1R2, IL1RAP, IL7R, IL2RG, IL21R, IL12RB1, IL1RA, IL1R3, GMCSFRA, IL12RB2, IL23R, IL15RA, IL2RB, IL4RA, IL13RA1, IL13RA2, CXCR3, CXCR4, CXCR7, CXCR2, CCR10).
B. Toll like receptors (TLR2, TLR3, TLR4, TLR5, TLR6, TLR7).
C. Immune markers (CD4, CD68, CD45, CD69, CD25, CD8, CD14, CD38, CD28).
D. Housekeeping genes (18s and GADPH).

3.2.3.5 Secondary PCR

PCR assay to quantify cytokine receptor expression was carried out using TaqMan™ PreAmp Master Mix (Thermofisher scientific, Massachusetts-USA).

3.2.4. Imaging kits and reagents

3.2.4.1 Tissue staining

Development of probes was carried out using RNAscope multiplex V2 fluorescent assay kit (ACDBio techne, California-USA). Staining of the probes was carried out using TSA Plus Fluorophorescent kit (PerkinElmer, Bucks-England). Preservation of the stained slide was carried out using ProLong Gold Antifade Moutant (Thermofisher scientific, Massachusetts-USA).

3.3. Collection and processing of cervical vaginal lavage

Self-collection method was used to collect cervical vaginal lavage. The study participants were given soft cups to insert into the vagina after providing informed consent before the interview session. The sample was collected over a period of 1.5 hours to 2 hours depending on the questionnaire the participant was engaged in after which the participant was given a falcon tube where they placed the SoftCup^{TM} and returned it to the research assistant. The soft cup was transported in a cooler box containing ice packs and cold water to maintain an optimum temperature of 4°C.

In the laboratory the falcon tube was unscrewed, and the soft cup rim pulled up using the broad end of a sterile 1000µl pipette tip as a spatula. The membrane was pushed inside out to allow the content to flow to the bottom of the tube, the SoftCup^{TM} was pushed back into the tube and 50µl of cold PBS was added into the falcon tube. The tube was capped and place in a swinging bucket rotor centrifuge, and centrifuge at 1500 revolutions per minute (rpm) for 5 min at 4°C (brakes off). The SoftCup^{TM} was carefully removed using a sterile 1000µl tip and discarded in a bucket containing Biocide. The tube containing SoftCup^{TM} secretions was recapped and centrifuged at 3000 rpm for 10 min at 4°C (brakes off) to separate the supernatant and the pellet. The supernatant was separated into a labelled cryovial in 50µl batches and diluted 5-fold using cold PBS. The samples were stored in a -80°C freezer.

The bottom of the falcon tube was tapped to disperse the cell pellet and resuspend with 1ml RNA later. The cell suspension was transferred into two pre-labeled cryovial and stored in a 4°C refrigerator overnight then transferred to a -80°C freezer the next day.

3.4. Collection and processing of urine

Midstream urine was collected in labelled urine cups. The samples were stored in a cooler and transported to the lab. Each sample was aliquoted into two 5ml storage tubes and stored in a -20 freezer.

3.5. Cytokine Bead Arrays

We quantified levels of 39 cytokines SoftCup™ supernatants diluted to a ratio of 1:10 using Bio-Rad kits outlined in section 3.2.1 of the methods section. The standard and quality controls were prepared by adding 781µl and 250µl of diluent respectively. The vials were vortexed for 5 seconds and incubated on ice for 30 minutes. The standard was serially diluted fourfold by adding 50µl of the reconstituted standard to 150µl of standard diluent HB the tube was vortexed then 50µl was transferred into another microcentrifuge tube containing 150µl of the diluent and vortexed. This procedure was repeated a further 6 times to create 8 serial dilutions. The coupled beads were vortexed for 30 seconds and diluted to 1x using 5,472µl assay buffer. The beads were vortexed and 50µl transferred to each well of the assay plate. The plate was washed two using a magnetic washer with 100µl Bio-plex wash buffer. The samples and standards were vortexed and 50µl of each was added to the plate. The plate was incubated in a dark room on a shaker for 1 hour at 50rpm. The plate was washed 3 times using 100µl wash buffer.

The antibodies were vortexed 10 minutes and briefly spun down. They were then diluted by adding 2,755µl detection antibody diluent, vortexed and 25µl was added to each well. The plate was covered and incubated in a dark room for 30 minutes at room temperature (RT) while being shaken at 50rpm. The plate was washed again 3 times as described above. The

streptavidin-PE was vortexed and briefly spun down. It was the diluted by adding 5,940µl assay buffer, vortexed again to mix well and 50µl was added to each well. The plate was covered and incubated in the dark for 10 minutes at RT while being shaken at 50rpm. The plate was washed as described above. The beads were then resuspended in 125µl assay buffer. The plate was covered and shaken at 50rpm for 30 seconds. The plate was read using Bio-Rad plate reader.

3.5.1. Data processing

Most (26/39) cytokines had >60% of specimens in the detectable range and were analyzed as log10-transformed continuous variables. The remaining 13/39 cytokines (IL28α, TNFβ, IL21, IL27, IL17E, IL5, IL4, IL2, IL9, IL22, GMCSF, IL17F and CCL19), were analyzed as binary variables. IL1Rα values were above the detection limit in >90% of samples and excluded from further analysis. The lower limit of quantification (LLOQ) was defined as the concentration in which the standard curve co-efficient of variation (CV) was >15%. All values below the LLOQ were imputed as the LOOQ/2. Intra-plate variability was determined using 6 sets of duplicate wells (CV range 9-11%). Inter-assay variation was assessed by running a common sample on each plate (CV range 7-17%).

3.6. Microbiome assay

3.6.1. Sample preparation

Cell pellets were removed from -80°C freezer and placed in a 4°C fridge overnight to thaw. The sample was vortexed. To homogenize the sample 300 µl of the sample and 200ul lysis buffer were added in a cryovial tube containing 0.5mm glass beads from VWR. The sample mixture was homogenized using an Omni bead mill homogenizer using the following cycle program repeated 3 times to ensure total homogenization:

- Speed 5 m/s for 45 seconds
- Rest for 2 minutes

The sample was spun down for 8000rpm for 2 minutes to separate the beads and the sample. The sample was the transferred into a new microcentrifuge tube.

3.6.2. DNA extraction

The extraction process was started by adding 20µl proteinase K to the sample. The sample was vortexed and incubated at 56°C for 10 min. We further added 200µl of 96% ethanol and mixed the sample by vortexing for 30 seconds. The mixture was pipetted into a DNeasy Mini spin column placed in a 2ml collection tube and spun at 8000 rpm for 1 minute. The flow through was discarded and the spin column placed in a new collection tube. We washed the sample by adding 500µl of wash buffer AW1 and spun the sample at 8000 rpm for 1 minute. The flow through was discarded and column placed in a new collection tube. The second wash was carried out by adding 500µl of wash buffer AW2, spun the sample at 14000 rpm for 3 minutes and both the flowthrough and collection tube were discarded. The column was placed in a new 1.5ml microcentrifuge tube, 100µl of buffer AE was added and the sample left to sit at room temperature for 15 minutes to elute the DNA. The column was spun down at 8000 rpm for 1 minute. Concentration was carried out by adding 500µl DNA binding buffer to the sample and mixing by vortexing. We transferred the sample into the provided Zymo-Spin™ Column and centrifuged for 30 seconds and discarding the contents of the collection tube. Washing was carried out by adding 200µl of DNA wash buffer to the column and spun down for 30 seconds. This step was repeat to ensure complete clean-up of the sample. The column was transferred to a clean 1.5 ml microcentrifuge tube and 50µl of DNA elution buffer was added to the column matrix. The sample was incubated for 15 minutes at room temperature and spun for 30 seconds to elute DNA.

3.6.3. Amplicon PCR and sequencing

The 16s primers were prepared by diluting 1µl of the 100µM stock solution in 99µl water. The master mix was prepared in a 5 ml tube using the Invitrogen Platinum II HotStart PCR Master mix Kit by combining the following reagents for 96 samples:

a. 2500 µl of Platinum II Master Mix
b. 1000 µl of GC enhancer

c. 500 µl of 1 micromolar (µM) 16S forward primer
d. 500 µl of 1 µM 16S reverse primer

Approximately 45µl of the master mix was added to each well of an Eppendorf semi-skirted 96 well plate first then 5µl of template was added to each well. Nuclease free water was added to the negative control well and DNA from *E. coli* sample was added to the positive control well. The plate was sealed and placed in a thermocycler with the following program:

a. 94°C for 2 minutes
b. 35 cycles of:

   94°C for 15 seconds

   60°C for 15 seconds

   68°C for 30 seconds

   68°C for 5 minutes
c. Hold at 4°C.

3.6.4. PCR cleanup

Approximately 32.5µl of AMPure XP beads to each well and mixed by pipetting up and down then incubated at room temperature for 5mins. The plate was placed on a magnetic stand until the supernatant cleared then the supernatant was discarded while the plate was still on the stand. Then 200µl of 80% EtOH was added to each well and the plate incubated on the magnetic stand for 30 seconds then the supernatant was discarded. This step was repeated step twice and the excess EtOH was removed with a P20 pipette. The beads were left to airdry while still on the magnetic stand. The plate was then removed from the magnetic stand, and 52.5µl of 10mM TRIS added to each well and mixed by pipetting up and down to resuspend the beads. The plate was incubated at room temperature for 2 minutes and returned on the magnetic plate to allow the supernatant to clear. The supernatant (50µl) was transferred to a new 96-well PCR plate.

3.6.5. Quality control

Quality control was carried out using D1000 TapeStation. The reagents were left to equilibrate to room temperature and the Screen Tapes were added into the TapeStation. Approximately 15µl buffer/5µl ladder (42 samples) were added to the first tube in a tube strip. We transferred 3µl of the buffer and 1µl sample in each well. The tube strips were capped, the plate sealed, while the strip was vortexed for 1 min and spun down. The plate was put in the plate holder and the tube strips were loaded into the machine. The tube containing the ladder was placed in position A and the samples run for 40 minutes. A band of around 550 base pairs (bp) was classified as a positive result.

3.6.6. Index PCR

A master mix for 96 samples was prepared using the following quantities:

a. 500µl 5X HiFi Buffer
b. 75µl 10 mM KAPA dNTP
c. 50µl KAPA HiFi DNA polymerase
d. 2900µl PCR grade water

Approximately 35µl of the master mix was transferred into each well of a semi-skirted 96 well plate while arranging the indices along the sides in numerical order as illustrated in the TruSeq Index Plate fixture. Then 5µl of Index 1 primer was added to each well in each row and subsequently 5µl of the appropriate index 2 primer for each well in each column. Then 5µl of template DNA was added to each well and mixed by pipetting up and down. The plate was with Microseal® A (clear plastic) and spun it down. PCR was performed on a thermocycler with the following program:

a. 95°C for 3 minutes
b. 8-12 cycles of:
    - 95°C for 30 seconds
    - 55°C for 30 seconds
    - 72°C for 30 seconds
    - 72°C for 5 minutes

c. Hold at 4°C

The samples were checked for a fragment size of 630bp using the TapeStation.

3.6.7. Library quantification

Qubit assay was set up for each of the sample by adding 195µl buffer and 5µl sample. The standards were prepared by adding 10µl of Standard 1 and 10µl of Standard 2 into their respective tubes and 190µl buffer was added. The samples and standards were vortexed briefly and measured using a Qubit machine.

3.6.8. Sample normalization

The average fragment size from the TapeStation assay was calculated and the Qubit concentrations were calculated from ng/µl to nM using the following equation:

$$\frac{660\ \text{g/mol} * \text{average library size}}{\text{"Concentration in "}\ \text{ng/ml}} * 10^6 = \text{concentration in nM}$$

To dilute the sample to 4nM, the total volume of the sample was divided by 4 nM to get the amount of sample that would give a concentration of 4nM.

A CSV file was generated by transferring the plate positions with their corresponding diluted concentrations onto the normalization CSV generator. Then the buffer and sample CSV files were transferred onto a USB and loaded it on the EPMotion computer and normalization protocol was started. A pooled amplicon library was created by adding 5µl of each 4 nM normalized sample.

3.6.9. Library Denaturation & MiSeq Loading

To prepare the reagents for the assay, the MiSeq cartridge was thawed in a water bath, heated the heat block to 96°C and prepared an ice bath with 3 parts ice and 1 part water. A 1:9 dilution of 1M TRIS was created by adding 10µl of 1M TRIS in 990 µl water to make a 10 mM TRIS. A dilution of NaOH was made by adding 200µl of 1N NaOH in 800 µl water to make a

0.2N NaOH. Equal volumes of pooled library and 0.2NaOH (5 µl each) were added into a 1.5ml microcentrifuge tube to make a 2 nM library. The library was vortexed, spun down and incubated for 5 minutes at room temperature. To create a 20 picomolar (pM) library, 990 µl HT1 was added to the denatured DNA. The library was further diluted by adding 300µl HT1 to 300µl of the 20pM library to make 10pM loading concentration. The library was inverted, pulse centrifuged and stored on ice. About 60µl of diluted PhiX was added to 540µl of the amplicon library to make up a total volume of 600µl at 10% PhiX concentration. The mixture was incubated at 96°C for 2 minutes, then placed on ice. The flow cell was rinsed with distilled water and dried with Kimwipe to remove dust. The library and PhiX was pipetted into cell 17 on the Miseq cartridge. Index PCR indices and the sample sheet were added into an Illumina expert manager. The flow cell, cartridge and incorporation buffer were loaded into the Miseq, and the run was started.

3.6.10. Bioinformatics

The FastQ file was loaded onto R version 24 and DADA2 was used to check the quality of the forward and backward reads. Trunclean was used to trim the forward reads at 280 base pairs and backward reads at 210 reads. Dereplication was carried out to condense the data by collapsing all the reads that encode the same sequence together and the reads were checked for any errors and merged. An amplicon sequence variant table (ASV) table which consists of a matrix with the sample names and the frequency of the sequence variants was constructed and chimeras removed from the reads. Chimeras are caused by ambiguous sequences which were not removed upstream. A check point was used to assess the number of reads excluded from each step in the pipeline. Taxonomy was assigned by training the data to assign both the genus and species classification using both RDP and SILVA database. An operational taxonomic unit (OTU) table was exported at this step as a table. The OTU table was reloaded into R and converted into a phyloseq object where for each sample the cumulative number of reads was calculated and those with less than 20,000 reads were excluded from further analysis. PHYLOSEQ was used to generate alpha and beta diversity indexes.

3.7. Cytokine receptor assay

3.7.1. RNA extraction

The 167 samples from chapter 1 whose cytokine measurements were available were used in this assay. The cell pellets were thawed in a 4°C fridge overnight. Approximately 150µl of the sample was transferred into a cryovial tube and 350µl of buffer RLT was added, the mixture was briefly vortexed then added into a QIAshredder placed in a 2ml collection tube and spun for 2 minutes at maximum speed. The homogenized lysate was then transferred into am AllPrep DNA spin column placed in a 2ml collection tube and spun at 10,000rpm for 30 seconds. Approximately 350µl of 70% ethanol to the flow-through and mixed by pipetting up and down. The mixture was transferred to and RNeasy spin column and spun it at 10,000 rpm for 15 seconds. The flow-through was discarded and 700µl of buffer RW1was added to the spin column. The sample was spun again at 10,000rpm for 15 seconds, the flow-through discarded, the collection tube tapped on paper towels and returned into the collection tube. For the second wash step 500µl of buffer RPE was added to the RNeasy spin column spun it again at 10,000 rpm for 15 seconds and discarded the flow-through. The step above was repeated and spun the column again at 10,000 rpm for 2 minutes. The RNeasy column was placed in a new collection tube and spun at full speed for 1 minute to eliminate buffer RPE then it was transferred to a 1.5ml collection tube. 50µl of RNase-free water was added to the column. The column was left to sit at room temperature for 15 minutes then later spun down at 10,000rpm for 1 minute. RNA was also extracted from pooled plasma of 5 study participants using the same procedure and used as the positive control for this assay.

3.7.2. RNA clean-up and concentration

Approximately 150µl of nuclease-free water was added to the RNA extract using to bring the total volume to 200µl. We added 100µl of Binding buffer and mixed thoroughly by pipetting the mixture up and down. About 300µl of 96%-100% ethanol was added to the sample and mixed thoroughly. The mixture was transferred to GeneJET RNA purification micro column and spun at 12,000 rpm for 60 seconds. The flow through was discarded and the spin column returned to the collection tube. In the first was step 700µl of wash buffer 1 was added to the spin column and spun at 12,000 rpm for 60 seconds, the flow through discarded and the spin column

returned in the collection tube. Approximately 700µl of wash buffer 2 was added to the column and it was spun for 12,000 rpm for 60 seconds and the flow through discarded. The step was repeated then the empty column was spun for 1 minute to remove any residual wash buffer. The spin column was transferred to 1.5ml collection tube, 10µl of nuclease-free water added and the spin column left to sit at room temperature for 15 minutes. The column was spun at 12,000 rpm for 1 minute to elute the RNA.

3.7.3. RNA quantification

The Nanodrop™ 2000/2000c was used to quantify the RNA and check for purity. The machine was blanked by adding 2µl nuclease-free water on the sample pedestal and the sampling arm lowered. The blanking program was initiated on the spectral measurement using the software on the computer and once the process was complete, the sample arm was raised and wiped both the arm and the pedestal using lint-free laboratory wipe. To measure the samples, we added 2µl of the RNA extract on the sample pedestal, closed the arm and initiated the spectral measurement program. We wiped down both the sample pedestal and arm using lint-free wipes before loading the next sample. This procedure was repeated for all the samples.

3.7.4. Primary PCR

The samples were amplified in batches of 22 including the positive and negative controls. The process was started by creating 2 master mix batches consisting of the following reagents:

Master mix 1

Component	x1 volume	x23 Volume
50 ng/ µl random hexamers	1 µl	23 µl
10 mM dNTP mix	1 µl	23 µl
DEPC-treated water	5 µl	115 µl
Total		161 µl

Master mix 2

Component	x1 volume	x23 Volume
5x SSIV Buffer	4 µl	92 µl
100 mM DTT	1 µl	23 µl
Ribonuclease Inhibitor	1 µl	23 µl
SuperScript™ IV Reverse Transcriptase	1 µl	23 µl
Total		161 µl

The components were mixed using a pipette and 7µl of the master mix was transferred into a pre-labelled 0.2ml PCR strip then 5µl of the samples was added into the tubes and mixed well by pipetting up and down several times. The mixture was heated at 65°C for 5 minutes then transferred the samples onto ice for 1 minute after which the second master mix was added to the reaction and briefly centrifuged. To anneal the RNA, the mixture was at 23°C for 10 minutes first then it was further incubated at 50-55°C for 10 minutes. The reaction was inactivated by incubating it at 80°C for 10 minutes. The amplified RNA was stored at -20°C until primary PCR for all the samples was complete.

3.7.5 Secondary PCR

This assay was carried out in batches of 48 including the positive and negative controls. A master mix was created using the following volumes:

Component	x1	x100
Taqman	5.0 µl	500 µl
Nuclease free water	2.6 µl	260 µl
Total		760 µl

Precisely 7.6µl of the master mix was added to half of the 96 well PCR plate then 2.4µl of the cDNA was added to the respective wells, pipetted up and down to mix the master mix and sample. The plate was spun down at 2000 rpm for 1 minute then transferred to a 334 well plate which was then sealed with a clear PCR plate cover and spun it down at 1000 rpm for 1 minute. The plate was later transferred to the Acufill™ to load the samples onto the open array plate. A CSV with the plate template of 48 samples was created and loaded in the sample folder in the Acufill™ software then the associated serial number of the open array plate was selected. The open array plate and tips were added on their respective sections in the machine, the seal on the sample plate was removed and the program started for the machine to load the samples into the Open array plate. The filled Open array plate was removed and quickly sealed with the cover. A presser was used to tighten the lid and oil was added to the sealed plate to maximum fill using a syringe in the screw opening. A screw was fixed in the screw opening and turned until it broke off. The excess oil was wiped from the surface of the plate with 70% alcohol and dry tissue. The Open array plate was loaded onto the QuantStudio™ 12K flex and the amplification program started.

3.7.6 Data processing

The results were assessed for percentage completeness i.e., detectability and 9 genes that had an undetectability rate of >20% and 5 samples where > 20% of the genes were undetectable were excluded from subsequent analysis. The LLOQ was calculated to fill in values of the genes that had a detectability rate of >80%. The relative expression was calculated by subtracting the CT value of the housekeeping gene from that of the genes measured. The data was the used for downstream analysis.

3.8 Tissue imaging

3.8.1 Cyro sectioning

The tissue blocks were placed in a cryostat chamber and allowed to equilibrate to chamber temperature for 30 minutes. OCT was applied on the metal sample stubs and the mould attached the tissue block facing upward opposite the adherence point. The stab was placed back into the

cryostat and allowed it to freeze. The blade was adjusted to 60µM and sectioned the block until the area with the tissue was reached then re-adjusted to 20µM to cut off thin sections which were mounted on pre-labelled slides.

3.8.2 Preparation of reagents

Wash buffer was prepared by adding 5.88L of distilled water to 2 bottles of prewarmed 120ml RNAscope® wash buffer in a large carboy and mixed well. The probes were warmed at 40°C for 10 minutes in a water bath and cooled to room temperature. C2 and C3 probes were briefly spun and pipetted 100µl of each of the 2 probes into a microcentrifuge tube. Five times volume of the initial probes (500µl) of C1 to the same tube and mixed them. TSA Plus Fluorophores stocks were prepared by diluting them with TSA buffer at a ratio of 1:750.

3.8.3 Staining

To carry out staining 200ml of fresh 10% Formalin was chilled to 4°C in a staining dish and the slides inserted to fix them for 15 minutes at 4°C. The slides were then dehydrated by placing them in 50% EtOH for 5 minutes at room temperature, then they were moved to a staining dish containing 70% EtOH for 5 minutes at room temperature then finally to a staining dish containing 100% EtOH for 5 minutes at room temperature. The last step twice was repeated again. The slides were air for 5 minutes and a hydrophobic barrier was drawn around the sample using a hydrophobic pen and allowed to air dry for 1 minute. A humidifying paper was placed in the HybEZ™ Humidity control tray and distilled water added to make the paper wet.

Approximately 6 drops of RNA scope Hydrogen Peroxide to cover the entire sample sections and the slides incubated on the humidity control tray at room temperature for 10 minutes. The hydrogen peroxide was removed the by flicking the slides and blotting off excess liquid using a paper towel. The slides were immersed in a rack filled with 1X PBS and washed 3 times. The dried slides were placed on the HybEZ™ Slide rack, 5 drops of Protease IV added to cover the sections with the tissue and the slides were incubated in the control tray for 30 minutes at room temperature. After the incubation period excess fluid was removed by flicking the slides, then they were washed with 1X PBS.

To hybridize the probe, slides were placed on the rack and 5 drops of the probe mix added to entirely cover the tissue section. The slide rack was inserted into the tray and incubated in the HybEZ™ oven at 40°C for 2 hours. The slides were then washed in 1X wash buffer for 2 minutes at room temperature twice. The slides were dried and returned onto the rack where 5 drops of RNAscope® Multiplex FL v2 Amp 1 were added to cover each slide. The slides were incubated again in the HbyEZ™ oven at 40°C for 30 minutes then washed the slides in 1X wash buffer as described in the steps above. The slides were returned to the rack and 5 drops of RNAscope® Multiplex FL v2 Amp 2 were added and the slides incubated at 40°C for 30 minutes. The slides were washed in a similar fashion as mentioned in the previous step, excess liquid removed from the slides, and 5 drops of RNAscope® Multiplex FL v2 Amp3 were added. The slides were incubated in the HybEZ™ oven at 40°C for 15 minutes, washed using 1X wash buffer in the steps outlined above and dried.

To develop the signals starting with HRP-C1 signal, 5 drops of RNAscope® Multiplex FL v2 HRP-C1 were added to cover the slides. The slides were incubated at 40°C for 15 minutes and washed the slides using 1X wash buffer for 2minutes at room temperature and repeated the wash step using fresh wash buffer. The slides were dried, 150 µl TSA Plus fluorescein added to each slide and incubated at 40°C for 30 minutes. The wash steps were repeated as outlined above, the slides dried, and 5 drops of RNAscope® Multiplex FL v2 blocker was added to cover the tissue sections. The slides were incubated in the oven for 30 minutes at 40°C and washed using the wash steps outlined above and excess liquid was removed from the slide using a paper towel.

To develop the second signal, 5 drops of RNAscope® Multiplex FL v2 HRP-C2 and the slides incubated for 15 minutes at 40°C. The slides were washed twice in 1X wash buffer for 2 minutes during each round, excess fluid removed from the slides using a paper towel, then 150 µl TSA Plus Cyanine 3 was added to each slide. The slides were incubated at 40°C for 30 minutes and the wash cycles repeated as described above. The blocker was added, and the slides incubated for 15 minutes at 40°C. Washing step with 1X wash buffer was repeated and finally added 5 drops of RNAscope® Multiplex FL V2 HRP-C3 to the slides and incubated the slides for 15 minutes at 40°C. The slides were washed and dried, then added 150 µl TSA Plus Cyanine

5 to each slide. The slides were incubated for 30 minutes at 40°C, the wash cycle was performed then 5 drops of the blocker were added. The slides were incubated at 40°C for 15 minutes, washed and dried as described above. The slides were counterstained by adding 4 drops of DAPI, the slides were incubated the at room temperature for 30 seconds. The DAPI was tapped out immediately and added 1-2 drops of ProLong Gold Antifade Moutant added. A cover slip was added on the slides, and they were left overnight in the dark.

3.8.4 Visualization of slides

The slides were visualized using confocal microscope by placing the slide inverted (side containing the sample facing down) onto the slide deck. The light path to the computer was blocked allowing only the light path to the eye piece to be visible. The DAPI light channel to focus the sample manually and once the nuclei of the tissue samples were visible, the light path was switched to the computer to continue with automated focusing and images taken.

3.9 Detection of sexually transmitted infections in urine

3.9.1 DNA extraction from urine

The urine samples were thawed overnight in a 4°C fridge. The samples were vortexed to mix them well. Approximately 300µl of the sample was transferred to a 1.5ml microcentrifuge tube. DNA extraction process detailed in section 3.6.2 was used to isolate DNA from the urine samples. Starting sample size was 1213 and sample exclusion as illustrated in figure 3 below was carried out due to failed reactions at various steps.

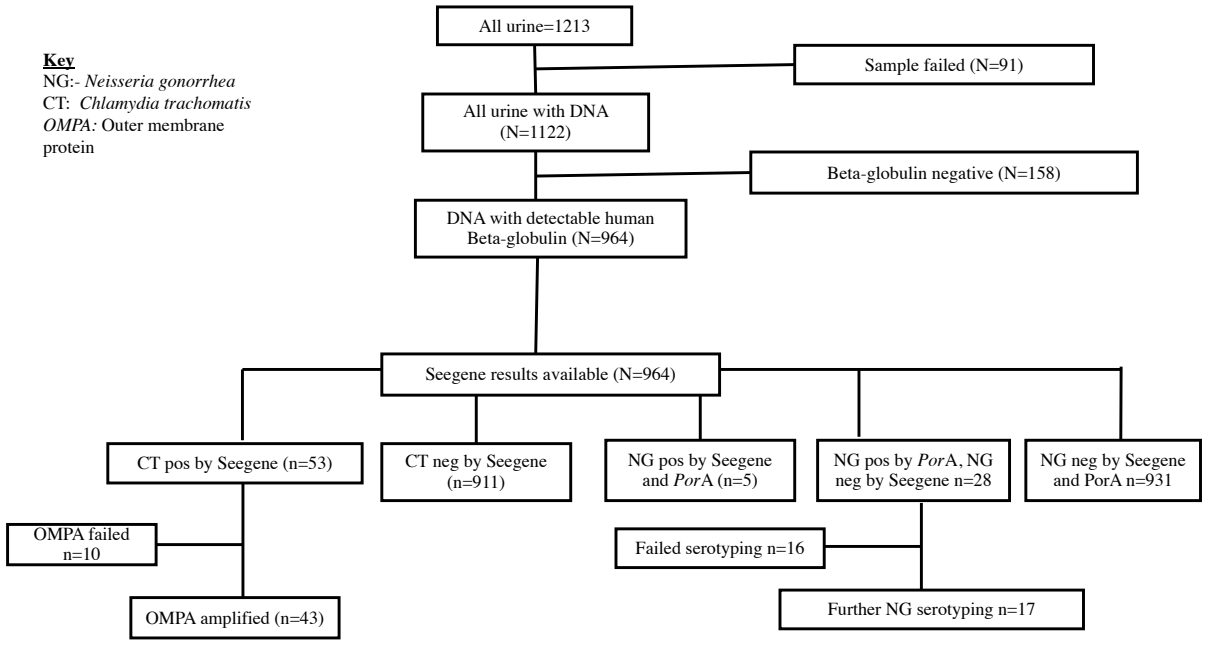


Figure 3. Consort diagram of STI sample processing

The figure above illustrates the sample steps due to various failed reactions and assays during the sample processing steps.

3.9.2 Detection of human β-globin gene to screen for DNA quality

Amplification of the human β-globin gene was carried out on all samples to confirm the presence of viable DNA in the extracts. The following primers with an expected output of 268 base pairs were used; HG1: 5' CAACTTCATCCACGTTCACC 3' and HG2: 5'GAAGAGCCAAGGACAGGTAC 3' with the following master mix preparations for each sample:

Component	Volume	Concentration
HG1	1.5 µl	0.5 mM
HG2	1.5 µl	0.5 mM
PCR Buffer	2.5 µl	

$MgCl_2$	5.0 µl	2.0 mM
dNTP's	5.0 µl	0.2 mM
Promega GoTaq Flexi Taq	0.15 µl	0.75 unit
Sterile H_2O	5 µl	
DNA sample	5.0 µl	
Total Volume	25 µl	

The following thermocycler parameters were used:

Step	Number of cycles	Temperature	Duration
Hot start	1	94°C	15 min
Cycles	40	94°C	0.5 min
		63°C	1.5 min
		72°C	1.5 min
Final extension		72°C	10 min
Hold		4°C	4 min

The results were detected using TapeStation as described in section 3.9.2 and samples with negative β-globin results were excluded from further assays.

3.9.3 Detection of sexually transmitted infections using Seegene Anyplex II

Samples with positive human β-globin results were considered fit for further assay and tested for STI using the Seegene kit. PCR master mix was prepared by combining the following reagents:

Component	x1	X96
5X STD6 ACE PM	4.0 µl	384 µl
STD IC	0.5 µl	48 µl

8- MOP Solution	2.5 µl	240 µl
2X Multiplex Master Mix	10 µl	960 µl
Total		1632 µl

The tube was then mixed by inverting up and down then briefly centrifuged. An aliquot of 17µl was transferred to each well on a PCR plate and 3µl of the sample was added to the respective wells. Water was used as a negative control while STD6 ACE PC was used as a positive control and 3µl of the controls were added to the respective wells. A total of 20 µl of sample and master mix was in each well. The plate was placed in a GeneAmp® 9700 PCR System preheated to 94℃. The following thermocycler schedule was used to carry out the amplification:

Step	Number of cycles	Temperature	Duration
Hot start	1	94℃	15 min
Cycles	40	94℃	0.5 min
		63℃	1.5 min
		72℃	1.5 min
Final extension		72℃	10 min

Detection was carried out using the TapeStation™ system. The ladder was prepared by adding 8µl of the ladder in a 0.2ml PCR tube. The samples were prepared by adding 2 µl of the PCR product and 6 µl of the loading buffer in individual 0.2ml PCR tubes the tubes were then vortexed then spun down. The ScreenTape DS12 was placed in the TapeStation system and tips added to the respective sections of the machine. The samples and ladder were added to the sample block and the program started. The results were visualized using the Screenplex™ software that shows the presence of a pathogen by positive sign (+) and negative by a negative sign (-) against the sample ID.

3.9.4 *Neisseria gonorrhea* sequence typing and antimicrobial resistance testing.

DNA extracted in section 3.9.1 of samples with a positive human β-globin was used to carry out this analysis. To identify single nucleotide polymorphism (SNPs) using whole genome sequencing library prep was carried out using Nextera DNA library preparation Kit (Illumina, San Diego, CA). The resulting reads were assessed then joined using Flash and assembled with SPAdes. Prokka was used for annotation whereby the average number of contigs selected was 147. Forwards and backward reads from each sample were joined and trimmed. The poor-quality reads were filtered using trimClean.pl. Mapping of the resulting high-quality reads was carried out using SMALT version 0.7.4 on to the *Gonorrhea* reference genome. The following criteria was used to exclude problematic variants; predicted phages, genomic islands, repetitive regions and 10 suspected highly recombinant regions. PhyML was used create a maximum likelihood tree of the core single nucleotide polymorphism positions. PhyoPart was used to assign the clades where a percentile distance value of 0.10 was used for all the clades except F.

Antimicrobial testing was carried out using *N. gonorrhea* multi antigen sequence typing (NG-MAST) PCR whereby the following primers were used in a nested PCR assay *porB* and *tbp*B (Supplementary table 1a). The master mix for the primary PCR was carried out using the following reagents and thermocycler conditions:

Component	x1	Concentration
2X TaqMan	12.5 µl	
*por*F1		900 nM
*por*F2		900 nM
*tbp*BF1		900 nM
*tbp*BR1		900 nM
Probe		250 nM
Template DNA	5 µl	1 ng/ µl
Total	25 µl	

Thermocycler conditions:

Step	Number of cycles	Temperature	Duration
Pre-heating	1	60°C	30s
Denaturation	1	95°C	10 min
Cycles	40	95°C	15s
		60°C	1 min
Final extension		60°C	30s

Similar reagent quantities, concentration and thermocycler conditions were used for the secondary PCR where *por*F2, *por*R2, *tbp*BF2 and *tbp*BR2 primers were used. For detection of cephalosporin sensitivity, the following primers were used in a single PCR reaction *por*A, *tbp*B, *pon*A, *por*B, *mtr*R and *pen*A (Supplementary Table 1b) with similar reagents and thermocycler concentration as listed above used in a one-step PCR reaction.

The results were analyzed and a quantification cycle value of 40 coupled with a relative fluorescence of >0.7 for *por*B was considered a positive result. The detection limit was arrived at by a 10-fold diluted *N. gonorrhea* control strains F62, WHO L and WHO K and amplifying the isolates in duplicates to record the lowest dilution that produced a positive result. Sensitivity and specificity were defined as follows: sensitivity is the percentage of DS containing SNP of interest while specificity is the percentage of susceptible isolates containing wild type (WT) allele. The following categories were designed for the isolates:

Status	Criteria 1	Criteria 2	Calculation
True positive (TP)	High minimum	SNP detected by	Sensitivity=TP/(FN+TP)
False positive (FP)	Low MIC	SNP detected by	*100
True negative (TN)	Low MIC	WT result	Specificity=TN/(FP+TP)
False negative (FN)	High MIC	WT result	*100

3.9.5 *Chlamydia trachomatis* sequence typing and antimicrobial resistance testing.

A nested PCR assay was used to amplify *C. trachomatis* serotypes. PCR master mix was made by combining the following reagents for each sample:

Component	x1	Concentration
2X KAPA HiFi HotStart	12.5 µl	
CT1 Primer	0.75 µl	10µM
CT5 Primer	0.75 µl	10µM
DNA lysate	5 µl	
DNAse/RNAse free water	6 µl	
Total	25 µl	

The following primary PCR conditions were used:

Step	Number of cycles	Temperature	Duration
Hot start	1	95°C	2 min
Cycles	35	98°C	20sec
		58°C	1 min
		72°C	2 min
Final extension		72°C	10 min
Hold		4°C	Infinity

Secondary PCR was carried out using the same reagent quantities tabulated above with different primers [D1 and D2 (Sequences in supplementary table 1)] of the same concentration, quantities and the same PCR conditions outlined above. Clean-up of PCR amplicons was carried out using a 1.8X Ampure clean-up kit as elaborated in section 3.6.4. Sequencing was carried out by adding 2.0 µl master of BigDye Sequencing Master Mix, 1.0 µl (Forward sequencing primer) 1.0 µl of D2 primer (Reverse sequencing primer) and 1 µl of the cleaned amplicon onto a PCR plate. The plate was briefly spun down and loaded onto a thermocycler for 35 cycles each consisting of the following schedule: 95°C for 12 seconds and 60°C for 55 seconds. Clean-up

was carried out using a 1.8X Ampure clean-up kit. Capillary electrophoresis was carried out by setting up the capillary array, adding the polymer and Sequencing Buffer into the ABI 3500 genetic analyzer to run. The data was then analyzed using the Sanger analysis modules to determine the serotypes.

3.10 Statistical Analyses

Statistical analysis for all the chapters was carried out using SPSS versions 24 and 25, MetaboAnalyst and R where applicable.

3.10.1 Impact of sex work on the microbiome in the female genital tract

Baseline characteristics were compared using analysis of variance (ANOVA) and χ^2 tests for continuous and categorical variables, respectively. Microbiome-related variables were defined in 3 main ways: (1) as categorical based on unsupervised hierarchical clustering[118]; (2) alpha diversity as a continuous variable, defined as above; and (3) beta diversity as continuous variables, including the first 3 principal coordinates in principal coordinates analysis. Hierarchical clustering of microbiome profiles was performed based on the Euclidean distance matrices, and the relative abundance of each genus within individual samples was generated using the Ward linkage method, which was visualized in a heatmap generated using MetaboAnalyst 3.0[162]. Spearman rank correlation was used to analyze associations between continuous microbiome, cytokine, and sexual activity variables. In certain cases where the data were non-normally distributed, such as the number of sex acts in the past week and time since the last sex, data were categorized and analyzed as predictors of alpha diversity in ANOVA, including Dunn post hoc test. Multiple comparisons were adjusted using False Discovery Rate (FDR) method of Benjamini and Hochberg, with False Discovery Rate (Q) = 5%. We used multivariable linear regression analyses to determine associations between the vaginal microbiome and study group, modelled as 3 categories, with alpha diversity as the outcome variable. Covariates including age, condom and contraception use, HIV and STI status, number of sexual partners in the past week, and practicing vaginal douching were included in some models. These were predetermined based on their potential to impact the mucosal milieu, and/or

their association with a study group. *P* values of $P < 0.05$ (two-sided) were considered statistically significant.

3.10.2 Correlation between cytokine receptor and bacterial vaginosis

We calculated the lower limit of quantification for each gene and used the value to impute the missing data of samples that had >80% of the genes detected. Relative expression (-2ΔCT) was calculated by subtracting the CT value of each gene from the CT value of the housekeeping gene and the result was used for downstream analysis. Unsupervised hierarchical clustering of cytokine receptor expression by microbiome groups was carried out using Euclidean distance matrices and visualized using MetaboAnalyst 3.0. We further used ANOVA with Tukey's post hoc tests to assess the difference in cytokine receptors between the microbiome groups. We created a statistical model to assess the impact of the combination of a lactobacillus dominant microbiome and elevated or down-regulated cytokine receptor expression on immune markers. We first stratified the participants into *Lactobacillus* dominance and non-dominance groups and used the median of cytokine receptors of interest to create high and low groups to assess the effect of specific cervicotype coupled with elevated or downregulated receptors on immune marker expression using ANOVA.

3.10.3 Impact of intravaginal behaviour associated with sex work on STIs.

We carried out descriptive analysis to summarize the participant demographics, frequencies of behaviour associated with sex work and frequency of STIs within the different groups. We further created a variable to capture recent STI symptoms reported by the participant in the last 3 months and assessed the frequency of their distribution between the groups. To assess the effect of behaviour associated with sex work on sexually transmitted infections we carried out univariate analysis and multivariable analysis with individual infections as the outcome variable. We picked predictor variables that have been previously associated with increased risk of STIs including alpha diversity, number of vaginal sex acts in the last week, number of partners in the last week, age, number of unprotected acts in the last week, belonging to the female sex worker or transactional group. After running a univariate analysis, we carried out a multivariate analysis by stepwise addition of each of the predictor variables to assess

whether they have an individual influence on each other. We assessed the overall frequency of *N. gonorrhea* subtypes and drug resistance profiles in the whole population without segregating them into different groups due to their low numbers. We also assessed the frequency of *C. trachomatis* subtypes in the different venue types.

3.10.4 Impact of group on the microbiome in the female genital tract

We compared the baseline characteristics of the study participants using ANOVA and Chi-square tests. We further assessed how individual biobehavioral variables impacted alpha diversity using ANOVA for categorical variables and Spearman's correlation for continuous variables. We defined microbiome-related variables as described in section 3.10.1. Previous studies related to the microbiome of the female genital tract have associated both inflammation and increased risk of HIV with having either a *Lactobacillus* dominant or non-*Lactobacillus* dominant state, we created a new variable that clustered individuals into the 2 groups. In this cohort we have detailed bio-behavioural epidemiological variables that include different types of sex partners; spouses, first-time paying clients, first-time paying clients, regular non-client sex partners and first-time non-client sex partners whose number of sex episodes that were both protected and unprotected were recorded, douching practices and their frequencies, use of substances to prepare for sex and their frequency of use and contraceptive use. We used these variables to assess their association with diverse microbiome we calculated the risk using univariate analysis which estimates the individual impact of an epidemiological variable on the outcome variable of interest. We further carried out a multivariate model in which there was stepwise addition of epidemiological variables to assess how the interaction of each variable impacts the output variable. To further query the interaction of the epidemiological variables and vaginal dysbiosis increased the risk of HIV we carried out causal modelling to assess the directionality of risk.

CHAPTER 4: Sex work is associated with increased vaginal microbiome diversity in young women from Mombasa, Kenya

4.1 Introduction

HIV is often transmitted across genital mucosa, where the virus must infect susceptible HIV target cells[163]. Before HIV can access these cells, the virus must first survive mucosal defences and cross the robust mucosal barrier that prevents HIV transmission during most exposures[164]. Genital inflammation defined by elevated inflammatory cytokines in genital secretions has been associated with HIV acquisition risk[165] and reduced efficacy of vaginally applied tenofovir[166]. However, the causes of genital inflammation remain poorly defined. Contributing upstream drivers likely include variables that have been associated with both increased HIV risk and elevated cytokines; these include sexually transmitted infections (STIs), abrasion, contraceptives, semen exposure, dysbiosis and vaginal douching[167,168].

In addition to the above, recent studies suggest that genital inflammation is strongly associated with bacterial communities dominated by anaerobes, such as *Gardnerella* and *Prevotella* spp., amongst many others[169–171]. These organisms thrive in the absence of healthy *Lactobacilli* (non-iners) spp., which if present help to maintain lactic acid production and a low vaginal pH. Microbiologically, this is often defined as bacterial vaginosis (BV), a condition that overlaps with molecularly-defined BV, and has been associated with an increased risk of HIV acquisition[110,172]. A recent meta-analysis estimated that Nugent-defined BV affects almost one-third of women in Sub-Saharan Africa[173], including asymptomatic presentation in up to 85% of cases[174].

Despite the well-defined association between BV and HIV risk, there are limited data on the influence of sex work and sexual activity on the composition of vaginal microbiota and resulting inflammation. One study of 32 adult women from Baltimore showed that sexual activity had a moderate influence on the composition of vaginal microbiota, independent of the menstrual cycle phase[133]. Multivariable analyses have shown that condom use, early sexual debut, age and history of STIs are correlated with molecular-BV (non-*Lactobacillus* dominance as defined by bacterial sequencing)[175]. However, few studies have focused on young women engaged in sex work, despite their increased risk of HIV acquisition[176,177]. More studies are required in female

sex workers (FSW) to capture the full spectrum of sexual activity, in the context of other variables, and its association with vaginal microbiome and inflammation profiles. In addition, most mucosal studies have focused on women ≥18 years of age, and therefore female genital tract immunology in younger women is poorly understood. The main objective of this study was to analyze the relationship between vaginal microbial community composition and mucosal immune milieu in young women in Kenya, including a subset <18 years of age, with varying patterns of sexual activity including a sub-group who self-identified as FSWs[178].

4.2 Results

4.2.1 Participant characteristics

Study participants included self-identified FSW (n = 72), transactional sex (n = 30), and non-FSWs (n = 66, Table 1). The median ages of FSW, transactional, and non-sex worker participants were 20 (IQR: 18-22), 19 (IQR: 17-21), and 20 (IQR: 18-22) years, respectively (p=0.081). A subset of participants (18%) were <18 years of age. Approximately one-third of participants had completed primary school. The number of sexual partners differed by study group, with FSW, transactional, and non-sex workers having medians of 6 (IQR 3-11), 1 (IQR 0-4), and 0 (IQR 0-1) sexual partners last week, respectively (p<0.001). The prevalence of HIV among FSW was 12.5% (9/72), 6.6% among the transactional group (2/30) and 4.5% among the non-sex worker group (3/66). Similar proportions had ≥1 bacterial STI, with 43.0% (31/72), 30.0% (9/30), and 31.8% (21/66) for FSW, transactional, and non-sex workers, respectively. The most prevalent STIs were *Mycoplasma hominis* and *Ureaplasma urealyticum* at 21 and 22%, respectively.

Table 1. Participant characteristics

	Non-FSW	Transactional	FSW	P value
N	66	30	72	
Age, median (IQR)	20 (18,22)	19 (17,21)	20 (18,22)	-
HIV status (n/N)	4.5% (3/66)	6.6% (2/30)	12.5%	-
Completed primary school	31.8% (21/66)	40.0% (12/30)	31.9%	-
Number of partners in the last	0 (0,1)	1 (0,4)	6 (3,11)	<0.001

Vaginal sex acts in the last	0 (0,1)	2 (0,6)	7 (3,17)	<0.001
Time since last sex in days,	15 (4, 60)	9 (1,30)	2 (1,4)	<0.001
STI positive (n/N)	31.8% (21/66)	30.0% (9/30)	43.1%	-
DMPA use	12.1% (8/66)	20.0% (6/30)	23.6%	-

4.2.2 Microbiome profiles including those associated with molecular-BV.

16S rRNA sequencing was used to determine the relative abundance of cervicovaginal bacteria at the genus and/or species level. Unsupervised hierarchical clustering categorized the operational taxonomic units (OTUs) into five clusters (Figure 4A). Because these were highly similar to what was defined by Anahtar et al., we employed a similar naming system. *Lactobacillus* spp. were further differentiated into *L. iners* and *L.* non-*iners*, reflecting the biological differences within this genus[170]. Cluster 1a (7%) was a mixture of *L. iners* and *L.* non-*iners* spp., while Cluster 1b (8%) was composed primarily of *L.* non-*iners*. Cluster 2, which accounted for 22% of the sample, was composed primarily of *L. iners*. In combination, Cluster 1a, 1b and 2 accounted for 37% of study participants (62/166). Bacterial communities associated with BV, including those dominated by *Gardenella* spp. (Cluster 3) and *Prevotella* spp. (Cluster 4) were characteristic of 63% (104/166) of participants. Principal coordinates analysis (PCoA) was used to compare beta diversity between clusters (Figure 4B). Consistent with unsupervised clustering, these data highlight the increased genetic diversity of Clusters 3 and 4.

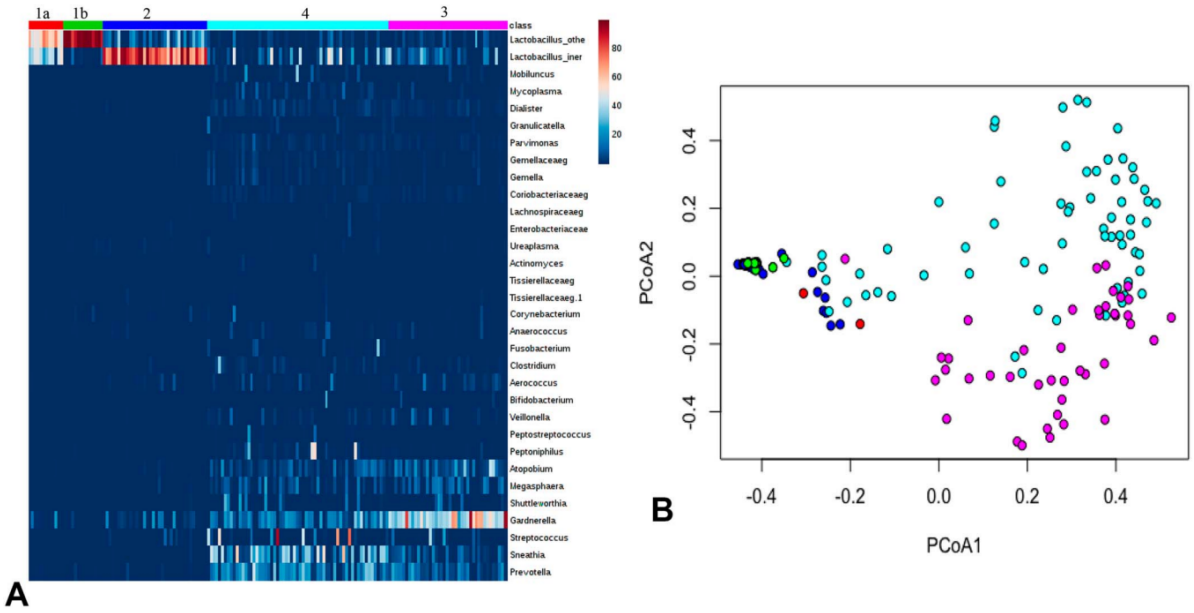


Figure 4. Cervicovaginal microbiota profiles in adolescent girls and young women from Mombasa, Kenya.

A) Unsupervised clustering of operational taxonomic unit (OTU) abundance into microbiome cluster. The heatmap is colour-coded by % abundance, with the name of each cluster summarized on the top of the graph. B) Principal coordinates analysis (PCoA) using unifrac distances between individuals. The colours in B) correspond to the clusters indicated in A), with communities dominated by *Prevotella* spp. (cyan bar/dots), *Gardenella* spp. (pink bar/dots), *Lactobacillus iners* (blue bar/dots), *Lactobacillus* non-*iners* (green bar/dots), and mixed *Lactobacillus* spp. (red bar/dots).

4.2.3 Associations between microbiome clusters and cervicovaginal cytokine expression

We next carried out a principal component analysis on cytokine concentrations. A large proportion (60% by PC1 and PC2) of the variance in cytokine levels was explained by microbiome clustering (Figure 5A). Comparing cytokine expression between different microbiome clusters identified several significant associations, including IFNα2, CCL5, IL-7, IL-6, IL-1α, IL-1β, TNF-α, CXCL10, and CXCL11 ($p<0.001$, ANOVA, Figure 5B). Most cytokines were present at higher concentrations in cluster 3 and 4, while chemokines CXCL10 and CXCL11 had the opposite trend. In support of this, we found modest correlations between alpha and beta diversity and higher cytokine concentrations: IL-10, IL-1α, IFN-γ, IL-13, IL-12p40, IL-7, IFN-α2, IL-15, IL-12p70, IL-1β, IL21, IL-23 and TNF-α ($p<0.05$, Table 2). In contrast, inverse

correlations were observed between alpha and beta diversity and a number of chemokines, including CXCL10, CXCL11, CXCL9, CCL20, and CCL19 ($p<0.05$).

Table 2. Spearman correlation between cytokines and alpha and beta diversity, ranked from strongest positive to strongest negative (for alpha diversity).

	Alpha diversity				Beta diversity		
Cytokine*	r	p-	q-	Associated	r	p-	q-
IL-12p40	0.390	<0.001	0.002	Yes	0.434	<0.001	0.002
IFN-α2	0.376	<0.001	0.002	Yes	0.442	<0.001	0.002
IL-13	0.325	<0.001	0.002	Yes	0.408	<0.001	0.002
TNF-α	0.318	0.001	0.002	Yes	0.343	<0.001	0.002
IL-23	0.317	0.001	0.002	Yes	0.297	0.001	0.002
IL-12p70	0.301	0.001	0.002	Yes	0.246	0.008	0.010
IL-1α	0.295	<0.001	0.002	Yes	0.393	<0.001	0.002
IL-15	0.289	0.002	0.003	Yes	0.315	0.001	0.002
IL-7	0.269	0.001	0.002	No	0.263	0.002	0.003
IFN-γ	0.254	0.006	0.008	No	0.203	0.03	0.032
IL-10	0.246	0.008	0.010	Yes	0.202	0.032	0.032
IL-21	0.184	0.05	0.053	No	0.285	0.002	0.003
IL-1β	0.128	0.174	0.174	Yes	0.222	0.017	0.019
MIP-3α	-0.229	0.014	0.016	Yes	-0.370	<0.001	0.002
MIP-3β	-0.271	0.004	0.006	Yes	-0.370	<0.001	0.002
IP-10	-0.290	0.001	0.002	Yes	-0.540	<0.001	0.002
MIG	-0.336	<0.001	0.002	Yes	-0.264	0.005	0.006
ITAC	-0.381	<0.001	0.002	Yes	-0.592	<0.001	0.002

* Modeled as $\log_{10}$ concentrations, only cytokines that were significant for at least one of the diversity measures are shown.

**Multiple comparisons adjustment using False Discovery Rate (FDR) method of Benjamini and Hochberg with FDR (Q)=5%

*** Each cytokine was log10 transformed and modeled as an outcome in multivariable linear regression, adjusted for alpha diversity of the vaginal microbiome, STI, HIV, age, number of sexual acts, DMPA, and study group.

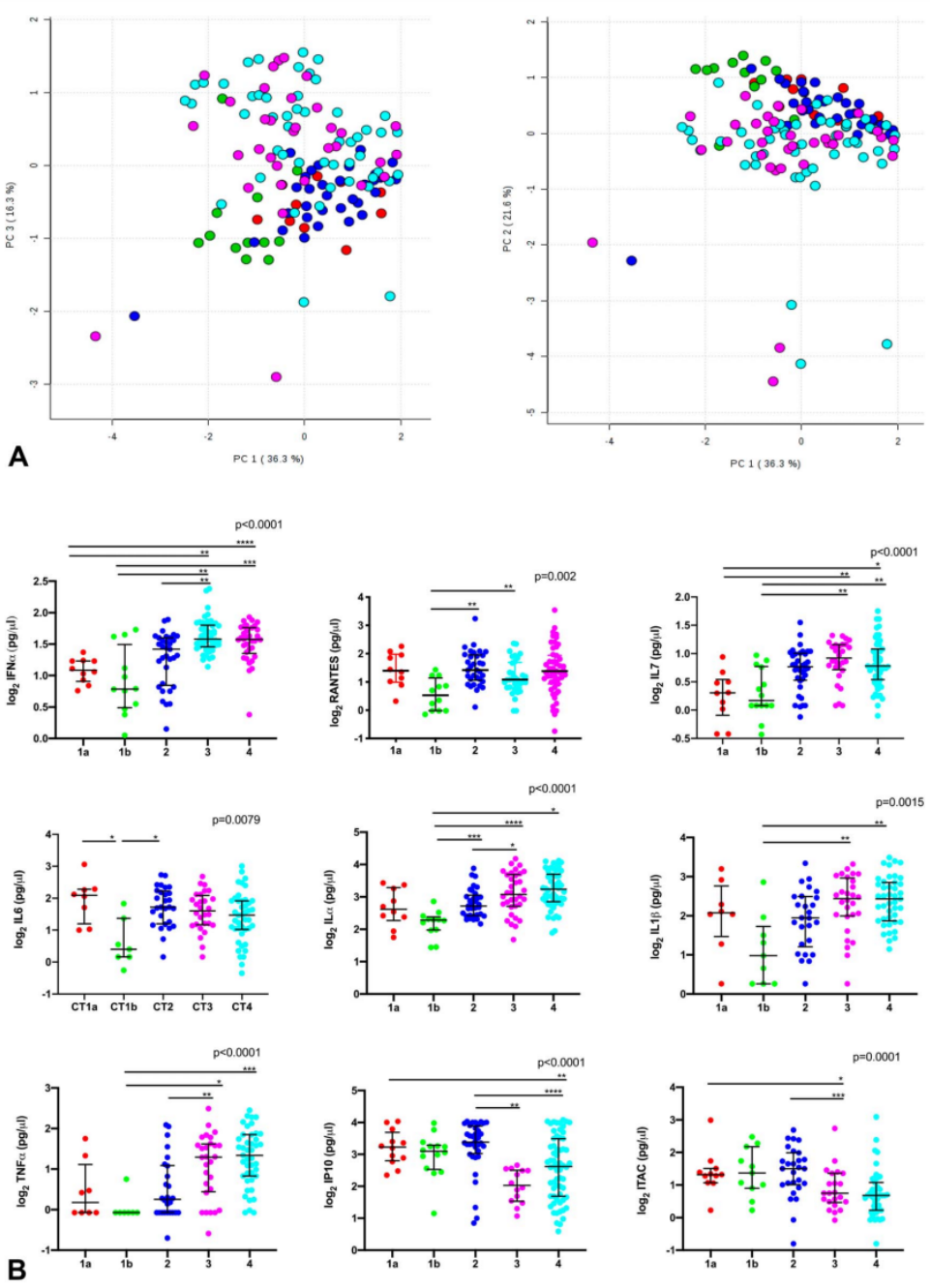


Figure 5. Relationship between vaginal microbiota and cervicovaginal cytokine/chemokine concentrations.

A) Principal component (PC) analysis showing unsupervised clustering of cervicovaginal (SoftCup) cytokine/chemokine variation by microbiome cluster. Each dot represents an individual colour-coded by cluster from Figure 4 and Figure 5A. The % variance captured is shown on the

axes for each principal component. B) Chemokine and cytokine concentrations in SoftCup fluid from individuals grouped by microbiome cluster. Lines indicate the median and interquartile range (IQR) for each dataset. Groups were compared using Kruskal-Wallis test with Dunn's post-hoc analyses.

4.2.4 Effect of age on microbiome diversity

Age did not correlate strongly with microbiome cluster, although a trend was observed for cluster 1b being more common in younger participants (ANOVA p=0.053). No association was observed between age and alpha diversity (beta = -0.012, 95% CI: -0.082 to 0.059, p=0.748, Table 3 and Figure 6). Similarly, no associations were observed between age at sexual debut or years of sexual activity and any microbiome cluster. There was an inverse trend between alpha diversity and age of sexual debut (ANOVA, p=0.0726), and women who had been sexually active for 6-10 years had higher alpha diversity compared to women with 1-5 years of sexual activity (Figure 6B). These data suggest that age, age at debut, and duration of sexual activity make relatively minor contributions to vaginal microbiome composition in the young women enrolled in our study.

Table 3. Multivariable linear regression analysis of variables associated with vaginal microbiota alpha diversity.

	Univariate analysis		Multivariate analysis	
Parameter	Exponentiated	P	Exponentiated	P value
Female sex worker	0.53 (0.16 – 0.90)	0.010	0.47 (0.05 – 0.90)	0.030
Transactional	0.28 (-0.20 – 0.76)	0.257	0.26 (-0.22 – 0.74)	0.288
Non-sex worker	Ref		Ref	
Age	-0.01 (-0.08 – 0.06)	0.748	-0.04 (0.11 – 0.03)	0.254
Any unprotected sex	-0.02 (-0.11 – 0.07)	0.661	-0.05 (0.15 – 0.06)	0.352
DMPA use	0.09 (-0.34 – 0.52)	0.677	0.01 (-0.44 – 0.46)	0.965
HIV positive	0.79 (0.45 – 1.44)	0.018	0.01 (-0.44 – 0.46)	0.015
STI positive	0.79 (0.45 – 1.12)	<0.001	0.80 (0.46 – 1.14)	<0.001
Number of acts last	0.00 (-0.02 – 0.02)	0.942	-0.01 (-0.04 – 0.03)	0.687
Practices vaginal	0.18 (-0.16 – 0.52)	0.306	0.15 (-0.19 – 0.49)	0.376

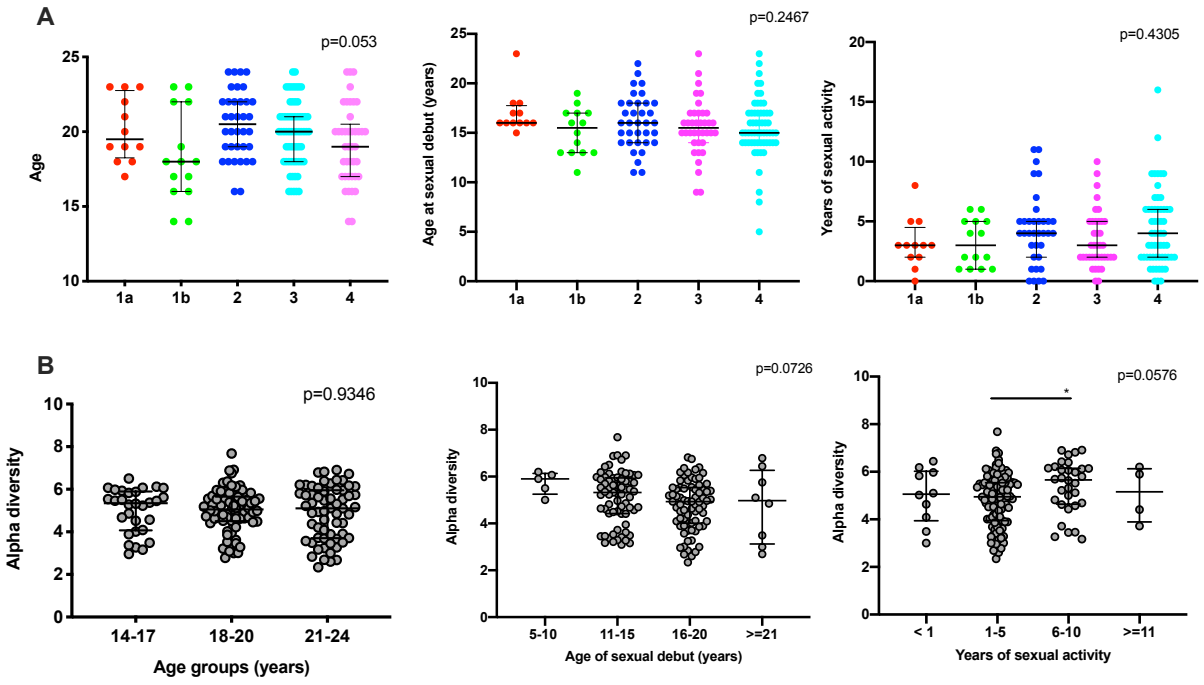


Figure 6. Effect of age and sexual behavior on vaginal microbiota.

A) Differences in age (years), age at sexual debut (years) and years of sexual activity between different CSTs groups (colour -coded as in Figure 1). B) Differences in alpha diversity between different age groups (14-17, 18-20 and 21-24 years of age), age of sexual debut (grouped as 5-10, 11-15, 16-20 and >=21 years of age) and years of sexual activity (grouped as <1, 1-5, 6-10 and >=11 years). Lines indicate the median and interquartile range (IQR) for each dataset. Groups were compared using Kruskal- Wallis test with Dunn's post-hoc analyses.

4.2.5 Effect of demographic and behavioral characteristics on microbiome profile

We next assessed whether self-identifying as an FSW was associated with vaginal microbiome alpha diversity in linear regression analysis (Table 3). Compared to the non-sex worker group, both the FSW and transactional group had higher alpha diversity. This was statistically significant for FSW vs non-FSW (beta=0.53, 95% CI: 0.16 to 0.90, p=0.01) but not non-FSW vs transactional (beta=0.28 95% CI: -0.20 to 0.76, p=0.257). The presence of an STI (p<0.001) and HIV infection (p=0.015) were also associated with alpha diversity. In multivariable linear regression analyses adjusted for age, DMPA use, unprotected sex, number of partners in the last week, vaginal douching, and HIV and STIs, alpha diversity remained significantly higher in the FSW group (beta=0.47, 95% CI: 0.05 to 0.90, p=0.03, Table 3). Similar results were obtained when the association between study group and alpha diversity was adjusted for time

since last sex.

We carried out bivariable analyses to assess whether the association between FSW and alpha diversity may be mediated by frequency of sex and/or rate of partner change. The number of sex acts with all partners and the number of partners in the past week both correlated with alpha diversity across all groups (Spearman rank correlation $r=0.215$, $p=0.006$ for number of sex acts; $r=0.206$, $p=0.008$ for number of partners). We then compared alpha diversity with the number of sex acts within each study group (Figure 7A). In general, alpha diversity increased with the number of sex acts, but this was most evident in the non-FSW who had 0 compared to 1-10 sex acts in the last week (ANOVA $p=0.04$). We further assessed the role of condom use on microbiome diversity, stratified by group (Figure 7B). Similar alpha diversity was observed for "always", "sometimes" and "never" condom users, although these participants generally all had higher alpha diversity than those who did not have any reported sex acts in the last week. In support of the finding that any sex in the past week was associated with alpha diversity, we observed a similar association between time since last sex and increased alpha diversity (ANOVA $p<0.001$, Figure 7C). These data suggest that both the amount and recentness of sexual activity may be upstream drivers of the association between the vaginal microbiome and engagement in sex work and/or transactional sex, leading to changes in the composition of vaginal microbial communities and resulting inflammatory profiles that have been associated with increased HIV acquisition risk.

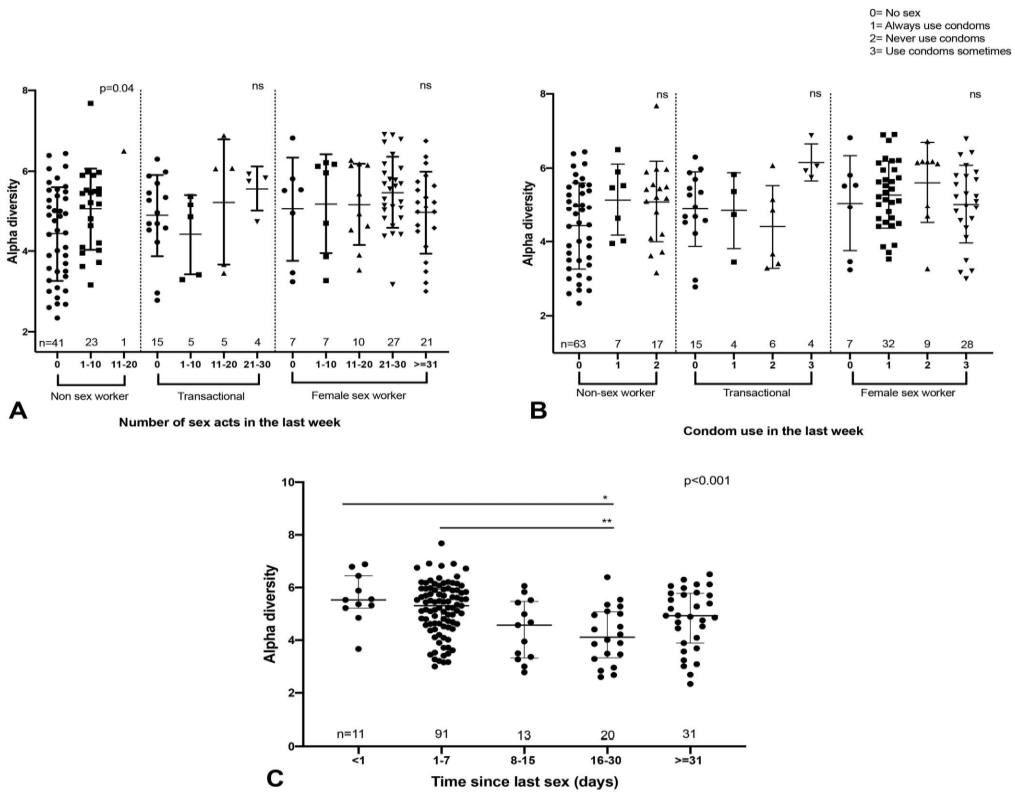


Figure 7. Association between recent sexual activity and alpha diversity.

A) Differences in alpha diversity within study groups (non-sex worker, transactional and FSW) based on number of sex acts in the preceding week. Number of sex acts are either 0 if no sex in the last week or grouped 1-10, 11-20, 21-30 and ≥ 30. B) Differences in alpha diversity within study groups based on condom use pattern in the preceding week, coded as 0 if no sex, and in categories of always, never, and sometimes using condoms. C) Differences in alpha diversity based on time since last sex, categorized as < 1, 1-7, 8-15, 16-30, and >30 days since last reported sex act. Lines indicate the median and interquartile range (IQR) for each dataset. Groups were compared using Kruskal-Wallis test with Dunn's post-hoc analyses.

4.3 Discussion

The cervicovaginal environment is an important determinant of HIV acquisition risk[179]. In sub-Saharan Africa, where two-thirds of global HIV transmission occurs, young women and FSW represent two important key populations for focusing HIV prevention[177]. Biological risk in these populations is incompletely understood, particularly in FSW who are new to sex work. Here we present a detailed characterization of the HIV-risk associated vaginal micro- environment in young women, including those <18 years of age. Our results show that self-reported sex work and recent sexual activity correlate with increased vaginal microbiome diversity, which in turn

explains a high proportion of the variance associated with cervicovaginal cytokine levels, including those associated with HIV acquisition[166,168]. This is not to say other variables, including STIs, don't also have strong cytokine effects. Similar to other studies[180], we observed reversed trends for pro-inflammatory cytokines such as IL- 1a and IL-1b, and chemokines such as CXCL10, which were increased and reduced in women with molecular-BV, respectively. Many factors may influence cytokine levels in the female genital tract; however, our study, in agreement with others, suggests that the commensal bacteria in this mucosa represent a dominant influence on cytokine milieu.

Many studies have demonstrated that molecular- and/or Nugent-BV is common in young women of African descent. A small proportion (<20%) of our participants had *Lactobacillus* non-iners spp. thought to be "optimal" for protection against HIV. This is in line with a study of South African women where only 14% carried this "healthy" microbiome[138]. Replication of this finding is important, demonstrating its generalizability to multiple regions of Africa[174,181,182]. Whether this high rate of molecular-BV is due to differences in genetic, environmental and/or behavioral factors, including diet, antibiotic use, and contraception methods, requires further research. The observation that BV has been associated with increased HIV risk and adverse reproductive outcomes underscore the need for HIV risk reduction efforts to include better methods to reduce inflammation and restore *Lactobacillus* dominant microbial communities in young women at high risk of HIV[99,173,183].

When certain microbial communities become established in the vaginal mucosa is difficult to ascertain, and this was not possible in the current cross-sectional study. However, the similar distribution of microbiome clusters and alpha diversity across all ages in our study (14-24 years) suggests that diverse vaginal microbiomes are common in young women under 18 years, in line with suggestions that the vaginal microbiome is likely "set" very early in life during puberty[184,185]. The extent to which a certain microbiome cluster is inherent to individual women remains unclear, but our data suggest that further investigation of host genetic associations with vaginal microbiota profiles is warranted[186].

Sexual activity is an important influence on the vaginal microbiome. Previous studies have shown that reporting a new sexual partner is associated with BV incidence[187]. A study by Ravel et al that sampled women frequently showed that sexual activity was one of the few consistent

associations of microbial diversity[133]. In our study sex work was associated with molecular-BV; this could be due to increased sexual activity, as supported by recent sex correlating with alpha diversity. While the sex work-microbiome association was independent of recent sex, sexual exposure is likely an important driver of this association. This is supported by the fact that the recent sex-alpha diversity association was strongest in the non-sex worker group. This might indicate that very few sex encounters can increase the incidence of molecular-BV, with additional sexual encounters having minimal effects. Although we controlled for many potential confounders in multivariable analysis, it remains possible that factors in addition to sexual activity, such as the type of partners, condom use, and/or intravaginal practices, also contribute toward FSWs having increased alpha diversity. Our results are in agreement with one previous study that observed an association between the vaginal microbiome and sex work[187]. In our study we were statistically underpowered to assess the correlation of the different sexual partners and alpha diversity. Other studies have noted that in women who have sex with men, their partner's penile microbiome is associated with the women's risk of incident BV. Presumably sex increases the risk of BV through the exchange of genital bacteria during intercourse, and sex with men specifically due to presence of anaerobic bacteria on the penis coronal sulcus[188]. Male circumcision reduces the abundance of anaerobic bacteria, leading to lower BV incidence in women with circumcised male partners[189] . There is a higher correlation between the species of microbiota between penile and vaginal samples from couples when the women have BV[190]. This suggests that the penile microbiota might have a significant effect on a partner's cervicovaginal microbiota. Semen has also been found to alter the immune profile in the female genital tract. In the CAPRISA 008 cohort where they assessed presence of human Y-chromosome detection in women (YcDNA) showed that the marker was associated with 11/48 cytokines they measured especially those of pro-inflammatory nature like IFNγ, IL12p70 and IP-10. In this cohort they also found that semen was associated with *P. bivia* {O.R, 95% CI:1.954, (1.312 – 2.911), p=0.006}. YcDNA in this study was associated with elevated levels MMP2 and TIMP4 which are markers of extracellular matrix damage[191]. MMP2 degrades the basement membrane of the epithelial matrix which facilitates migration of neutrophils and leukocytes therefore activating inflammation[192]. TIMP4 is a regulator of MMP2 therefore its elevated levels may be due to its efforts to regulate MMP2[191,192].

BV is also impacted by other exterior factors like condom use, douching and friction from sexual acts. Douching with water was associated with alpha diversity in our study (p=0.036) and we found a weak trend with douching using store bought products (p=0.079). Douching had been found to be a common practice worldwide being reported by approximately 29% - 92% women worldwide despite it being against medical advice[193]. Reasons mainly given by women for douching include its ability to enhance cleanliness, it's found to be socially acceptable and it is considered as a way of maintaining cleanliness during menses and after sex[193,194]. Douching has been associated with adverse health outcomes including cervical cancer, preterm birth, and bacterial vaginosis as it alters the physiology of the tissues in the FGT. It has also been associated with some of the commonly occurring sexually transmitted diseases e.g., the odds of having *C. trachomatis* were found to be 2.29 times higher among women who reported that they douched at any given point 12 months before visiting a clinic compared to those that did not report any douching in a study carried out among women in U.S.A[195]. We did not find any significant correlation between use of substances to prepare for sex and BV. Other studies found weak trends between use of substances to tighten the vagina and vagina flora {O.R, 95% CI:1.29, (0.98 – 1.711), p=0.072}[196]. We postulate that the use of foreign substances in the female genital tract work by physically displacing the normal vaginal flora and altering the pH therefore creating a suitable environment for other bacterial populations to establish colonies. Most of the foreign substances that are introduced during these practices are composed of chemicals which may be aggressive on the epithelial cells thus changing the composition of the cells and the barrier integrity. Confirmation of these theories by the use of mice models would make a significant contribution to the field towards understanding the chemical processes that are changed by these practices.

Our study had some limitations. Although our sample size was relatively small, we selected specimens randomly from a larger study that used population-representative sampling[178], increasing the generalizability of the findings. A larger study would be required to better understand how diversity of partner types (casual vs regular, paid vs unpaid, etc.), volume, and the importance of condom and/or semen exposure influence these microbiome associations. Sexual activity was self-reported via face-to-face interviews and therefore subject to social-desirability bias and under-reporting. The intention of this analysis was to profile the most

abundant bacterial communities in our study population rather than a more detailed examination of bacterial function. However, for the vaginal microbiome, clear groupings by abundance category are evident, and are highly associated with immunological profiles including those that predict increased HIV acquisition risk[166,197].

In conclusion, our data show that molecular-BV is common in young Kenyan women and may be an important driver of HIV risk-associated inflammation. Epidemiologically, this key population has amongst the highest HIV incidence rates in the region, emphasizing the need to better understand their risk profiles. Our study covers a wide range of sexual frequency, with many measures of sex work and/or recent sex correlated with molecular- BV. A key finding is that sex work exposure itself may increase the proportion of women with an HIV susceptible phenotype. This could be highly relevant to HIV transmission; if sex work induces microbiota changes, this could increase the likelihood of infection if the next sexual partner is HIV infected and viremic. These data further underscore the urgent need for better strategies to reduce the burden of BV, particularly in young women in eastern and southern Africa.

CHAPTER 5: Characterization of cytokine receptors in the cervicovaginal cells.

5.1 Introduction

The female genital tract is colonized by microbial populations that provides protection against new infections, in part by creating and maintaining a hostile, high pH environment[198]. Change in bacterial populations from lactobacillus to non-lactobacillus dominance leads to a condition known as bacterial vaginosis that has been associated with increased risk to sexually transmitted infections and negative reproductive health outcomes[199,200] . In sub-Saharan Africa, microbial dysbiosis affects approximately 25-80% of women. Although treatment with metronidazole is often efficacious, this is associated with a recurrence rate of approximately 40% within 12 months of treatment[53,180,201]. Several bio-behavioral factors including douching, sex work, and recent sexual activity have been found to be associated with diverse vaginal microbiome populations by diagnosis of both Nugent method and molecular-BV[101,202,203].

The epithelial layer is the first line of defence against invading pathogens. It provides a physical barrier through the tight junctions, intracellular pathogen receptors, antigen presentation, secretion of cytokines and chemokines and production of antimicrobial factors[204–206] . Epithelial cell cultures have been found to inhibit growth of *Candida albicans* and *Neisseria gonorrhea* without having any impact on *Lactobacillus crispatus* which is an optimal commensal microbiome[205] . Toll-like receptors (TLRs) which are expressed in Antigen Presenting Cells (APCs) are located in close proximity to epithelial cell environment, detect different components of microbes, and stimulate an appropriate immune response through cytokine production. This enhances maturation of different immune cells inducing an adaptive immune response[198,207,208] . Detection of mRNA transcripts of TLRs 1-9 are evidence that they are expressed in different sections of the female reproductive tract and in different disease states[209,210] . In the case of HIV exposed individuals, Leister et al found differential expression among women with different stages of disease where there was upregulation of TLR6, TLR7 and TLR8 transcripts among women with CD4 counts less than 200 cells/ml and higher elevation of TLR2, TLR3 AND TLR4 mRNA in women classified as having AIDs compared to uninfected an indication that infection impact expression of TLRs[211]. Benjelloun et al showed that stimulation of different TLRs increases production of some cytokines like IL6, GMCSF, receptors like IL2R and IL1RA, and chemokines like CCL4[212] .

Numerous studies have shown that individuals with molecular-BV have increased levels of pro-inflammatory cytokines that may underpin BV's increased risk of HIV acquisition[110,181,213] . Fewer studies have assessed BV's impact on expression of cytokine receptors, toll-like receptors, and immune marker transcripts in the female genital tract. All cytokines signal to immune cells via their cytokine receptors, which are typically cell-surface glycoproteins that allow the transduction of signals resulting in immune responses. Changes in the physiology and function of these receptors can have downstream impact on the functions of the immune system[214,215] . Genetic deficiencies in cytokine receptors have been associated with primary severely combined immunodeficiencies which impacts susceptibility to some diseases. Mutations in IL2Rα have been associated with poor T-cell functioning while deficiencies in IFNγR1 have been associated with loss of responsiveness to IFNγ[216] . Cytokine signalling is crucial to the functioning of the immune system, and it impacts multiple downstream immunological processes. Cytokine receptor downregulation is common way in which the immune system dampens its signaling and this inhibits cytokine expression as without attachment to receptors signalling cannot occur.

There are two types of cytokine receptors: type I and type II, both of which signal through the Janus kinases and signal transducers, and activators of transcription (JAK-STAT) pathway. One important difference is that cytokines in the Type I family utilize additional ligands including an alpha chain, beta chain, gamma chain, gp130 or homodimeric receptor to complete signalling[217,218] . In this study we quantified expression levels of a wide range of cytokine and toll-like receptors (TLR) in cervical vaginal immune and epithelial cells to determine how different vaginal microbiome communities impact their expression.

5.2 Results

5.2.1 Study participants

A total of 96 participants were included in this analysis: 34 non-sex workers, 19 in the transactional sex group, and 43 female sex workers. The median age of non-sex workers was 20 (16-22); for the transactional group, 18 (17-20); and for FSW group, 20 (19-23). Approximately three-quarters of all study participants (73.9%) completed primary school. Approximately 15%

of participants (14/96) had a sexually transmitted infection. Prevalence of HIV was 7.29% (7/96, Table 1). More than half of the participants (54.1%) practiced vaginal douching. Lack of use of condom use was reported among 37.8% of the participants who had sex last week.

Table 4. Characteristics of the study participants in the cytokine receptor sub-study

	Non-FSW	Transactional	Female sex	p value
N	66	30	72	
Age, Median (IQR)	20 (18,22)	19 (17,21)	20 (18,22)	-
HIV status (n/N)	4.5%	6.6% (2/30)	12.5% (9/72)	-
Completed	31.8%	40.0%	31.9%	-
Number of partners in the last week of sex	0 (0, 1)	1 (0, 4)	6 (3, 11)	<0.001
Vaginal sex acts in the last week,	0 (0, 1)	2 (0, 6)	7 (3, 17)	<0.001
Time since last sex in days, median (IQR)	15 (4, 60)	9 (1, 30)	2 (1, 4)	<0.001
STI positive (n/N)	31.8%	30.0% (9/30)	43.1%	-
DMPA use	12.1%	20.0% (6/30)	23.6%	-

FSW: female sex worker; IQR: interquartile range; DMPA: depo-medroxyprogesterone acetate; STI: sexually transmitted infection; HIV: human immunodeficiency virus

5.2.2 Associations between receptor expression and their ligands.

We carried out bivariate analyses to assess whether there is an association between cytokines and their receptors. There was no correlation between most of the receptors we and their ligands. IL1αa and IL1βb which were both positively correlated with IL1R1 (r=0.285**, p=0.005 and r=0.256*, p=0.013 respectively) whereas MIP1βb was inversely correlated with CCR1 (r= -0.247*, p=0.015, Table 5). Both IL7 and IL9 were negatively correlated with IL2RG.

GMCF was negatively correlated with GMCFRα and IL2 was also negatively correlated with IL2Rβ.

Table 5. Association between cytokines and their respective receptors.

Ligand	Receptor	P value	R value
TNFα	TNFRSF1α	0.121	-0.161
	TNFRSF1β	0.709	-0.309
IFNα2	IFNαR1	0.676	0.043
	IFNαR2	0.945	0.007
IL1α	IL1R1	0.005	0.285**
	IL1RαP	0.068	0.188
	IL1R2	0.502	-0.069
IL1β	IL1R1	0.013	0.256*
MIP1β	CCR1	0.015	-0.247*
MIP3α	CCR6	0.878	0.016
MIP3β	CCR7	0.837	0.022
ITAC	CXCR3	0.869	-0.017
MIG	CXCR3	0.509	-0.069
IP10	CXCR3	0.870	-0.017
IFNγ	IFNγR1	0.195	0.135
	IFNγR2	0.907	-0.012
IL10	IL10R α	0.670	-0.045
	IL10R β	0.529	-0.066
Ligand	Receptor	P value	R value
IL7	IL7R	0.665	0.045
	IL2RG	0.019	-0.238*
IL21	IL2RG	0.249	-0.120
IL9	IL2RG	0.641	-0.049
IL12P70	IL12Rβ1	0.027	0.228*
GMCF	GMCFRα	0.142	-0.152
IL2	IL2Rβ	0.036	-0.216*

5.2.3 Microbiome associations with receptor expression

We'd previously used hierarchical clustering to classify the OTUs into 5 microbiome cervicotypes; mixed *lactobacillus spp* (CST 1a*), Lactobacillus non-iners* (CST 1b)*, Lactobacillus iners* (CST 2), *Provetella spp* (CST 3) and *Gardenella spp* (CST 4)[101]. Most participants had diverse microbial populations with 40.6% (39/96) having primarily *Provetella spp* and 26.6% (25/96) having primarily *Gardenella spp*.; the remainder had various combinations of various *Lactobacillus* spp. Further clustering of cytokine receptor expression by microbiome groups showed that participants who had *Provetella* -dominant and *Lactobacillus non-iners* cervicotypes (CST) had down regulated expression of most of their cytokine receptors (Figure 8A and B).

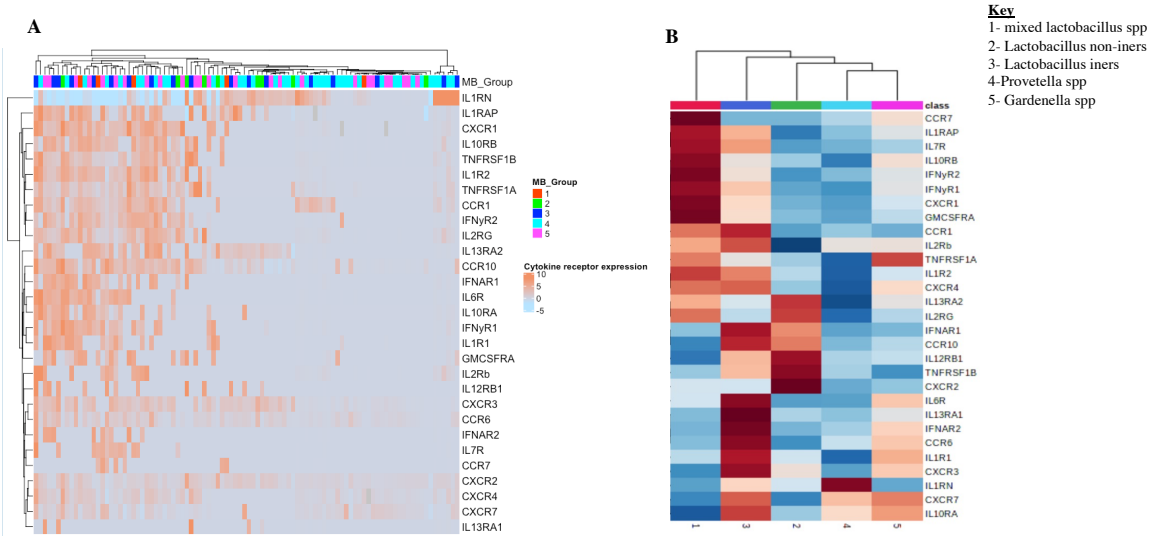


Figure 8. (A and B). Heatmap representing the relationship between cytokine receptor expression and microbiome groups.

Cytokine receptor expression was significantly upregulated among women with lactobacillus dominant microbiome (CST 1 and 3) while it was significantly downregulated in women who had a diverse microbiome (CST4 and 5) and women with *Lactobacillus* non-*iners* bacterial populations.

We further tested associations between individual CSTs and cytokine receptor expression and found that CXCR3, CXCR2, CCR10, CCR7, IFNAR2 and TLR6 were differentially expressed amongst the different CSTs. These receptors were downregulated among the non-

Lactobacillus dominant *spp* (p<0.05). CXCR3, IFNAR2 and CCR10 were downregulated in participants with *Provetella spp* compared to those with *Lactobacillus iners* (p=0.005, 0.022 and 0.014). CXCR2 was highly down regulated among participants with CST 3 and CST 4 compared to participants with CST1b (p=0.005 and p=0.019 respectively). For CCR7, the biggest difference was between CST 1a and CST 2 (p=0.033) (Figure 8C).

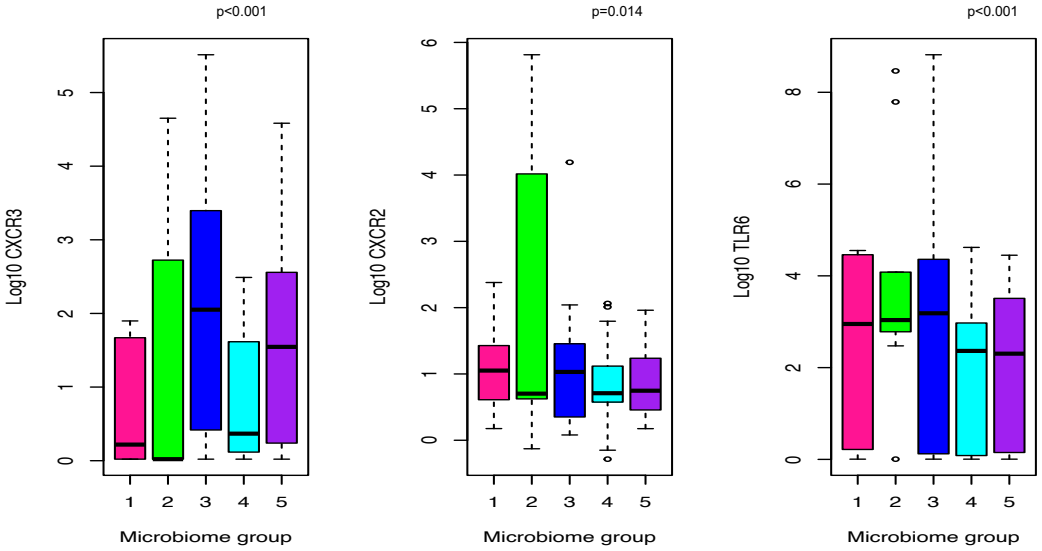


Figure 9. Receptors expression in microbiome groups.

Expression of CXCR2 was downregulated among participants colonized by non-*Lactobacillus* dominant bacterial populations (*Provetella spp* and *Gardnerella spp*). A similar observation was noted with TLR6 whereas the expression of CXCR3 was significantly down regulated in *Lactobacillus* non-*iners* compared to *Lactobacillus iners.*

5.2.4 Effect of behavior associated with sex work on receptor expression.

We further assessed if any epidemiological variables correlated with receptor expression, particularly receptors that were associated with molecular BV. We found positive correlations between time since last sex and IFNαR1, IL7R, GMCSFRα, IL6R, IL1RAP, IFNαR1, TNFRSF1β, TLR2 and TLR4 (p<0.05) an indication that more recent sex is associated with increased receptors. Current age was positively correlated with IL1R1 (p<0.05) thus younger individuals had more IL1R1. We found a positive correlation between age and IL1R1 (p=0.025, r= 0.229*) (Table 6). Since we did not observe any correlation when number of partners and sex

acts in the last week of sex were assessed individually, we related a composite variable of the two and found that there was a negative correlation with IFNgR1, IFNgR2, IFNαR1, CXCR1, IL10Rb, TNFRSF1b, IL2RG, GMCFR α, IL7R, TLR2 and TLR4 ($p<0.05$) (Table 6). An indication that less partners and sex events in the last week of sex is associated with decreased levels of these cytokines. Having a non-*Lactobacillus* dominant microbiome was associated with downregulated CXCR3, CXCR2, CCR10, CCR7(not shown) and IFNAR2(not shown) expression ($p<0.05$) (Figure 9a). An indication that molecular BV is an important factor in dictating the immune environment of the female genital tract. Being in the FSW and transactional group was associated with lower expression of CXCR3, CCR10, IL10Rb and IL6R (not shown), TLR2(not shown) ($p<0.05$) (Figure 9b).

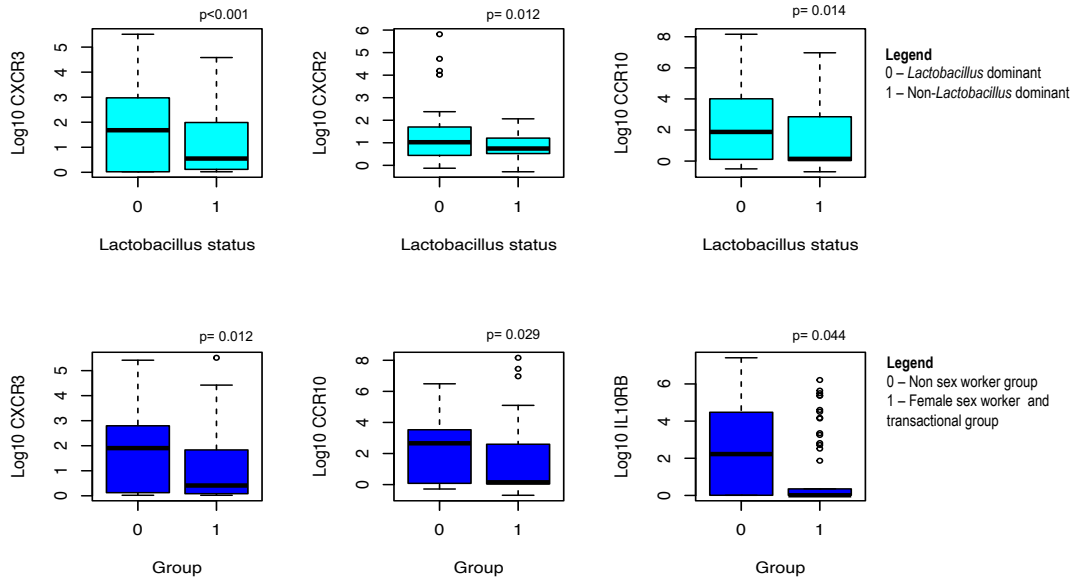


Figure 10 (A and B). Cytokine receptor expression in different microbiome status and group. A) Participants with a non-lactobacillus dominant status have elevated levels of CXCR3, CXCR2 and CCR10. B) Participant in the non sex worker group similarly have elevated levels of CXCR3, CCR10 and IL10Rb.

Table 6. Correlation between variables associated with sex work and receptors.

	Number of partners*Number of sex		Time since last sex	
Receptor	P value	R	P value	R
IFNγR1	0.012	-0.255*	0.038	0.219*
IFNγR2	0.033	-0.217*	-	-
IL10Rβ	<0.001	-0.346*	-	-
TNFRSF1β	0.008	-0.270**	0.10	0.270*
IL2RG	0.007	-0.274**	0.010	0.270*
GMCFRα	0.042	-0.208*	0.037	0.220*
IL7R	0.001	-0.331**	<0.001	0.433*
TLR2	0.001	-0.325*	<0.001	0.351*
TLR4	0.039	-0.211*	0.012	0.263*
CXCR1	0.041	-0.211*	-	-
IFNαR1	0.020	-0.237*	0.005	0.296*
IL1RAP	-	-	0.044	0.214*
IL6R	-	-	0.003	0.310*
IL10Rβ	-	-	0.002	0.315*
Il13Rα2	-	-	0.048	0.209*

5.2.4 Effect of a *Lactobacillus* or non-*Lactobacillus* dominant microbial state and cytokine receptor expression on immune markers.

Given the strong microbiome-cytokine receptor associations, we stratified the participants according to their *Lactobacillus* status which has been shown to be associated with increased pro-inflammatory immune cells in the female genital tract by previous study done by our group (Chapter 4) and by other researchers. We further correlated the expression of cytokine receptors and immune markers in the female genital tract. In this case, since we do not have immune cell measurements, we have used gene expressions of specific parental markers like CD3, CD14, etc.

as proxies of immune cell expression[219–221]. In participants who had had a non-*Lactobacillus* dominant microbiome we observed a positive correlation between some of the immune markers and cytokine receptors e.g., CD4 and receptors; IFNAR1, IFNαR2, CCR6, CCR10, CCR7 ($p<0.05$), CD45 and CXCR2 ($p=0.001$, R=0.391**). One of the interesting observations is that most of the receptors were negatively correlated with CD14 (Figure 10). Among the participants with Lactobacillus dominant microbiome fewer correlations which were positive were observed. CD4 was positively correlated with CXCR3, CCR10, CCR6; CD14 with CXCR2 and CXCR4, CD68 and GMCFSRα, CD69 and CCR10 and GMCFSRα, while CD25 was positively correlated with most of the cytokines; IFNα R1, CXCR2, CXCR4, CCR10 and TLR6 ($p< 0.05$).

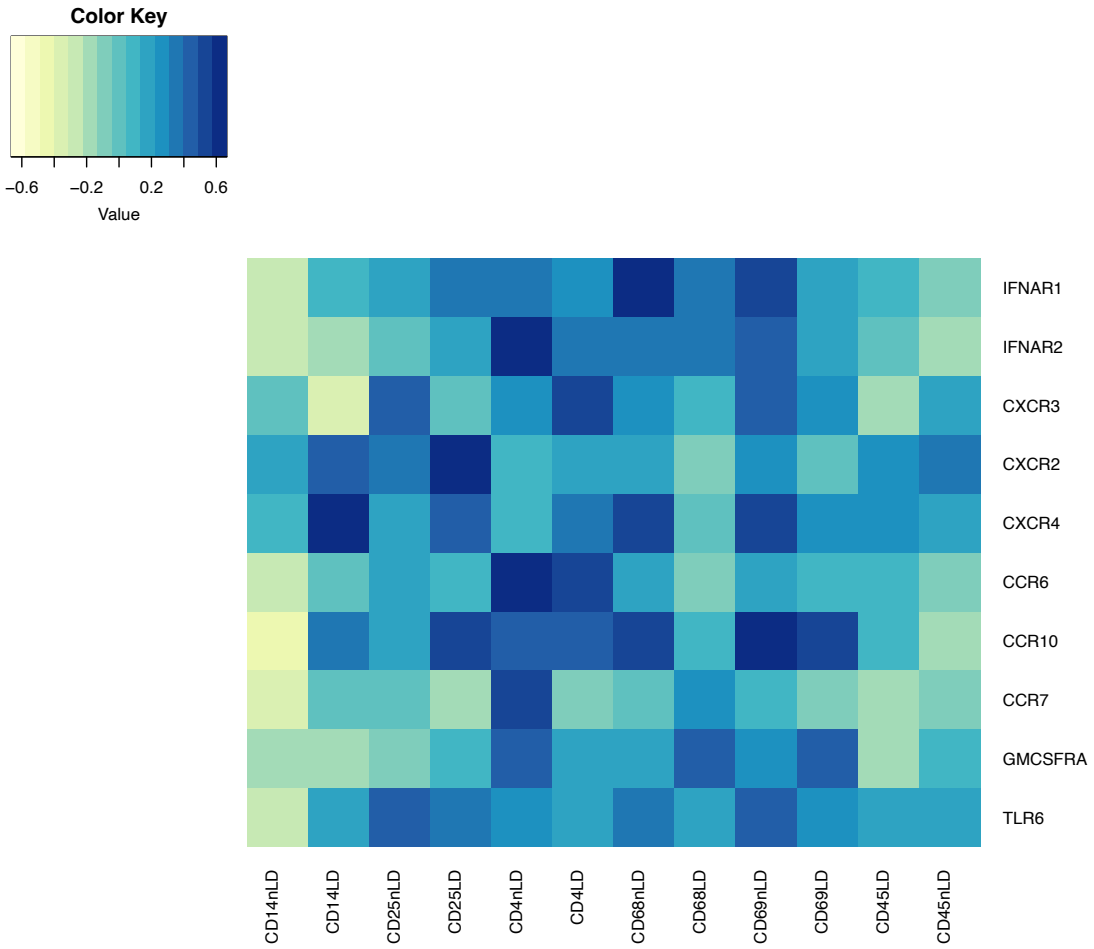


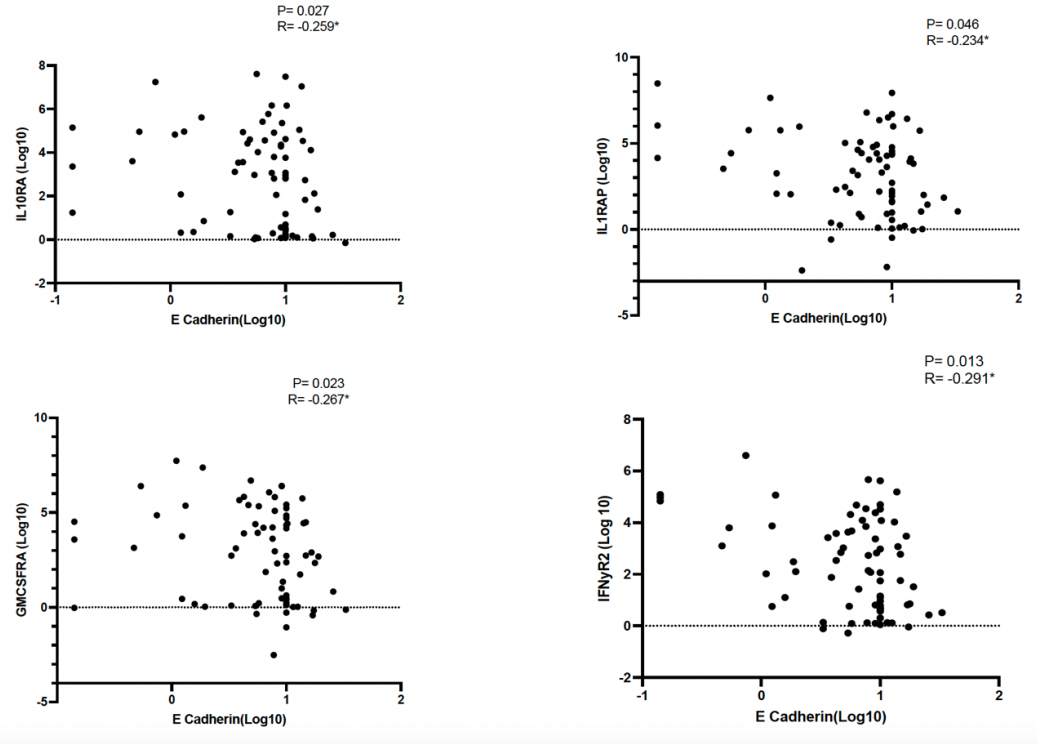
Figure 11. Correlations between cytokines and receptors in *Lactobacillus* and non-*Lactobacillus* microbiome states in the female genital tract.

Among participants with a *Lactobacillus* dominant microbiome, there is upregulation of receptors associated with inflammation (blue boxes) whereas among participants with non-*Lactobacillus* dominant microbiome there is a downregulation of immune markers and pro-inflammatory cytokines (Yellow to light blue boxes).

5.2.5 Correlation between barrier marker and immunological markers in the female genital tract

We assessed the impact of epidemiological variable on barrier integrity and found that participants in the female sex worker and transactional group have lower E-cadherin compared to those in the female sex worker group (p=0.015). We did not find any correlation with other epidemiological variables like time since last sex, cumulative vaginal acts, number of partners.

We analyzed the correlation between soluble E-cadherin with immune markers in the female genital tract. Interestingly we found that there was a negative correlation with the following cytokine receptors; IL10RA, IL1RAP, GMCSFRA, IFNYR2 (Figure 12a) but there was no correlation with their corresponding cytokines. In this analysis we also found that participants with increased levels of IL7, IL33 and IL12P40 had decreased levels of soluble E-cadherin (Figure 12b). E-cadherin also had a negative correlation with TLR7 and CD69 (Figure 12c). We did not find any correlation with the microbiome groups and any STI infection (P>0.05).



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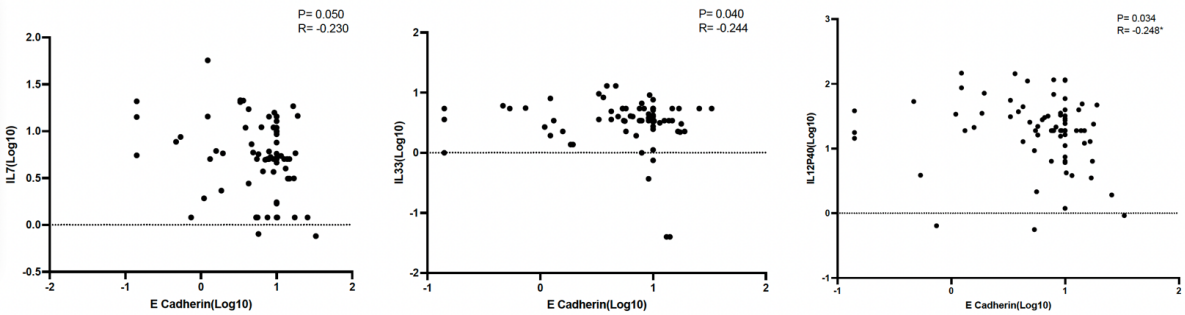

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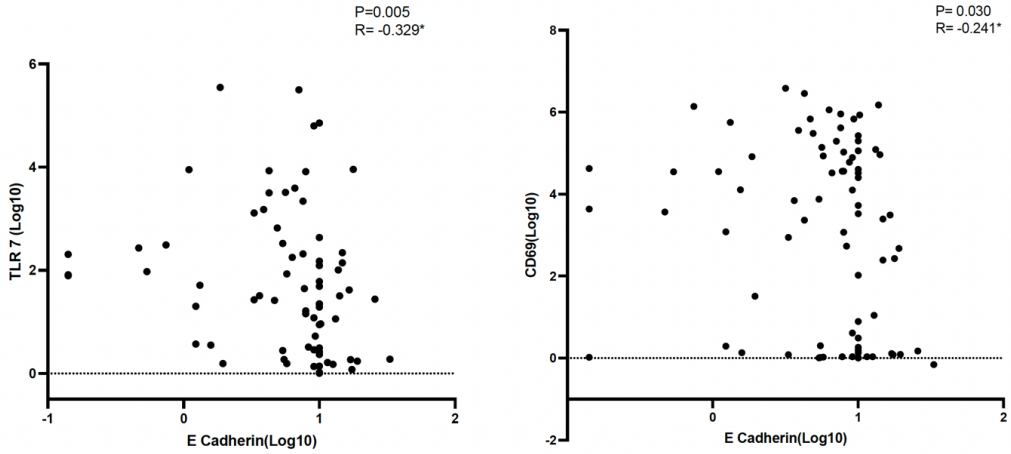

C

Figure 12. Correlation plots between E-cadherin and receptors.

Figure 12a. There is a negative correlation between cytokine receptors and immune markers.

Figure 12b. Shows the negative correlation between IL7 and IL12p40. We did not find correlations with any other cytokines.

Figure 12 c. Shows that we found a negative correlation between IL7 and CD69.

5.2.6 Receptor mRNA expression in tissue samples

To better understand the impact of inflammation on receptor expression we took carried out imaging in a sample that had high pro inflammatory cytokine levels and carried out imaging of two of the highly expressed receptors from our assay CXCR2, CXCR3 and E-cadherin which is a marker of barrier integrity. From the images (figure 13a), there was low expression of CXCR3 which was completely asked when the image was merged (figure 13b). There was significant expression of both CXCR2 and E-cadherin.

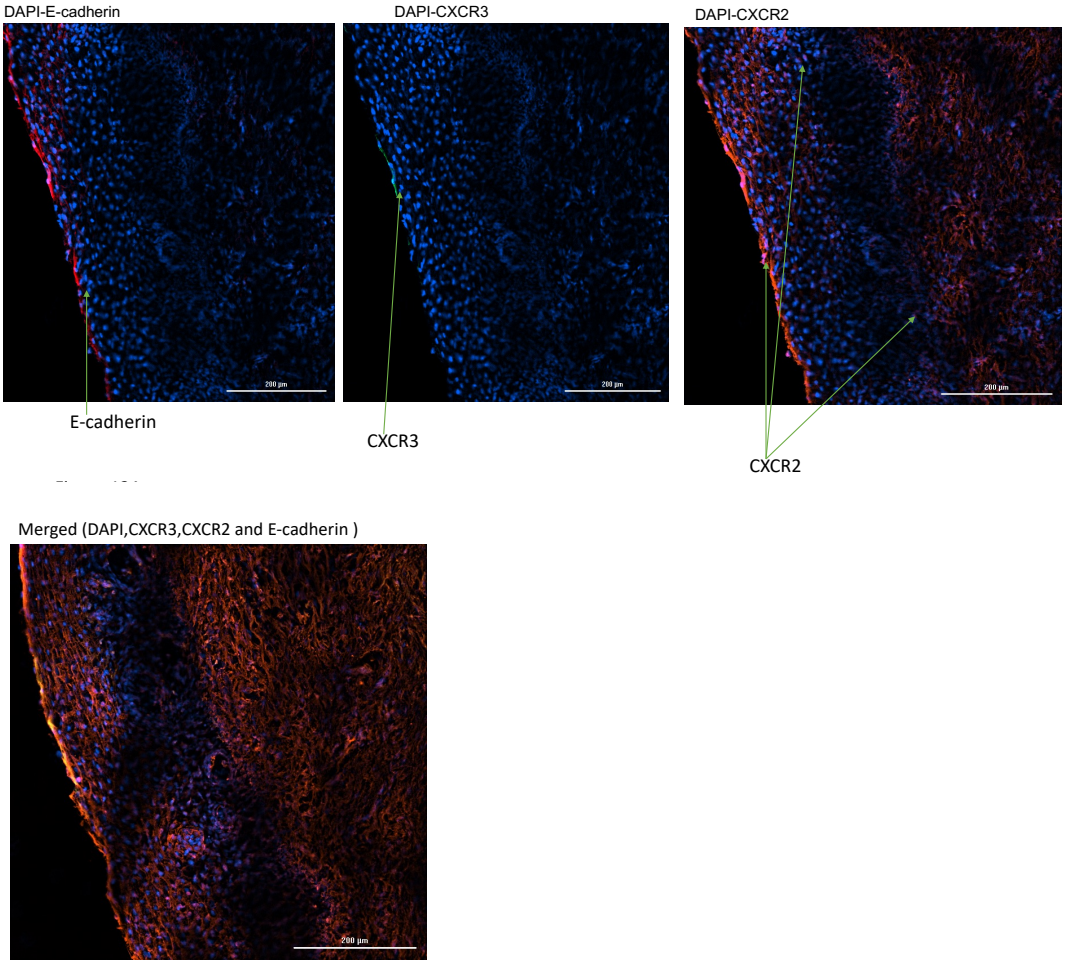

Figure 13. Images showing the expression of receptors in female genital tract biopsy samples.

In figure 13 a. there is sufficient expression of E-cadherin and CXCR2 whereas there is low expression of CXCR3. When the three images are merged in figure 13 b. CXCR3 is masked by the strong expression of CXCR2 and E-cadherin.

5.3 Discussion and conclusion

HIV infections among adolescent girls and young remain high in Sub-Saharan Africa, and therefore understanding the immunological factors that increase the risk of HIV acquisition is an important step in developing better HIV prevention options. Many studies have shown that the increase in pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF-α, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β) coupled with a molecular BV have been associated with increased HIV acquisition, even in the presence of topical PREP[166,197,222] . While many studies measure soluble cytokines,

fewer have focused on measuring cytokine receptors in the female genital tract and how intravaginal behavior impacts their expression. Here we characterize a combination of cytokine receptors, some TLRs and immune markers among AGYW at increased risk of HIV acquisition.

In this study, we found that most cytokine receptors were downregulated in AGYW with non-*Lactobacillus* dominant microbiomes. This is the first paper that describe the correlation between microbiome and cytokine receptors in the female genital tract. Previous studies that have focused on the role of cytokine receptors in sexually transmitted diseases, showing that CXCR3 deficiency in mice infected by *Chlamydia trachomatis* alters the Th1 responses, thus blocking the trafficking of T cells to the genital mucosa. In this study they showed that the CXCR3 deficient mice models were unable to clear *C. Trachomatis* infection compared to mice with intact CXCR3 signaling[223] . CXCR2 is expressed early in infection and has been known to be responsible for recruitment of neutrophils to the site of infection to assist in bacterial clearance, though excess activation of neutrophils is detrimental. In studies investigating the role of CXCR2 in *Chlamydia muradium* infection showed that migration into the upper female genital tract is slower in mice that are CXCR2-/- homozygous[224,225] . In women with recurrent UTI lower levels of CXCR2 are associated with recurrence of infection[226]. In our study we see downregulation of CXCR2 in participants with non-Lactobacillus dominant microbiome. A possible explanation may be that the bacterial species colonizing the female genital tract constantly downregulate the expression of CXCR2 to easily re-establish infection. This could be one of the reasons to the observation that bacterial vaginosis recurs in 80% of the women[201].

Epithelial cells play a major role in preventing disease through the rigidity of the tight junctions which are responsible for securing the intracellular spaces and by expressing TLRs that detect invading pathogens[208,227]. Anton et al found that *G. Vaginalis* is associated with the breakdown of epithelial barrier and activation of TLR2[126]. In our study we found that among participants non-*Lactobacillus* dominant CST there was a positive correlation between CXCR3 and CD69 which is a marker of early T cell activation and it had been found to be expressed in tissue memory cells specifically CD8+ and CD4+ cells[228]. Similarly high expression of CXCR3 has been found in activated memory and effector T cells. Thus, together these results suggest that a non-*Lactobacillus* dominant CST is associated with activation of T cells in the FGT. Previous studies have associated non-*Lactobacillus* dominant microbiome with increased risk of HIV

acquisition and subsequent treatment of bacterial vaginosis has been associated with downregulated CD4 levels[229]. In our study we found that most of the receptors associated with pro-inflammatory cytokines (IFNαR1, IFNαR2, CCR6, CCR10, CCR7, GMCSFRA) are correlated with CD4 among participants with non-*Lactobacillus* dominant CST. There could be two reasons that explain this observation; either the bacteria are avoiding a host response to achieve better colonization, or the host is trying to avoid damage.

An interesting observation is that some of the receptors were downregulated female sex workers. We have previously shown in a multivariable model that sex work was associated with increased alpha diversity[101] . Sexual activity has been found to be an important factor in influencing the composition of vaginal microbiome but there are no studies that have assessed its impact on cytokine receptors[174,230] . One hypothesis is that sexual activity is associated with exposure to different semen compositions, thus having a downstream effect on cytokine receptors. We also show that most of the receptors and their ligands did not correlate in a bivariate analysis apart from IL1α, IL1β and MIP1β. This does not underscore the fact that there could be other explanations better explored by mechanistic studies to better explain this phenomenon. One hypothesis is that since receptors present a binding site for the cytokines after which subsequent immune reactions follow, there is a possibility that once attachment occurs there could be immediate down regulation of the receptors. Another question is whether the receptors are stimulated by presence of the receptors, or they are always upregulated in anticipation of attachment to their ligands. From the images generated from a sample with elevated pro inflammatory levels we show that the expression of CXCR3 is low whereas there is significant expression of CXCR2 which is a marker of neutrophils. Its high expression is an indication of neutrophil infiltration. There was significant expression of E-cadherin although not consistently through the outer layer of the tissue. This may be an indication of decreased levels of E-cadherin in inflammatory condition.

BV has been known to have an impact on barrier integrity therefore allowing infiltration of pathogens beneath the epithelial layer thus increasing susceptibility to infection. In a study by Schwecht el al used VK2 cell model, short fatty chain acids (SCFA) and lactic acid (LA) to mimic the impact of the metabolites produced in eubiotic and dysbiotic environment on the barrier integrity and inflammation. They found that in wells inoculated with high amounts of

SCFA compared to LA there was high infiltration of FITC-dextan dye, elevated expression of tight junction proteins ZO-1, demoglein-1, E-cadherin, and this environment resulted in translocation of Nf-Kβ, elevated levels of TNF-α. These observations show that metabolites from BV play a role in increasing epithelial cell permeability by breaking down tight junction proteins and induces a pro-inflammatory pathway. They also found higher concentration of LA in dysbiotic conditions resulted in amelioration of the conditions caused in that environment. LA was associated with supressed production of TNF-α, upregulation of anti-inflammatory gene IL-1RA and upregulation of tight junctions[231]. BV has also been found to elevate immune cells some which are known to damage the tissue barrier integrity when they remain elevated for longer period e.g., neutrophils. Neutrophils are among the first type of immune cells that are released during an infection and immediately undergo apoptosis once they have cleared the infection. Prolonged presence of neutrophils has been associated with tissue damage through reactive oxygen species and neutrophil proteases. Cheu et al found that nLD samples from their studies were characterised by elevated frequency of C45+ cells which are key in the activation of neutrophils. In this study they found that nLD samples had low expression of CD16 and Caspase-3 both which indicate a mechanism through which elongated survival and functioning of neutrophils instead of their natural process of undergoing apoptosis. They also observed that when neutrophils were co-culture BV and non-BV samples, it resulted in higher infiltration dye in the BV when observed through transepithelial electric resistance microscope confirming their role in reducing barrier integrity[232]. In our study we observed a positive correlation between CD45 expression among nLD and CXCR2 and CXCR4 all of which are known to play a key role in activation of neutrophils. This shows that there is a need for further investigation of the correlation of this expression using cell lines to determine whether the correlation grows stronger when the cells are exposed for longer periods in BV conditions. Such a model can also be used to determine whether a stronger correlation between CD45 and the two receptors eventually results in loss of barrier integrity. As our study was cross sectional, I postulate that in a longitudinal study we will be able to determine how behaviour impacts barrier integrity using a combination of lab-based experiments and mathematical modelling when the expression of these receptors are measured at each of the timepoints of sample collection.

Our data represent the complexity of human mucosa and suggest the importance of further understanding how non-*Lactobacillus* dominant microbiome is associated with cytokine receptors and immune markers in the female genital tract. Despite having a small sample size, we observed a consistent pattern of cytokine receptor expression associated with nLD, suggestive of a large effect. One possible conclusion is that downregulating cytokine receptors in nLD may be beneficial in limiting immune cell influx, and failure to do so might result in increased HIV risk attributable to inflammation or due to recruitment of HIV targets. It is important to carry out future longitudinal studies to further understand how the changes in microbiome would impact receptors and immune markers in the female genital tract.

In conclusion, our study uncovers a strong association between vaginal microbiota in the female genital tract and not only cytokines but also their receptors, which are key modulators in immune signaling at the mucosal site. Understanding how cytokine receptor expression relates to the risk of HIV infection in this population, which is highly vulnerable to HIV, yet the biological drivers of increased risk are not yet well understood. The dysbiotic microbiota may be downregulating the receptor expression to evade detection by the immune system or the host may be downregulating the expression of these receptors to prevent further damage of the of the female genital mucosa. There is a need to understand how the interplay between dysbiotic microbiota and receptors affect barrier immunity and increase the susceptibility to infection.

CHAPTER 6: Prevalence and risk factors for sexually transmitted infections among AGYW from Mombasa, Kenya.

6.1 Introduction

In 2012 the WHO estimated that 357 million people worldwide were infected with 4 of the curable sexually transmitted infections (STIs); chlamydia, syphilis, gonorrhea and trichomoniasis, for an estimated 1 million that new STIs daily [233]. Many Sub-Saharan countries rely on syndromic management of STIs, even though most of these infections are asymptomatic or mildly symptomatic; this poses a major challenge in efficient diagnosis and management of these infections. In addition, there are limited studies that assess the prevalence of STIs among adolescent girls and young women (AGYW) in Sub-Saharan Africa. A recent meta-analysis showed that AGYW from high-risk populations in Eastern Africa are at higher risk of contracting STI compared to women aged between 25-49 years. The high disease burden was attributable to behavioral risk factors [53]. Varied prevalence estimates have been reported for the common STIs, with *Chlamydia trachomatis* infections ranging between 3.1 to 15.0%, *Neisseria gonorrhea* 1.0 to 1.8%, and *Trichomonas vaginalis* 5.0 to 13.6% [54,55,57–59,234]. Younger women below 25 years who practice condomless sex, have multiple partners have a higher incidence of *C. trachomatis* infections compared to older women[235,236].

STIs have been associated with increased risk of HIV acquisition, potentially by damaging the mucosal layer, increasing levels of pro-inflammatory cytokines and recruiting immune cells that are readily infected by HIV[198]. In several cohort studies both symptomatic and asymptomatic infections have been found to elicit strong immune responses [155,222]. This was particularly noted in *C. trachomatis* and *N. gonorrhea* infections whereby the levels of pro-inflammatory cytokines were much higher than other infections [138,237,238]. In addition to the direct increase of HIV risk there exist other co-factors like molecular BV that play major roles in increasing STI acquisition risk. Changes in the microbial environment of the female genital tract from a lactobacillus dominant to a mixed flora community has a synergistic effect on increasing both the incidence and pathogenesis of gonorrhea and chlamydia infections[239]. *Lactobacillus* species provide an acidic environment thus rendering the female genital tract inhabitable to other microorganisms, but a shift in the microbiota results in an environmental condition that allows other microorganisms to thrive [240,241]. Studies have also shown that lactobacillus species have the

ability to inhibit and displace *N. gonorrhea* from epithelial cells [242]. In addition to being potential drivers of HIV epidemics, STIs contribute toward adverse reproductive health outcomes like pelvic inflammatory disease, pre-term birth and infertility [243,244].

Africa is known to have relatively high prevalence of both gonorrhea and chlamydia infections compared to other regions in the world[245]. Despite this, very little is known about the circulating subtypes of *N. gonorrhea* and *C. trachomatis* infections among sexually AGYW in Kenya in higher prevalence regions. In addition, there is an important knowledge gap regarding the type of drug resistance pseudogenes circulating within this group. The main aim of this study is to characterize the molecular distribution of *N. gonorrhea*, *C. trachomatis* and the drug resistance patterns of *N. gonorrhea* infections among AGYW from Mombasa, Kenya with heterogeneous sexual exposure.

6.2 Results

6.2.1 Participant characteristics

There were a total of 964 participants were included in this study. The median age was 19 years (IQR: 17,21) and there was no difference between the groups. Only 13.5% (123) of the participants reported having a regular income. Among the participants, 22.3% (215) reported that they had completed secondary school education. Most participants were recruited from venue-based hotspots which are hotels, bars, or restaurants where the participants go to seek out clients (83.2%, n=803) compared to 16.7% (n=161) participants who were from non-venue-based hotspots which are the streets or online platforms. Few participants (6.1%, n=59) reported recent STI treatment less than 3 months prior to sampling (Table 1a). Only one-third (34.5%, n=333) of participants reported currently experiencing any STI-related symptom, with 12.2% (n=118) reporting current vaginal discharge (Table 7).

Table 7. Characteristics of participants in the STI sub-study

		All	Study groups		
			Non-sex	Transactional	Female sex
Age		19 (17,21)	19 (17,21)	19 (18,21)	20 (18,22)
Completed secondary school		22.3% (215)	22.5% (118)	33.8% (48)	16.0% (49)
Regular income		13.5% (123)	12.0% (63)	8.5% (12)	16.1% (48)
Married		0.8% (8)	1.1% (6)	0.6% (1)	0.3% (1)
Recent STI treatment (<=3		6.1% (59)	5.4% (28)	7.7% (11)	6.7% (20)
HIV status		5.2% (50)	3.4% (18)	2.1% (3)	9.7% (29)
Hot spot type	Venue based	83.2% (803)	82.6% (432)	81.0% (115)	85.6% (256)
	Non-venue	16.7% (161)	17.4% (91)	19.0% (27)	14.4% (43)
Current symptoms (within the last 3 months)					
Discharge		12.2 (118)	9.2% (48)	16.9% (24)	15.4% (46)
Genital ulcers		1.8% (17)	1.1% (6)	2.8% (4)	2.3% (7)
Fever with lower abdominal		12.0% (116)	8.6% (45)	13.4% (19)	17.4% (52)
Itching		4.8% (47)	4.7% (25)	6.3% (9)	4.3% (13)
Smelly urine		0.5% (5)	0.19% (1)	0.7% (1)	1.0% (3)
Pain while urinating		3.11% (30)	2.6% (14)	2.8% (4)	4.0% (12)

6.2.2 Prevalence of sexually transmitted infections

We first used the Seegene kit to screen for STIs among and found that 30.7% (296) of the participants tested positive for an STI but the prevalence of *N. gonorrhea* compared to trends in Sub-Saharan Africa. We further carried out screening for *N. gonorrhea* using an in-house PCR for *Por*A on all those that tested positive for b-globin. Approximately 9.6% (93) had multiple infections. The most common infection was *U. urealyticum* at 18.9% followed by *M. hominis* at 14.9%. Those that had the lowest prevalence were *N. gonorrhea* 0.5% (n=5) and *M. genitalium* 0.4% (n=4) (Table 2). There was no difference in the distribution of STIs among the groups apart from *T. vaginalis,* which was highest in the female sex worker group at 4.6%. A total of 33

samples tested NG-positive for this assay, for a prevalence of 3.4%, which is a 5-fold increase in the prevalence of *N. gonorrhea* as per Seegene. All further assays were carried out using the results from PorA assay. Both non-sex worker and female sex worker groups had the higher prevalence of *N. gonorrhea* 3.6% (n=19) and 4.0% (n=12), respectively, compared to the transactional sex group 1.4% (n=2).

Table 8. Distribution of sexually transmitted infections within the groups by Seegene kit

	All	Study groups		
		Non-sex	Transactional	Female sex
Mycoplasma genitalium	0.4 (4)	9.2% (2)	0	0.6% (2)
Mycoplasma hominis	14.9% (114)	14.9% (78)	13.3% (19)	15.7% (47)
Ureaplasma urealyticum	18.9% (183)	15.4% (81)	21.8% (31)	23.7% (71)
Trichomonas vaginalis	2.5% (24)	1.3% (7)	2.1% (3)	4.6% (14)
Chlamydia trachomatis	5.5% (53)	4.9% (26)	7.7% (11)	5.3% (16)
Neisseria gonorrhea	0.5% (5)	0.3% (2)	1.4% (2)	0.3% (1)
Prevalence of *Neisseria gonorrhea* by *PorA* assay				
	3.4% (33)	3.6% (19)	1.4% (2)	4.0% (12)

6.2.3 Impact of behavior associated to sex work on sexually transmitted infection.

We further assessed epidemiological associations of having any STI, e.g., testing positive for any of the 6 microorganisms in the panel. A univariate model was first carried out whereby alpha diversity (OR 2.18, 95% CI: 1.49 to 3.20, p<0.001) and older age (OR 1.06/year, 95% CI: 1.00 to 1.12, p=0.041) were the only associations of having an STI. In a stepwise multivariable model, only alpha diversity remained associated with any STI infection (adjusted OR 2.38, 95% CI: 1.56 to 3.62, p<0.001). Number of vaginal acts and number of sex partners were associated with *N. gonorrhea* in the unadjusted model (OR 1.05/sex act, 95% CI: 1.06 to 1.09), p=-0.028) and OR 1.05/partner (95% CI: 1.01 to 1.10, p=0.023), respectively. These were not significant following adjustment in a multivariable model. For *C. trachomatis*, no epidemiologic associations were

observed. Being in the FSW and/or transactional group was a risk factor for *T. vaginalis* infection in unadjusted (OR 2.99, 95% CI: 1.23 to 7.27, p=0.016 but not adjusted model.

Table 9. Factors associated with increased risk of STI acquisition.

Any STI				
Risk variable	**Univariate analysis**		**Multivariate analysis**	
	Odds Ratio (95% C.I)	P	Odds Ratio (95% C.I)	P
Alpha diversity	2.181 (1.489 – 3.196)	<0.001	2.380 (1.564 – 3.622)	<0.001
Number of vaginal acts	1.016 (0.991 – 1.043)	0.213	1.055 (0.831 – 1.339)	0.661
Number of unprotected	1.021 (0.954 – 1.093)	0.541	0.945 (0.739 – 1.209)	0.654
Age	1.058 (1.002 – 1.117)	0.041	1.132 (0.961 – 1.335)	0.138
Number of sex partners	1.023 (0.997 – 1.050)	0.084	1.005 (0.791 – 1.278)	0.965
FSW*Transactional	1.279 (0.974 – 1.681)	0.077	0.931 (0.382 – 2.267)	0.875
N. gonorrhea				
Alpha diversity	0.831 (0.370 – 1.869)	0.655	1.412 (0.281 – 7.082)	0.675
Number of vaginal acts	1.048 (1.005 – 1.092)	0.028	0.868 (0.568 – 1.327)	0.514
Number of unprotected	0.896 (0.633 – 1.269)	0.536	0.562 (0.225 – 1.402)	0.217
Age	0.948 (0.825 – 1.089)	0.451	0.638 (0.284 – 1.433)	0.276
Number of sex partners	1.050 (1.007 – 1.095)	0.023	2.063 (0.893 – 4.768)	0.090
FSW*Transactional	0.771 (0.379 – 1.569)	0.473	0.002 (0.000 – 4.196)	0.214
C. trachomatis				
Alpha diversity	1.056 (0.538 – 2.070)	0.875	1.053 (0.529 – 2.097)	0.882
Number of vaginal acts	1.019 (0.974 – 1.065)	0.417	1.048 (0.637 – 1.723)	0.854
Number of unprotected	1.008 (0.882 – 1.152)	0.903	0.986 (0.605 – 1.607)	0.955
Age	1.026 (0.919 –	0.646	1.012 (0.742 – 1.379)	0.941
Number of sex partners	1.019 (0.974 – 1.066)	0.422	0.949 (0.575 – 1.567)	0.837
FSW*Transactional	1.259 (0.723 – 2.194)	0.415	1.168 (0.215 – 6.350)	0.857

T. vaginalis				
Alpha diversity	0.974 (0.425 – 2.233)	0.951	0.888 (0.374 - 2.111)	0.789
Number of vaginal acts	1.015 (0.950 – 1.085)	0.654	1.090 (0.641 -1.853)	0.750
Number of unprotected	0.979 (0.764 – 1.254)	0.867	0.883 (0.442 -1.764)	0.725
Age	0.974 (0.829 – 1.144)	0.749	0.800 (0.524 -1.222)	0.303
Number of sex partners	1.017 (0.951 – 1.086)	0.628	0.914 (0.533 - 1.568	0.744
FSW*Transactional	2.987 (1.227 – 7.274)	0.016	3.210 (0.294 -3.5062)	0.339

6.2.4 Distribution of *Neisseria gonorrhea* and drug resistant mutations

Only 17 samples that had tested positive for *N. gonorrhea* and had DNA remaining could be typed, and 10/17 were non-typable while we were able to successfully type 7 samples. The distribution of the typed samples was as follows; 2 were ST8949, 2 were ST355,1 was ST20890, 1 was ST6154 and 1 was ST19093 (Table 3). In both groups there were 10 and 8 non-typeable samples respectively. Despite observing a few subtypes in our study their distribution was random among the three groups. Some were exclusively distributed in the non-sex worker group including ST 355, ST 19093, ST 6154, and ST 20890 while only 1 subtype; ST 8949 was in the FSW group. The non-typeable subtypes were equally distributed between the non-sex worker and the female sex worker group. Similar, trend was observed in the allele distribution where most of them were in the non-sex worker group while only one 5286 pro allele and one 15 *tpb*B allele were in the female sex worker group. Most of the serotypes were distributed among the venue based hot spot while only ST 8949 was found in the non-venue-based type of hotspot. We further assessed drug resistant mutation in 16 samples that were subtyped and found that 19% (3) were resistant to cephalosporins while 44% (7) were resistant to ciprofloxacin. There were no drug resistant mutations against Azithromycin among the samples.

6.2.5 Distribution of *Chlamydia trachomatis* subtypes

All those samples that tested positive with the Seegene kit were subtyped by amplification of the outer membrane protein gene (MOMP). Out of the 43 positive samples 40 were typable.

The most prevalent subtype was G at 36% followed by E at 33.33%. Subtype K had the lowest prevalence at 13.88%. Two of the participants had 2 serotypes while only one of the participants had both Neisseria and chlamydia infections. We further assessed the distribution of the serotypes in the different types of hotspots and found that most of the serotypes were found in the venue-based hotspots and only D (n=2), E (n=2) and G (n=3) were found in the non-venue-based hotpots. The participants that had been infected with 2 serotypes were from the venue-based hotspots and were from the non-sex worker and transactional groups respectively.

6.2.6 Impact of STI infection on the immunological environment of the female genital tract

To assess the impact of having any of the STIs on the cytokines and cytokine receptors we carried out further analysis on a subset of the data that had both cytokine and cytokine receptors measurement. Participants with an STI had low expression of CXCR1 and IL2RG (Figure 12) but we did not find any significant correlation with cytokines. We also found that participants with infection had low expression of TLR 2, TLR6 and CD69 (Figure 12). We further created a complex variable that combined participants with good microbiome and STI infection and those with bad microbiome and STI infection and assessed how these groups associate with immunological markers in the female genital tract. In participants who had a lactobacillus dominant microbiome and an STI infection there was downregulated expression of both IL6 and IFNγ. There were no other significant correlations with all other cytokines and receptors. Among the 34 participants who had diverse microbiome and STI infection we only observed a correlated with the following receptors IFNRyR1, IL1R2, IL2Rg, CXCR3, CXCR2, GMCSFRA, TLR6 and the following immune markers CD45 and CD69 (Figure 13) where there was down regulation of these markers. We did not find any correlation between any of the STI groups with barrier marker E-cadherin.

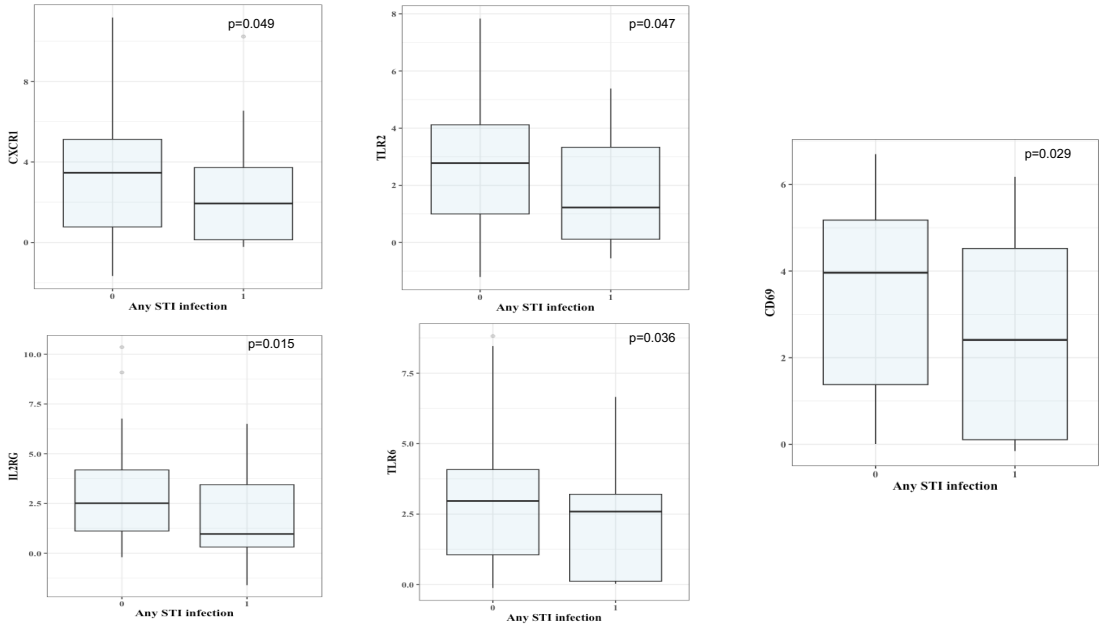

Figure 14. Receptor expression in participants with any STI infection

The box plots above show that participants with any of the STI infections had reduced expression of CXCR1, IL2Rg, TLR2, TLR6 and CD69.

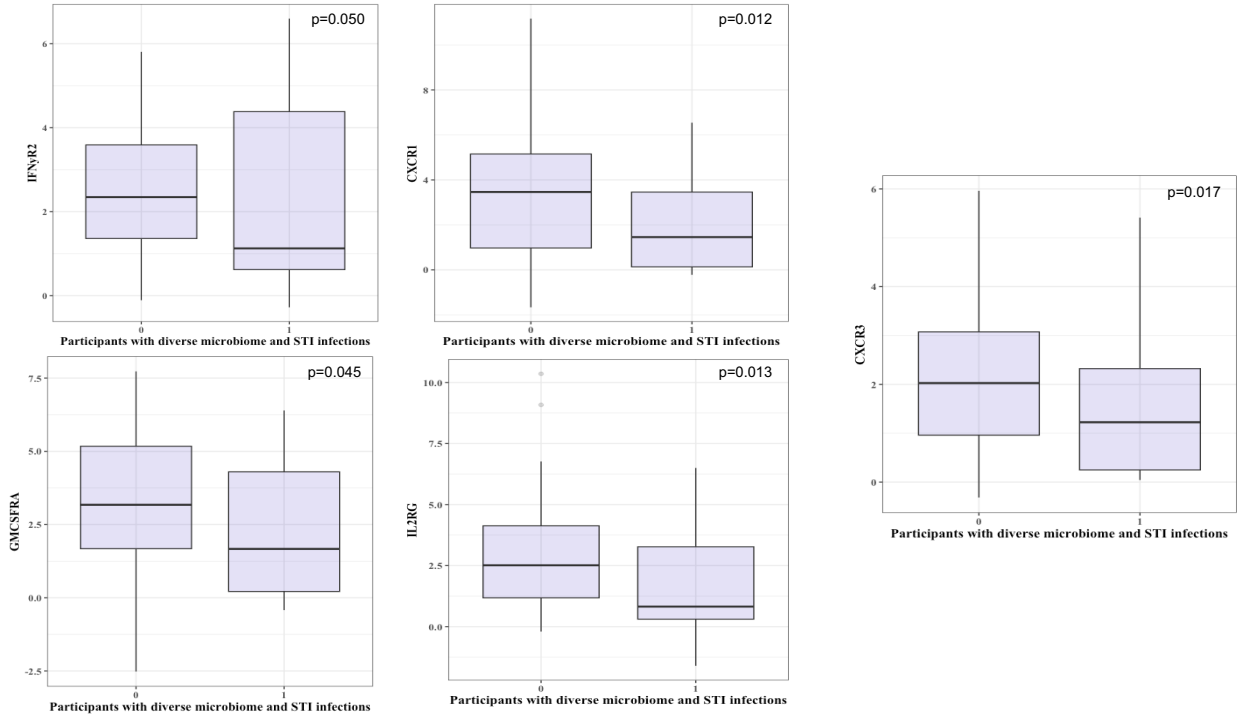

Figure 15. Receptor expression in participants with both STI infection and diverse microbiome.

Participants who had both diverse microbiome and STI infections had reduced expression of receptors, TLRs, and immune markers. TLR2 and TLR6 shown above are associated with the detection of bacterial infection.

6.3 Discussion

With most of the studies in Sub-Saharan Africa focused on reporting the seroprevalence of both *N. gonorrhea* and *C. trachomatis* infections, our study provides information about the circulating serotypes of the two microorganisms and the drug resistance mutations associated with *N. gonorrhea*. Our study is the first to report on circulating serotypes of both microorganisms among adolescent girls and young women from Kenya. When our isolates were compared to other Kenyan isolates in the pubMLST database [246], we found a high diversity of *N. gonorrhea* subtypes in our study than previously reported. A previous study that analyzed *N. gonorrhea* isolated from MSM in Coastal Kenya reported the presence of ST 1893, ST 1903, ST 1599 and ST 11366 as the most prevalent yet very distinct from those found in the UK[247]. Similarly, a study in South Africa found unique serotypes different from those isolated in the study mentioned previously and WHO reference sequences[248].

The high diversity recorded from the few studies that have characterized *N. gonorrhea* from this region indicates a need to characterize more isolates instead of only reporting the prevalence, given that Sub-Saharan Africa still has the highest number of *N. gonorrhea* infections worldwide. The prevalence rate of *C. trachomatis* infections in our study is similar to what has been reported in regional studies (3.1% to 3.4%), but much lower than studies from South Africa which reported a prevalence range of 5.5% to 11%. An overall prevalence of the infections in some systematic reviews have been reported to be between 5.5% to 7.8%[249,250]. We also found a diverse distribution of *C. trachomatis* serotypes - K, G, E, F, Ia and D, with serotype G having the highest prevalence, contrary to other studies that reported a higher prevalence of serotype E[251,252]. The serotypes found in our study are associated with genital and neonatal infections.

Both STI and diverse microbiome work synergistically to establish colonies for example BV causing bacteria produce metabolites like tryptophan that are important in growth of STI.

Their presence has also been linked to elevated pro-inflammatory cytokines and damaging of the epithelial barrier[198] . In this study we found that people with STI had downregulated expression of some of the cytokine receptors. Similar results were also observed among participants who had both STI and diverse microbiome. This may be an escape mechanism by the microorganism or a self-protection mechanism by the host as observed in chapter 5's discussion of this thesis.

Our study has several limitations. The Seegene kit was not sensitive enough thus, we had to incorporate a second screening method to countercheck the prevalence of STIs. Unfortunately, not all the microorganisms identified have alternative screening tests; therefore, we were limited to two microorganisms. Information about frequency or use antimicrobial use was not collected. This would have offered us a glimpse of its correlation with drug resistance patterns. In conclusion, our study shows the importance of incorporating screening of STIs among AGYW to understand better how these infections increase the risk of HIV acquisition. There is a high diversity of both *N. gonorrhea* and *C. trachomatis* infections circulating in Mombasa. Lack of comprehensive STI data from AGYW underscores the need to shift from a syndromic-based diagnosis of STI to screening-based diagnosis within the Kenyan healthcare system. In addition, future STI network studies would give useful information on how transmission of infections within sexual networks.

Chapter 7. General discussion

Studies have shown that females bear a disproportionately high burden of HIV in Sub-Saharan Africa, and this is highly seen among females under the age of 25 years who acquire the infection much earlier than their male counterparts and are 3 times more likely to acquire the infection. For many years scientist have been trying to understand the biological and behavioral factors that play a major role in contributing to the increased risk of HIV acquisition among women. The center of this research has been understanding the impact of microbiome and sexually transmitted infections in the female genital tract. In our study we have shown that presence of microbial population in the female genital tract is associated with increased levels of pro inflammatory cytokines which have been previously associated with increased risk of HIV acquisition. These findings are similar to those that have been previously published by other research groups. In the CAPRISA 004 cohort the Masson et al identified 9 pro inflammatory cytokines and chemokines that were associated with increased risk of HIV seroconversion; MIP-1β, IP-10, TNF-α IL-8, IL-1β, MCP-1, IL-1α, MIP-1α, and IL-6 where having elevated levels of at least 5 of these cytokines were likely to seroconvert to HIV [Odds ratio (95% confidence interval)] 3.2 (1.3–7.9), p = .014 [197]. McKinnon et al showed that in the presence of elevated levels of proinflammatory cytokines tenofovir gel does not confer any protection [166]. In our study we found that the different cytokine level was explained by microbiome clustering (figure 5) and subsequently having a diverse microbiome was associated with increased pro inflammatory cytokines [101]. These findings are similar to those reported by other groups in different cohorts, Anahtar et al found that in a cohort of South African who participated in both cross-sectional and longitudinal studies, there was a correlation between microbiome diversity and increased proinflammatory cytokines [138]. Similarly in a study of HIV-exposed seronegative (HESN) and unexposed women by Ponnan et al, they found that HESN group had a highly diverse microbiome compared to unexposed individuals with *Gardnerella* having the highest permutational multivariate analysis of variance coefficient. When they investigated the correlation between natural killer (NK) cells expressing natural cytotoxicity receptors (NCR) such as NKp30, NKp44 and NKp46, killer inhibitory receptors (KIRs) and microbial diversity they found a positive correlation an indication of recruitment activity of immune cells that are crucial in early control of HIV infection thus reducing the risk of infection [253]. In a study by

Ssemaganda et al that focused on female sex workers from Nairobi Kenya, the group noted that *Provetella spp* was negatively correlated with T regulatory cells (Tregs) which are important in the reduction of inflammation [254]. They did not find a correlation with any other bacteria associated with non-optimal vaginal microbiome. They concluded that Tregs could be modulating the inflammatory response of the bacteria themselves rather than their presence in the mucosa.

One of the striking observations during our study period is that there is a lot of information around correlation between non-optimal vaginal microbiome, cytokines, and immune cells but none of the groups have really focused on how the microbiome impacts cytokine receptor gene expression. Cytokines signal through receptors thus forming an important piece of the immune modulation pathway. In our study we made several interesting observations: there was downregulation among participants with non-optimal vaginal microbiome possible mechanism; only IL1α and IL1β were positively correlated with their ligands, while MIP1β was inversely correlated; Some of the behavior associated with sex work were positively correlated with receptors like IFNYR1, IL1RAP, IL6R, and negatively correlated with CXCR3, GMCSFRA, IL7R, TLR2; among participants with non-*Lactobacillus* dominant microbiome and elevated receptors like CXCR3, CXCR2 were mostly positively correlated with immune marker (chapter 5). These results taken together show how the vaginal microbiome in combination with behavior may have an impact on receptor expression of both cytokines, toll like receptors and immune markers although in-depth studies are required to confirm these observations. We postulate that understanding of the factors that disrupt the binding affinity of cytokines and its receptors is a crucial part of understanding the correlates of HIV prevention and make an important step in its preventive measures. In a review Gorby et al noted that the stability of the bond between the cytokine and its receptor has been found to be a crucial step in influencing the downstream immune signaling. This phenomenon has been understood through studying the interferon whereby it was found that if IFNαR2 variants that are altered to mimic the binding affinity of IFNβ towards IFNαR1 result in antiproliferative properties. When we mirror back these observations to our results and postulate that the microbiome may have the ability to change the binding affinity of a cytokine to its receptor and use it as an escape mechanism; a hypothesis that needs to be studied further. In the same review viruses like Ebstein barr virus (EBV) are known

to clone viral IL-10 which counter the effects of human IL-10 thus downregulating the inflammatory response towards it [255]. We postulate that the microbiome may have an ability to either directly impact the signaling pathway in maybe a similar fashion as described in the viral ability or indirectly by changing the metabolic pathways, the tissue environment and that plays a crucial role in the signaling cascade.

In our study we found that the rate of the common STIs was low among AGYW compared to results from a similar age group specifically from South Africa. One of the striking observations is that we had much higher rates of *M. hominis* and *U. urealyticum* organisms that have caused a debate on whether they can be classified as real infections of not. *M. genitalium* was quite low among the participants. While M. genitalium is known to cause pelvic inflammatory disease, M. hominis and *U. urealyticum* are both considered to be persistent and my cause severe infection but in most cases, they saliently occur in the bladder and may have serious impactions in pregnant women. Descension of *U. urealyticum* into the female genital tract has resulted in its isolation in cases of bacterial vaginosis [256]. In our study we found that have a diverse microbial population was greatly associated with *U. urealyticum* and *M. hominis* in both univariate and multivariate models. Both microorganisms were correlated C. *trachomatis* infections and not *N. gonorrhea* infections ($p<0.05$) whereas *U. urealyticum* was correlated with both, *T. vaginalis* ($p=0.016$)*, M. genitalium* ($p=0.027$) while *M. hominis* was only correlated with*, T. vaginalis* ($p<0.001$) and not *M. genitalium.* Alpha diversity was found to be a driver of any of the STI infections even after controlling for other sex related behaviors. In a review by Lewis et al, it highlights studies that have assessed the implications of BV on STI and found that individuals with a Nugent score greater than 3 have been associated with increased risk of STI acquisition; Nugent 4 was associated with 4-fold increase of gonorrhea infection while Nugent 3 was associated with chlamydia infection. This reviews the importance of an individual's social network and diet in shaping the microbiome of the female genital tract [257]. Similarly, when we looked at STI status as a predictor of Alpha diversity in Chapter 4 it remained one of the main predictors after controlling for HIV and behaviors; a possible indication that the 2 are synonymous. Women with *C. trachomatis* have been shown to have increased alpha diversity Shannon diversity's index and evenness; $p<0.05$ with Lactobacillus being the most common dominant genera among the healthy participants whilst a more diverse genera was isolated

among infected individuals [258]. In a study assessing the microbial diversity among women with symptomatic vs asymptomatic *N. gonorrhea* infections found that *Lactobacillus* species was a dominant taxon among women who were asymptomatic but gonorrhea positive without other co-infections. This taxon was in lower frequencies among women with symptomatic *gonorrhea* infections. In this study microbial diversity was also noted among individuals with co-infections whose samples were dominated with CT4 which was the diverse microbiota. In this study, diversity is a contributor of *gonorrhea* symptoms [259]. The results from these 2 studies are an example of other cohort studies that have found increased risk of STI among participants with BV [260]. In our review paper we highlight an important opinion of a possible symbiotic relationship between STI and BV as highlighted in section 1.7.6 of this thesis. One of the missing explanations is whether the displacement of healthy bacteria occurs before establishment of a sexually transmitted infection, or the infection gets established first then due to the aggressiveness of the bacteria there is displacement of the healthy microbiota; a phenomenon that needs to be well studied. Our study also shows that having an STI impacts the expression of certain receptors in a univariate model but this effect waters down in a multivariate model where only GMCSFRA remains relevant. This effect disappears when microbiome is added into the multivariable model. To our knowledge this is the first study to highlight the impact of STI on receptors in the female genital tract.

Both intravaginal and behavior associated with sexual activities is one of the major drivers of the immunological factors that impact the female genital tract. We found that having multiple partners and number of vaginal acts in the past week were influencers of diverse microbiome. A study by Bradshaw et al that investigated the possibility of BV being impacted by behavior showed that even having sex with the same partner even after treatment of bacterial vaginosis was a risk factor for recurrent BV[261]. There has been investigations on the transfer of microbiome between sex partners whereby studies have shown that BV recurs after treatment when the participant engages in sexual activity with the same partner they had pre treatment[261–263]. A model by Keynon et al established that transmission between males and females is highly dependent on sexual networks despite the race of the individual. Among women who maintained a shorter sexual network BV remains between the partners and spreads only when the both the partners engage in sexual activities with other new partners[264]. Ma et al in a mathematical

modelling study that analyzed the transfer of microbiome in partners who had attended an infertility clinic found that the exchange of microbiome is a random effect whose probability may be mathematically determined but not predicted[265]. Taken together these mathematical models confirm the results in our univariate analysis in chapter 4 where recency in sexual activity and number of acts in a univariate model were associated with alpha diversity.

7.2 Experimental limitations and future directions

This study enabled us to make very neat observations regarding the interaction between the intravaginal behavior, microbiome and STI there were several limitations. The study design was cross-sectional therefore implying that we were not able to observe the changes in microbial diversity in the different phases of menstrual period, how behavioral changes over time, the cycle of STI infections and recovery impact microbiome. We sequenced microbiome using the 16s approach, despite the ability of this method to give a semblance of the bacterial composition available there are several shortcomings associated with this method; this method does not have the ability to comprehensively allow scientists to get to the species level of some of the microbial populations especially those that belong to *Gardnerella* genera. More research has been ongoing and recently there has been suggestions of methods like Cpn60 and shotgun method which allow further teasing out of species level, but all these have their own shortcoming. These is also no golden approach when it comes to the bioinformatics pipeline analysis, this leaves the scholar to explore and use the most popular bioinformatics pipelines used by other authors. The method used to run the samples and the pipeline used to analyze the sequences always result in slight differences of the classification of CSTs. As described by McKinnon et al in their review there is no standardized method of naming the microbial populations in the female genital tract, a synchronized naming system in the future would be helpful to scholars and avoid further confusion [110].

For the first time we described the distribution of cytokine receptors and immune markers in the female genital tract and measure how microbiome impacts the receptors. This is a very novel section of the study though some of these observations would be better explained in a mechanistic study where each of the factors can be added either to cell lines or mice models to

make further conclusions. In this study we used Seegene™ kit to assess the STI prevalence in our cohort. We later realized that the kit may not be as sensitive due to the low N. gonorrhea positives. This shows that qPCR kits should not always be trusted, and it is important to carry tests with more than one kit where funds are available to confirm the data. Laboratories that are focused on measuring the STI prevalence should also come up with inhouse assays that can be used to countercheck the prevalence an approach that we were able to use for *Neisseria* only since we did not have any other in-house assays designed for the other microorganisms. Antibiotic use in Kenya is not tightly regulated thus there is over the counter mis use of antibiotics. This is a major contributor to antibiotic resistance and may also have an influence of the actual prevalence of STIs. In our questionnaire we missed an opportunity to enquire about the most recent use of unprescribed antibiotics a variable that would have assisted us in determining whether it influences the low prevalence of STIs despite there being records of higher prevalence in similar regions like South Africa among AGYW.

7.3 Future directions

Despite this being a cohort study, the results from this study have set precedence for the designing of much more complicated follow up studies that may be useful in adding context to the observations made. The microbiome is impacted by several behavioral and hormonal factors we suggest that in future studies longitudinal cohorts should be used. We typically suggest that a 12 month follow up approach of the cohort with collection of both epidemiological data and samples occurring at least every 2 months for processing. This period would allow the observation of changes in behavior, hormones and would allow pattern of changes in sex partners and their increase of decrease to be studied in detail. Sex hormones have been found to have an impact on the response to infectious disease. In a typical month the females body undergoes different fluctuations of hormones due to the menstrual cycle. The levels of estrogen rise during the luteal phase till the ovulation period which is marked by high luteinizing hormone and lack of fertilization results in the drop of estrogen and increase of progesterone during the follicular phase. Both estrogen and progesterone have been found to impact the responses of Th1/Th2 immune cells. Sex steroids have been found to influence the immune responses thus

having downstream impact on metabolism, growth, and virulence factors of bacterial infections. For instance, women are more susceptible to STIs, and they are more likely to have lengthy infectious period without appropriate diagnosis as described in the review by Dias *et al*[266]. A detailed questionnaire that captures the day of the cycle, sexual and intravaginal behavior of the participants will be administered. Approximately 3ml of blood will be drawn from the participants into a red top tubes and hormone levels measured using the Alinity Iä according to the manufacturer's instruction. CVL samples will be collected from the participants using soft cup and chemokines as described in Aida et al[101]. Statistical analysis will be carried out using R.

In this study we also noted very interesting observations around cytokine receptor expression. We acknowledge that since this is a cohort study and some of these observations may be influenced by multifaceted factors given that the reactions of the immune system may be a reaction to an interconnection of various factors. Therefore, it is important to confirm our observations with *in vitro* studies. VK2 cells have been widely used in *in vitro* assays to study different questions that improve the understanding of the morphological immune correlates of the female genital tract for instance Zalenskaya et al used the cells to measure gene expression in a study designed to assess how new microbicide candidates can either induce inflammation or reduce it in the presence of *Lactobacillus* spp and *Provetella bivia* [267]. In this prospective study I propose that the cells would be cultured to sufficient confluency and inoculated with different concentrations of microbial species e.g., *L. crispatus*, *L. iners*, *P. bivia*, *G. vaginalis* and PBS as a control can be inoculated with the cells for 24 hours. The same panel of cytokine receptors and immune markers measured using gene expression assay and the differential expression of the receptors can be calculated across the different concentrations. In this assay, the interrelationship between related cytokine receptors and immune markers can also be studied. Another set of cells can be grown to confluency and replicates of each set to depict different durations of hours post-infection i.e. 12 hours, 24 hours, 36 hours and 48 hours. The wells will be inoculated with the different microorganisms and gene expression will be measured at the different time points. I will carry out a Multistate Markov model to assess how the relative abundance of the microbiome impacts cytokine receptors and immune markers across a continuous period.

L. iners has been isolated in quantifiable amounts in different stages of BV infections. Previous findings that assessed women's vaginal fluids concluded that increased levels of *L.*

iners could be found in both BV-positive and BV-negative samples. In some studies, it is postulated that *L. iners* is the last microorganism to be displaced in a diverse environment although the exact mechanisms have not yet been well understood. Bacteria contain S-layer proteins which are crystalline arrays of self-reassembling protein on bacterial cell surface that are not well conserved thus they differ among different species. In pathogenic bacteria they are known to contribute to the adhesion properties of the microorganisms[268]. Analysing the difference in the S-layers among the lactobacillus species may be one approach to understanding the delayed displacement of *L. iners*. This may be carried out *L. iners* and *L. crispatus* by culturing the bacteria in Man Rogosa Sharpe (MRS) broth under anaerobic conditions at 42 °C. The cells will be harvested by centrifugation at 603 × *g* for 15 min, washed twice with PBS (pH 7.4). They will be agitated for 1h following the addition of 5 M LiCl, then centrifuged at 5478 × *g* for 20 min at room temperature[269]. The supernatants containing the LiCl-extracted S-layer proteins will be dialyzed against purified water at room temperature for 24 h under agitation. The Slps in the clear supernatant will be evaluated by SDS-PAGE 12% and quantified using Mini-PROTEANÔ Tetra vertical electrophoresis system.

7.4 Conclusion

This study highlights 3 important factors that modulate the immune milleu of the female genital tract. 1) behavior associated with sex work does not only influence the composition of microbial population but also the receptor expression. 2) It is also associated with increased pro inflammatory cytokines. 3) Both same partner and multiple partners play an important role in the transmission of BV. At the center of these activities' behavior takes a major role. Thus, an indication that behavior is a fundamental contribution to prevention of STI and its modulation may play an important role in reducing HIV infections among adolescent girls and young women. The multidimensional approach of this thesis contributes major preliminary findings among AGYW setting up the pace for further research in the field of HIV prevention among an understudied population who are at a higher risk of HIV acquisition.

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Appendices
Appendix 1. Questionnaire 1.

INTRODUCTION

Hello, my name is _____ and I am working on a project with _____ (local organization) and the University of Manitoba, Canada. We are conducting a survey among young women that asks about various issues related to their sex practices and sexual health. We would very much appreciate your participation in this survey. The information you provide will help us improve the sexual health services for you and your peers in this area. All the information you provide will be kept confidential and will not be shared with anyone other than members of our project team.

This survey will ask questions that might be difficult for you to answer. If there are questions you find uncomfortable answering, please just let me know and we can move on to the next question. You may also stop the interview at any time. However, we hope that you will participate in this survey because your views are important to us.

At this time, do you have any questions you want to ask me about the survey?

May I begin the interview now?

Habari, jina langu ni_____na nafanya kazi katika mradi wa_____ (local organization) na Chuo Kikuu cha Manitoba Canada. Tunafanya utafiti miongoni mwa wanawake wadogo ambao unauliza kuhusu maswala mbalimbali yanayohusiana na mienendo yao ya ngono na afya ya uzazi. Tutashukuru sana kushiriki kwako katika huu utafiti. Habari utakayotupatia itatusaidia kuboresha huduma za afya ya uzazi kwako wewe na marika wako katika eneo hili. Habari yote utakayotupatia itawekwa siri na haitapeanwa kwa mtu mwengine kando na wafanyikazi katika mradi huu.

Katika utafiti huu tutauliza maswali ambayo huenda yakawa magumu kwako kujibu. Ikiwa kuna maswali amabayo utajihisi wasiwasi kujibu, tafadhali nijulishe na tutaendelea na swali lifuatalo. pia unaweza kukatiza mahojiano haya wakati wowote. Hata hivyo, tunatarajia utashiriki katika utafiti huu kwa sababu maoni yako ni muhimu sana kwetu.

Kwa wakati huu, una maswali yoyote ambayo ungependa kuniuliza kuhusiana na utafiti huu?

Sasa naweza kuanza mahojiano

SECTION I: REGISTRATION DATA

LOCALE ID

County/District/Oblast:_____ Code.....☐☐

Division/City/Town/Village:_____ Code.....☐☐

Zone:_____ Code.....☐

Hotspot where participant was recruited:_____

RESPONDENT ID

Respondent ID Number.....☐☐☐☐☐☐☐☐☐

INTERVIEW INFORMATION

Interviewer Name (Print):_____ Interviewer Code.....☐☐

Interviewer Signature:_____ Date of Interview:_____ (dd/mm/yyyy)

Start time of White Booklet:_____ End time of White Booklet:_____

Respondent agrees to participate via written consent: YES.....☐ NO.....☐

Colour booklet selected: YELLOW ☐ BLUE ☐ PINK ☐

Result of interview.....☐

(Interview completed = 1; Respondent withdrew, Interview partially completed= 2; Respondent did not meet inclusion criteria = 3; Respondent was a duplicate = 4)

Data checked by				
	Code	Name (Print)	Signature	Date of

				Data Check/Entry
Supervisor				(dd/mm/yyyy)
Quality Assurance Officer				(dd/mm/yyyy)
Data Entry Clerk				(dd/mm/yyyy)

SECTION II: SOCIO-DEMOGRAPHIC INFORMATION

No.	QUESTIONS	CODING CATEGORIES	SKIP
201***	When is your birthday? *Siku yako ya kuzaliwa ni lini?*	DAY..... ☐☐ MONTH..... ☐☐ YEAR..... ☐☐☐☐	
202	How old were you on your last birthday? *Ulikuwa na miaka mingapi siku yako ya kuzaliwa ya mwisho?* Probe and estimate if not known. *If younger than 14 years or older than 24 years, TERMINATE the interview.*	AGE IN YEARS..... ☐☐ DON'T KNOW..... 8 8	
203	Have you ever had sex? *Je, umeshawahi kufanya ngono?* *If respondent answers "NO," TERMINATE the interview.*	YES..... 1 ... NO..... 0 → END .	

204	Can you read and write? *Unaweza kusoma na kuandika?*	YES..... 1 ... NO..... 0 .	
205 ***	What is the highest level that you completed in school? *Ni kiwango gani cha juu ambacho umemaliza shule?*		
	For use in <u>Kenya</u> and <u>India</u> *If respondent answers 3, 5, 7, 8 or 9, ask respondent "when" schooling was completed and mark it on the timeline.*	NEVER RECEIVED FORMAL EDUCATION..... 1 ATTENDED PRIMARY SCHOOL, BUT DID NOT COMPLETE..... 2 COMPLETED PRIMARY SCHOOL..... 3 ATTENDED HIGH SCHOOL, BUT DID NOT COMPLETE..... 4 COMPLETED HIGH SCHOOL..... 5 ATTENDED VOCATIONAL SCHOOL AFTER HIGH SCHOOL, BUT DID NOT COMPLETE..... 6 COMPLETED VOCATIONAL SCHOOL AFTER HIGHSCHOOL..... 7 COMPLETED COLLEGE..... 8 COMPLETED UNIVERISTY..... 9	

For use in <u>Ukraine</u> *If respondent answers U3, U5, U7, U8, U9, U10 or*	NEVER ATTENDED SCHOOL..... U1 UNFINISHED PRIMARY SCHOOL (LESS THAN 4 YEARS)..... U2	

	U11, ask respondent "when" schooling was completed and mark it on the timeline.	FINISHED PRIMARY SCHOOL (4 YEARS)..... UNFINISHED MIDDLE SCHOOL (LESS THAN 9 YEARS)..... FINISHED MIDDLE SCHOOL (9 YEARS)..... UNFINISHED HIGH SCHOOL (LESS THAN 11 YEARS)..... FINISHED HIGH SCHOOL (11 YEARS)..... VOCATIONAL EDUCATION..... UNDERGRADUATE EDUCATION (JUNIOR SPECIALIST)..... BASIC HIGHER EDUCATION (BACHELOR)..... HIGHER EDUCATION (SPECIALIST, MASTER)...	U3 U4 U5 U6 U7 U8 U9 U10 U11	
206	Are you currently a student receiving formal education at an institution? *Kwa sasa wewe ni mwanafunzi anayepokea elimu rasmi katika taasisi?*	YES..... NO.....	1 0	
207	Do you currently have a regular source of income? *Je kwa sasa uko na mapato ya mara kwa mara (mshahara)?*	YES..... NO.....	1 0	
208	What is your current marital status? *Ni nini hali yako ya ndoa kwa sasa?*	SINGLE, NEVER MARRIED..... MARRIED, AND LIVE WITH SPOUSE..... MARRIED, BUT NOT LIVING WITH SPOUSE..... NOT CURRENTLY MARRIED, BUT LIVE WITH A SEX PARTNER (COMMON LAW).....	1 → 212 2 → 209 3 → 210 4 → 212	

		WIDOWED..... 5 ..... DIVORCED / 6 SEPARATED.....	21 1
209** **	a) How many years ago was it when you were first married? *Ni miaka mingapi iliyopita uliolewa mara ya kwanza?* *If respondent answers "more than 2 years ago",* *fill in part a) only.* *If respondent answers "2 years ago or less",* *fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBMER..... 88	21 2
	b) If 2 years ago or less, in what month and year were you first married? *Kama ni chini ya miaka miwili iliyopita au zaidi, ni mwezi na mwaka gani ulikuwa umeolewa mara ya kwanza?*	MONTH..... ☐☐ ..... YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	212
210	<u>For respondents who are married, not separated but not living with spouse</u> When did you and your spouse stop living together? *Ni lini wewe na mume wako mliacha kuishi pamoja?*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐	212
211** **	When did you become widowed/divorced/ separated? *Ni lini ulikuwa mjane /mtalikiwa/tengana?*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐	

212	What is your religion? *Dini yako ni gani?*			
	For use in Kenya	ROMAN CATHOLIC.....	K1	
		PROTESTANT / OTHER CHRISTIAN.....	K2	
		MUSLIM.....	K3	
		NO RELIGION.....	K4	
		OTHER (SPECIFY) _____ ___	K5	
	For use in India	HINDU.....	IN1	
		MUSLIM.....	IN2	
		CHRISTIAN.....	IN3	
		SIKH.....	IN4	
		BUDDHIST / NEO-BUDDHIST.....	IN5	
		JAIN.....	IN6	
		NO RELIGION.....	IN7	
		OTHER (SPECIFY) _____ ___	IN8	
	For use in Ukraine	CHRISTIAN ORTHODOX.....	U1	
		CHRISTIAN CATHOLIC.....	U2	
		CHRISTIAN PROTESTANT.....	U3	
		ISLAM.....	U4	
		JUDAISM.....	U5	
		NO RELIGION.....	U6	

		OTHER (SPECIFY) _____ U7 ___	

213	For use in Kenya Which tribe do you belong to or identify as? *Wewe ni wa kabila au unajitambulisha na kabila gani?*	EMBU..... K1 ..... KALENJIN..... K2 ..... KAMBA…… K3 …… KIKUYU…… K4 …… KISII…… K5 …… LUHYA…… K6 ……. LUO…… K7 …… MASAI……..…… K8 …….. MERU…… K9 …….. MIJIKENDA/SWAHILI…… K10 ……. SOMALI…… K11 …….. TAITA/TAVETA…… K12 …….. OTHER (SPECIFY) _____ K15 ___	
	For use in India Which caste were you born into?	CASTE _____ DON'T KNOW..... IN8 8 NO ANSWER..... IN9 9 ..	
	For use in Ukraine	UKRAINIAN..... U1	

	Which ethnic group do you belong to or identify as?	RUSSIAN..... U2 ..... OTHER (SPECIFY) _____ U3 ___	
214	Over the past 6 months, in which neighbourhood or zone do you sleep on most nights? *Katika muda wa miezi sita iliyopita ni mahili au eneo gani unalala nyakati za usiku?*	_____ ____	

SECTION III: SEXUAL BEHAVIOURS AND PRACTICES

III-A. FIRST SEX			
No.	QUESTIONS	CODING CATEGORIES	SKIP
Now I'm going to ask you some questions about sexual behaviours. *Sasa nitakuuliza maswali kuhusu tabia za ngono*			
301***	How old were you when you had your first menstrual period? *Ulikuwa na miaka mingapi ulipopata damu (hedhi) yako ya mwezi ya kwanza?*	AGE IN YEARS..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
302	Are you menstruating right now? *Je sasa uko na damu ya mwezi (hedhi)?*	YES..... 1 ..... NO..... 0	

303	What was the start date of your current/last period (i.e. the first day of bleeding)? *Ni lini tarehe ya kwanza (siku ya kwanza) ya damu yako (hedhi) ya sasa na ya mwisho?*	MONTH..... ☐☐ ☐☐ DAY.....	
304***	a) How many years ago did you first have vaginal or anal sex?	NUMBER OF MONTHS AGO..... ☐☐ ☐☐	

	Ni miaka mingapi iliyopita ulifanya ngono ya uke au ya mkundu kwa mara ya kwanza? *If respondent answers "more than 2 years ago",* *fill in part a) only.* *If respondent answers "2 years ago or less',* *fill in parts a) and b).*	NUMBER OF YEARS AGO..... (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBMER..... 88	
	b) If 2 years ago or less, in what month and year did you first have sex? *Kama ni chini ya miaka miwili iliyopita au zaidi, ni mwezi na mwaka gani ulifanya ngono mara ya kwanza*	MONTH..... ☐☐ YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	
	c) Was that first sex event forced against your will? *Je ulilazimishwa bila kutaka kufanya kitendo cha kwanza cha ngono?*	YES..... 1 NO..... 0	
305	At that time when you first had sex, was this person older than you, younger than you, or about the same age as you? *Wakati ulipofanya ngono mara ya kwanza, je mtu huyo alikuwa mkubwa wako, mdogo wako au umri sawa na wewe?*	OLDER..... 1 → 306 YOUNGER..... 2 ABOUT THE SAME AGE AS ME..... 3 → 307 DON'T KNOW / DON'T REMEMBER..... 88	
306	How much was this person older than you? *Mtu huyu alikuwa mkubwa wako kwa kiasi gani?* READ OUT ANSWERS	10 OR MORE YEARS OLDER..... 1 LESS THAN 10 YEARS OLDER..... 2 OLDER BUT UNSURE HOW MUCH..... 3	

307	a) Did this first sex event involve: *Je tukio hili la kwanza la ngono lilihusisha:* ✓ *Tick all that apply.*	b) Was a condom used? *Je kondomu ilitumika?*		
	i) Vaginal sex?..... ..... *Ngono ya uke?* ☐	i) YES..... ☐ NO..... ☐ ...		
	ii) Anal sex?..... ..... *Ngono ya mkundu?* ☐	ii) YES..... ☐ NO..... ☐ ...		
308	Did you receive any gifts or money for that first sex event? Examples of gifts may be clothes, food, a place to stay, alcohol etc. *Je ulipokea zawadi au pesa kwa tukio hilo la kwanza la ngono? Mifano ya zawadi yaweza kuwa nguo, chakula, pahali pa kuishi, pombe, n.k.*	YES..... 1 ..... NO..... 0 → 310		
309	What did you receive for that first sex event? *Ulipokea nini kwa hilo tukio la kwanza la ngono?* MULTIPLE ANSWERS POSSIBLE	MONEY..... A ..... FOOD..... B ..... CLOTHES..... C ..... A PLACE TO STAY..... D		

		ALCOHOL.....	E	
		.....		
		DRUGS.....	F	
		.....		
		PAID SCHOOL FEES.....	G	
		FREE RIDE.....	H	
		OTHER (SPECIFY) _____ ___	I	
		DON'T KNOW / DON'T REMEMBER.....	Y	
310	a) Who arranged that first sex event? *Nani alipanga tukio hilo la kwanza la ngono?*	ME.....	1	
		.....		
		THE PERSON I HAD SEX WITH.....	2	
		MUTUALLY DECIDED BETWEEN MYSELF AND PERSON I HAD SEX WITH.....	3	312
		SOMEONE ELSE.....	4	
	b) If the first sex event was arranged by someone else, who was this person? *Kama tukio hilo la kwanza la ngono lilipangwa na mtu mwengine, mtu huyo alikuwa nani?*	HUSBAND / SPOUSE / BOYFRIEND.....	1	
		PARENTS / GUARDIANS.....	2	
		OTHER FAMILY MEMBERS.....	3	
		FRIEND.....	4	
		.....		
		NEIGHBOUR.....	5	
		.....		
		OTHER (SPECIFY) _____ ___	6	
		DON'T REMEMBER.....	88	

311	What did this person receive for arranging your first sex event.	NOTHING.....	A	
		.....		
		MONEY.....	B	
		.....		

	Mtu huyo alipokea nini kwa kupanga tukio lako la kwanza la ngono? MULTIPLE ANSWERS POSSIBLE	FOOD..... CLOTHES..... A PLACE TO STAY..... ALCOHOL..... DRUGS..... OTHER (SPECIFY) _____ DON'T KNOW / DON'T REMEMBER.....	C D E F G H Y	
312	At the time, did you feel coerced or deceived (tricked) into having sex with that man? *Wakati huo, je ulihisi kulazimishwa au kudanganywa ili ufanye ngono na mwanaume huyo?*	YES..... NO.....	1 0	
313	After you had your first sex with that man, did you have sex with him again on another occasion? *Baada ya kufanya ngono mara ya kwanza na mtu huyo, je uliwahi kufanya naye ngono tena mara nyingine?* PROMPT IF REPSONDENT ANSWERS 'YES"	YES, HE BECAME MY BOYFRIEND..... YES, HE BECAME MY HUSBAND..... YES, HE BECAME A REGULAR SEX PARTNER WHO GAVE ME MONEY, GIFTS, GOODS OR OTHER RESOURCES IN EXCHANGE FOR SEX.... YES, I HAD SEX WITH HIM AGAIN ON OTHER OCCASION(S) BUT WE DIDN'T CONTINUE AFTER THAT..... NO.....	1 2 3 4 0	
314	Have you ever had sex with a man with the expectation that you would receive money, gifts, goods or other resources in return?	YES..... NO.....	1 0	→ 317

	Umeshawahi kufanya ngono na mwanaume ukiwa na matarajio kwamba utapokea pesa, zawadi, bidhaa au vitu vyengine baadaye?		
315***	a) How many years ago was it when you first had sex with a man for receiving money, gifts, goods or other resources in return? *Ni miaka mingapi iliyopita ulifanya ngono mara ya kwanza na mwanaume kwa ajili ya kupokea pesa, zawadi, bidhaa au vitu vyengine?* *If respondent answers "more than 2 years ago",* *fill in part a) only.* *If respondent answers "2 years ago or less",* *fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBMER..... 88	
	b) If 2 years ago or less, in what month and year did you first have sex with a man for receiving money, gifts, goods or other resources in return? *Kama ni chini ya miaka miwili iliyopita au zaidi, ni mwezi na mwaka gani ulifanya ngono mara ya kwanza kwa ajili ya kupokea pesa, zawadi, bidhaa au vitu vyengine?*	MONTH..... ☐☐ YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	
316	What type of gifts, goods or other resources did you receive that first time?	MONEY..... A FOOD..... B CLOTHES..... C	

	Ni aina gani za zawadi au vitu vyengine ulipokea wakati huo wa kwanza? MULTIPLE ANSWERS POSSIBLE	A PLACE TO STAY..... D ALCOHOL..... E DRUGS..... F PAID SCHOOL FEES..... G FREE RIDE..... H OTHER (SPECIFY) _____ I ___ DON'T KNOW / DON'T REMEMBER..... Y	
317	Have you ever had sex with a man where the price of sex was negotiated before the sex event? *Je umewahi kufanya ngono na mwanaume ambapo kiwango cha pesa ki lizungumzwa kabla ya tukio la ngono?*	YES..... 1 NO..... 0 →	See Instr. belo w
318***	a) How many years ago was it when you first had sex with a man where the price of sex was negotiated before the sex event? *Ni miaka mingapi iliyopita ulipofanya ngono na mwanaume mara ya kwanza wakati kiwango cha pesa kilzungumziwa kabla ya kitendo cha ngono?* *If respondent answers "more than 2 years ago",* *fill in part a) only.* *If respondent answers "2 years ago or less",* *fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBMER..... 88	

	b) If 2 years ago or less, in what month and year did you first have sex with a man where the price of sex was negotiated before the sex event? *Kama ni chini ya miaka miwili iliyopita au zaidi, ni mwezi na mwaka ulifanya ngono mara ya kwanza na mwanaume ambapo kiwango cha pesa kilizungumziwa kabla ya kitendo cha ngono?*	MONTH..... ☐☐ ..... YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	See Instr. below

INTERVIEWER: *Follow the instructions below and circle the colour booklet selected.*		
Question 314	Question 317	CONTINUE WITH
NO	NO	YELLOW booklet
YES	NO	BLUE booklet
NO	YES	Question 319
YES	YES	Question 319

No.	QUESTIONS	CODING CATEGORIES	SKIP
You told me that you have received money in exchange for sex and that sometimes the price of sex was negotiated before the sex event. I want you to think back to that period of time when you first received money in exchange for sex. The questions I am going to ask you are about those first sex partners who paid you to have sex, whether or not the price of sex was negotiated before the sex event, and then about some of the other sex partners during that time – that is, after a man first paid you to have sex. *Uliniambia umewahi kupokea pesa kwa kushiriki ngono na kwamba mara nyengine bei ya ngono huzungumzwa kabla ya kitendo cha ngono. Ningependa ukumbuke kipindi cha hapo awali wakati ulipokea pesa kwa mara ya kwanza kwa kushiriki ngono. Maswali nitakayokuuliza yanahusu wale washiriki wako wa kwanza waliokulipa kushiriki ngono na, kama au si bei ya ngono ilizungumziwa kabla ya kitendo cha ngono, na kisha kuhusu baadhi ya washiriki wako wakati huo – hii ni kumaanisha, baada ya mwanaume kukulipa mara ya kwanza ili kufanya ngono nao.*			
319	How did you meet the first man who gave you money in exchange for sex?	ARRANGED BY HUSBAND / SPOUSE / 1	

	For example, was the meeting arranged by someone? Did you meet him somewhere on your own? *Ni vipi ulikutana na mwanaume wa kwanza aliyekulipa kufanya ngono naye?* *Kwa mfano, kukutana huko kulipangwa na mtu? Je ulikutana naye pekee yako mahali fulani?*	BOYFRIEND..... 1 ARRANGED BY PARENTS / GUARDIAN..... 2 ARRANGED BY OTHER FAMILY MEMBERS..... 3 ARRANGED BY FRIEND..... 4 ARRANGED BY PIMP / MANAGER..... 5 MET THROUGH INTERNET ON MY OWN..... 6 MET THROUGH MOBILE PHOHE ON MY OWN..... 7 MET ON STREET ON MY OWN..... 8 MET AT ENTERTAINMENT VENUE (BAR, DISCO, CLUB, CAFE, SAUNA ETC.) ON MY OWN..... 9 MET AT HOTEL / LODGE ON MY OWN..... 10 MET AT WORK ON MY OWN..... 11 MET AT SCHOOL ON MY OWN..... 12 MET AT CHURCH / MOSQUE ON MY OWN..... 13 MET AT BUS STOP ON MY OWN..... 14 MET AT MY / HIS HOME..... 15 OTHER (SPECIFY) _____ 16 DON'T REMEMBER..... 88	
320	Did he physically force you to have sex with him? *Je alitumia nguvu kukulazimisha kushiriki ngono na yeye?*	YES..... 1 NO..... 0	

321	a) Was this first man who paid you to have sex a paying client with whom the price of sex was negotiated before the sex event? *Je mwanaume huyu wa kwanza kukupatia pesa ili mfanye ngono, alikuwa mteja wa kulipa ambaye bei ya ngono ilizungumziwa kabla ya tukio la ngono?*	YES..... 1 ..... NO..... 0	
	b) Did he pay you right after the sex event? *Je alikulipa mara tu baada ya tukio hilo la ngono?*	YES..... 1 ..... NO..... 0	
322	Now let's think about the FIRST 10 (OR FIRST SEVERAL) SEX PARTNERS who gave you money in exchange for sex, how did you meet them? For example, were these meetings arranged by someone? Did you meet them somewhere on your own? *Sasa, tufikirie kuhusu washirika wako 10 WA KWANZA (AU 10 KADHAA) wa ngono ambao walikupatia pesa kwa kushiriki ngono nao, ulikutana nao vipi?* *Kwa mfano, kukutana huku kulipangwa na mtu? Ulikutana nao pekee yako mahali fulani?* MULTIPLE ANSWERS POSSIBLE	ARRANGED BY HUSABAND / SPOUSE / BOYFRIEND..... A ARRANGED BY PARENTS / GUARDIAN..... B ARRANGED BY OTHER FAMILY MEMBERS..... C ARRANGED BY FRIEND..... D ARRANGED BY PIMP / MANAGER..... E MET THROUGH INTERNET ON MY OWN..... F MET THROUGH MOBILE PHOHE ON MY OWN..... G MET ON STREET ON MY OWN..... H MET AT ENTERTAINMENT VENUE (BAR, DISCO, CLUB, CAFE, SAUNA ETC.) ON MY OWN..... I MET AT HOTEL / LODGE ON MY OWN..... J	

		MET AT WORK ON MY OWN..... K MET AT SCHOOL ON MY OWN..... L MET AT CHURCH / MOSQUE ON MY OWN..... M MET AT BUS STOP ON MY OWN..... N MET AT MY / HIS HOME..... O OTHER (SPECIFY) _____ p ___ DON'T REMEMBER..... Y	

323	Sometimes when men pay women to have sex with them, the women may be paid at each visit, per hour, or per sex act. Thinking back to the FIRST 10 (OR FIRST SEVERAL) SEX PARTNERS who gave you money in exchange for sex, *overall*, were you mostly paid: *Saa nyengine wanaume wanapowalipa wanamke kushiriki ngono nao, wanawake wanaweza kulipwa kwa kila tembezi, kila saa, au kwa kila tukio la ngono. Sasa, ukifikiri nyuma kuhusu washirika wako 10 WA KWANZA (AU 10 KADHAA) wa ngono ambao walikupatia pesa kwa kushiriki ngono nao, kwa wastani, ulilipwa sana:*		
	a) per visit? *Kwa kila tembezi?*	YES.....☐ NO.....☐	
	b) per hour? *Kwa saa?*	YES.....☐ NO.....☐	
	c) per vaginal or anal sex act? *Kwa kila tukio la ngono uke au mkundu?*	YES.....☐ NO.....☐	
	d) every few days as if receiving wages? *Kwa kila siku chache kama ambae unapokea mapato?*	YES.....☐ NO.....☐	
324	Some women who receive money in exchange for sex only get to keep some of the money and	NO ONE ARRANGED MY SEX ENCOUNTERS; I KEPT ALL OF THE MONEY..... 1	

	the rest of the money goes to the person who arranged the exchange. *On average*, how much of the money did you keep when you first began receiving money for sex for the FIRST 10 (OR FIRST SEVERAL) SEX PARTNERS ? *Wanawake wengine ambao hupokea pesa kwa kushiriki ngono hupata tu kujiwekea kiasi fulani cha pesa na zengine huenda kwa mtu anayepanga kukutana huko.* *Kwa wastani, ni kiasi gani cha pesa hizo ulijiwekea ulipoanza kupokea pesa kwa ajili ya ngono KWA WASHIRIKI WAKO WA 10 (AU KADHAA) WA NGONO?*	NONE..... 2 ..... LESS THAN HALF..... 3 HALF..... 4 ..... MORE THAN HALF BUT NOT ALL..... 5 ALL..... 6	

	Many questions that I'm going to ask you from now on are about different sex partners that you may have. These men may include paying clients, non-client sex partners, husband, spouse and boyfriends. PAYING CLIENTS are men with whom the price of sex is negotiated before the sex event and by whom money is often paid before or immediately after the sex event. NON-CLIENT SEX PARTNERS are men with whom you have sex, with the expectation that you would receive money, gifts or other resources in return but the price of sex is often not negotiated upfront and is implicitly understood. Is the distinction between the different types of sex partners clear? May we continue? *Maswali mengi ambayo nitakuuliza kuanzia sasa ni kuhusu washiriki wako wa ngono anaweza kuwa nao. Hawa wanaweza kuwa wateja wa kulipa, washiriki wa ngono wasio wa kulipa, mume wako au rafiki wa kiume. WATEJA WA KULIPA ni wanaume ambao bei ya ngono huwa imezungumzwa kabla tukio la ngono na sana sana pesa hulipwa tu baada ya tukio hilo la ngono. WASHIRIKI WA NGONO WASIO WATEJA ni wanaume amboo hufanya nao ngono ukiwa na matarajio ya kwamba utapokea pesa, zawadi au vitu vyengine baadaye na bei ya ngono haizungumzwa na inaeleweka.* *Je hii tofauti baina ya washiriki hawa tofauti wa ngono inaeleweka? Tunaweza endelea?*		
325	Thinking back to those FIRST 10 (OR FIRST SEVERAL) SEX PARTNERS who gave you money	NUMBER OF PAYING CLIENTS..... ☐☐	

	in exchange for sex, how many were paying clients with whom the price of sex was negotiated before the sex event? *ffikiria nyuma kuhusu washirika wako 10 WA KWANZA (AU 10 KADHAA) WA NGONO ambao walikupatia pesa kwa kushiriki ngono nao, wangapi walikuwa wateja wa kulipa ambao bei ya ngono ilijazungumzwa kabla ya kitendo cha ngono?*	DON'T KNOW / DON'T REMEMBER..... 88	
326***	How old were you when you took your first paying client with whom the price of sex was negotiated before the sex event? *Ulikuwa na miaka mingapi ulipokuwa na mteja wa kulipa wa kwanza ambaye bei ya ngono ilizungumzwa kabla ya tukio la ngono?*	AGE IN YEARS..... ☐☐ I HAVE NEVER HAD PAYING CLIENTS ..... 77 (→ Continue with BLUE booklet Question C339) → C339 DON'T REMEMBER..... 88	
327	For how long have you been taking paying clients? *Umekuwa na wateja wa kulipa kwa mda gani?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS..... ☐☐ NUMBER OF MONTHS..... ☐☐ NUMBER OF YEARS..... ☐☐	

328 ***	Thinking back to that FIRST MONTH after you had your first paying client, did the money from your paying clients cover your living expense in that first month? *Fikiria nyuma kuhusu MWEZI WA KWANZA baada ya kuwa na mteja wa kulipa wa kwanza, je pesa hizo kutoka kwa wateja wako wa kulipa ziligharamia maisha yako ya kuishi mwezi huo wa kwanza?* READ OUT ANSWERS	NONE OF THE LIVING EXPENSES..... 1 LESS THAN HALF OF THE LIVING EXPENSES.... 2 HALF..... 3 MORE THAN HALF..... 4 ALL..... 5 DON'T KNOW / DON'T REMEMBER..... 88	
329	Thinking back to that FIRST MONTH after you had your first paying client, with how many DIFFERENT MEN, including your paying clients, non-client partners, husband and boyfriends, did you have sex (vaginal, anal or both)? *Fikiria nyuma kuhusu ule MWEZI WA KWANZA baada ya kuwa na mteja wako wa kwanza wa kulipa, ni WANAUME TOFAUTI wangapi, ikiwemo wateja wa kulipa, mshiriki wa ngono asiye wa kulipa, mume na rafiki wa kiume; ulifanya ngono nao (ya uke, mkundu au kote)?*	NUMBER OF DIFFERENT MEN..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 →	331

330	Thinking back to that FIRST MONTH after you had your first paying client, you said you had sex with ___ [fill in answer from question 329 above] DIFFERENT MEN. With how many of them did you have: *Fikiria nyuma kuhusu MWEZI HUO WA KWANZA baada ya kuwa na mteja wakulipa wa kwanza, ulisema umefanya ngono na ___ [*fill in answer from question 329 above*] WANAUME TOFAUTI. Ni wangapi kati yao ulifanya nao*: *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*				
	a) <u>both vaginal and anal</u> sex? <u>*ngono uke na ya mkundu*</u>*?*			With how many of these men did you negotiate the price of both vaginal and anal sex before the sex event? *Ni wangapi kati ya hao mlizungumzia bei ya ngono ya uke na mkundu kabla ya kitendo cha ngono?*	
		DK/DR	NA	DK/DR	NA
	b) <u>only vaginal</u> sex? <u>*ngono ya uke pekee?*</u>			With how many of these men did you negotiate the price of vaginal sex before the sex event? *Ni wangapi kati yao mlizungumzia bei ya ngono ya uke kabla ya kitendo cha ngono?*	
		DK/DR	NA	DK/DR	NA
	c) <u>only anal</u> sex? <u>*Ngono ya mkundu pekee?*</u>			With how many of these men did you negotiate the price of anal sex before the sex event? *Ni wangapi kati yao mlizungumzia bei ya ngono ya mkundu kabla ya kitendo cha ngono?*	
		DK/DR	NA	DK/DR	NA
331	Presently, do you consider yourself a sex worker? *Je sasa wewe hujichukulia kama mbangaizaji?*	YES..... 1 → B335 ..... (→Continue with PINK booklet QUESTION B335)			
		NO..... 0			
332	Were you ever a sex worker? *Umeshawahi kuwa mbangaizaji?*	YES..... 1 → B333 ..... (→Continue with PINK booklet QUESTION B333)			
		NO..... 0 ..... (→ Continue with BLUE booklet)			

Appendix 2. Questionnaire 2

LOCALE ID

County/District/Oblast:_____ Code.....☐☐

Division/City/Town/Village:_____ Code.....☐☐

Zone:_____ Code.....☐

Hotspot where participant was recruited:_____

RESPONDENT ID

Respondent ID Number.....☐☐☐☐☐☐☐☐☐

INTERVIEW INFORMATION

Interviewer Name (Print):_____ Interviewer Code.....☐☐

Interviewer Signature:_____ Date of Interview:_____

(dd/mm/yyyy)

Start Time of Pink Booklet:_____ End Time of Pink Booklet:_____

III-B TIME AFTER FIRST SEX – GROUP 3			
No.	QUESTIONS	CODING CATEGORIES	SKIP
B333***	c) How many years ago did you stop sex work?	NUMBER OF MONTHS AGO.....☐☐	

	Uliwacha kubangaiza miaka mingapi iliyopita? If respondent answers "more than 2 years ago", fill in part a) only. If respondent answers "2 years ago or less", fill in parts a) and b).	NUMBER OF YEARS AGO..... (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) 88 DON'T REMEMBER.....	
	d) If 2 years ago or less, in what month and year did you stop sex work? *Kama ni chini ya miaka 2 au zaidi, ni mwezi na mwaka gani uliwacha kazi ya kubangaiza?*	MONTH..... YEAR..... DON'T REMEMBER..... 88	
B334	Do you think you will take on paying clients again in the future? *Je unafikiri utachukua wateja wa kulipa tena siku za usoni?*	YES..... 1 ..... 2 UNSURE..... 0 ..... NO.....	

B335***	a) How many years ago did you first consider yourself a sex worker? *Ni miaka mingapi iliyopita ulianza kujichukulia kama mbangaizaji kwa mara ya kwanza?* If respondent answers "more than 2 years ago", fill in part a) only. If respondent answers "2 years ago or less", fill in parts a) and b).	NUMBER OF MONTHS AGO..... NUMBER OF YEARS AGO..... (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) 88 DON'T REMEMBER.....	
	b) If 2 years ago or less, in what month and year		

	did you first consider yourself a sex worker? *Kama ni chini ya miaka 2 au zaidi iliopita, ni mwezi na mwaka gani ulipoanza kujichukulia kama mbangaizaji kwa mara ya kwanza?*	MONTH..... ..... YEAR.....☐☐☐☐ DON'T REMEMBER.....	
B336***	a) How many years ago did you first enter sex work? *Ni miaka mingapi imepita tangu uanze kazi ya kubangaiza?* If respondent answers "more than 2 years ago", fill in part a) only. If respondent answers "2 years ago or less", fill in parts a) and b).	NUMBER OF MONTHS AGO.....☐☐ NUMBER OF YEARS AGO.....☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBER..... 88	
	b) If 2 years ago or less, in what month and year did you first enter sex work? *kama ni chini ya miaka 2 au zaidi iliopita, ni mwezi na mwaka gani ulianza ubangaizaji?*	MONTH.....☐☐ YEAR.....☐☐☐☐ DON'T REMEMBER..... 88	
B337	For how long have/had you been in sex work? *Umekuwa ukibangaiza kwa mda gani?* [If < 1 month, record in days.	NUMBER OF DAYS.....☐☐ NUMBER OF MONTHS.....☐☐ NUMBER OF YEARS.....☐☐	

	If >1 month and <1 year, record in months. If >1 year, record in years.]	DON'T KNOW / DON'T REMEMBER.....	

B338	Thinking back to when you took your FIRST PAYING CLIENT, at that time: *Ukifikiria hapo awali ulipopata MTEJA WAKO WA KWANZA WA KULIPA, wakati huo, ulikuwa:* READ OUT ANSWERS.	
	a) Were you married?..... ..... *ulikuwa umeolewa?* ☐ b) Were you divorced/separated?..... ..... *ulikuwa umetalakiwa/umetengana?* ☐ c) Were you widowed?..... ..... ulikuwa mjane*?* ☐ d) Had you left home?..... ..... *Ulikuwa umehama nyumbani?* e) Were you living with your parents?..... *Ulikuwa unaishi na wazazi wako?* f) Other (specify)?_____ _____ ☐ ✓ *Tick all that apply.*	

	nyengine (fafanua)?	
B339***	How did you meet your PAYING CLIENTS in the FIRST MONTH OF SEX WORK and in the LAST MONTH OF SEX WORK? *Tick all that apply in each column.* *Ni vipi ulikutana na WATEJA WAKO WA KULIPA katika MWEZI WA KWANZA na wa MWISHO WA UBANGAIZAJI?* INTERVIEWER*: Ask respondent when the last month of sex work was and mark it on the timeline.*	
	A. FIRST MONTH OF SEX WORK	B. LAST MONTH OF SEX WORK
	a) Arranged by spouse/boyfriend ?..... *Ilipangwa na mume/rafiki wa kiume?* ☐	..... ☐
	b) Arranged by parents/guardians ?..... *Ilipangwa na wazazi/walezi?* ☐	..... ☐
	c) Arranged by other family members?..... *Ilipangwa na watu wengine kwenye familia* ☐	..... ☐
	d) Arranged by friend?..... ..... *Ilipangwa na rafiki?*	....
	e) Arranged by pimp/manager?.... ..... *Ilipangwa na wakala/meneja?* ☐	..... ☐
		☐

f) Through the internet on your own?..... *Kupitia mtandao wewe mwenyewe?*	....	☐
g) Through mobile phone on your own?..... *Kupitia simu ya rununu wewe wenyewe?*	....	☐
h) On the street on your own?..... .. *Mtaani wewe mwenyewe?*	....	☐
i) At an entertainment venue (bar, disco, club, cafe, sauna etc.) on your own?..... *Katika eneo la burudani wewe mwenyewe?*	..... ☐	☐
j) At hotel/lodge on your own?..... *Kwa mkahawa/lodge wewe mwenyewe?*	Other (Specify)? ☐ _____	☐
k) At work on your own?..... ..... *Kazini wewe mwenyewe?*		☐
l) At school on your own?.....		☐

	Shuleni wewe mwenyewe? m) At church/mosque on your own?..... *Kanisani/mskikiti ni wewe mwenyewe?* n) At bus stop on your own?..... *Kwa kituo cha basi wewe mwenyewe?* o) At my/his home?..... *Kwangu/kwake nyumbani?* p) Other (Specify)?_____ *Nyengine (fafanua)?*	☐ ☐ ☐ ☐
B340	In question B339 above, of all the different places and ways you had met PAYING CLIENTS, which was the most common for the FIRST MONTH OF SEX WORK and the LAST MONTH OF SEX WORK. *Indicate answer using letters.* *Katika swali la B339, kati ya maeneo yote tofauti na njia ulizokutana na wateja wa kulipa, ni mahali gani ulitumia sana sana kwa MWEZI WA KWANZA WA UBANGAIZAJI na kwa MWEZI WA MWISHO WA UBANGAIZAJI?*	
	A. FIRST MONTH OF SEX WORK..... ☐ *MWEZI WA KWANZA WA UBANGAIZAJI*	B. LAST MONTH OF SEX WORK..... ☐ .... *MWEZI WA MWISHO WA UBANGAIZAJI*
B341	Can you tell me the name of that most common place you met PAYING CLIENTS in the LAST MONTH OF SEX WORK?	

	Unaweza kuniambia jina la pahali pa kawaida ulikuwa unakutana na wateja wako wa kulipa katika MWEZI WA MWISHO WA UBANGAIZAJI?
	NAME OF VENUE_____ DON'T REMEMBER..... ☐ _____

B342	In the FIRST MONTH OF SEX WORK and the LAST MONTH OF SEX WORK, did you have a pimp, a Manager/madam or did you work by yourself? *Tick all that apply in each column.* *Katika MWEZI WA KWANZA WA UBANGAIZAJI na MWEZI WA MWISHO WA UBANGAIZAJI, ulikuwa na wakala/meneja/bibie ama ulifanya kazi kivyako?*		
	A. FIRST MONTH OF SEX WORK	B. LAST MONTH OF SEX WORK	
	a) HAD A PIMP..... ..... *Nilikuwa na wakala* ☐ b) HAD A MANAGER..... ..... *Nilikuwa na Manega* ☐ c) WORKED BY MYSELF..... .... *Nilifanya kazi kivyangu* ☐	..... ☐ .... ☐ ..... ☐ .... ☐ .... ☐	
B343	At the time when you first started sex work overall, did you feel coerced or deceived (tricked) into having sex with the FIRST 10 (OR FIRST SEVERAL) PAYING CLIENTS? *Wakati ule ulipoanza kufanya kazi ya kubangaiza kwa mara ya kwanza kwa jumla, ulijihisi kulazimishwa au kudanganywa kufanya mapenzi na WATEJA 10 WAKULIPA WA KWANZA*	YES..... 1 NO..... 0	

	(AU KADHAA WA KWANZA)?		
B344	In the FIRST MONTH OF SEX WORK, was the amount of money you received from your paying clients in exchange for sex less, more or about the same as what you are paid in the LAST MONTH OF SEX WORK? *Katika MWEZI WA KWANZA WA KUBANGAIZA, kiwango cha pesa ulichopokea kutoka kwa wateja wako wa kulipa kwa kushiriki ngono na wao kilikua kidogo, zaidi au sawa na kile ulicholipwa MWEZI WA MWISHO WA UBANGAIZAJI*?	LESS..... 1 MORE..... 2 ABOUT THE SAME..... 3 DON'T KNOW / DON'T REMEMBER..... 88	

B345	In the FIRST MONTH OF SEX WORK and the LAST MONTH OF SEX WORK, did the money from your paying clients cover your living expenses in that month? *Katika MWEZI WA KWANZA WA UBANGAIZAJI na MWEZI WA MWISHO WA UBANGAIZAJI, je pesa ulizopokea kutoka kwa wateja wa kulipa zilisimamia matumizi yako ya kuishi katika mwezi huo?* READ OUT ANSWERS. *Tick one in each column.*	
	A. FIRST MONTH OF SEX WORK	B. LAST MONTH OF SEX WORK
	a) NONE OF THE LIVING EXPENSES..... *HAKUNA*	☐ ☐ ☐

	b) LESS THAN HALF OF THE LIVING EXPENSES..... *CHINI YA NUSU* c) HALF..... ..... *NUSU* d) MORE THAN HALF..... *ZAIDI YA NUSU* e) ALL..... ..... *ZOTE* f) DON'T KNOW / DON'T REMEMBER..... *SIJUI/SIKUMBUKI*	....☐..... ....☐..... ....☐..... ....☐.....	☐ ☐ ☐ ☐
B346	Have some PAYING CLIENTS ever requested or tried to have anal sex with you? *Kunao baadhi ya WATEJA WAKO WAKULIPA wamewahi kukuomba au kujaribu kufanya ngono ya mkundu na wewe?*	YES…… ……..... 1 NO…… ……. 0	
B347	Thinking back to the FIRST MONTH OF SEX WORK, with how many DIFFERENT MEN, including your paying clients, non-client sex partners, husband, spouse and boyfriends, did you have sex (vaginal, anal or both)? Just as a reminder – NON-CLIENT SEX PARTNERS are men with whom you have sex, with the expectation that you would receive money, gifts or other resources in return but the price of sex is often not negotiated upfront and is implicitly understood.	NUMBER OF DIFFERENT MEN IN THE FIRST MONTH OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	B349 →

	Ukifikiria kuhusu ule MWEZI WA KWANZA WA UBANGAIZAJI, ni WANAUME WANGAPI TOFAUTI, ukiweka pamoja na wateja wako wakulipa, wateja wasio kulipa, mume na rafiki wa kiume, ulifanya nao ngono (ya uke, mkundu au zote?) *Ili kukumbusha – WASHIRIKI WANGONO AMBAO SI WATEJA ni wanaume ambao umefanya nao ngono ukitarajia ya kwamba utapokea pesa, zawadi au vitu vyengine baadaye lakini mara nyingi bei ya ngono haijazungumziwa na inaeleweka*		

B348	Thinking back to the FIRST MONTH OF SEX WORK, you said you had sex with _____ *[fill in answer from question B347 above]* DIFFERENT MEN. With how many of them did you have: *Ukufikiria kule nyuma katika MWEZI WA KWANZA WA UBANGAIZAJI ulisema ulifanya ngono na _____ [fill answer from question B347 above] WANAUME TOFAUTI. Ni wangapi kati yao ulifanya nao:* *[DK/DR = DON'T KNOW/DON'T REMEMBER; NA = NOT APPLICABLE]*								
	a) <u>Both vaginal and anal</u> sex? <u>*Ngono ya uke na ya mkundu?*</u>		How many of these men were paying clients? *Wangapi kati ya wanaume hawa walikuwa wateja wa kulipa?*			How many of these men were non-client sex partners? *Wangapi kati ya wanaume hawa walikuwa washiriki wa ngono ambao si wateja?*			
		DK/DR	NA		DK/DR	NA		DK/DR	NA
	b) <u>Only vaginal</u> sex? <u>*ngono uke pekee*</u>*?*		How many of these men were paying clients? *Wangapi kati ya wanaume hawa walikuwa wateja wa kulipa?*			How many of these men were non-client sex partners? *Wangapi kati ya wanaume hawa walikuwa washiriki wa ngono ambao si wateja?*			
		DK/DR	NA		DK/DR	NA		DK/DR	NA

	c) <u>Only anal</u> sex? *<u>Ngono ya mkundu pekee?</u>*		How many of these men were paying clients? *Wangapi kati ya wanaume hawa walikuwa wateja wa kulipa?*			How many of these men were non-client sex partners? *Wangapi kati ya wanaume hawa walikuwa washiriki wa ngono ambao si wateja?*		
	DK/DR	NA		DK/DR	NA		DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)							
B349	In the FIRST MONTH OF SEX WORK, how many PAYING CLIENTS did you have in a TYPICAL WEEK? *Katika MWEZI WA KWANZA WA KUBANGAIZA, ni WATEJA WANGAPI WA KULIPA ulikuwa nao KWA WIKI YA KAWAIDA?*		NUMBER OF PAYING CLIENTS IN A TYPICAL WEEK IN THE FIRST MONTH OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88					
B350	In the FIRST MONTH OF SEX WORK, on average, how many sex acts did you have per client visit? *Katika MWEZI WA KWANZA WA KUBANGAIZA, kwa kawaida, ni vitendo vingapi vya ngono ulifanya na kila mteja unapompata?* *[DK/DR = DON'T KNOW / DON'T REMBMBER; NA = NOT APPLICABLE]*							
	a) Average number of vaginal sex acts per client visit? *Kwa kawaida idadi ya vitendo vya ngono ya uke kila unapokutana na mteja?*			If more than 1 vaginal sex act per visit, on average, for how many of these vaginal sex acts was a condom NOT used? *Ni vitendo vingapi kondomu HAIKUTUMIKA?* *[Skip if average number of vaginal sex act per visit =1]*		
		DK/DR	NA		DK/DR	NA
	b) Average number of anal sex acts per client visit? *Kwa kawaida idadi ya vitendo vya ngono ya mkundu kila unapokutana na mteja?*			If more than 1 anal sex act per visit, on average, for how many of these anal sex acts was a condom NOT used *Ni vitendo vinagapi kondomu HAIKUTUMIKA?* *[Skip if average number of anal sex act per visit =1]*		
		DK/DR	NA		DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)					
B351	In the LAST MONTH OF SEX WORK, on average, how many sex acts did you have per client visit? *Katika MWEZI WA MWISHO WA KUBANGAIZA, kwa kawaida, ulifanya vitendo vingapi vya ngono kila mteja unapompata?* *[DK/DR = DON'T KNOW / DON'T REMBMBER; NA = NOT APPLICABLE]*							

	a) Average number of vaginal sex acts per client visit? *Kwa kawaida idadi ya vitendo vya ngono kwa uke kila unapokutana na mteja?*			If more than 1 vaginal sex act per visit, on average, for how many of these vaginal sex acts was a condom NOT used? *Ni vitendo vingapi kondomu HAIKUTUMIKA?* *[Skip if average number of vaginal sex act per visit =1]*		
		DK/DR	NA		DK/DR	NA
	b) Average number of anal sex acts per client visit? *Kwa kawaida, idadi ya vitendo vya ngono ya mkundu kila unapokutana na mteja?*			If more than 1 anal sex act per visit, on average, for how many of these anal sex acts was a condom NOT used? *Ni vitendo vingapi kondomu HAIKUTUMIKA?* *[Skip if average number of anal sex act per visit =1]*		
		DK/DR	NA		DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)					

B352	Some women only get to keep some of the money from their paying clients in exchange for sex and the rest of the money goes to the person who arranged the exchange. On average, in the LAST MONTH OF SEX WORK, how much of the money did you keep? *Wanawake wengine hujiwekea kiwango fulani cha pesa wanayopata kutoka kwa wateja wanaoshiriki nao ngono na kiwango kingine huenda kwa mtu aliyewakutanisha.* *Kwa kawaida, katika MWEZI WA MWISHO WA KAZI YA*	NO ONE ARRANGES MY SEX ENCOUNTERS; 1 I KEPT ALL OF THE MONEY..... 2 NONE..... 3 ..... 4 LESS THAN HALF..... 5 HALF..... 6 ..... MORE THAN HALF BUT NOT ALL..... ALL.....	

	KUBANGAIZA, ni kiasi gani cha pesa ulijiwekea?		
B353	In the LAST MONTH OF SEX WORK, how many paying clients directly reached you for sex on your mobile phone? *Katika MWEZI WA MWISHO WA KAZI YA KUBANGAIZA ni wateja wangapi wakulipa walikufikia moja kwa moja kupitia simu yako ya rununu?*	NUMBER OF MOBILE PHONE CLIENTS..... ☐☐ DON'T KNOW / UNSURE..... 88	
B354	In the LAST MONTH OF SEX WORK, how many paying clients directly reached you for sex via the Internet? *Katika MWEZI WA MWISHO WA KAZI YA KUBANGAIZA, ni wateja wangapi wakulipa waliwasiliana nawe kupitia mtandao?*	NUMBER OF INTERNET CLIENTS..... ☐☐ DON'T KNOW / UNSURE..... 88	
B355	When was the LAST TIME you had sex with a PAYING CLIENT? *Ni lini MWISHO ulifanya ngono na MTEJA WA KULIPA?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ IF ≥ 1 YEAR AGO, GO TO QUESTION B356. → B356 IF < 1 YEAR AGO, SKIP TO QUESTION B357. → B357	
B356	In the LAST MONTH OF SEX WORK, how many paying clients did you have? *Katika MWEZI WA MWISHO WA KUBANGAIZA, ulikuwa na wateja wangapi wakulipa?*	NUMBER OF PAYING CLIENTS IN THE LAST MONTH OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	B364

B357	How many PAYING CLIENTS did you have sex (vaginal, anal or both) with: *Ni WATEJA WANGAPI WA KULIPA ulifanya nao ngono* (*ya uke, mkundu au zote*):		
	a) In the LAST DAY OF SEX WORK? *Katika SIKU YA MWISHO YA KUBANGAIZA?*	NUMBER OF PAYING CLIENTS IN THE LAST DAY OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
	b) In the LAST WEEK OF SEX WORK? *Katika WIKI YA MWISHO YA KUBANGAIZA?*	NUMBER OF PAYING CLIENTS IN THE LAST WEEK OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
	c) In the LAST MONTH OF SEX WORK? *Katika MWEZI WA MWISHO WA KUBANGAIZA?*	NUMBER OF PAYING CLIENTS IN THE LAST MONTH OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 IF RESPONDENT HAD "00" PAYING CLIENTS IN THE LAST WEEK OF SEX WORK OR SELECTED "DK/DR" FOR PART b), SKIP TO QUESTION B360. →	B360
B358	INTERVIEWER: *Look back to question B357b above and fill in the blank below.* Of the _____ PAYING CLIENTS with whom you had sex in the LAST WEEK OF SEX WORK, how many were REGULAR CLIENTS? Regular clients are clients with whom you had sex on more than one occasion. *Kati ya WATEJAWA KULIPA - _____ ambao umeshiriki ngono nao katika WIKI YA MWISHO YA KUBANGAIZA ni wangapi*	NUMBER OF REGULAR PAYING CLIENTS IN THE LAST WEEK OF SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 IF RESPONDENT HAD "00" REGULAR PAYING CLIENTS IN THE LAST WEEK OF SEX WORK OR SELECTED "DK/DR", SKIP TO QUESTION B360. →	B360

	walikuwa WATEJA WA KAWAIDA? *Wateja wa kawaida ni wateja ambao umeshiriki nao ngono zaidi ya mara moja.*		

B359	Of those REGULAR PAYING CLIENTS in the LAST WEEK OF SEX WORK, with how many of these men did you have: *Kati ya WATEJA WA KULIPA WA KAWAIDA katika WIKI YA MWISHO YA KUBANGAIZA, ni wangapi kati ya wanaume hawa ulifanya nao:* *[DK/DR = DON'T KNOW / DON'T REMBMBER; NA = NOT APPLICABLE]*				
	a) Both vaginal and anal sex? *Ngono ya uke na mkundu?*			With how many of them was a condom NOT used? *Ni wangapi HAIKUTUMIA kondomu nao?*	
		DK/DR	NA	DK/DR	NA
	b) Only vaginal sex? *Ngono ya uke pekee?*			With how many of them was a condom NOT used? *Ni wangapi HAIKUTUMIA kondomu nao?*	
		DK/DR	NA	DK/DR	NA
	c) Only anal sex? *Ngono ya mkundu pekee?*			With how many of them was a condom NOT used? *Ni wangapi HAIKUTUMIA kondomu nao?*	
		DK/DR	NA	DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)				
B360	Think of your LAST REGULAR PAYING CLIENT: *Fikiria kuhusu MTEJA WAKO WA KULIPA WA KAWAIDA WA MWISHO:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*				
	a) How many vaginal sex acts did you have with him in the LAST MONTH OF SEX WORK?			How many of those times was a condom NOT used?	

	Ni vitendo vingapi vya ngono ya uke mlifanya naye KATIKA MWEZI WA MWISHO WA KUBANGAIZA?			*Ni mara ngapi kati ya hizo kondomu HAIKUTUMKIA?*		
		DK/DR	NA		DK/DR	NA
	b) How many <u>anal</u> sex acts did you have with him in the LAST MONTH OF SEX WORK? *Ni vitendo vingapi vya ngono ya mkundu mlifanya naye KATIKA MWEZI WA MWISHO WA KUBANGAIZA?*			How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMKIA?*		
		DK/DR	NA		DK/DR	NA
	I DIDN'T HAVE ANY REGULAR PAYING CLIENTS IN THE LAST MONTH OF SEX WORK..... ☐ (tick)			I NEVER HAD ANAL SEX..... ☐ (tick)		

B361	INTERVIEWER: *Do calculation here.* You told me you had _____ *[fill in answer from question B357b]* paying clients in the last week of sex work and _____ *[fill in answer from question B358]* were regular paying clients. That would mean that in the last week of sex work, _____ were first-time paying clients. *Uliniambia ulikuwa na wateja [[fill in answer from question B357b] katika wiki yako ya mwisho ya kubangaiza na _____ [fill in answer from question B358] walikuwa wateja wa kawaida. Hii inamaanisha kwamba katika wiki yako ya mwisho ya kubangaiza, _____ walikuwa wateja wa kulipa mara ya kwanza.* IF RESPONDENT HAD '00" FIRST-TIME PAYING CLIENTS IN THE LAST WEEK OF SEX WORK, SKIP TO QUESTION B363.	
	a) Is this correct? *Hii ni sawa?*	YES..... 1 ⟶ B36 ..... 2 NO..... 0 .....
	b) If incorrect, how many FIRST-TIME PAYING CLIENTS did	NUMBER OF FIRST-TIME PAYING CLIENTS ☐☐

	you have in the LAST WEEK OF SEX WORK? *Ikiwa si sahihi, ni WATEJA WA KULIPA WA MARA YA KWANZA wangapi ulikuwa nao katika WIKI YA MWISHO YA KUBANGAIZA*?	IN THE LAST WEEK OF SEX WORK.....

B362	Of those FIRST-TIME PAYING CLIENTS in the LAST WEEK OF SEX WORK, with how many of these men did you have: *Kati ya wale WATEJA WA KULIPA WA MARA YA KWANZA katika WIKI YA MWISHO YA KUBANGAIZA, ni wangapi kati ya wanaume hawa ulifanya nao:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*				
	a) Both vaginal and anal sex? *Ngono ya uke na mkundu?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*	
		DK/DR	NA	DK/DR	NA
	b) Only vaginal sex? *Ngono ya uke pekee?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*	
		DK/DR	NA	DK/DR	NA
	c) Only anal sex? *Ngono ya mkundu pekee?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*	
		DK/DR	NA	DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)				

B363	Think of your LAST FIRST-TIME PAYING CLIENT: *Fikiria kuhusu MTEJA WAKO WA MWISHO WA KULIPA WA MARA YA KWANZA:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*				
	a) How many vaginal sex acts did you have with him? *Ni vitendo vingapi vya ngono ya uke mlifanya naye?*			How many of those times was a condom NOT used? *Ni mara ngapi kondomu HAIKUTUMIKA?*	
		DK/DR	NA	DK/DR	NA

	b) How many <u>anal</u> sex acts did you have with him? *Ni vitendo vingapi vya ngono ya mkundu mlifanya naye?*			How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I DON'T HAVE ANY FIRST-TIME PAYING CLIENTS..... (tick) ☐			I NEVER HAD ANAL SEX..... (tick) ☐		
B364	Now think back to when you first entered sex work. In the 1 MONTH BEFORE you entered sex work, how many DIFFERENT NON-CLIENT SEX PARTNERS did you have sex with – again these are men with whom you have sex, with the expectation that you would receive money, gifts or other resources in return but the price of sex is often not negotiated upfront and is implicitly understood? *Sasa fikiria kule nyuma ulipoingia katika kazi ya ubangaizaji. KATIKA MWEZI 1 KABLA ya uingie kwenye ubangaizaji, ni WASHIRIKI WANGAPI TOFAUTI WA NGONO AMBAO SI WATEJA ulifanya nao ngono – tena hawa ni wanaume ambao umefanya nao ngono ukiwa na matarajio ya kupokea pesa, zawadi au vitu vingine baadaye lakini mara nyingi bei ya ngono hajadiliwi kabla na inaeleweka*	NUMBER OF NON-CLIENT SEX PARTNERS IN THE ONE MONTH BEFORE SEX WORK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88				
B365	In the FIRST MONTH OF SEX WORK, how many DIFFERENT NON-CLIENT SEX PARTNERS did you have?	NUMBER OF NON-CLIENT SEX PARTNERS IN FIRST MONTH OF SEX WORK..... ☐☐				

	Katika MWEZI WA KWANZA WA KUBANGAIZA, ni WANAUME WANGAPI TOFAUTI WASIO WATEJA ulifanya nao?	DON'T KNOW / DON'T REMEMBER..... 88	

B366	Now I am going to ask you about ALL THE OTHER MEN with whom you had sex. These men may include NON-CLIENT SEX PARTNERS (i.e. men with whom you have sex, with the expectation that you would receive money, gifts or other resources in return but the price of sex is often not negotiated upfront and is implicitly understood) as well as HUSBAND, SPOUSE or BOYFRIENDS. How many OTHER MEN did you have sex with: *Sasa nitakuuliza maswali kuhusu WANAUME WOTE WENGINE ambao umefanya nao ngono. Wanaume hawa ni pamoja na WASHIRIKI WA NGONO WASIO WATEJA (k.m. wanaume unaofanya nao ngono ukiwa na matarajio ya kwamba utapokea pesa, zawadi au vitu vyengine baadaye lakini mara nyingi bei ya ngono haijajadiliwa mbeleni na inaeleweka) pamoja na BWANA AU RAFIKI WA KIUME* *Ni WANAUME WENGINE wangapi umefanya nao ngono:*		
	a) In the LAST DAY? *Katika SIKU YA MWISHO?*	NUMBER OF OTHER SEX PARTNERS IN THE LAST DAY..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
	b) In the LAST WEEK? *Katika WIKI YA MWISHO?*	NUMBER OF OTHER SEX PARTNERS IN THE LAST WEEK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
	c) In the LAST MONTH? *Katika MWEZI WA MWISHO?*	NUMBER OF OTHER SEX PARTNERS IN THE LAST MONTH..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 IF RESPONDENT HAD "00" OTHER SEX PARTNERS IN THE LAST WEEK OR SELECTED "DK/DR" IN PART b), SKIP TO QUESTION B370.	B370

B367	INTERVIEWER: *Look back to question B366b above and fill in the blank below.* Of the _____ OTHER SEX PARTNERS in the LAST WEEK, how many non-client sex partners did you have sex with who gave you money, gifts or other resources in exchange for sex? *Kati ya WASHIRIKI WAKO WENGINE WA NGONO katika WIKI ILIYOPITA, ni washiriki wangapi wa ngono wasio wateja ulifanya nao ngono na wakakupatia pesa, zawadi au vitu vyengine kwa ajili ya kufanya ngono?*	NUMBER OF NON-CLIENT SEX PARTNERS IN THE LAST WEEK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 IF RESPONDENT HAD "00" NON-CLIENT SEX PARTNERS IN THE LAST WEEK OR SELECTED "DK/DR", SKIP TO ~~QUESTION~~ B370. →	B370

B368	INTERVIEWER: *Look back to the question B367 above and fill in the blank below.* Of those_____ non-client sex partners in the LAST WEEK that gave you money, gifts or other resources in exchange for sex, how many are REGULAR SEX PARTNERS with whom you had sex on more than one occasion? *Kwa wale washiriki wa ngono _____ wasio wa kulipa katika WIKI YA MWISHO waliokupatia pesa, zawadi au vitu vyengine kwa ajili ya ngono, ni wangapi ni WASHIRIKI WA NGONO WA KAWAIDA ambao ulifanya nao ngono zaidi ya mara moja?*	NUMBER OF REGULAR NON-CLIENT SEX PARTNERS IN THE LAST WEEK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 IF RESPONDENT HAD "00" REGULAR NON-CLIENT SEX PARTNERS IN THE LAST WEEK OR SELECTED "DK/DR", SKIP TO QUESTION B370. →	B370

B369	Of those REGULAR, NON-CLIENT SEX PARTNERS in the LAST WEEK, with how many of these men did you have: *Kati ya WASHIRIKI WAKO WA NGONO WA KAWAIDA, WASHIRIKI WA NGONO WASIO WATEJA katika WIKI YA MWISHO, ni wangapi kati yao ulifanya:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	d) Both vaginal and anal sex? *Ngono ya uke na ya mkundu?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	e) Only vaginal sex? *Ngono ya uke pekee?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	f) Only anal sex? *Ngono ya mkundu pekee?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)					
B370	Think of your LAST REGULAR NON-CLIENT SEX PARTNER: *Fikiria kuhusu MSHIRIKI WAKO WA MWISHO WA KAWAIDA WA NGONO ASIYE MTEJA:* *[DK/DR = DON'T KNOW / DON'T REMEMBER]*					
	a) How many vaginal sex acts did you have with him in the LAST MONTH? *Ni vitendo vingapi vya ngono ya uke mlifanya naye katika MWEZI WA MWISHO?*			How many of those times was a condom NOT used? *Ni mara ngapi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) How many anal sex acts did you have with him in the LAST MONTH? *Ni vitendo vingapi vya ngono ya mkundu mlifanya naye katika MWEZI WA MWISHO?*			How many of those times was a condom NOT used? *Ni mara ngapi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I DIDN'T HAVE ANY REGULAR ☐			☐		

	NON-CLIENT SEX PARTNERS IN THE LAST MONTH..... (tick)	I NEVER HAD ANAL SEX..... (tick)
B371	INTERVIEWER: *Do calculation here.* You told me you had _____ *[fill in answer from question B367]* non-client sex partners in the last week and _____ *[fill in answer from question B368]* were regular non-client sex partners. That would mean that in the last week, _____ were first-time non-client sex partners. *Uliniambia ulikuwa na washiriki wa ngono wasio wateja _____ [[fill in answer from question B367] katika wiki ya mwisho na _____ [fill in answer from question B368] walikuwa washiriki wa ngono wa kawaida wasio wateja. Hii inamaanisha kwamba katika wiki ya mwisho _____ walikuwa washiriki wa ngono wasio wateja wa mara ya kwanza.* IF RESPONDENT HAD "00" FIRST-TIME NON-CLIENT SEX PARTNES IN THE LAST WEEK, SKIP TO QUESTION B373.	
	a) Is this correct? *Hii ni sawa?*	YES..... 1 → B372 NO..... 0
	b) If incorrect, how many FIRST TIME NON-CLIENT SEX PARTNER did you have in the LAST WEEK? *Kama si sawa, ni washiriki wa wangapi wa ngono wa MARA YA KWANZA AMBAO SI WATEJA ulifanya nao ngono WIKI YA MWISHO?*	NUMBER OF FIRST-NON-CLIENT SEX PARTNERS IN THE LAST WEEK..... ☐☐

B372	Of those FIRST-TIME NON-CLIENT SEX PARTNERS in the LAST WEEK, with how many of these men did you have: *Kati ya WASHIRIKI WA NGONO WA KWANZA WASIO WATEJA katika WIKI YA MWISHO, ni wangapi kati ya hawa wanaume ulifanya:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) <u>Both vaginal and anal</u> sex? <u>*Ngono ya uke na ya mkundu*</u>*?*			With how many of them was a condom NOT used? *Ni wangapi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) <u>Only vaginal</u> sex? <u>*Ngono ya uke pekee?*</u>			With how many of them was a condom NOT used? *Ni wangapi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA

	c) Only anal sex? *Ngono ya mkundu pekee?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)					
B373	Think of your LAST FIRST TIME NON-CLIENT SEX PARTNER: *Fikiria kuhusu MSHIRIKI WAKO WA NGONO WA MWISHO, ASIYE MTEJA WA KWANZA: [DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many vaginal sex acts did you have with him? *Ni vitendo vingapi vya ngono ya uke mlifanya naye?*			How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) How many anal sex acts did you have with him? *Ni vitendo vingapi vya ngono ya mkundu mlifanya naye?*			How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I DON'T HAVE ANY FIRST-TIME NON-CLIENT SEX PARTNERS..... ☐ (tick)			I NEVER HAD ANAL SEX..... ☐ (tick)		
B374	Thinking of your LAST NON-CLIENT SEX PARTNER – this is the last man with whom you had sex, with the expectation that you would receive money, gifts or other resources in return but the price of sex is often not negotiated upfront and is implicitly understood, how did you first meet him? For example, was the meeting arranged by someone? Did you meet him somewhere? *Ukifikiria kuhusu mshiriki wako wa ngono wa MWISHO AMBAYE SI MTEJA – huyu ni yule*			ARRANGED BY SPOUSE / BOYFRIEND..... 1 ARRANGED BY PARENTS / GUARDIANS..... 2 ARRANGED BY OTHER FAMILY MEMBERS..... 3 ARRANGED BY FRIEND..... 4 ARRANGED BY PIMP / MANAGER..... 5 MET THROUGH INTERNET ON MY OWN..... 6 MET THROUGH MOBILE PHOHE ON MY OWN..... 7 MET ON STREET ON MY OWN..... 8 MET AT ENTERTAINMENT VENUE (BAR, DISCO, CLUB, CAFE, SAUNA ETC.)		

	mwanaume wa mwisho ambaye mlifanya naye ngono ukiwa na mataraji kuwa utapokea zawadi au vitu vyengine baadaye lakini mara nyingi bei ya ngono haizungumziwi mbeleni na inaeleweka, ulikutana naye vipi mara ya kwanza? *Kwa mfano, kukutana huko kulipangwa na mtu? Ulikutana naye mahali fulani?*	ON MY OWN..... 9 MET AT HOTEL / LODGE ON MY OWN..... 10 MET AT WORK ON MY OWN..... 11 MET AT SCHOOL ON MY OWN..... 12 MET AT CHURCH / MOSQUE ON MY OWN..... 13 MET AT BUS STOP ON MY OWN..... 14 MET AT MY / HIS HOME..... 15 OTHER (SPECIFY) _____ 16 DON'T REMEMBER..... 88	

B375***	I would like you to think back to the first time a NON-CLIENT SEX PARTNER gave you money, gifts or other resources in exchange for sex. In the 1 MONTH AFTER you had sex with him for the first time, how many other DIFFERENT NON-CLIENT SEX PARTNERS did you have? *Ningependa ufikirie nyuma kuhusu ile mara ya kwanza MSHIRIKI WA NGONO ASIYE WAKULIPA alikupatia zawadi au vitu vyengine kwa ajili ya kufanya ngono. Katika MWEZI 1 BAADA ya kufanya ngono naye mara ya kwanza, ni WASHIRIKI WANGAPI WA*	NUMBER OF NON-CLIENT SEX PARTNERS IN THE ONE MONTH AFTER FIRST NON-CLIENT SEX PARTNER..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	

	NGONO WASIO WATEJA TOFAUTI umekua nao?		
B376	Now let's think about your husband, spouse or boyfriends. How many of these men did you have sex with: *Sasa tufikirie kuhusu mume au rafiki yako wa kiume. Ni wangapi kati yao umefanya nao ngono:*		
	a) In the LAST DAY? *Katika SIKU YA MWISHO?*	NUMBER OF HUSBAND / SPOUSE / BOYFRIENDS IN THE LAST DAY..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
	b) In the LAST WEEK? *Katika WIKI YA MWISHO?*	NUMBER OF HUSBAND / SPOUSE / BOYFRIENDS IN THE LAST WEEK..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
	c) In the LAST MONTH? *Katika MWEZ I WA MWISHO?*	NUMBER OF HUSBAND / SPOUSE / BOYFRIENDS IN THE LAST MONTH..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 IF RESPONDENT HAD "00" HUSBAND, SPOUSE OR BOYFRIENDS IN THE <u>LAST WEEK</u> OR SELECTED "DK/DR" IN PART b), SKIP TO QUESTION B379. →	B379

B377	In the LAST WEEK, of your husband, spouse or boyfriends, with how many of these men did you have: *Katika WIKI YA MWISHO, kati ya mume au rafiki yako wa kiume, ni wangapi kati yao umefanya nao:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) <u>Both vaginal and anal</u> sex? *Ngono ya uke na ya mkundu?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) <u>Only vaginal</u> sex? *<u>Ngono ya uke pekee?</u>*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA

	c) Only anal sex? *Ngono ya mkundu pekee?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I NEVER HAD ANAL SEX..... ☐ (tick)					
B378	Of your husband, spouse or boyfriends with whom you had sex LAST WEEK, what are their relationships to you? *Kati ya mume au rafiki yako wa kiume ambaye ulifanya naye ngono WIKI YA MWISHO, ni nini uhusiano wao na wewe?* MULTIPLE ANSWERS POSSIBLE	HUSBAND / SPOUSE..... A BOYFRIEND..... B ..... C OTHER (SPECIFY) _____ ___				
B379	Think of your LAST HUSBAND, SPOUSE or BOYFRIEND: *Fikiria kuhusu MUME AU RAFIKI YAKO WA KIUME WA MWISHO:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many vaginal sex acts did you have with him in the LAST MONTH? *Ni vitendo vingapi vya ngono ya uke ulifanya naye katika MWEZI WA MWISHO?*			How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) How many anal sex acts did you have with him in the LAST MONTH? *Ni vitendo vingapi vya ngono ya mkundu ulifanya naye katika MWEZI WA MWISHO?*			How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	I DIDN'T HAVE HUSBAND, SPOUSE OR BOYFRIEND IN THE LAST MONTH..... ☐ (tick)			I NEVER HAD ANAL SEX..... ☐ (tick)		

SECTION IV: PERCEPTION/DISCLOSURE OF SEX WORK

No.	QUESTIONS	CODING CATEGORIES
B401	Who among your friends and/or family know you are/were a sex worker? *Ni nani kati ya rafiki zako na/au familia wanajua wewe ni/ulikuwa mbangaizaji?* INTERVIEWER: *If respondent answers "YES" to any of the following categories, ask if she told that person. Tick all that apply. [NA = NOT APPLICABLE]*	

	i) Does this person know? *Je huyu mtu anajua?*			ii) Did you tell them? *Ulimwambia?*		
a) MOTHER / *MAMA*	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐
b) FATHER / *BABA*	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐
c) GUARDIAN / *MLEZI*	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐
d) BROTHER(S) / SISTER(S) *KAKA/DADA (ZANGU)*	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐
e) OTHER RELATIVE(S) *JAMAA WENGINE*	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐

	f) FRIENDS / ROOMMATES / NEIGHBOURS MARAFIKI / UNAOISHI NAO / MAJIRANI	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐
	g) HUSBAND / SPOUSE / BOYFRIENDS MUME / RAFIKI WA KIUME	YES ☐	NO ☐	NA ☐	YES ☐	NO ☐	NA ☐

B402	Presently, how many other women do you know personally who are in sex work? *Kwa sasa, ni wanawake wangapi wengine unaowajua kibinafsi wanabangaiza?*	NONE..... 1 1 TO 5..... 2 6 TO 15..... 3 16 TO 30..... 4 MORE THAN 30..... 5

B403	Some people think that sex work is easy, lucrative and desirable. Others think that sex work is difficult, hard-earned and undesirable. On a scale from 1 to 10, do you think... *Watu wengine hufikirii kuwa kazi ya kubangaiza ni rahisi, ya faida na ni ya kutamanika. Wengine wanadhani kuwa kazi ya kubangaiza ni ngumu, haina pesa na si ya kutamanisha. Katika mizani ya 1 hadi 10, unafikiri...*	
	Sex work is: *Ubangaizaji ni?* 	Score

	a) Unpleasant/*Mbaya* Pleasant/S*io mbaya* ⟷	
	b) Non-prestigious/ Prestigious/*Ya kifahari* *Si ya kifahari* ⟷	
	c) Hard-earned money/ Easy money/ *Rahisi kupata pesa* ⟷ *Ngumu kupata pesa*	
	d) Dangerous/*hatari* Not dangerous/*si hatari* ⟷	
	e) Does not lead to lead to desirable desirable lifestyle lifestyle ⟷ Can *Haiwezi kumpatia mtu* *Inaweza kumpatia mtu* *maisha anayotamani* *maisha anayotamani*	

SECTION V. ALCOHOL AND ILLICIT DRUG USE

No.	QUESTIONS	CODING CATEGORIES	SKIP
B501	In the LAST MONTH, how often did you consume alcohol? *Katika MWEZI WA MWISHO, ulitumia pombe mara ngapi?*	LESS THAN 1 TIME A MONTH..... 1 1 TO 3 TIMES A MONTH..... 2 1 TO 3 TIMES A WEEK..... 3 ALMOST EVERYDAY..... 4 EVERYDAY..... 5 I NEVER CONSUMED BEVERAGES CONTAINING ALCOHOL.....77 →	B511
B502	In the LAST MONTH, was there at least one occasion where you consumed 5 or more alcoholic drinks (binge-drank)?	YES..... 1 ..... 0 NO.....	

	Katika MWEZI WA MWISHO, kuna angalau tukio moja ambapo ulitumia vinywaji 5 au zaidi vya pombe (kulewa)?		

B503	In the LAST MONTH, how many times did you get drunk (under the influence of alcohol)? *Katika MWEZI WA MWISHO, ni mara ngapi ulilewa?(kama umekunywa pombe)?*	0 TIMES..... 1 → B511 1 TO 3 TIMES..... 2 4 TO 6 TIMES..... 3 7 TO 10 TIMES..... 4 MORE THAN 10 TIMES..... 5	

B504	In the LAST MONTH, how often were *you* drunk when you had sex with: Katika *MWEZI WA MWISHO, ni mara ngapi ulikuwa mlevi ulifanya ngono na:*					
	(Tick one for each partner type)	Regular paying clients *Mteja wa kulipa wa kawaida*	First-time paying clients *Mteja wa kulipa wa mara ya kwanza*	Regular non- client sex partners *Mteja wa kawaida asiye wa kulipa*	First-time non-client sex partners *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	ALWAYS/*KILA MARA* (9-10 TIMES OUT OF 10)					
	OFTEN/*MARA NYINGI* (7-8 TIMES OUT OF 10)					
	SOMETIMES/*MARA NYINGINE* (3-6 TIMES OUT OF 10)					
	SELDOM/*NADRA* (1-2 TIMES OUT OF 10)					
	NEVER/SIJAWAHI					

	(0 TIMES OUT OF 10)					
	NOT APPLICABLE					

B505	Think of the LAST TIME you had sex with this type of partner when *you* were drunk, did you have vaginal sex with him? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki ukiwa mlevi, je ulishiriki ngono ya uke na yeye?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					

B506	Think of the LAST TIME you had sex with this type of partner when *you* were drunk, was a condom used for vaginal sex? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki wakati ukiwa mlevi, je kondomu ilitumika kwa ngono ya uke?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					

B507	Think of the LAST TIME you had sex with this type of partner when *you* were drunk, did you have anal sex with him? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshirika wakati ukiwa mlevi, je ulishiriki ngono ya mkundu na yeye?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					
	I NEVER HAD ANAL SEX..... ☐ (tick)					

B508	Think of the LAST TIME you had sex with this type of partner when *you* were drunk, was a condom used for anal sex? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki wakati ukiwa mlevi, je kondomu ilitumika kwa ngono ya mkundu?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida*	A first-time non-client sex partner *Mshiriki wa ngono asiye*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*

		wa kawaida		*asiye wa kulipa*	*mteja wa mara ya kwanza*	
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					
	I NEVER HAD ANAL SEX..... ☐ (tick)					
B509	Think of the LAST TIME you had sex with this type of partner when *you* were drunk, did he physically hurt you? *FikiriaMARA YA MWISHO ulipofanya ngono na aina hii ya mshiriki ukiwa mlevi, je alikuumiza kimwili?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					

B510	Think of the LAST TIME you had sex with this type of partner when *you* were drunk, did he force you to have sex with him when you were not willing? *Fikiria MARA YA MWISHO ulipofanya ngono na aina hii ya mshiriki ukiwa mlevi, je alikulazimisha kufanya ngono na yeye ijapokuwa ulikuwa hutaki?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					
B511	In the LAST MONTH, how often was *this type of partner* drunk when you had sex with them: *Katika MWEZI WA MWISHO, ni mara ngapi aina ya mteja huyu alikuwa mlevi ulipofanya ngono naye?*					
	(Tick one for each partner type)	Regular paying clients *Mteja wa kulipa wa kawaida*	First-time paying clients *Mteja wa kulipa wa mara ya kwanza*	Regular non- client sex partners *Mteja wa kawaida asiye wa kulipa*	First-time non-client sex partners *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	ALWAYS/ *KILA MARA* (9-10 TIMES OUT OF 10)					
	OFTEN/*MARA NYINGI* (7-8 TIMES OUT OF 10)					
	SOMETIMES/*MARA NYINGINE* (3-6 TIMES OUT OF 10)					

	SELDOM/*NADRA* (1-2 TIMES OUT OF 10)					
	NEVER/SIJAWAHI (0 TIMES OUT OF 10)					
	NOT APPLICABLE					

B512	Think of the LAST TIME you had sex with this type of partner when *he* was drunk, did you have vaginal sex with him? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki wakati akiwa mlevi, je kondomu ilitumika kwa ngono ya uke?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					
B513	Think of the LAST TIME you had sex with this type of partner when *he* was drunk, was a condom used for vaginal sex? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki wakati akiwa mlevi, je kondomu ilitumika kwa ngono ya uke?*					
	(Tick one for each	A regular paying client	A first-time paying client	A regular non- client sex partner	A first-time non-client sex partner	Your husband, spouse or boyfriend

	partner type)	*Mteja wa kulipa wa kawaida*	*Mteja wa kulipa wa mara ya kwanza*	*Mteja wa kawaida asiye wa kulipa*	*Mshiriki wa ngono asiye mteja wa mara ya kwanza*	*Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					

B514	Think of the LAST TIME you had sex with this type of partner when *he* was drunk, did you have anal sex with him? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki wakati akiwa mlevi, je ulishiriki ngono ya mkundu na yeye?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					

	I NEVER HAD ANAL SEX..... ☐ (tick)					
B515	Think of the LAST TIME you had sex with this type of partner when *he* was drunk, was a condom used for anal sex? *Fikiria MARA YA MWISHO uliposhiriki ngono na aina hii ya mshiriki wakati akiwa mlevi, je kondomu ilitumika kwa ngono ya mkundu?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					
	I NEVER HAD ANAL SEX..... ☐ (tick)					

B516	Think of the LAST TIME you had sex with this type of partner when *he* was drunk, did he physically hurt you? *Fikiria MARA YA MWISHO ulipofanya ngono na aina hii ya mshiriki akiwa mlevi, je alikuumiza kimwili?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *shiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					

	DON'T REMEMBER					
	NOT APPLICABLE					

B517	Think of the LAST TIME you had sex with this type of partner when *he* was drunk, did he force you to have sex with him when you were not willing? *Fikiria MARA YA MWISHO ulipofanya ngono na aina hii ya mshiriki akiwa mlevi, je alikulazimisha kufanya naye ngono wakati hukua unataka?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					

B518 ***	Now I'm going to ask you some questions related to drug use. How old were you when you first used drugs other than those required for medical reasons (to make you high)? *Sasa nitakuuliza maswali kuhusiana na matumizi ya dawa za kulevya.* *Ulikuwa na miaka mingapi wakati ulipotumia dawa za kulevya mara ya kwanza mbali na zile zinazohitajika kwa sababu za matibabu (kukufanya ulewe)?*	AGE IN YEARS..... ☐☐ I NEVER USED DRUGS OTHER THAN FOR MEDICAL REASONS..... 77 → B535 DON'T KNOW / DON'T REMEMBER..... 88	

B519	How did you take your first drug? *Ulitumia vipi dawa yako ya kulevya ya kwanza?*	INJECTED (WITH A SYRINGE)..... 1 WITHOUT INJECTING (SMOKED, SNORTED, SWALLOWED, CHEWED ETC.)..... 2 DON'T KNOW / DON'T REMEMBER..... 88	
B520***	a) How many years ago did you first inject drugs? *Ni miaka mingapi iliopita ulipoanza kujidunga dawa za kulevya kwa mara ya kwanza?* *If respondent answers "more than 2 years ago", fill in part a) only.* *If respondent answers "2 years ago or less", fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) I NEVER INJECTED DRUGS..... 77 → B531 DON'T REMEMBER..... 88	
	b) If 2 years ago or less, in what month and year did you first inject drugs? *Kama ni chini ya miaka 2 iliopita, ni mwezi na mwaka gani ulipoanza kujidunga dawa za kulevya?*	MONTH..... ☐☐ YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	
B521	In the LAST MONTH, how often do you inject drugs? *Katika MWEZI WA MWISHO ni mara ngapi ulijidunga dawa za kulevya?*	MORE THAN 3 TIMES A DAY..... 1 2 TO 3 TIMES A DAY..... 2 ONCE A DAY..... 3 MORE THAN 3 TIMES A WEEK..... 4 2 TO 3 TIMES A WEEK..... 5 ONCE A WEEK..... 6 ONCE A MONTH..... 7 LESS THAN ONCE A MONTH..... 8	

		I STOPPED INJECTING DRUGS..... 77 DON'T REMEMBER..... 88	
B522	When was the LAST TIME you injected drugs? *Ni lini MARA YA MWISHO ulijidunga dawa za kulevya ilikuwa lini?*	TODAY.....1 YESTERDAY..... 2 LAST WEEK..... 3 LAST MONTH..... 4 LAST YEAR..... 5 OTHER (SPECIFY) _____6 DON'T REMEMBER..... 88	

B523	How long have/had you been injecting drugs? *Umekuwa/ulikuwa ukijidunga dawa za kulevya kwa muda gani?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS..... ☐☐ NUMBER OF MONTHS..... ☐☐ NUMBER OF YEARS..... ☐☐ 8 99 DON'T KNOW / DON'T REMEMBER..... NO ANSWER..... .	
B524 ***	How old were you when you first shared a needle, syringe, or other injecting equipment with someone else? *Ulikuwa miaka mingapi mara ya kwanza ulipotumia sindano pamoja na mtu mwengine, bomba au kifaa chengine cha kujidunga?*	AGE IN YEARS..... ☐☐ I NEVER SHARED NEEDLES, SYRINGE, OR OTHER INJECTING EQUIPMENT..... 77 DON'T KNOW / DON'T REMEMBER..... 88	→B529
B525		1	

	In the LAST MONTH, how often did you lend another person your used needle/syringe? *Katika MWEZI WA MWISHO, ni mara ngapi ulimpatia mtu mwengine sindano/bomba uliotumia?*	ALWAYS (9-10 TIMES OUT OF 10..... 2 OFTEN (7-8 TIMES OUT OF 10)..... 3 SOMETIMES (3-6 TIMES OUT OF 10)..... 4 SELDOM (1-2 TIMES OUT OF 10)..... 5 NEVER (0 TIMES OUT OF 10).....	
B526	In the LAST MONTH, how often did you inject with another person's used needle/ syringe? *Katika MWEZI WA MWISHO, ni mara ngapi umejidunga na sindano/bomba iliyotumiwa na mtu mwengine?*	ALWAYS (9-10 TIMES OUT OF 10..... 1 OFTEN (7-8 TIMES OUT OF 10)..... 2 SOMETIMES (3-6 TIMES OUT OF 10)..... 3 SELDOM (1-2 TIMES OUT OF 10)..... 4 NEVER (0 TIMES OUT OF 10)..... 5	
B527	In the LAST MONTH, how often did you use another person's used spoon, mixing container or filter when preparing a drug for injection? *Katika MWEZI WA MWISHO, ni mara ngapi umetumia kijiko, kifaa cha kuchanganyia au chujio la mtu mwengine wakati wa kutayarisha dawa kwa kujidunga?*	ALWAYS (9-10 TIMES OUT OF 10..... 1 OFTEN (7-8 TIMES OUT OF 10)..... 2 SOMETIMES (3-6 TIMES OUT OF 10)..... 3 SELDOM (1-2 TIMES OUT OF 10)..... 4 NEVER (0 TIMES OUT OF 10)..... 5	

B528	In the LAST MONTH, with whom did you share needles, syringes or other injection equipment when you injected with someone else:

	Katika MWEZI WA MWISHO, ni nani ulitumia naye sindano, mabomba au vifaa vyengine vya kujidunga wakati ulipojidunga pamoja na mtu mwengine:			
	a) HUSBAND/SPOUSE /BOYFRIENDS?..... ..... *MUME/MCHUMBA /RAFIKI YA KIUME?* b) PAYING CLIENTS?..... ..... *WASTIRI / WATEJA?* c) NON-CLIENT SEX PARTNERS?..... ..... *WASHIRIKI WA NGONO WASIO WATEJA?* d) FAMILY MEMBERS?..... ..... *WATU WA FAMILIA?* e) FRIENDS?..... ..... *MARAFIKI?* f) FELLOW FEMALE SEX WORKERS?..... . *WABANGAIZAJI WENZAKO WA KIKE*? g) STRANGERS?..... ..... *WATU USIOWAJUA?* h) OTHER (SPECIFY)?_____ _____ *WENGINEO (FAFANUA)?*		☐ ✓ *Tick all that apply.* ☐ ☐ ☐ ☐ ☐ ☐	
B529	In the LAST MONTH, how often did you inject with a new unused needle/syringe?	ALWAYS (9-10 TIMES OUT OF 10..... OFTEN (7-8 TIMES OUT OF 10).....	1 2 3 4 5	

	Katika MWEZI WA MWISHO, ni mara ngapi ulijidunga na sindano mpya ambayo haijatumiwa?	SOMETIMES (3-6 TIMES OUT OF 10)..... SELDOM (1-2 TIMES OUT OF 10)..... NEVER (0 TIMES OUT OF 10).....	
B530	When was the LAST TIME you obtained new unused needles/syringes? *Ni lini MARA YA MWISHO ulipata sindano mpya ambayo haijawahi tumiwa?*	TODAY..... 1 YESTERDAY..... 2 LAST WEEK..... 3 LAST MONTH..... 4 LAST YEAR..... 5 NEVER..... 6 OTHER (SPECIFY) _____ 7	

B531	Are you currently using opioid substitution therapy? *Je kwa sasa unatumia tiba mbadala ya opioid? (opioid ni tiba inayotumika badala ya madawa ya kulevya)*	YES..... 1 NO..... 0	→B533
B532	Have you ever used opioid substitution therapy? *Umeshawahi kutumia tiba mbadala ya opioid?*	YES..... 1 NO..... 0	
B533	In the LAST MONTH, how often were *you* high from injecting and/or non-injecting drugs when you had sex with this type of sex partners: *Katika MWEZI WA MWISHO, ni mara ngapi ulikuwa umelewa kutokana na dawa za kujidunga/au za kutojidunga uliposhiriki ngono na aina hii ya washiriki wa ngono:*		

	(Tick one for each partner type)	Regular paying clients *Mteja wa kulipa wa kawaida*	First-time paying clients *Mteja wa kulipa wa mara ya kwanza*	Regular non- client sex partners *Mteja wa kawaida asiye wa kulipa*	First-time non-client sex partners *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	ALWAYS/ *KILA MARA* (9-10 TIMES OUT OF 10)					
	OFTEN/*MARA NYINGI* (7-8 TIMES OUT OF 10)					
	SOMETIMES/*MARA NYINGINE* (3-6 TIMES OUT OF 10)					
	SELDOM/ *NADRA* (1-2 TIMES OUT OF 10)					
	NEVER/SIJAWAHI (0 TIMES OUT OF 10)					
	NOT APPLICABLE					

B534	Think of the LAST TIME you had sex with this type of partner when *you* were high from injecting and/or non-injecting drugs, was a condom used? *Fikiria ile MARA YA MWISHO ulipofanya ngono na aina hii ya mshiriki ukiwa umelewa kutokana na dawa za kujidunga/au za kutojidunga je kondomu ilitumika?*					
	(Tick one for each partner type)	A regular paying client *Mteja wa kulipa wa kawaida*	A first-time paying client *Mteja wa kulipa wa mara ya kwanza*	A regular non- client sex partner *Mteja wa kawaida asiye wa kulipa*	A first-time non-client sex partner *Mshiriki wa ngono asiye mteja wa mara ya kwanza*	Your husband, spouse or boyfriend *Mume wako au rafiki wa kiume*
	YES					
	NO					
	DON'T REMEMBER					
	NOT APPLICABLE					

B535	Of your LAST 10 PAYING CLIENTS, how many of them injected/inject drugs? *Kati ya WATEJA WAKO 10 WA KULIPA WA MWISHO, ni wangapi walijidunga/hujidunga dawa za kulevya?*	NUMBER OF PAYING CLIENTS WHO INJECT(ED) DRUGS..... ☐☐ DON'T KNOW / UNSURE..... 88 DON'T REMEMBER..... 89	
B536	Of your LAST 10 NON-CLIENT SEX PARTNERS – these are men with whom you have sex, with the expectation that you would receive money, gifts or other resources in	NUMBER OF NON-CLIENT SEX PARTNERS WHO INJECT(ED) DRUGS..... ☐☐ DON'T KNOW / UNSURE..... 88	

	return but the price of sex is often not negotiated upfront and is implicitly understood, how many of them injected/inject drugs? *Kati ya WASHIRIKA WAKO 10 WA MWISHO WA NGONO WASIO WATEJA – hawa ni wanaume ambao umefanya nao ngono ukiwa na matarajio ya kupokea pesa, zawadi au vitu vyengine lakini mara nyinigi bei ya ngono haijazungumzwa mbeleni na inaeleweka, ni wangapi walijidunga/wanajindunga dawa za kulevya?*	DON'T REMEMBER..... 89	
B537	Of your LAST 10 HUSBANDS, SPOUSES OR BOYFRIENDS, how many of them injected/inject drugs? *Kati ya MABWANA AU MARAFIKI WA KIUME 10 WA MWISHO, ni wangapi walijidunga/wanajidunga dawa za kulevya?*	NUMBER OF HUSBANDS, SPOUSES OR BOYFRIENDS WHO INJECT(ED) DRUGS..... ☐☐ DON'T KNOW / UNSURE..... 88 DON'T REMEMBER..... 89	

SECTION VI: VIOLENCE

No.	QUESTIONS	CODING CATEGORIES	SKIP
The questions I am going to ask you now are about physical and sexual violence that you might have experienced from the hands of men with whom you have had sex. *Maswali nitakayokuuliza sasa yanahusu vurugu za kimwili na ngono ambazo huenda umepitia mikononi mwa wanaume ambao umeshiriki ngono nao.*			
B601***	a) Think of the FIRST TIME when you were physically hurt by a sex partner. How many years ago was that?	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐	

	Kumbuka MARA YA KWANZA ulipoumizwa mwilini na mshiriki wa ngono. Ni miaka mingapi iliyopita? *If respondent answers "more than 2 years ago",* *fill in part a) only.* *If respondent answers "2 years ago or less",* *fill in parts a) and b).*	(IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) I HAVE NEVER EXPERIENCED PHYSICAL VIOLENCE..... 77 → B612 DON'T REMEMBER..... 88	
	b) If 2 years ago or less, in what month and year were you first physically hurt by a sex partner? *Kama ni chini ya miaka 2 iliopita, ni mwezi na mwaka gani ulipoumizwa na mshiriki wa ngono kwa mara ya kwanza?*	MONTH..... ☐☐ YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	
B602***	When was the LAST TIME a sex partner physically hurt you? *Ni lini MARA YA MWISHO uliumizwa mwilini na mshirika wa ngono?* [If < 1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ THE LAST TIME A SEX PARTNER PHYSICALLY HURT ME WAS ALSO MY FIRST TIME; I HAVE ONLY BEEN PHYSICALLY HURT ONCE..... 77 DON'T KNOW / DON'T REMEMBER..... 88	

B603	Who physically hurt you the FIRST TIME and the LAST TIME? *Ni nani alikuumiza mwilini MARA YA KWANZA na MARA YA MWISHO?* *Tick one in each column. [NA = NOT APPLICABLE]*		
	A. FIRST TIME	B. LAST TIME	
	a) Regular paying client..... *Mteja wa kulipa wa kawaida*	....	NA…… ……..
	b) First-time paying client..... *Mteja wa kulipa wa mara ya kwanza*	....	NA…… ……..
	c) Regular non-client sex partner..... *MshirikI wa ngono asiye mteja wa kawaida*	....	NA…… ……..
	d) First-time non-client sex partner..... *Mshirika wa ngono asiye mteja wa mara ya kwanza*	....	NA…… ……..
	e) Husband / spouse / boyfriends..... *Mume/ rafiki wa kiume*	....	NA…… ……..
	f) Other (Specify) *Mwengine (FAFANUA)* _____	Other (Specify) *Mwengine (FAFANUA)* _____	NA…… ……..
B604	Think of the FIRST TIME and the LAST TIME when you were physically hurt by a sex partner. *Fikiria ile MARA YA KWANZA na YA MWISHO uliumizwa mwilini na mshiriki wa ngono.* *Tick one in each column. [DR = DON'T REMEMBER; NA = NOT APPLICABLE]*		

	FIRST TIME	
	a) i) At that time, did he have vaginal sex with you? *Wakati huo, ulishiriki ngono ya uke naye?* YES NO.. ☐☐ DR..... ☐ NA..... ☐	ii) Was a condom used for vaginal sex? *Je kondomu ilitumika?* YES.... ☐ NO..... ☐ DR..... ☐ NA..... ☐
	b) i) At that time, did he have anal sex with you? *Wakati huo, ulishiriki ngono ya mkundu naye*? YES NO.. ☐☐ DR..... ☐ NA..... ☐	ii) Was a condom used for anal sex? *Je kondomu ilitumika?* YES.... ☐ NO..... ☐ DR..... ☐ NA..... ☐

	LAST TIME	
	c) i) At that time, did he have vaginal sex with you? *Wakati huo, alishiriki ngono ya uke na wewe?* YES..... NO☐☐ DR.....☐ NA.....☐	ii) Was a condom used for vaginal sex? *Je kondomu ilitumika?* YES....☐ NO.....☐ DR.....☐ NA....☐
	d) i) At that time, did he have anal sex with you? *Wakati huo, ulishiriki ngono ya mkundu naye?* YES..... NO☐☐ DR.....☐ NA.....☐	ii) Was a condom used anal sex? *Je kondomu ilitumika?* YES....☐ NO.....☐ DR.....☐ NA....☐

B605	In the LAST MONTH, of the men with whom you had sex, how many of them physically hurt you? *Katika MWEZI WA MWISHO, kati ya wanaume ulioshiriki nao ngono,*	NUMBER OF MEN.....☐☐ DON'T REMEMBER..... 88	

	ni wangapi kati yao walikuumiza mwili?	IF RESPONDENT ANSWERS "00" OR "DR", SKIP TO QUESTION B607. →	B607

B606	Of the _____[*fill in answer from question B605 above*] men who physically hurt you in the LAST MONTH, how many of these men were: *Kati ya wanaume [fill in answer from question B605 above] waliokuumiza katika MWEZI WA MWISHO, ni wangapi kati ya hawa walikuwa:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) Regular paying clients? *Wateja wa kulipa wa kawaida*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) First-time paying clients? *Wateja wa mara ya kwanza?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) Regular non-client sex partners? *Mshiriki wa ngono wa kawaida asiye mteja?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	d) First-time non-client sex partner? *Mteja asiye wa kulipa wa mara ya kwanza*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	e) Husband / spouse / boyfriends? *Mume/ rafiki wa kiume?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA

	f) Other (Specify)? *Mwengine (FAFANUA)?* _____			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
B607	In the FIRST MONTH OF SEX WORK, of the men with whom you had sex, how many of them physically hurt you?	NUMBER OF MEN.....				

	Katika MWEZI WA KWANZA WA KUBANGAIZA, kati ya wanaume uliofanya ngono nao, ni wangapi walikuumiza mwili?	DON'T REMEMBER..... 88 IF RESPONDENT ANSWERS "00" OR "DR", SKIP TO QUESTION B609. →	B609

B608	Of the _____[*fill in answer from question B607 above*] men who physically hurt you in the FIRST MONTH OF SEX WORK, how many of these men were: *Kati ya _____[fill in answer from question B607 above] wanaume waliokuumiza mwili katika MWEZI WA KWANZA WA KUBANGAIZA, ni wangapi kati ya wanaume hao walikuwa:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) Regular paying clients? *Wateja wa kulipa wa kawaida ?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) First-time paying clients? *Wateja wa kulipa wa mara ya kwanza?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) Regular non-client sex partners? *Mshiriki wa ngono wa kawaida asiye mtej?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	d) First-time non-client sex partners? *Mshiriki wa kwanza wa ngono asiye mteja?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	e) Husband / spouse / boyfriends? *Mume/ rafiki wa kiume?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	f) Other (Specify)? *Mwengine (FAFANUA)?* _____			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA

B609	Think of your LAST REGULAR PAYING CLIENT: *Fikiria kuhusu MTEJA WA KULIPA WA KAWAIDA WA MWISHO :*

	[DK/DR = DON'T KNOW / DON'T REMEMBER; NOT APPLICABLE]					
	a) How many times did he physically hurt you in the LAST MONTH? *Ni mara ngapi alikuumiza mwilini katika MWEZI ULIOPITA?*			b) How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) How many times did he threaten or verbally abuse you in the LAST MONTH? *Ni mara ngapi alikutishia au kukutukana katika MWEZI ULIOPITA?*					
		DK/DR		NA		
B610	Think of your LAST REGULAR NON-CLIENT SEX PARTNER. *Fikiria kuhusu MTEJA WAKO WA MWISHO WA KAWAIDA ASIYE LIPA.* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many times did he physically hurt you in the LAST MONTH? *Ni mara ngapi alikuumiza mwilini katika MWEZI ULIOPITA?*			b) How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizo kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) How many times did he threaten or verbally abuse you in the LAST MONTH? *Ni mara ngapi alikutishia au kukutukana katika MWEZI MWEZI WA MWISHO?*					
		DK/DR		NA		
B611	Think of your HUSBAND, SPOUSE or BOYFRIEND. *Fikiria kuhusu MUME WAKO/RAFIKI YAKO WA KIUME.* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many times did he physically hurt you in the LAST MONTH? *Ni mara ngapi alikuumiza mwili katika MWEZI WA MWISHO?*			b) How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) How many times did he threaten or verbally abuse you in the LAST MONTH? *Ni mara ngapi alikutishia au kukutukana katika MWEZI WA MWISHO?*					
		DK/DR		NA		

Now I am going to ask you some questions about sexual violence that you might have experienced. *Sasa nitakuuliza maswali kadhaa kuhusu dhuluma ya kijinsia ambayo huenda umepitia.*			
B612	Think of that FIRST TIME when the man forced you to have sex with him when you were not willing. Was that man your first sex partner? *Fikiria ile MARA YA KWANZA mwanaume alikuazimisha kufanya ngono naye wakati hukua unataka. Mwanaume huyo alikuwa mshiriki wako wa ngono wa kwanza?*	YES..... 1 NO..... 0 NO ONE HAS EVER FORCED ME TO HAVE SEX WITH HIM..... 77 → B624	
B613***	a) How many years ago was it when this man forced you to have sex with him when you were not willing? *Ni miaka mingapi iliopita wakati mwanaume huyu alikulazimisha kufanya ngono naye wakati hukua unataka?* *If respondent answers "more than 2 years ago", fill in part a) only.* *If respondent answers "2 years ago or less", fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBER..... 88	
	b) If 2 years ago or less, in what month and year did this man force you to have sex with him when you were not willing? *Kama ni chini ya miaka 2 iliopita, ni mwezi na mwaka gani mwanaume huyu alikulazimisha kufanya ngono naye wakati hukua unataka?*	MONTH..... ☐☐ YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	
B614***		☐☐	

	When was the LAST TIME a man forced you to have sex with him when you were not willing? *Ni lini MARA YA MWISHO mwanaume alikulazimisha kufanya ngono naye wakati hukua unataka?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ THE LAST TIME A MAN FORCED ME TO HAVE SEX WITH HIM WAS ALSO MY FIRST TIME; I HAVE ONLY HAD FORCED SEX ONCE..... 77 DON'T KNOW / DON'T REMEMBER..... 88	

B615	Who forced you to have sex with him the FIRST TIME and the LAST TIME when you were not willing? *Ni nani alikulazimisha kufanya ngono naye MARA YA KWANZA na MARA YA MWISHO wakati hukua unataka?* *Tick one in each column.*		
		A. FIRST TIME	B. LAST TIME
	a) Regular paying client..... *Mteja wa kulipa wa kawaida*	☐	.... ☐ NA…… …….. ☐
	b) First-time paying client..... *Mteja wa kulipa wa mara ya kwanza*	☐	.... ☐ NA…… …….. ☐
	c) Regular non-client sex partner..... *Mteja wa kawaida asiye kulipa*	☐	.... ☐ NA…… …….. ☐
	d) First-time non-client sex partner.....	☐	.... ☐ NA…… …….. ☐

	Mshirika wa ngono asiye mteja wa mara ya kwanza e) Husband / spouse / boyfriends..... *Mume/ rafiki wa kiume* f) Other (Specify) *Mwengine (FAFANUA)* _____ _____	.... Other (Specify) _____ __

B616	Think of the FIRST TIME and the LAST TIME when a man forced you to have sex with him when you were not willing. *Ukifikiria ile MARA YA KWANZA na MARA YA MWISHO mwanaume alikulazimisha kufanya ngono naye wakati hukua unataka.* *Tick one in each column. [DR = DON'T REMEMBER]*	
	FIRST TIME	
	a) i) At that time, did he have vaginal sex with you? *Wakati huo, alifanya ngono ya uke na wewe?* YES..... NO ☐☐ DR..... ☐ NA..... ☐	ii) Was a condom used for vaginal sex? *Je kondomu ilitumika kwa ngono ya uke?* YES.... ☐ NO..... ☐ DR..... ☐ NA..... ☐
	b) i) At that time, did he have anal sex with you? *Wakati huo, alifanya ngono ya mkundu na wewe?* YES NO ☐☐ DR..... ☐ NA..... ☐	ii) Was a condom used for anal sex? *Je kondomu ilitumika kwa ngono ya nyuma?* YES.... ☐ NO..... ☐ DR..... ☐ NA..... ☐

	. . . .	

	LAST TIME	
	c) i) At that time, did he have vaginal sex with you? *Wakati huo, alifanya ngono ya uke na wewe?* YES..... NO☐☐ DR.....☐ NA.....☐	ii) Was a condom used for vaginal sex? *Je kondomu ilitumika kwa ngono ya uke?* YES....☐ NO.....☐ DR.....☐ NA.....☐
	d) i) At that time, did he have anal sex with you? *Wakati huo, alifanya ngono ya mkundu na wewe?* YES..... NO☐☐ DR.....☐ NA.....☐	ii) Was a condom used for anal sex? *Je kondomu ilitumika kwa ngono ya nyuma?* YES....☐ NO.....☐ DR.....☐ NA.....☐
B617		☐☐

	In the LAST MONTH, how many men forced you to have sex with them when you were not willing? *Katika MWEZI WA MWISHO, ni wanaume wangapi walikulazimisha kufanya ngono nao wakati hukua unataka?*	NUMBER OF MEN..... DON'T REMEMBER..... 88 IF RESPONDENT ANSWERS "00" OR "DR", SKIP TO QUESTION B619. →	B619

B618	Of the _____[*fill in answer from question B617 above*] men who forced you to have sex with them when you were not willing in the LAST MONTH, how many of these men were: *Kati ya wanaume _____[fill in answer from question B617 above] ambao walikulazimisha kufanya ngono nao katika MWEZI WA MWISHO, ni wangapi kati ya wanaume hao walikua:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) Regular paying client? *Wateja wa kulipa wa kawaida?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) First-time paying client? *Wateja wa kulipa wa mara ya kwanza?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) Regular non-client sex partners? *Mshirika wa ngono asiye mteja wa kawaida?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	d) First-time non-client sex partners? *Mshirika wa ngono asiye mteja wa kawaida*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	e) Husband / spouse / boyfriends? *Mume/ rafiki wa kiume?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA

	f) Other (Specify)? *Mwengine (FAFANUA)* _____ _____	With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*

		DK/DR	NA		DK/DR	NA
B619	In the FIRST MONTH OF SEX WORK, how many men forced you to have sex with them when you were not willing? *Katika MWEZI WAKO WA KWANZA WA KUBANGAIZA, ni wanaume wangapi walikulazimisha kufanya ngono nao wakati hukua unataka?*	NUMBER OF MEN..... DON'T REMEMBER..... 88 IF RESPONDENT ANSWERS "00" OR "DR", SKIP TO QUESTION B621. → B621				
B620	Of the _____[*fill in answer from question B619 above*] men who forced you to have sex with them when you were not willing in the FIRST MONTH OF SEX WORK, how many of these men were: *Kati ya [fill in answer from question B619 above] wanaume ambao walikulazimisha kufanya ngono nao katika kipindi cha MWEZI WA KWANZA WA KUBANGAIZA, ni wangapi kati ya wanaume hao walikua:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLI CABLE]*					
	a) Regular paying clients? *Wateja wa kulipa wa kawaida?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	b) First-time paying clients? *Wateja wa kulipa wa mara ya kwanza?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	c) Regular non-client sex partners? *Mshiriki wa ngono asiye mteja wa kawaida?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	d) First-time non-client sex partners? *Mshiriki wa ngono asiye mteja wa mara ya kwanza?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	e) Husband / spouse / boyfriends? *Mume/ rafiki wa kiume?*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
	f) Other (Specify)? *Mwengine (FAFANUA)*			With how many of them was a condom NOT used? *Ni wangapi kati yao kondomu HAIKUTUMIKA?*		

	_____ _____					
		DK/DR	NA		DK/DR	NA

B621	Think of your LAST REGULAR PAYING CLIENT. *Fikiria kuhusu MTEJA WAKO WA MWISHO WA KAWAIDA WA KULIPA:* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many times did he force you to have sex with him in the LAST MONTH when you were not willing? *Ni mara ngapi alikulazimisha kufanya naye ngono MWEZI WA MWISHO wakati hukua unataka?*			b) How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
B622	Think of your LAST REGULAR NON-CLIENT SEX PARTNER. *Fikiria kuhusu MTEJA WAKO MWISHO WA KAWAIDA ASIYE WAKULIPA.* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many times did he force you to have sex with him in the LAST MONTH when you were not willing? *Ni mara ngapi alikulazimisha kufanya naye ngono katika MWEZI WA MWISHO wakati hukua unataka?*			b) How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
B623	Think of your HUSBAND, SPOUSE or BOYFRIEND. *Fikiria kuhusu MUME WAKO/RAFIKI YAKO WA KIUME.* *[DK/DR = DON'T KNOW / DON'T REMEMBER; NA = NOT APPLICABLE]*					
	a) How many times did he force you to have sex with him in the LAST MONTH when you were not willing? *Ni mara ngapi alikulazimisha kufanya naye ngono katika MWEZI WA MWISHO wakati hukua unataka?*			b) How many of those times was a condom NOT used? *Ni mara ngapi kati ya hizi kondomu HAIKUTUMIKA?*		
		DK/DR	NA		DK/DR	NA
B624 ***	Think of the FIRST TIME when you were physically assaulted or arrested by law enforcement (e.g. police, *sungu sungu* etc.) while	AGE IN YEARS..... ☐☐				

	you were working as a sex worker. How old were you? *Fikiria ile MARA YA KWANZA ulinyanyaswa au kukamatwa na watekelezaji sheria (k.m. polisi, sungu sungu n.k.) ukiwa kwa kazi ya ubangaizaji. Ulikuwa na miaka mingapi?*	I HAVE NEVER ENCOUNTED AND HAVE NEVER BEEN PHYSICALLY ASSAULTED OR ARRESTED BY LAW ENFORCEMENT..... 77 I HAVE ENCOUNTERED BUT HAVE NEVER BEEN PHYSICALLY ASSAULTED OR ARRESTED BY LAW ENFORCEMENT……..…… …… 78 DON'T REMEMBER…… ……. 88	701

B625 ***	Think of the LAST TIME when you were physically assaulted or arrested by law enforcement while you were working as a sex worker. How old were you? *Fikiria kuhusu ile MARA YA MWISHO ulinyanyaswa au kukamatwa na watekelezaji sheria (k.m. polisi, sungu sungu n.k.) ukiwa kwa kazi ya ubangaizaji. Ulikuwa na miaka mingapi?*	AGE IN YEARS..... ☐☐ THE LAST TIME I WAS PHYSICALLY ASSAULTED OR ARRESTED BY LAW ENFORCEMENT WAS ALSO MY FIRST TIME; I HAVE ONLY BEEN PHYSICALLY ASSAULTED OR ARRESTED BY LAW ENFORCEMENT ONCE..... ..... 77 DON'T REMEMBER…… ……. 88	

B626	In the FIRST MONTH OF SEX WORK and the LAST MONTH OF SEX WORK, how many times did law enforcement: *Katika MWEZI WA KWANZA na WA MWISHO WA KUBANGAIZA, ni mara ngapi watekelezaji sheria:*		
	A. FIRST MONTH OF SEX WORK	B. LAST MONTH OF SEX WORK	
	a) Physically assaulted you?..... *Walikudhulumu mwili?* ☐☐	..... ☐☐	
	b) Arrested you while you were working ☐☐	..... ☐☐	

	as a sex worker?..... . *Walikukamata ukiwa unafanya kazi ya kubangaiza?*	

SECTION VII: SEXUALLY TRANSMITTED AND BLOOD-BORNE INFECTIONS

No.	QUESTIONS	CODING CATEGORIES	SKIP
701 ***	When was the LAST TIME you were treated for sexually transmitted infections (STI) other than HIV? *Ni lini MARA YA MWISHO ulitibiwa magonjwa ya zinaa kando na virusi vya UKIMWI?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ 88 I WAS NEVER TREATED FOR AN STI..... DON'T REMEMBER.....	} 703
702	What was/were the reason(s) you were treated for STI the LAST TIME? *Nini ilikuwa/zilikuwa sababu za wewe kutibiwa magonjwa ya zinaa (STI) MARA YA MWISHO?* MULTIPLE ANSWERS POSSIBLE	I HAD VAGINAL DISCHARGE AND / OR GENITAL ULCERS..... A A SEX PARTNER HAD GENITAL DISCHARGE AND / OR ULCERS..... B C D A SEX PARTNER TESTED POSITIVE FOR STI..... E TREATMENT WAS OFFERED TO ME..... F I WAS PREGNANT..... Y OTHER (SPECIFY) _____ ___ DON'T KNOW / DON'T REMEMBER.....	
703 ***		☐☐	

	When was the LAST TIME you tested for sexually transmitted infections (STI)? *Ni lini MARA YA MWISHO ulipimwa magonjwa ya zinaa?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ I NEVER TESTED FOR AN STI..... DON'T REMEMBER.....	77 707
704	What was/were the reason(s) you tested for STI the LAST TIME? *Nini ilikuwa/zilikuwa sababu za wewe kupimwa magonjwa ya zinaa MARA YA MWISHO?* MULTIPLE ANSWERS POSSIBLE	I HAD VAGINAL DISCHARGE AND / OR GENITAL ULCERS..... A A SEX PARTNER HAD GENITAL ULCERS / DISCHARGE..... B A SEX PARTNER TESTED POSITIVE FOR STI..... C TEST WAS OFFERED TO ME.....D I WAS PREGNANT..... E PEER ASKED ME TO GET TESTED..... F PEER EDUCATOR ASKED ME TO GET TESTED.....G HEALTH CARE PROVIDER ADVISED ME TO GET TESTED..... H ROUTINE CHECK-UP..... I OTHER (SPECIFY) _____ J DON'T KNOW / DON'T REMEMBER..... Y	
705	How many times were you tested for an STI in the LAST YEAR (even if you had no symptoms)?	NUMBER OF TIMES..... ☐☐ 88	

	Ulipimwa magonjwa ya zinaa mara ngapi MWAKA ULIOPITA (hata kama ulikuwa huna dalili?)	DON'T KNOW / DON'T REMEMBER.....	
706 ***	When was the FIRST TIME you tested for an STI? *Ni lini MARA YA KWANZA ulipimwa ugonjwa wa zinaa?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ THE LAST TIME I TESTED FOR AN STI WAS ALSO MY FIRST TIME; I HAVE ONLY BEEN TESTED ONCE..... 77 DON'T KNOW / DON'T REMEMBER..... 88	

707	Do you currently have the following symptoms? *Je kwa sasa uko na dalili zifuatazo?*		
	a) Abnormal genital discharge *Uchafu usio wa kawaida kwa sehemu za siri*	YES..... ☐	NO..... ☐
	b) Genital ulcers *Vidonda kwa sehemu za siri*	YES..... ☐	NO..... ☐
	c) Fever with lower abdominal pain *Homa na maumivi ya tumbo*	YES..... ☐	NO..... ☐
	d) Other (Specify) *Nyingine (fafanua)* _____ _____		

708***	When was the LAST TIME you tested for hepatitis C? *Ni lini MARA YA MWISHO ulipimwa Hepatitis C?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ I NEVER TESTED FOR HEPATITIS C..... 77 I DON'T KNOW WHAT HEPATITS C IS..... 78 DON'T KNOW / DON'T REMEMBER..... 88	801
709	What was/were the reason(s) you tested for hepatitis C the LAST TIME? *Nini ilikuwa/zilikuwa sababu ya/za wewe kupimwa Hepatitis C MARA YA MWISHO?* MULTIPLE ANSWERS POSSIBLE	I WAS SICK..... A A SEX PARTNER WAS SICK..... B A SEX PARTNER TESTED POSITIVE FOR HEPATITIS C..... C I SHARED NEEDLES WITH OTHER PEOPLE..... D AN INJECTION DRUG PARTNER WAS SICK..... E AN INJECTION DRUG PARTNER TESTED POSITIVE FOR HEPATITIS C..... F TEST WAS OFFERED TO ME..... G I WAS PREGNANT..... H PEER ASKED ME TO GET TESTED..... I PEER EDUCATOR ASKED ME TO GET TESTED.. J HEALTH CARE PROVIDER ADVISED ME TO GET TESTED..... K . OTHER (SPECIFY)	

		_____ L ___ DON'T KNOW / DON'T REMEMBER..... Y	

710 ***	When was the FIRST TIME you tested for Hepatitis C? *Ni lini MARA YA KWANZA ulipimwa Hepatitis C?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ THE LAST TIME I TESTED FOR HEP C WAS ALSO MY FIRST TIME; I HAVE ONLY BEEN TESTED ONCE..... 77 DON'T KNOW / DON'T REMEMBER..... 88	

SECTION VIII EXPOSURE TO HIV PREVENTION INTERVENTIONS

No.	QUESTIONS	CODING CATEGORIES	SKIP
801	Now I would like to ask you a few questions about the HIV prevention programs in _____ (name of county/district/ oblast). Are you aware of any NGOs, CBOs or FBOs working on the prevention of HIV/AIDS for young women AND/OR female sex workers in _____ *Sasa ninegependa kukuuliza maswali machache kuhusiana na miradi ya kuzuia maambukizi ya virusi vya UKIMWI* _____	YES..... 1 ..... NO..... 0 → 816	

	Unajua NGO, CBO, au FBO zozote zinazo fanya kazi ya kuzuia maambukizi ya Virusi vya UKIMWI miongoni mwa wanawake wadogo NA/AU wabangaizaji wa kike _____?		
802	How old were you when you first knew about this (these) NGO(s), CBO(s) or FBO(s)? *Ulikuwa na miaka mingapi mara ya kwanza ulipojua kuhusu hizi NGO, CBO au FBO?*	AGE IN YEARS..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	

803 ***	a) How many years ago were you first contacted by peers/staff from an NGO, CBO or FBO? *Ni miaka mingapi iliopita ulifikiwa mara ya kwanza na marika/wafanyikazi wa NGO, CBO au FBO?* *If respondent answers "more than 2 years ago", fill in part a) only.* *If respondent answers "2 years ago or less", fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) I HAVE NEVER BEEN CONTACTED BY PEERS/ 77 → 816 STAFF FROM AN NGO, CBO OR FBO..... 88 DON'T KNOW / DON'T REMEMBER.....	
	b) If 2 years ago or less, in what month and year were you first contacted by peers/staff from an NGO, CBO or FBO?	MONTH..... ☐☐ YEAR..... ☐☐☐☐	

	Kama ni chini ya miaka 2 au zaidi, ni mwezi na mwaka gani ulifikiwa kwa mara ya kwanza na marika/wafanyikazi kutoka NGO, CBO au FBO?	DON'T KNOW / DON'T REMEMBER..... 88	
804 ***	Approximately how long after you had your first paying client were you first contacted by peers/staff from an NGO, CBO OR FBO? *Ni kama mda gani baada ya kuwa na mteja wako wa kwanza wa kulipa ulipowasiliana ulifikiwa na marika /wafanya kazi wa NGO, CBO au FBO kwa mara ya kwanza?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AFTER..... ☐☐ NUMBER OF MONTHS AFTER..... ☐☐ NUMBER OF YEARS AFTER..... ☐☐ BEFORE MY FIRST PAYING CLIENT..... 77 DON'T KNOW / DON'T REMEMBER..... 88	
805 ***	Approximately how long after you first considered yourself a sex worker were you first contacted by peers/staff from an NGO, CBO OR FBO? *Ni kama mda gani tangu ujichukulie kama mbangaizaji marika/wafanyikazi wa NGO, CBO au FBO waliwasiliana nawe mara ya kwanza?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AFTER..... ☐☐ NUMBER OF MONTHS AFTER..... ☐☐ NUMBER OF YEARS AFTER..... ☐☐ BEFORE I FIRST CONSIDER MYSELF A SEX WORKER…… 77 …….. DON'T KNOW / DON'T REMEMBER..... 88	

806	Are you registered with an NGO, CBO, and FBO?	YES..... 1	

	Je umesajiliwa na NGO, CBO, na FBO?	NO..... 0	
807	When was the FIRST TIME you saw a demonstration on correct use of a male condom by a peer educator, outreach worker or staff from an NGO, CBO or FBO? *Ni lini MARA YA KWANZA uliona maonyesho ya matumizi sahihi ya kondomu ya kiume ilifanywa na mwalimu wa marika, mhudumu wa afya wa mashinani au mfanyikazi wa NGO, CBO au FBO?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ I NEVER SAW A DEMONSTRATION ON CORRECT MALE CONDOM USE BY AN NGO, CBO OR FBO..... 77 DON'T KNOW / DON'T REMEMBER..... 88	
808 ***	When was the FIRST TIME you were given clean needles/syringes by a peer/worker from an NGO, CBO or FBO? *Ni lini MARA YA KWANZA ulipewa sindano/bomba safi na marika/wafanyikazi kutoka NGO, CBO au FBO?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ I WAS NEVER GIVEN CLEAN NEEDLES / SYRINGES FROM AN NGO, CBO OR FBO..... 77 ..... I DON'T NEED CLEAN NEEDLES / SYRINGES.... 78 I DON'T USE INJECTION DRUGS..... 79 DON'T KNOW / DON'T REMEMBER..... 88	

809	Have you ever used any clinics run by an NGO, CBO or FBO? *Umeshawahi tembelea kliniki zinazoendeshwa na NGO, CBO au FBO?*	YES..... 1 ..... NO..... 0 → 816	
810 ***	Approximately how long after you had your first paying client did you first use any clinics run by an NGO, CBO or FBO? *Ni kama mda gani baada ya kuwa na mteja wako wa kwanza wa kulipa ulitembelea kwa mara ya kwanza kiniki zinazoendeshwa na NGO, CBO au FBO?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AFTER..... ☐☐ NUMBER OF MONTHS AFTER..... ☐☐ NUMBER OF YEARS AFTER..... ☐☐ BEFORE MY FIRST PAYING CLIENT..... 77 DON'T KNOW / DON'T REMEMBER..... 88	
811 ***	Approximately how long after you first considered yourself a sex worker did you first use any clinics run by an NGO, CBO or FBO? *Ni kama mda gani tangu ujichukulie kama mbangaizaji ulitembelea kwa mara ya kwanza kiniki zinazoendeshwa na NGO, CBO au FBO?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AFTER..... ☐☐ NUMBER OF MONTHS AFTER..... ☐☐ NUMBER OF YEARS AFTER..... ☐☐ BEFORE I FIRST CONSIDERED MYSELF A SEX WORKER..... 77 ..... DON'T KNOW / DON'T REMEMBER..... 88	
812	How many times have you used these clinics to see the doctor in the LAST MONTH?	NUMBER OF CLINIC VISITS LAST MONTH..... ☐☐ 88	

	Ni mara ngapi umetembelea kliniki hii (hizi) kumuona daktari katika kipindi cha MWEZI WA MWISHO?	DON'T KNOW / DON'T REMEMBER.....	
813 ***	When was the FIRST TIME you used the services in any drop-in centres run by an NGO, CBO or FBO? *Ni lini MARA YA KWANZA ulipotembelea kituo cha drop-in kinachoendeshwa na NGO, CBO au FBO?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ THE NGO, CBO OR FBO I VISIT DOESN'T HAVE A DROP-IN CENTRE..... 77 THE NGO, CBO OR FBO I VISIT T HAS A DROP-IN CENTRE BUT I NEVER VISITED THE DROP-IN CENTRE..... 78 DON'T KNOW / DON'T REMEMBER..... 88	
814	Are you a peer worker/peer educator of an NGO, FBO or CBO? *Je wewe ni mfanyakazi/mwalimu wa marika katika NGO, CBO au FBO?*	YES..... 1 NO..... 0 DON'T KNOW..... 88	

815	You said you have been contacted by staff of an NGO, CBO or FBO and/or you have visited an NGO, CBO or FBO. What are the services that you received from this/these NGO, CBO or FBO program(s) in the LAST MONTH? *Umesema umewahi fikiwa na mfanyakazi wa NGO, CBO au FBO/au kutembelea NGO, CBO au FBO. Ni huduma gani ulipokea kutoka kwa miradi ya hii/hizi NGO, CBO au FBO MWEZI WA MWISHO?* INTERVIEWER: *Do not read the answers. Record all answers.*	SERVICES *Tick "YES" or "NO" for each option.*	YES	NO	
		CONDOMS			
		HEALTH CHECK-UP			
		HIV EDUCATION / COUNSELING			
		TESTING FOR STI			
		TESTING FOR HIV			
		FREE MEDICINE FOR STI			
		FREE MEDICINE FOR GENERAL HEALTH PROBLEMS			
		CLEAN NEEDLES / SYRINGES			
		OPIOID SUBSTITUTION THERAPY			
		MEMBERSHIP IN SELF-HELP GROUP			
		HELP FOR VIOLENCE			
		TRAINING / MEETINGS			
		OTHER (SPECIFY) _____			
		NO ANSWER	99		
816	Do you think your chances of getting HIV/AIDS are great, moderate, small or no risk at all? *Unadhani uwezekano wako wa kupata virusi vya UKIMWI ni mdogo, kiasi, mkubwa au hakuna hatari kabisa?*	NO RISK AT ALL…….	1		
		SMALL……	2		
		MODERATE……....	3		
		GREAT……	4		
		UNSURE……	5		
		I HAVE HIV/AIDS…….……....	6		
817 ***	When was the LAST TIME you got tested for HIV? *Ni lini MARA YA MWISHO ulipimwa HIV?*	NUMBER OF DAYS AGO.....	☐☐		
		NUMBER OF MONTHS AGO.....	☐☐		

	[If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF YEARS AGO..... ☐☐ I HAVE NEVER BEEN TESTED FOR HIV..... 77 DON'T KNOW / DON'T REMEMBER..... 88 NO ANSWER..... 99	1001

818 ***	When was the FIRST TIME you tested for HIV? *Ni lini MARA YA KWANZA ulipimwa HIV?* [If <1 month, record in days. If >1 month and <1 year, record in months. If > 1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ THE LAST TIME I TESTED FOR HIV WAS ALSO MY FIRST TIME; I HAVE ONLY BEEN TESTED FOR HIV ONCE…… 77 DON'T KNOW / DON'T REMEMBER..... 88	

819	Where did you get tested for HIV the FIRST TIME and the LAST TIME? *Ulipimwa wapi HIV MARA YA KWANZA na MARA YA MWISHO?* *Tick one in each column.*		
	A. FIRST TIME	B. LAST TIME	
	a) Public/government facility..... ☐	..... ☐	NA..... ☐

	b) NGO, CBO or FBO facility through its outreach worker.....	☐	.....	☐	NA.....	☐
	c) Private facility.....	☐	.....	☐	NA.....	☐
	d) Other (Specify)? _____	☐	Other (Specify)? _____	☐	NA.....	☐
	e) DON'T KNOW / DON'T REMEMBER.....	☐	.....	☐	NA.....	☐

820 What were the reason you got tested for HIV the FIRST TIME and the LAST TIME?
Ni sababu gani za wewe kupimwa virusi vya HIV MARA YA KWANZA na MARA YA MWISHO?
Tick all that apply in each column.

A. FIRST TIME		B. LAST TIME			
a) I wanted to know.....	☐	.....	☐	NA.....	☐
b) I was sick.....	☐	.....	☐	NA.....	☐
c) A sex partner was sick.....	☐	.....	☐	NA.....	☐
d) A sex partner tested positive for HIV.....	☐	.....	☐	NA.....	☐
e) Test was offered to me.....	☐	.....	☐	NA.....	☐
f) Peer asked me to get tested.....	☐	.....	☐	NA.....	☐

	g) Peer educator asked me to get tested.....	☐	....	☐ ☐	NA..... ...	☐ ☐
	h) I was pregnant.....		....		NA..... ...	
	i) I had tuberculosis (TB).....	☐	....	☐	NA..... ...	☐
	j) As a part of a research study I participated in.....	☐	....	☐	NA..... ...	☐
	k) Doctor/ health care provider advised me to get tested.....	☐	....	☐	NA..... ...	☐
	l) Other (Specify)? _____ _____	☐	Other (Specify)? _____ __	☐	NA..... ...	☐
	m) DON'T KNOW / DONT REMEMBER.....	☐	....	☐	NA..... ...	☐

821	Did you receive your test result when you were tested for HIV the FIRST TIME and the LAST TIME? *Ulipokea matokeo yako MARA YA KWANZA na MARA YA MWISHO ulipopimwa virusi vya UKIMWI?* *Tick one in each column.*					
	A. FIRST TIME		B. LAST TIME			
	YES.....	☐	YES.....	☐	NA..... ...	☐
	NO.....	☐	NO..... ...	☐	NA..... ...	☐
822	Between the first HIV test and the last HIV test, how many		NUMBER OF TIMES.....	☐☐		

	other times were you tested for HIV? *Kati ya mara yako ya kwanza na ya mwisho kupimwa virusi vya UKIMWI, ni mara ngapi tena umepimwa virusi vya UKIMWI?*	DON'T KNOW / DON'T REMEMBER..... 88	
823	How many times did you have an HIV test in the LAST YEAR? *Ni mara ngapi umepimwa virusi vya UKIMWI katika MWAKA WA MWISHO?*	NUMBER OF TIMES..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88	
824	Would you be willing to tell me the result of your last HIV test? *Ungependelea kuniambia matokeo ya kipimo cha virusi vya UKIMWI ya mwisho?*	POSITIVE..... 1 NEGATIVE..... 0 NOT WILLING TO DISCLOSE..... 99 → 1001	825

825	Whom did you tell about your HIV results? *Ni nani ulimwambia kuhusu majibu yako ya HIV?* MULTIPLE ANSWERS POSSIBLE	NO ONE..... A IMMEDIATE FAMILY..... B OTHER RELATIVES..... C SPOUSE..... D BOYFRIEND..... E PIMP / MANAGER..... F PEER..... G PEER EDUCATOR..... H HEALTH CARE PROVIDER..... I	

		OTHER (SPECIFY) _____ I ___ DON'T REMEMBER..... Y	

SECTION IX HIV TREATMENT AND CARE

Only for respondents who reported a positive HIV status.

No.	QUESTIONS	CODING CATEGORIES	SKIP
901 ***	a) How many years ago did you test positive for HIV? *Ni miaka mingapi iliopita ulipopatikana na virusi vya UKIMWI?* *If respondent answers "more than 2 years ago", fill in part a) only.* *If respondent answers "2 years ago or less", fill in parts a) and b).*	NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ (IF LESS THAN 2 YEARS AGO, FILL IN THE NUMBER OF MONTHS.) DON'T REMEMBER..... 88	
	b) If 2 years ago or less, in what month and year did you test positive for HIV? *Kama ni chini ya miaka 2 iliopita, ni mwezi na mwaka gani ulipogunduliwa kuwa na HIV?*	MONTH..... ☐☐ ..... YEAR..... ☐☐☐☐ DON'T REMEMBER..... 88	
902	Are you part of a HIV support group? *Uko katika kikundi chochote cha kuinuana cha walio na virusi vya UKIMWI*?	YES..... 1 ..... NO..... 0 ..... NO ANSWER..... 99 ..	

903	Are you registered with an HIV treatment and care centre? *Je umesajiliwa katika kituo cha matibabu ya virusi vya UKIMWI na utunzi?*	YES..... 1 NO..... 0 NO ANSWER..... 99	→904 906

904 ***	When did you first register with an HIV treatment and care centre? *Ni lini ulijisajili na kituo cha matibabu na utunzi wa virusi vya UKIMWI kwa mara ya kwanza?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 NO ANSWER..... 99	
905 ***	Approximately how long after you found out your HIV results did you first register with an HIV treatment and care centre? *Ni kama mda gani baada ya kujua matokeo ya virusi vya UKIMWI ulijisajili na kituo cha matibabu na utunzi kwa mara ya kwanza?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AFTER..... ☐☐ NUMBER OF MONTHS AFTER..... ☐☐ NUMBER OF YEARS AFTER..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 NO ANSWER..... 99	
906	To determine when people should begin treatment for HIV, a test called CD4 is done. Have you been	YES..... 1 NO, AND I AM NOT SCHEDULED TO HAVE MY CD4 LEVELS CHECKED..... 2	→907

	tested to learn what your CD4 levels are? *Ilikubainisha lini watu wanapaswa kuanza matibabu ya virusi vya UKIMWI, kipimo kinachoitwa CD4 hufanywa. Je umeshawahi kupimwa ili kujua kiwango chako cha CD4?*	NO, BUT I AM SCHEDULED TO HAVE MY CD4 LEVELS CHECKED..... 3 DON'T KNOW / DON'T REMEMBER..... 88 NO ANSWER..... 99	908
907	What was your last CD4 count? *Kipimo chako cha mara ya mwisho cha CD4 kilikuwa vipi?*	LAST CD4 COUNT..... ☐☐☐☐ I DID NOT RECEIVE RESULTS OF MY LAST CD4 TEST..... 77 DON'T KNOW / DON'T REMEMBER..... 88 NO ANSWER..... 99	
908	Have you heard of antiretroviral therapy (the medicines used to treat HIV)? *Umeshawahi kusikia kuhusu madawa ya kupunguza makali ya virusi vya UKIMWI?*	YES..... 1 NO..... 0	

909	Were you ever offered antiretroviral therapy by a doctor or healthcare provider to treat HIV? *Umeshawahi kupewa madawa ya kupunguza makali ya virus vya UKIMWI na daktari au*	YES..... 1 →	910
		NO..... 0	
		NO ANSWER..... 99	1001

	mhudumu wa afya ili kutibu virusi vya UKIMWI?		
910	Were you ever started on antiretroviral therapy? *Je uliwahi kuanzishiwa madawa ya kupunguza makali ya virusi vya UKIMWI?*	YES..... 1 ..... NO..... 0 ..... NO ANSWER..... 99 ..	→911 1001
911 ***	When were you started on ART? *Ulianzishwa lini madawa ya kupunguza makali ya virusi vya UKIMWI?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS AGO..... ☐☐ NUMBER OF MONTHS AGO..... ☐☐ NUMBER OF YEARS AGO..... ☐☐ DON'T REMEMBER..... 88 NO ANSWER..... 99 ..	
912	Are you currently taking antiretroviral medications? *Kwa sasa unatumia dawa za kupunguza makali ya virusi vya UKIMWI?*	YES..... 1 ..... NO..... 0 ..... DON'T KNOW..... 88 NO ANSWER..... 99 ..	→915 →913 1001
913	If no, why did you stop your antiretroviral therapy? *Ikiwa ni la, ni kwanini uliwacha matibabu yako ya kupunguza makali ya virusi vya UKIMWI?* MULTIPLE ANSWERS POSSIBLE	I HAD SIDE EFFECTS FROM THE MEDICATION (SPECIFY SIDE EFFECTS)_____ A I RAN OUT OF MEDICATION..... B MY DOCTOR TOLD ME TO STOP..... C IT'S TOO DIFFICULT FOR ME TO GET TO THE CLINIC TO OBTAIN MEDICATION..... D	

		IT'S TOO FAR FOR ME TO GET TO THE CLINIC TO OBTAIN MEDICATION..... E I TOOK SOME MEDICATION THEN I STOPPED BECAUSE I STARTED TO FEEL BETTER..... F THE MEDICATION IS NOT WORKING..... G I'M TAKING OTHER TREATMENT (SUCH AS TRADITIONAL HERBS) THAT WILL MAKE ME BETTER..... H I DON'T FEEL SICK..... I I'M CURED OF HIV..... J OTHER (SPECIFY)_____ K _____ DON'T KNOW..... Y NO ANSWER..... Z	

914 ***	For how long did you take your antiretroviral medications before stopping? *Ulitumia dawa zako za kupunguza makali ya virusi vya UKIMWI kwa muda gani kabla kuwacha?* [If <1 month, record in days. If >1 month and <1 year, record in months. If >1 year, record in years.]	NUMBER OF DAYS..... ☐☐ NUMBER OF MONTHS..... ☐☐ NUMBER OF YEARS..... ☐☐ DON'T KNOW / DON'T REMEMBER..... 88 NO ANSWER..... 99	1001
915		☐	

	In the LAST WEEK, how many days did you miss taking your antiretroviral medications? *Katika WIKI YA MWISHO, ni siku ngapi umekosa kutumia dawa zako za kupunguza makali ya virusi vya UKIMWI?*	NUMBER OF DAYS MISSED LAST WEEK..... DON'T REMEMBER..... 88	
916	How often do you pick up your antiretroviral medications from clinics or pharmacies? *Wewe huchukua dawa zako za kupunguza makali ya virusi vya UKIMWI kutoka kwa kliniki au duka la dawa mara ngapi?*	WEEKLY..... 1 MONTHLY..... 2 ONCE EVERY 2 MONTHS..... 3 ONCE EVERY 3 MONTHS..... 4 OTHER (SPECIFY) _____ 5 NO ANSWER..... 99	
917	From where did you get the antiretroviral medications the LAST TIME? *Ni wapi ulipata dawa zako za kupunguza makali ya virusi vya UKIMWI MARA YA MWISHO?*	THROUGH COMMUNITY HEALTH WORKERS..... 1 PUBLIC / GOVERNMENT ANTIRETROVIRAL TREATMENT CENTRE..... 2 NGO / CBO / FBO FACILITY..... 3 PRIVATE FACILITY..... 4 OTHER (SPECIFY) _____ 5 DON'T KNOW / DON'T REMEMBER..... 88	

SECTION X. REPRODUCTIVE HEALTH

No.	QUESTIONS	CODING CATEGORIES	SKIP
1001	Have you ever been pregnant? *Umeshawahi kuwa na mimba?*	YES..... 1 ..... NO..... 0 → 1013	
1002	Are you pregnant now? *Kwa sasa uko na mimba?*	YES..... 1 ..... NO..... 0	
1003 ***	*Think of your FIRST AND LAST PREGNANCY. Fikiria kuhusu MIMBA YAKO YA KWANZA NA YA MWISHO.*		
	How old were you when you found out you were pregnant? *Ulikuwa na miaka mingapi ulipogundua una mimba?* INTERVIEWER: *Mark first and last pregnancy on the timeline, if applicable.*	A. FIRST PREGNANCY AGE IN YEARS..... ☐☐ DON'T REMEMBER..... 88 MY LAST PREGNANCY WAS ALSO MY FIRST PREGNANCY; I HAVE ONLY BEEN PREGNANT ONCE..... ☐ ✓ *Tick if this applies.*	B. LAST PREGNANCY ..... ☐☐ .. .. 88 ..
1004	Have you ever given birth? *Umeshawahi kuzaa?*	YES..... 1 ..... NO..... 0 → 1010	
1005	How many children have you given birth to who are alive (but may or may not live with you)? *Ni watoto wangapi umezaa ambao wako hai (lakini wanaweza kuwa wanaishi au hawaishi na wewe)?*	NUMBER OF LIVE BIRTHS..... ☐☐	
INTERVIEWER: *Look back at the question 1002 above. If the respondent is not currently pregnant, then ask about the last pregnancy.*			
1006	Did you receive an HIV test during this/your last pregnancy?	YES..... 1 ..... NO..... 0	

	Ulipimwa virusi vya UKIMWI wakati wa mimba hii/mimba yako ya mwisho?	DON'T KNOW / DON'T REMEMBER..... 88	
1007	Did you receive the results of the HIV test during this/your last pregnancy? *Ulipata matokeo ya yako ya virusi vya UKIMWI wakati wakati wa mimba hii/mimba yako ya mwisho?*	YES..... 1 NO..... 0 DON'T KNOW / DON'T REMEMBER..... 88	
1008	Did you receive any special drugs which were explained by health care providers to reduce transmission of the HIV virus to the baby during this/your last pregnancy? *Ulipokea dawa zozote maalum kupunguza makali ya maambukizi ya virusi vya UKIMWI kwa mtoto wakati wa wakati wa mimba hii/mimba yako ya mwisho?*	YES, I TOOK SPECIAL DRUGS..... 1 NO, I DID NOT TAKE SPECIAL DRUGS..... 0 I DON'T HAVE HIV..... 77 DON'T KNOW / DON'T REMEMBER..... 88	

1009	Where did you give birth the LAST TIME? *Ulijifungua wapi MARA YA MWISHO?*	MY HOME WITH TRADITIONAL BIRTH ATTENDANT..... 1 MY HOME WITHOUT TRADITIONAL BIRTH ATTENDANT..... 2 SOMEONE ELSE'S HOME WITH TRADITIONAL BIRTH ATTENDANT..... 3 SOMEONE ELSE'S HOME WITHOUT TRADITIONAL BIRTH ATTENDANT..... 4 PUBLIC / GOVERNMENT FACILITY..... 5	

		PRIVATE / NGO / CBO / FBO FACILITY..... 6 OTHER (SPECIFY) _____ 7 ___ I AM PREGNANT NOW BUT I DO NOT KNOW WHERE I WILL GIVE BIRTH..... 77 DON'T REMEMBER..... 88	

Women sometimes have pregnancies which do not end in a live born child. That is, a pregnancy can be ended early by an abortion, a miscarriage or a stillbirth. I will now ask you some questions about abortion.
Wakati mwengine wanawake hupata momba ambazo haziishii kwa kuzaliwa mtoto aliyehai. Hii nikusema, mimba inaweza kutamatishwa mapema kupitia kutoa mimba, kuharibika kwa mimba au kuzaa mtoto aliyefariki. Sasa nitakuuliza maswali mengine kuhusu utoaji mimba.

1010	How many abortions have you had? *Umetoa mimba ngapi?*	NUMBER OF ABORTIONS..... ☐☐ I NEVER HAD ANY ABORTIONS..... 77 → 1013 DON'T REMEMBER..... 88 NO ANSWER..... 99 → 1013	

1011 ***	Think of your FIRST AND LAST ABORTION. *Fikiria kuhusu mimba MARA YAKO YA KWANZA NA MWISHO uliyotoa*		
		A. FIRST ABORTION	B. LAST ABORTION
	How old were you then? *Ulikuwa na miaka mingapi wakati huo?*	AGE IN YEARS..... ☐☐ DON'T REMEMBER..... 88	..... ☐☐ 88
	INTERVIEWER: *Mark first and last abortion on the timeline, if applicable.*	MY LAST ABORTION WAS ALSO MY FIRST ABORTION; I HAVE ONLY HAD ONE ABORTION..... ☐ ✓ *Tick if this applies.*	

1012	Where was the LAST ABORTION performed?	MY HOME..... 1	

	UTOAJI WA MIMBA wa mwisho ilifanyiwa wapi?	SOMEONE ELSE'S HOME..... PUBLIC / GOVERNMENT FACILITY..... PRIVATE / NGO / CBO / FBO FACILITY..... UNLICENSED CLINIC (E.G. QUACK)..... OTHER (SPECIFY) _____ ___ DON'T REMEMBER.....	2 3 4 5 6 88	
1013	There are various ways or methods that can be used to delay or avoid a pregnancy. What method(s) of contraception do you currently use (i.e. the last one month)? *Kuna njia mbali mbali ambazo zinaweza kutumiwa kuchelewesha au kuzuia kupata mimba* *Ni njia ipi (gani) ya kuzuia kupata mimba unatumia kwa sasa (katika mwezi wa mwisho)?* MULTIPLE ANSWERS POSSIBLE	FEMALE STERILIZATION (I.E. TUBES TIED)..... BIRTH CONTROL PILLS..... INTRAUTERINE DEVICES (IUD)..... INJECTABLES..... IMPLANTS..... CONDOMS..... LACTATIONAL AMENORRHEA METHOD (I DIDN'T USE CONTRACEPTION BECAUSE I WAS BREASTFEEDING)..... RHYTHM METHOD..... WITHDRAWAL..... EMERGENCY CONTRACEPTION..... OTHER (SPECIFY) _____ ___ I AM TRYING TO GET PREGNANT.....	A B C D E F G H I J K X	

		I AM NOT USING ANY METHODS TO AVOID PREGNANCY.....	Y	
1014***	How old were you when you first used contraception? *Ulikuwa na miaka mingapi ulipotumia njia ya kupanga uzazi mara ya kwanza?*	AGE IN YEARS.....	☐☐	
		I HAVE NEVER USED ANY CONTRACEPTION....	77 →	1016
		DON'T REMEMBER.....	88	
1015	What method of contraception did you first use? *Ni njia ipi ya kupanga uzazi ulitumia kwanza?* MULTIPLE ANSWERS POSSIBLE	FEMALE STERILIZATION (I.E. TUBES TIED).....	1	
		BIRTH CONTROL PILLS.....	2	
		INTRAUTERINE DEVICES (IUD).....	3	
		INJECTABLES.....	4	
		IMPLANTS.....	5	
		CONDOMS.....	6	
		LACTATIONAL AMENORRHEA METHOD (I DIDN'T USE CONTRACEPTION BECAUSE I WAS BREASTFEEDING).....	7	
		RHYTHM METHOD.....	8	
		WITHDRAWAL.....	9	
		EMERGENCY CONTRACEPTION.....	10	
		OTHER (SPECIFY) _____	11	
1016	Women sometimes clean inside their vaginas (i.e. douche) for a number of reasons. In the LAST MONTH, how often did you clean	I NEVER CLEAN INSIDE MY VAGINA.....	1 →	1018
		LESS THAN 1 TIME A MONTH.....	2	
		1 TO 3 TIMES A MONTH.....	3	

	inside your vagina? *Wakati mwengine wanawake husafisha (kuosha) ndani ya uke wao kwa sababu kadhaa. Katika MWEZI WA MWISHO ni mara ngapi ulisafisha ndani ya uke wako?*	1 TO 3 TIMES A WEEK..... ALMOST EVERYDAY..... EVERYDAY.....	4 5 6	
1017	In the LAST MONTH, what did you use to clean inside your vagina? *Katika MWEZI WA MWISHO ulitumia nini kusafisha ndani ya uke wako?* MULTIPLE ANSWERS POSSIBLE	WATER..... ..... SOAP..... ..... VINEGAR..... ..... PERFUME / FRAGRANCES..... TRADITIONAL HERBS..... STORE-BOUGHT SOLUTIONS..... OTHER (SPECIFY) _____ ___	A B C D E F G	
1018	In the LAST MONTH, how often did you insert/use other substances inside your vagina to prepare for sex? *Katika MWEZI WA MWISHO, Ni mara ngapi uliingiza/kutumia vitu vingine ndani ya uke wako ili kujitayarisha kwa ngono?*	I NEVER INSERT / USE ANY SUBSTANCES INSIDE MY VAGINA TO PREPARE FOR SEX..... LESS THAN 1 TIME A MONTH..... 1 TO 3 TIMES A MONTH..... 1 TO 3 TIMES A WEEK..... ALMOST EVERYDAY..... EVERYDAY.....	1 2 3 4 5 6	→ END
1019	In the LAST MONTH, what other substances did you insert/use inside your vagina to prepare for sex? *Katika MWEZI WA MWISHO, ni vitu gani vingine*	DETERGENTS..... ..... TRADITIONAL HERBS / LEAVES..... POWDER..... ..... BURNED INCENSE FOR DRYING VAGINA.....	A B C D	

	uliingiza/kutumia ndani ya uke wako ili kujitayarisha kwa ngono? MULTIPLE ANSWERS POSSIBLE	LEMON..... ..... CLOTH, GAUZE OR PAPER..... VAGINAL LUBRICANT..... FEMALE CONDOMS..... MICROBICIDE..... ..... OTHER (SPECIFY) _____ ___	E F G H I J	END

FINAL INSTRUCTIONS FOR INTERVIEWER:

- Carefully review the completeness of the contents of the questionnaire and respondent's answers.
  - Make sure "Locale ID", "Respondent ID" and "Interviewer Information" matches on the white booklet, the colour booklet and the BIOLOGICAL COMPONENT REFERRAL SHEET on the last page of the questionnaire.
  - Staple or fasten together the white booklet and colour booklet.
- Clarify any doubts or questions the respondent has about HIV/AIDS. Record respondent's questions below.

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

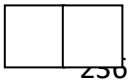
- Ask the respondent if she would like to be referred to an HIV program in her area. If the respondent agrees, provide her with the relevant contact information and record the name of the referred program below.

_____
_____
_____
_____
_____
_____
_____
_____

- With the respondent, complete the BIOLOGICAL COMPONENT REFERRAL SHEET on the last page of the questionnaire.
- Tear off the BIOLOGICAL COMPONENT REFERRAL SHEET and give it to the respondent. Escort the respondent to a private room where biological samples will be collected.
- When the respondent returns with the signed BIOLOGICAL COMPONENT REFERRAL SHEET following collection of biological samples, thank the respondent and provide honorarium.

BIOLOGICAL COMPONENT REFERRAL SHEET

LOCALE ID



County/District/Oblast:_____ Code.....
____

City/Town/Village:_____ Code.....
_____

Zone:_____ Code.....
_____

Hotspot where participant was recruited:_____

RESPONDENT ID

Respondent ID Number.....

To be completed by interviewer

The respondent consents to provide the following biological samples:

✓	Tick all that apply.
	Venous blood
	Dried blood spot §
	Urine
	Vaginal secretion collected via soft cup

§ Not needed if respondent consents to provide venous blood sample.

☐ The respondent consents to be tested for HIV.

☐ The respondent DOES NOT consent to be tested for HIV.

To be completed by clinical officer/nurse counselor

The following biological samples have been collected from the respondent:

✓	Tick all that apply.
	Blood – EDTA tube
	Blood – PAXgene tube
	Dried blood spot §
	Urine
	Vaginal secretion collected via soft cup

§ Not needed if respondent consents to provide venous blood sample.

HIV Rapid Test Results: (+/-)

☐ First test – Determine
☐ Confirmatory test – First Response
☐ Tie-breaker test – Uni-Gold

CONCLUSION: _____

X _____ _____

Clinical Officer / Nurse Counselor Name (Print) Date of Collection (dd/mm/yyyy)

X _____ AM / PM

Clinical Officer / Nurse Counselor Signature Time of Collection

Appendix 3. Permission to use published sections of my co-authored review article

RE: Request for permission to use the article; Mechanisms of sexually transmitted infection-induced inflammation in women: implications for HIV risk in my thesis.

Alberto Rossi <alberto.rossi@iasociety.org>
Thu 8/17/2023 2:00 AM
To:Ruth Mwatelah <mwatelar@myumanitoba.ca>
Cc:JIAS Editorial Office <editorial@jiasociety.org>

Dear Ruth Mwatelah,

Thanks for your message.

JIAS, and therefore your publication, operates under the CC BY - Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which allows users to copy, distribute, transmit, and adapt an article or parts of it **as long as the original source is attributed** – both for commercial and non-commercial reuse.

You may therefore use the figure and section of the discussion, and you will have to provide the appropriate reference (*e.g.* “Taken from…” “Adapted from…”).

I hope this answers your question and please let me know if you need any further clarification.

Best regards,

Alberto

Alberto Rossi, PhD
JIAS Managing Editor
Journal of the International AIDS Society

Appendix 4. Permission to use published material (Chapter 4) from Journal

Case #01948220 - Request for permission to use the article; Sex Work Is Associated With Increased Vaginal Microbiome Diversity in Young Women From Mombasa, Kenya. in my thesis

noreply@salesforce.com <noreply@salesforce.com>
on behalf of
customercare@copyright.com <customercare@copyright.com>
Wed 8/9/2023 9:04 AM
To:Ruth Mwatelah <mwatelar@myumanitoba.ca>

Dear Ruth Mwatelah,

Thank you for contacting Copyright Clearance Center's RightsLink service and for your request for copyright permission for use of your article "Sex Work Is Associated With Increased Vaginal Microbiome Diversity in Young Women From Mombasa, Kenya" in your thesis. RightsLink provides permission for the reproduction and distribution of copyrighted materials in print and electronic formats on behalf of copyright owners who list their titles with us.

If you were to process your request via RighstLink you would see the following messaging from the publisher, Wolters Kluwer:

> Sex Work Is Associated With Increased Vaginal Microbiome Diversity in Young Women From Mombasa, Kenya
>
> **Author:** Aida Sivro, Ruth Mwatelah, Cheli Kambaran, et al
> **Publication:** JAIDS: Journal of Acquired Immune Deficiency Syndrome
> **Publisher:** Wolters Kluwer Health, Inc.
>
> **Date:** Sep 1, 2020
> Copyright © 2020, Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.
> License Not Required
> Wolters Kluwer policy permits only the final peer-reviewed manuscript of the article to be reused in a thesis. You are free to use the final peer-reviewed manuscript in your print thesis at this time,