HIV Drug Resistance-associated Mutations among ART-naïve Female Sex Workers in Nairobi, Kenya

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DECLARATION

This dissertation is my original work and has not, to the best of my knowledge, been presented anywhere else. All resources and materials used or quoted have been indicated and acknowledged appropriately. This thesis is submitted in partial fulfillment for the award of a Master of Science Degree in Medical microbiology at the University of Nairobi.

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DEDICATION

To Wairimu and Clement, my parents, Luis and Natasha, my siblings, and my friend Nancy.

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And to God, for His strength, wisdom, and favor. Through Him, everything is possible.

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LIST OF ABBREVIATIONS

3TC – lamivudine

ABC – Abacavir

APV – Amprenavir

ATV – Atazanavir

AZT – Zidovudine

BIC – Bictegravir (BIC)

DOR – Doravirine

DRMs – Drug Resistance Mutations

DRV – Darunavir

DTG – Dolutegravir

EFV – Efavirenz

ETV – Etravirine (ETV)

EVG – Elvitegravir

FPV – Fosamprenavir

FSWs – Female Sex Workers

FTC – Emtricitabine

IDV – Indinavir

INSTI – Integrase Strand Transfer Inhibitors

KPs – Key populations

LMICs – Low- and Middle-Income Countries

LPV – Lopinavir

MIDR – Molecular and Infectious Diseases Reference Laboratory

MRVs – missing minority resistance variants

NFV – Nelfinavir

NNRTIs – Non-nucleoside Reverse Transcriptase Inhibitors

NRTIs – Nucleotide Reverse Transcriptase Inhibitors

NVP – Nevirapine

PIs – Protease Inhibitors

PWID – people who inject drugs

RAL – Raltegravir

RPV – Rilpivirine (RPV)

RTIs – Reverse Transcriptase Inhibitors

RTV – Ritonavir

SQV – Saquinavir

SWs – Sex Workers

TDF – Tenofovir Disoproxil Fumarate

TPV – Tipranavir

UNITID – University of Nairobi Institute of Infectious and Tropical Diseases

UoN – University of Nairobi

WHO – World Health Organization

OPERATIONAL DEFINITIONS

Acquired Drug Resistance: Resistance that occurs in treatment-experienced individuals when viral replication is not completely suppressed and resistance mutations emerge.

Circulating Recombinant Forms: Products of genetic recombination between different subtypes.

Drug resistance loci: The position within a gene where resistance-conferring mutations occur.

Genetic Barrier: Number of mutations required to confer resistance.

HIV Drug Resistance: The ability of HIV to replicate in the presence of ART.

Key Populations: people who, for one reason or another, are more vulnerable to HIV infection because they engage in high-risk behaviors such as injecting drugs, or because they are marginalized by society and are fearful of accessing HIV services.

Minority Resistant Variants: Variants of HIV that emanate from the error-prone replication processes of HIV reverse transcriptase and which bear mutations that may confer drug resistance.

Polymorphisms: Variations within a gene.

Pre-Treatment Drug Resistance: Resistance that occurs in either treatment-naive or experienced individuals who are initiating or re-initiating first-line treatment respectively.

Primary Mutations: Mutations that are selected first during therapy.

Quasispecies: A population of viruses possessing different gene structures often due to mutations.

Secondary Mutations: Mutations that develop after primary mutations and result in increased fitness of the virus.

Transmitted Drug Resistance: occurs in individuals who are treatment-naive typically resulting from infection with a virus that already harbors drug resistance mutations.

Treatment-naive: Having no prior exposure to treatment.

ABSTRACT

Background: HIV is a public health concern issue worldwide, especially with the emergence of HIV drug resistance (HIVDR). People with HIVDR are prone to treatment failure and have a higher chance of transmitting HIV than those with the wild type. There is a knowledge gap regarding HIVDR patterns amongst Female sex workers (FSWs) in Nairobi. Some FSWs are infected with strains bearing resistance mutations to recommended first-line Anti-retroviral therapy (ART).

Methods: To evaluate HIV Drug Resistance-associated Mutations (DRMs) among ART-naive FSWs in Kenya, we characterized HIVDR on samples collected from 158 ART-naive FSWs between November 2020 and April 2021. Viral RNA was extracted from plasma and amplified by RT-PCR, then the Sanger sequencing technique was used to sequence the samples, and HIV DRMs associated with reverse transcriptase inhibitors and protease inhibitors were identified using the Stanford HIV database.

Results: We successfully sequenced 64 of the 158 samples. The mean age and baseline CD4 counts were 36 (22–58) years and 369.2 (23– 1113) cells/mm^3 respectively. Of the 64 samples genotyped, 22 (34.4%) had detectable DRMs. Major reverse transcriptase inhibitors mutations were observed in 32.8% (21/64) of the FSWs. The prevalence of nucleoside reverse transcriptase inhibitors DRMs was 6.3% (4/64), non-nucleoside reverse transcriptase inhibitors DRMs was 32.8% (21/64) and protease inhibitors DRMs was 4.7% (4/64). There were 3 FSWs (4.7%) with DRMs to both tenofovir and lamivudine and 14 (21.5%) with high-level resistance to efavirenz. A majority (17/22,77.3%) of the FSWs who had DRMs had CD4 counts below 500 cells/mm^3. The predominant subtypes were A1 67.2% (43/64) and D 15.6% (10/64).

Conclusion: There is a high prevalence of pretreatment drug resistance (PDR) among ART-naive FSWs. Drug resistance testing and surveillance may need to be implemented for the general population. We also recommend a nationwide PDR survey to investigate the causes of PDR, and effective measurements needed for efficient population-based interventions.

CHAPTER ONE: INTRODUCTION

1.1 Introduction

HIV is a worldwide public health issue, especially in low- and middle-income countries (LMICs). Most significantly affected among the LMICs are those in the sub-Sahara where two-thirds of the planet's proportion of People Living with HIV (PLHIV) are (UNAIDS, 2019). There are 37.9 million PLHIV worldwide and Kenya had 1.3 million of these as of 2018 (UNAIDS, 2019). PLHIV divert a lot of their finances to medical care, and eventually even on funeral expenses (National AIDS and STI Control Program, 2018; Veenstra & Whiteside, 2005). Besides, the economy commits a lot of its resources to the fight against HIV, causing a significant financial and labor transfer (Trapero-Bertran & Oliva-Moreno, 2014). Consequently, HIV has the effect of reducing available labor productivity, thereby decreasing national productivity, especially in LMICs (Dixon et al., 2001).

HIV transmission occurs mainly through sexual intercourse, sharing of needles, blood transfusion, and by mother-to-child transmission (MTCT) (Patel et al., 2014). Key populations (KPs) including men who have sex with men (MSM), people who inject drugs (PWIDs), and sex workers account for 54% of new HIV infections globally and 14% of new infections in Kenya (National AIDS Control Council, 2019; UNAIDS, 2019). Female sex workers (FSWs) in particular are 21-times more likely to acquire HIV compared to the general population (UNAIDS, 2019; Vandenhoudt et al., 2013). This risk is further compounded if the female sex workers (FSWs) are PWIDs (Wolf et al., 2013). Fifteen percent of all HIV infections worldwide among women can be attributed to female sex work (Wolf et al., 2013).

Antiretroviral therapy (ART) is an essential tool in our arsenal against HIV (Bure et al., 2015). These drugs target essential viral enzymes and other essential viral components consequently inhibiting their actions. These drugs include Nucleoside Reverse Transcriptase inhibitors (NRTIs) and non-Nucleoside Reverse Transcriptase inhibitors (NNRTI) that inhibit Reverse Transcriptase (RT), Protease Inhibitors (PIs) that inhibit protease and Integrase Strand Transfer Inhibitors (INSTIs) that inhibit integrase. Current WHO guidelines endorse dolutegravir (DTG) with two NRTIs as the first-line ART of choice for PLHIV in all populations (WHO, 2019c). For first-line therapy, 2018 ART guidelines in Kenya indicate Abacavir together with lamivudine and lopinavir for treatment of children below 15 years,

and tenofovir and lamivudine together with either dolutegravir or efavirenz for treatment of adults (National AIDS and STI Control Programme, 2018).

The United Nations Program on HIV/AIDS was set to curb AIDS as a public health concern by 2030 by scaling up access to ART to all PLHIV. This has decreased HIV- related mortality by 52% since 2010 (UNAIDS data 2021). The emergence of HIV drug resistance (HIVDR) however threatens the progress of this milestone in the fight against HIV. The level of pre-treatment drug resistance (PDR) to NNRTIs exceeded 10% in 12 out of 18 participating countries in a recent survey. Amongst sex workers in Kenya, there was a 22% prevalence of HIVDR, and PDR was highest among treatment-naïve FSWs compared to the other sex workers (Sampathkumar et al., 2014; Silverman et al., 2018; WHO, 2019a).

Efforts on understanding HIV-1 incidence and patterns among KAPs including FSWs will be crucial in the fight to curb HIV (Gounder et al., 2016). Novel treatment and vaccine development strategies are also quite reliant on knowledge of existing and emerging viral variants, and the transmission networks of circulating variants can allude from studies on FSWs (Chimukangara et al., 2019; Gounder et al., 2016).

This study aims to describe the patterns of pretreatment HIV drug resistance-associated mutations to NRTIs, NNRTIs, and PIs among treatment-naïve FSWs in Nairobi, Kenya.

1.2 Problem statement

People with HIVDR are prone to treatment failure, and consequently have poor health outcomes, become subjected to less tolerable & costlier regimen changes, and have a higher chance of transmitting the resistant strain to others. In Kenya, a 10% prevalence of PDR was noted among individuals initiating ART (Silverman et al., 2018). There is little knowledge of the existing drug resistance mutations (DRMs) within the circulating strains, especially on FSWs who happen to be major players in HIV transmission dynamics. This study will try to highlight the pretreatment drug resistance patterns among selected FSWs in Nairobi.

1.3 Justification

Characterizing the ***pol*** region among treatment naïve FSWs will provide important information on DRMs of HIV-1 variants in this key population. The results obtained from this study are intended to aid policy-making on the formulation of ART treatment guidelines and steer clinicians towards efficient individual therapy for their patients, thereby resulting in

less pill burden and ART regimen change-associated costs. HIVDR transmission dynamics data from the study will also provide insight into the effectiveness of existing public health interventions, and also help guide the formulation of efficient cluster-informed strategies for early detection and prevention of DRMs. Knowledge of circulating variants will also be important to local researchers working on probable novel drugs and vaccine designs against HIV-1.

1.4 Research questions

1. Which ***pol*** drug resistance mutations are present among ART-naïve FSWs in Nairobi?
2. What are the frequency and distributions of the DRMs?
3. Which HIV subtypes are in circulation among ART-naïve FSWs in Nairobi?

1.5 Objectives

1.5.1 Broad objectives

To describe pretreatment HIV drug resistance mutations associated with NNRTIs, NRTIs, and PIs in treatment-naïve female sex workers in Nairobi.

1.5.2 Specific objectives

1. To characterize the ***pol*** region, particularly the RT and protease sequences of HIV-1 virus among treatment-naïve female sex workers using Sanger population sequencing approaches and determine the prevalence of the various NRTI, NNRTI, and PI-associated mutations.
2. To determine the HIV-1 subtypes using the ***pol*** sequences with the COMET subtyping tool.
3. To investigate the genetic relatedness of HIV ***pol***-RT and protease sequences from objective 1 and other previously sequenced African HIV-1 ***pol*** isolates downloaded from the LANL database using MEGA 11 software.

1.6 Conceptual framework

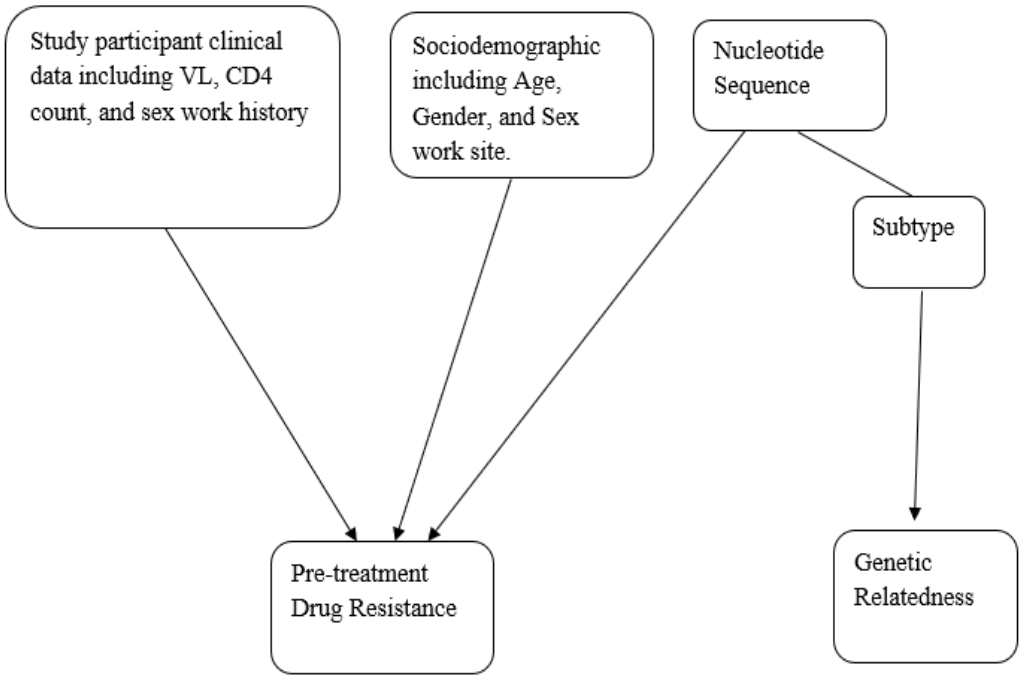

Figure 1.1: Conceptual Framework. The independent variables are the participants' sociodemographic data and the nucleotide sequence. The dependent variables are pre-treatment drug resistance and genetic relatedness. The subtype is an intermediate outcome.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction to HIV

The Human Immunodeficiency Virus (HIV) is a member of the Retroviridae family and the genus lentivirus. It has two genetically and antigenically distinct viruses; HIV-1 and HIV-2 (Seitz, 2016). These two are morphologically indistinguishable and exhibit the same modes of transmission and life cycle patterns but have significantly different geographical distribution patterns and rates of transmission & disease progression (Lemey et al., 2003). Some contrasting clinical manifestations between the two have been reported (Nyamweya et al., 2013). HIV-1 is more easily transmitted between individuals and progresses faster to acquired immunodeficiency syndrome (AIDS) than HIV-2 (Marlink et al., 1994). HIV-1 is distributed worldwide whereas HIV-2 predominantly occurs in West African countries (Campbell-Yesufu & Gandhi, 2011).

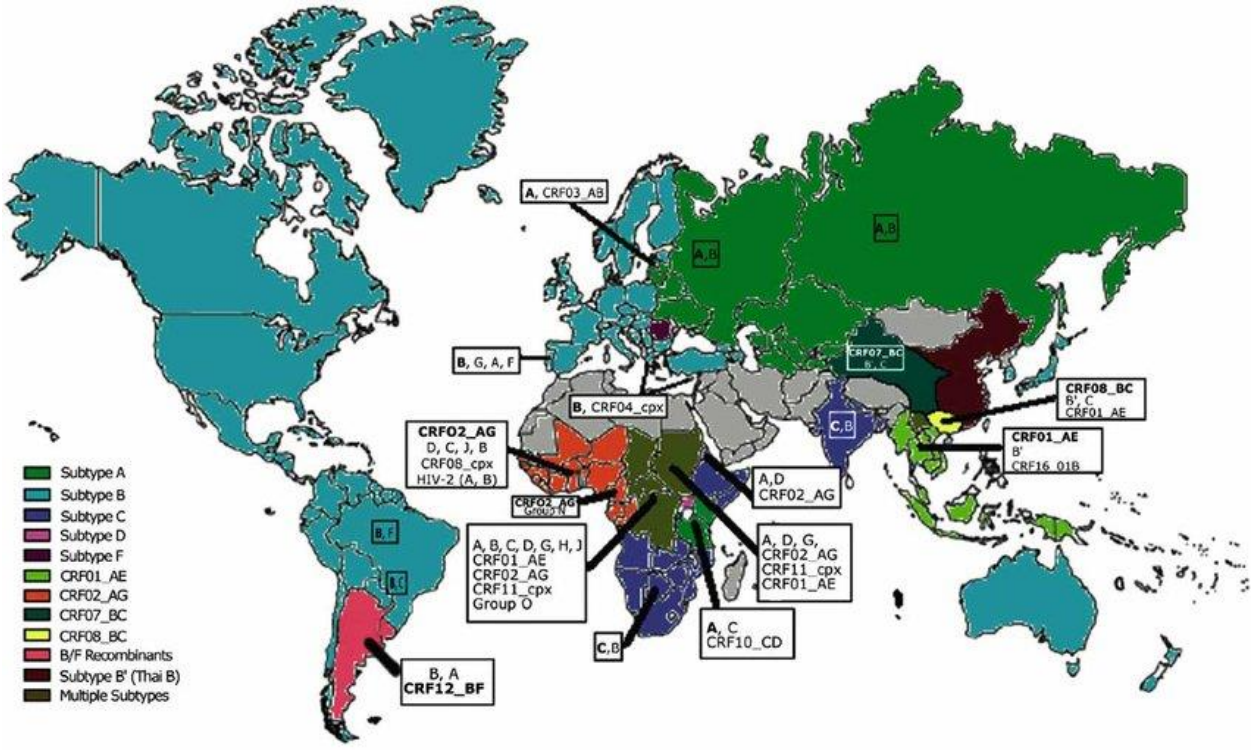

Figure 2.1: Geographic distribution of HIV-1 subtypes. Colors have been used to distinguish subtypes. Secondary subtypes, groups, and CRFs are shown in the text boxes (Butler et al., 2006).

HIV-1 has four groups; M (Major), N (non-M, non-O), O (Outlier), and P, with M being at the helm of the HIV-1 pandemic (Bbosa et al., 2019a; Shao & Williamson, 2012). HIV-2 is

divided into groups A-H (Seitz, 2016). HIV-1 group M is grouped into subtypes; A-D, F-H, J, K & L. Subtype A has sub-subtypes A1 and A2, and subtype F has sub-subtypes F1 and F2 (Seitz, 2016; Shao & Williamson, 2012; Yamaguchi et al., 2019). HIV Subtypes also show a defined geographical distribution and can further combine to form circulating recombinant forms (CRFs) and unique recombinant forms (Seitz, 2016). There are currently more than 70 CRFs described worldwide (Bbosa et al., 2019b). Subtype B prevails in Europe, America, and Australia. In Kenya, subtype A prevails but subtypes D, C and G have also been previously isolated (Gounder et al., 2016; Khoja et al., 2008).

2.2 Pathogenesis of HIV infection

2.2.1 HIV transmission

HIV is most frequently transmitted sexually upon introduction of virally infected seminal, vaginal or rectal fluids into susceptible mucous membranes (Moir et al., 2011; Raimondo et al., 2010). Other routes of transmission include needle-sharing in people who inject drugs (PWIDs), percutaneous, direct inoculation into the bloodstream as is the case during blood transfusion and from mother-to-child (MTCT) either in-utero, during childbirth or while breastfeeding (Moir et al., 2011). HIV transmission is contingent on the biological properties of the virus, its concentration in the transmission body fluid, and host susceptibility (Raimondo et al., 2010). Besides, various acts of exposure to the virus confer relative risks of infection (Patel et al., 2014). The order of transmission risk is highest in blood transfusion, MTCT, receptive anal intercourse, PWID, and least in percutaneous needle stick injuries (Patel et al., 2014). Sexual transmission poses a higher risk for the receptive counterpart than the insertive partner, and anal intercourse exacerbates the risk in such encounters (Patel et al., 2014). People with Sexually Transmitted Infections (STIs) especially those with ulcerative lesions such as syphilis have a significantly higher probability of getting infected with HIV (Mahlen et al., 2012).

HIV infection has two clinical phases; acute and chronic.

2.2.2 Phase I: Acute/Early infection

Immediately following viral entry during the primary exposure, resident antigen-presenting dendritic cells at the mucosa process and present HIV to $CD4^+$ T-lymphocytes at the lymph

nodes (Abbas et al., 2017). During this antigen presentation process, the virus may be transmitted to activated CD4+ T-lymphocytes through receptor-dependent mechanisms wherein the virus rapidly replicates resulting in $CD4^+$ T-lymphocytes death (Abbas et al., 2017; Raimondo et al., 2010). This viral replication stage is reliant on host cell transcription factors made in activated CD4+ lymphocytes (Mahlen et al., 2012). Infected cells die as the virus then propagates via draining lymphatic nodes into the bloodstream and gut-associated lymphoid tissue (GALT) (Abbas et al., 2017; Mahlen et al., 2012; Raimondo et al., 2010). Extensive exhaustion of CD4+ lymphocytes in blood and the GALT happens as viremia aggressively progresses (Moir et al., 2011). Peak viremia occurs at about 3 – 4 weeks post-exposure, during which an acute HIV syndrome with defined flu-like symptoms presents, and includes manifestations such as headache, fever, sore throat, generalized lymphadenopathy, and body rashes (Mahlen et al., 2012; Moir et al., 2011). The adaptive immune response is activated and the CD8+ response coupled with neutralizing antibodies to HIV substantially reduces the plasma viremia over the next couple of weeks to a defined set point (Mahlen et al., 2012; Moir et al., 2011).

2.2.3 Chronic/ Clinical latency phase

The spread of the virus to the peripheral tissues, especially the peripheral lymph nodes and GALT induces viral latency during which there is low-level continuous virus replication (Abbas et al., 2017; Mahlen et al., 2012; Moir et al., 2011; Raimondo et al., 2010). Consequently, a clinical latency phase occurs and the patient becomes asymptomatic for a variable duration based on the immune system's ability to ward off opportunistic microbes albeit as CD4+ T-cells especially Th17 lymphocytes progressively wane (Moir et al., 2011). A slow progression of the disease occurs, characterized by chronic decimation of the lymphoid architecture, and AIDS-related complex (ARC) which manifests as persistent symptoms including fevers, fatigue, weight loss, and lymphadenopathy may occur (Mahlen et al., 2012; Raimondo et al., 2010). Eventually, this culminates in AIDS when CD4+ lymphocyte numbers fall below 200 cells/µl (Mahlen et al., 2012; Moir et al., 2011).

2.2.4 AIDS

AIDS is the final phase of infection. Individuals become severely immunocompromised and are frequently faced with a vast array of severe opportunistic infections and neoplastic

diseases due to the severe drop in T-helper cells numbers and impaired immune system (Abbas et al., 2017; Mahlen et al., 2012). The most prominent infections include pneumocystis pneumonia, candidiasis, cryptococcosis, histoplasmosis, toxoplasmosis, cryptosporidiosis, tuberculosis, and Kaposi sarcoma among others (Mahlen et al., 2012; Raimondo et al., 2010). Such people become wasted and may present with diffuse lymph node swelling, respiratory and gastrointestinal symptoms, and central nervous system (CNS) manifestations such as dementia (Abbas et al., 2017; Mahlen et al., 2012; Raimondo et al., 2010).

2.3 Clinical staging

Clinical staging of HIV occurs after the establishment of a HIV infection by rapid antibody, enzyme immunoassay, or PCR tests (WHO, 2007). Clinical staging by WHO classifies HIV into four stages; stage 1 to stage 4 (WHO, 2007). The occurrence of at least one of the classical features is a requisite for placement into any stage.

Stage one patients are asymptomatic and may present with persistent generalized lymphadenopathy for over six months (WHO, 2007). The second clinical stage is a mild symptomatic stage that involves marginal weight loss typically not above 10% of the total body weight, and an array of recurring upper respiratory tract infections including sinusitis, tonsillitis, popular pruritic eruptions, pharyngitis, sporadic oral ulcerations and fungal nail infections (WHO, 2007). Stage three patients present with advanced symptoms as their immune systems deteriorate further. Patients undergo massive weight loss of over 10% of their total body weight in addition to neutropenia, chronic thrombocytopenia, unexplained anemia, chronic fever, and chronic diarrhea for more than a month, and oral manifestations such as oral candidiasis, oral leukoplakia, periodontitis and gingivitis (WHO, 2007). Opportunistic bacterial infections begin to set in, pulmonary tuberculosis being the most prevalent among others that engender a cocktail of infections including meningitis, bacteremia, and pneumonia (WHO, 2007). The hallmark of the last clinical stage, stage four, is the occurrence of AIDS. Patients undergo extensive body wasting (HIV wasting syndrome) and a wide array of recurrent severe disseminated opportunistic infections including extrapulmonary tuberculosis, severe bacterial pneumonia, HIV encephalopathy, disseminated mycosis, disseminated non-tuberculous mycobacterial infection cryptococcosis,

cryptosporidiosis, non-typhoidal salmonella bacteremia and malignancies including Kaposi sarcoma (WHO, 2007).

2.4 HIV genome

HIV is a diploid positive-sense RNA virus (Mahlen et al., 2012). The genome is sandwiched between long terminal repeats (LTR)(Mahlen et al., 2012). Three structural genes and six accessory genes are sandwiched between these LTRs. The structural genes are ***Gag***, ***Pol,*** and ***Env***. ***Gag***, a group-specific gene, is the first gene reading frame after the LTR on the 5' end (Seitz et al., 2016). It encodes outer core membrane proteins; the p24 capsid protein, the p17 matrix protein, and the p7 nucleocapsid protein (Seitz et al., 2016). Adjacent to ***gag*** is the ***pol*** which codes for three enzymes; RT, protease, and integrase. After ***pol*** is ***env*** from which the surface proteins gp120 and gp41 are derived (Seitz et al., 2016).

Accessory viral genes are next in the reading frame after ***env***. These are ***vif***, ***vpr***, ***vpu***, ***tat***, ***rev,*** and ***nef*** (Seitz et al., 2016). HIV-2 encodes ***vpx*** instead of ***vpu*** (Seitz et al., 2016).

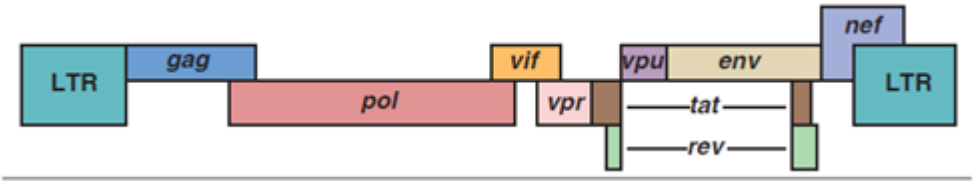

Figure 2.2: Illustration of the HIV-1 genome

The 3' and 5' ends of the genome contain LTRs that are sites for host transcription factors. Following the 5' LTR is ***gag*** that encodes viral nucleocapsid core and matrix proteins. ***pol*** gene follows closely and from it stems the viral enzymes IN, RT and PR. The ***env*** gene that codes for the glycoproteins gp120 and gp41 lies between the genes that code for regulatory factors ***vif***, ***vpr***, ***vpu***, ***tat***, ***rev,*** and ***nef*** (Abbas et al., 2017).

2.5 HIV life cycle

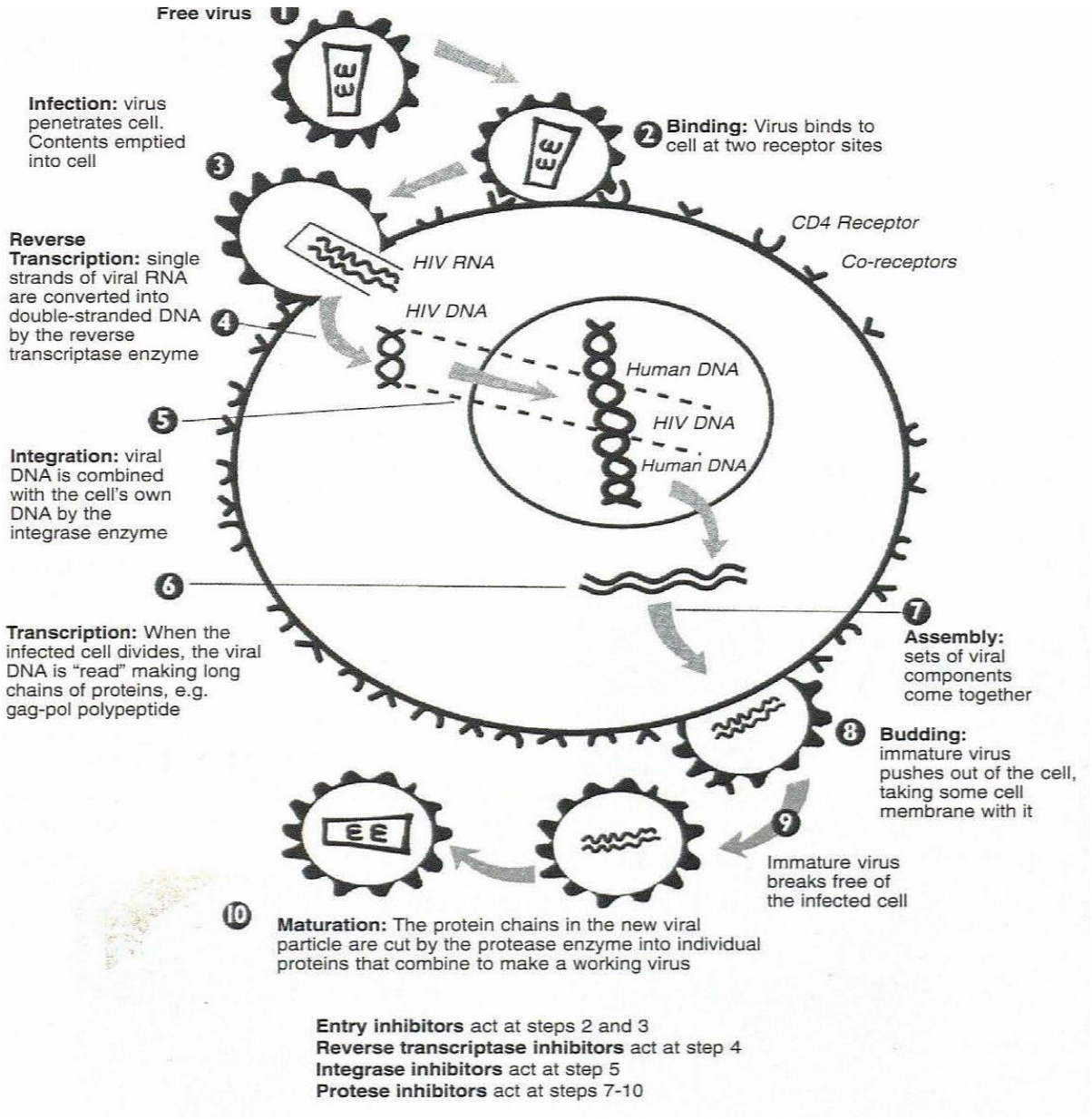

Figure 2.3: A demonstration of the HIV life cycle and ARV targets (Miller, 2002).

The HIV life cycle begins with attachment to susceptible host cells. HIV typically infects CD4+ T-lymphocytes and macrophages, but dendritic cells and astrocytes can be infected too (Seitz et al., 2016). The gp120 of an incoming mature virion attaches to the host cell CD4 receptor, causing a conformational change that permits gp120 to bind a chemokine co-receptor, CCR5 or CXCR4 (Esposito & Craigie, 1999; Ferguson et al., 2002; Mahlen et al., 2012; Raimondo et al., 2010; Seitz et al., 2016). The co-receptor onto which the virion

attaches defines viral tropism. Subsequently, the N terminal fusion peptide (FP) of gp41 moves and inserts into the host cell membrane, essentially causing the cell membrane and viral envelope to merge, thereby permitting the virus to enter the cell (Chen, 2019; Ferguson et al., 2002; Seitz et al., 2016).

Following entry, viral enzymes are activated, which in turn initiate the viral replicative processes (Abbas et al., 2017; Raimondo, n.d.). The nucleocapsid is degraded and its contents are released into the cytoplasm. RT reverse transcribes the viral genome into a cDNA strand while the RNAase H activity of RT simultaneously breaks down the RNA. RT then transforms the single-stranded cDNA into a double-stranded DNA, which together with integrase is transported into the nucleus via nucleopores (Ferguson et al., 2002; Seitz et al., 2016). Integrase then integrates the viral DNA into the host cell, a step that establishes the virus in the host cell as a persistent infection (Seitz et al., 2016).

When the host cells get activated, viral DNA gets transcribed into a single full-genome-length transcript that undergoes alternative splicing (Abbas et al., 2017). Cytoplasmic translation of the spliced viral mRNAs occurs in two stages; the early and the late phase. Early-phase translation yields regulatory proteins whereas late-phase translation yields structural proteins (Abbas et al., 2017; Sundquist & Kräusslich, 2012). The latter is translated as polyproteins, Pr160GagPol and Pr55Gag. Pr160GagPol is cleaved by protease to yield RT, protease, and integrase while Pr55Gag is cleaved to yield core and matrix proteins p7, p24, and p17 (Abbas et al., 2017; Ferguson et al., 2002; Miller, 2002). The viral genome and enzymes are then packaged within the p7 protein forming a nucleoprotein complex that buds from the host cell as a mature virion (Abbas et al., 2017; Mahlen et al., 2012; Seitz et al., 2016).

2.6 Genetic variation

HIV-1 undergoes rapid genetic evolution owing to events in its propagation cycle such as high rates of mutations and recombination during reverse transcription and a rapid viral turnover in infected cells, resulting in a highly divergent epidemic (Lihana et al., 2009; Shao & Williamson, 2012). The HIV RT has no proofreading ability and is therefore error-prone, incorporating approximately one incorrect nucleotide per round of transcription, and consequently, a broad range of quasispecies (Seitz et al., 2016). These mutations may confer an evolutionary advantage or disadvantage. The advantage occurs when these mutations confer an ability to evade immune responses mounted against them or even confer resistance

to drugs. Mutations in essential segments of key structural proteins may however result in both replication-incompetent or replication-efficient mutants (Ferguson et al., 2002; Seitz et al., 2016).

2.7 Reverse Transcriptase

The HIV ***pol*** gene is transcribed in host cells and one of its end products is RT, a dimer composed of two subunits, p66 and p51, both of which share a single amino terminus and have 560 amino acids and 440 amino acids respectively (Sarafianos et al., 2008). Two p66 domains are spatially distinct; a polymerase and an RNase-H domain, whereas p51 merely performs a structural role (Sarafianos et al., 2008; Singh et al., 2014). P51 and the p66 polymerase domain are each further structured into four subdomains; palms, fingers, connection, and thumb. For the polymerase, amino acids 1-85 and 118-155 form the fingers, 86-117 and 156-236 form the palm, 237-318 form the thumb, and the connection spans through amino acids 319-426 (Sarafianos et al., 2008). Nucleic acid binding occurs on a cleft formed by the polymerase and RNase-H, with the floor of the cleft being the connection and thumb subdomains of p51 (Sarafianos et al., 2008; Singh et al., 2014). As such, substrate nucleic acids interact with both the polymerase and RNase-H active sites. The α-helices of p66 thumb assist in the insertion of nucleic acid substrates through associations engaging the template strands and the primer, while the DNA primer grip composed of the p66 β12- β13 hairpin in HIV-1 RT assists in positioning the 3'-OH of the primer at the polymerase catalytic site (Sarafianos et al., 2008). The latter is a highly preserved motif (Sarafianos et al., 2008). Three catalytic Mg^{2+}-binding carboxylates form the polymerase catalytic site; D110, D185 & D186. D185 and D186 are part of the highly preserved YXDD motif (Sarafianos et al., 2008; te Velthuis, n.d.). R72, K65, Y115, and Q151 are other conserved residues important in dNTP binding (Sarafianos et al., 2008).

Nucleotide polymerization begins when a nucleic acid substrate, primer, and template bind at the nucleotide-binding site (N site), forming a three-layered composite, thus inducing an alteration of the p66 thumb from a closed to an open structure (Sarafianos et al., 2008). Consequently, the p66 fingers hold the incoming dNTP and position it such that it faces the 3'-OH of the primer, the α-phosphate of the dNTP, and the polymerase active site. The primer and the dNTP are then linked via a phosphodiester bond. The growing nucleotide

chain is then translocated to free the N site thus allowing processive DNA synthesis (Sarafianos et al., 2008).

2.8 Protease

The HIV protease is a ***pol*** gene product made of two identical 99-amino acids subunits (homodimer) (Ghosh et al., 2016; Kneller et al., 2019; Miao et al., 2018; A. M. J. Wensing et al., 2010). It is an aspartic protease whose substrate binding cleft contains catalytic aspartate residues from each monomer (Kneller et al., 2019; A. M. J. Wensing et al., 2010). Two malleable glycine-dense β-sheets form a fold over the catalytic site that closes when a substrate is bound and opens to allow substrate entry or release (Ghosh et al., 2016; Koch, 2017). The substrate interacts with at least seven amino acids on the enzyme's active site denoted as P4 to P1 and P1' to P4' (Ghosh et al., 2016). The amide bond on the substrate is hydrolyzed between p1 and P1' residues. Each subsite of the enzyme is highly specific, thereby defining substrate side chain preference (Ghosh et al., 2016).

2.9 HIV treatment

HIV is still without a cure, but ART has been fundamental in managing HIV patients. ART entails a cocktail of antiretroviral drugs (ARVs) which suppress HIV replication thereby effectively reducing plasma viral load to undetectable levels (Arts & Hazuda, 2012). ART improves CD4 counts in patients thus positively revamping the immune system (Arts & Hazuda, 2012; Autran et al., 1997). Essentially, ART has the effect of improving patients' health and hence prolonging their lives. ART is an important tool in reducing HIV-related morbidity and deaths, especially among those who initiate therapy early and are adherent (Arts & Hazuda, 2012; Rai et al., 2018).

2.9.1 ART classification

At present, HIV drugs can be broadly grouped into six categories based on their mechanisms of action: Entry inhibitors/co-receptor antagonists, fusion inhibitors, NRTIs, NNRTIs, PIs, and INSTIs (Arts & Hazuda, 2012; Singh et al., 2014). Entry inhibitors obstruct the binding of viral envelope proteins to either the CD4 T-lymphocytes receptor or the chemokine co-receptors (Arts & Hazuda, 2012; Briz et al., 2006). An example in this class is Maraviroc, a CCR5 receptor antagonist that prevents gp120 from binding CCR5, thereby precluding

membrane fusion and entry (Arts & Hazuda, 2012). Enfurvitide is a fusion inhibitor that binds to the HR2 region of gp41, thereby preventing a conformational change important in HIV fusion with the host cell membrane (Briz et al., 2006). NRTIs and NNRTIs act by targeting the activity of the enzyme reverse transcriptase. NRTIs are prodrugs that require host cellular machinery for phosphorylation, just like cellular nucleotides. They however lack a free 3'OH required for nucleotide polymerization and therefore once activated by cellular kinases, enact their activity by causing chain termination (Arts & Hazuda, 2012). NNRTIs act by binding RT and causing a spatial conformation change of its active site, consequently inhibiting its activity (Arts & Hazuda, 2012). Currently, the list of FDA-approved NRTIs includes abacavir (ABC), zidovudine (AZT), lamivudine (3TC), emtricitabine (FTC), and tenofovir disoproxil fumarate (TDF) while approved NNRTIs include efavirenz (EFV), nevirapine (NVP), doravirine (DOR), etravirine (ETV) and rilpivirine (RPV) (Holec et al., 2017; Wang et al., 2019). Protease inhibitors enact their effect by blocking its catalytic site thereby preventing it from cleaving precursor polyproteins into solitary functional proteins, thus disengaging the viral assembly process before budding (Arts & Hazuda, 2012). The FDA currently approves use of the following PIs: ritonavir (RTV), lopinavir (LPV), atazanavir (ATV), saquinavir (SQV), indinavir (IDV), nelfinavir (NFV), darunavir (DRV), amprenavir (APV), fosamprenavir (FPV), and tipranavir (TPV) (Ghosh et al., 2016). Lastly, INSTIs which are the most recent class of drugs act by targeting the strand transfer activity of the enzyme. This results in a halt to the proviral DNA integration process (Arts & Hazuda, 2012). Five INSTIs have been FDA-approved; raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG), bictegravir (BIC), and Cabotegravir (Markham, 2018, 2020; Orkin et al., 2020).

2.10 HIV treatment guidelines

HIV treatment guidelines recommend the use of HIV drugs as a cocktail of ARVs with different molecular mechanisms of action to enhance the overall efficiency and durability of therapy (WHO, 2019b; World Health Organization, 2015). Current ART programs worldwide follow the universal test-and-treat policy for all PLHIV (World Health Organization, 2015). These are categorized into stratified groups based on treatment scheduling and patient response to the drugs i.e., first-line, second-line, and third-line regimens. For first-line therapy, recent Kenyan guidelines indicate the use of Zidovudine dosed with Lamivudine and Nevirapine for infants, Abacavir in combination with lamivudine

and Efavirenz for children under 15 years, and Tenofovir and Lamivudine in combination with either Dolutegravir or Efavirenz for treatment of all above 15 years old (National AIDS and STI Control Programme, 2018; WHO 2018).

2.11 HIV Drug Resistance (HIVDR)

HIV Drug Resistance (HIVDR) is the ability of HIV to replicate even when subjected to ARVs (WHO, 2019a). This is usually a result of mutations on the genes coding for viral components acted upon by the ARVs. These mutations are mainly due to RT errors that are exacerbated by the rapid viral turnover and often due to recombination occurring within infected cells (Ghosh et al., 2016). Such mutations can be primary or secondary; Primary mutations reduce the ability of the drug to bind the target enzyme, while secondary mutations supplement primary mutations by improving the fitness of variants with primary mutations (Holec et al., 2017). Primary mutations result in an increased drug dosage required to inhibit the enzyme, whereas secondary mutations have a minimal inhibitory effect on the drug's activity (WHO, 2012). HIVDR is classified into three categories; acquired drug resistance (ADR), transmitted drug resistance (TDR), and pre-treatment drug resistance (PDR) (WHO, 2019a). ADR is the capability of the virus to divide as a consequence of drug selection pressure due to mutations that occur in treatment-experienced individuals (Coetzee et al., 2017; WHO, 2019a). TDR occurs in treatment-naïve individuals who get infected with a virus already bearing HIVDR-related mutations (WHO, 2019a). PDR occurs when viral replication remains unsuppressed in treatment-naïve persons initiating ART or on treatment-experienced patients re-initiating ART, and as such, PDR can be TDR, ADR, or both (Chimukangara et al., 2019; WHO, 2019a).

2.11.1 Mechanisms of NRTI resistance

The NRTIs indicated as part of first-line treatment in Kenya are Tenofovir, Lamivudine, and Abacavir. Tenofovir is indicated as part of the ART backbone in adults, while lamivudine can be used across all ages (National AIDS and STI Control Programme, 2018). Having been the backbone of most first and second-line ART regimens, and owing to their long-term use, numerous resistance patterns to NRTIs have occurred over time (Holec et al., 2017). NRTI resistance occurs by either of two mechanisms caused by mutations: reversal of chain termination and NRTI discriminatory mutations (Arts & Hazuda, 2012; Clutter et al., 2016;

Iyidogan & Anderson, 2014). Reversal of chain termination occurs by excision of the NRTI-triphosphate from the growing chain by ATP-dependent phosphorolysis (Clutter et al., 2016; Sluis-Cremer et al., 2015). Such DRMs also called primer unblocking mutations or thymidine analog mutations (TAMs) and include M41L, D67N, K70R, L210W, T215F/Y, and K219Q/E (Arts & Hazuda, 2012; Clutter et al., 2016; Holec et al., 2017; Iyidogan & Anderson, 2014). The second mechanism entails discriminatory mutations that reduce the NRTI binding affinity whilst permitting efficient binding of host cell dNTPs thereby allowing the virus to proliferate (Arts & Hazuda, 2012; Holec et al., 2017). Such mutations include M184V, L74V, Q151M, and K65R (Arts & Hazuda, 2012; Clutter et al., 2016).

Table 2. 1: Common clinically noteworthy NRTI-resistance-associated mutations. The highest extent of decreased susceptibility to indicated NRTIs are highlighted in bold red, while those that decrease NRTI susceptibility or virologic response are indicated in bold normal color font. Plain text mutations decrease susceptibility when they occur simultaneously with other NRTI resistance-associated mutations (A. Wensing, 2019).

	184	**65**	**70**	**74**	**115**	**41**	**67**	**70**	**210**	**215**	**219**	**69**	**151**
Consensus	**M**	**K**	**K**	**L**	**Y**	**M**	**D**	**K**	**L**	**T**	**K**	**T**	**Q**
3TC	**VI**	**R**										**Ins**	**M**
FTC	**VI**	**R**										**Ins**	**M**
ABC	VI	**R**	**E**	**VI**	**F**	L			W	FY		**Ins**	**M**
DDI	VI	**R**	**E**	**VI**		L			W	FY		**Ins**	**M**
TDF	***	**R**	**E**		F	L		R	W	FY		**Ins**	**M**
D4T	***	**R**	**E**			**L**	**N**	**R**	**W**	**FY**	QE	**Ins**	**M**
ZDV	***	***	*	*		**L**	**N**	**R**	**W**	**FY**	QE	**Ins**	**M**

2.11.2 Mechanisms of NNRTI resistance

Efavirenz and nevirapine are the NNRTIs currently indicated as part of the ART regimen used in Kenya (National AIDS and STI Control Programme, 2018). Several mechanisms are involved in the occurrence of NNRTI resistance, but all are caused by mutations. Amassing of mutations in the NNRTI binding pocket which in turn affects NNRTI binding by three mechanisms (Sluis-Cremer et al., 2015). First of all, by impairing interactions between NNRTIs and the NNRTI binding pocket (Lai et al., 2016; Sluis-Cremer et al., 2015). The ubiquitous DRM in this category is the K103N mutation (Sluis-Cremer et al., 2015). This mutation is largely responsible for reduced susceptibility to EFV and NVP. Secondly, some

mutations establish steric barriers to NNRTI binding, the most common DRM in this category being the Y181C mutation that reduces the association between the NNRTI and the NNRTI-binding pocket (Sluis-Cremer et al., 2015). Lastly, mutations that establish or remove inter-residue interactions in the NNRTI-binding pocket thereby impeding the capability of another residue in the pocket sandwich the drug. This often results in a structure that is unable to bind with sufficient specificity (Sluis-Cremer et al., 2015). An example of such a mutation is the Y188L mutation. Other frequently occurring NNRTI mutations include L100I, K101E/P, V106A/M, G190A/S/E, and M230L all of which cause resistance to NVP. Cross-resistance is also a defining feature in NNRTIs (Antinori et al., 2002; Melikian et al., 2014).

Table 2. 2: Common clinically noteworthy NNRTI-resistance associated mutations. Decreased susceptibility and virologic response to indicated NNRTIs are most significant among the mutations highlighted in bold red, while those that decrease NNRTI susceptibility or virologic response are in the bold normal color font. Plain text mutations decrease susceptibility when they occur simultaneously with other NNRTI resistance-associated mutations (A. Wensing, 2019).

	100	101	103	106	138	181	188	190	230
Consensus	**L**	**K**	**K**	**V**	**E**	**Y**	**Y**	**G**	**M**
DOR	I	EP		**AMI**		CIV	**L**HC	**SE**	**L**
EFV	**I**	E**P**	**NS**	A**M**		CIV	**LC**H	**ASE**	**L**
ETR	**I**	E**P**			AGKQ	**C**I**V**	L	AS**E**	**L**
NVP	**I**	**EP**	**NS**	**AM**		**CIV**	**LCH**	**ASE**	**L**
RPV	**I**	**EP**			**AGKQ**	**CIV**	**L**	AS**E**	**L**

2.11.3 Mechanisms of PI resistance

The PIs currently indicated in Kenya's first-line regimen is Lopinavir which is indicated for children between 4 weeks to 3 years old (National AIDS and STI Control Programme, 2018) The occurrence of PI resistance has largely been attributed to RT errors (Ghosh et al., 2016). Besides, the rapid low-fidelity transcription coupled with random recombination and the evolutionary pressure of PIs exacerbate the introduction of mutants born of reverse transcription errors (Deeks & Volberding, 1997; Ghosh et al., 2016). Most of these PR-related mutations are single amino acid substitutions, with rare occasions of insertions being

reported (Koch, 2017). PR mutations are classified into two; primary and secondary (Ghosh et al., 2016; A. M. J. Wensing et al., 2010). Unlike primary mutations that involve substitutions in the PR active site and hence directly affect the substrate binding cleft, secondary mutations occur further away from the active site and do not have a marked effect on the phenotypic resistance outcome (Ghosh et al., 2016; A. M. J. Wensing et al., 2010). Primary mutations cause resistance by either of these three mechanisms; substitutions that alter drug interactions, lower dimer stability, and cause deficiencies in autoprocessing (Koch, 2017). Substitutions that alter drug interactions reduce PI binding affinity and include D30N, V32I, I47V, G48V, I50V, Val82, and I84V (Koch, 2017). Those that lower dimer stability are: L24I, I50V, and F53L (Koch, 2017). Mutants bearing L76V have defects in autoprocessing and lowered dimer stability (Koch, 2017). PI-associated mutations have also been noted in the *gag* protein (Maguire et al., 2002).

Table 2. 3: Most noteworthy frequently occurring PI resistance-associated mutations. Phenotypic resistance and/or decreased virologic response to PI treatment is most significant when mutations highlighted in bold red are present (A. Wensing, 2019).

	30	**32**	**33**	**46**	**47**	**48**	**50**	**54**	**76**	**82**	**84**	**88**	**90**
Consensus	**D**	**V**	**L**	**M**	**I**	**G**	**I**	**I**	**L**	**V**	**I**	**N**	**L**
ATV/r		I	F	IL	V	**VM**	**L**	VTALM		ATFS	**V**	**S**	**M**
DRV/r		**I**	F		**VA**		**V**	**LM**	**V**	F	V		
FPV/r		**I**	**F**	**IL**	**VA**		**V**	VTA**LM**	**V**	ATS**F**	**V**		**M**
IDV/r		**I**		**IL**	V			**VTA**LM	**V**	**AFTS**	**V**	S	**M**
LPV/r		**I**	F	IL	V**A**	VM	**V**	**VTALM**	**V**	**AFTS**	**V**		M
NFV	**N**		F	**IL**	V	**VM**		**VTALM**		**AFTS**	**V**	**DS**	**M**
SQV/r						**VM**		**VTA**LM		AT	**V**	S	**M**
TPV/r		I	F	IL	**VA**			**VAM**		**TL**	**V**		

2.11.4 Mechanisms of INSTI resistance

Current ART guidelines in Kenya recommend dolutegravir as part of the standard first-line ART for adults, and Raltegravir as an alternative to Nevirapine in children (National AIDS and STI Control Programme, 2018). INSTIs were the most recently ARVs approved for ART (Anstett et al., 2017). However, there are drug-resistance mutations to the first-generation INSTIs raltegravir and elvitegravir (Anstett et al., 2017; Arts & Hazuda, 2012; Fantauzzi & Mezzaroma, 2014). These have largely been due to single-point mutations that occur due to

the low-fidelity transcription by RT (Arts & Hazuda, 2012). These mutations occur in the integrase active site, consequently conferring reduced susceptibility to RAL, and include N155H, Q148H/R/K, and Y143C/R (Arts & Hazuda, 2012). Concomitant occurrence of these mutations can result in cross-resistance between RAL and EVG (Fantauzzi & Mezzaroma, 2014). Differences between subtypes have also been noted to contribute to INSTI the development of resistance (Santoro & Perno, 2013). Mutations for Dolutegravir (DTG) have not been well studied (Arts & Hazuda, 2012).

2.12 Drug Resistance Testing (DRT)

HIV drug resistance testing (DRT) involves tests used to screen for the presence of DRMs in HIV patients. DRT can be done by either of two approaches; phenotypic or genotypic (Clutter et al., 2016). Phenotypic DRT is done *in vitro* to measure the ARV concentration required to repress viral replication in cell culture. ARV susceptibility refers to the amount of ARV concentration that impedes viral replication by 50% (IC50) that is related to the IC50 of a reference strain susceptible to the drug of interest after which a ratio is used to indicate a ratio termed the 'fold change' (Clutter et al., 2016). Most susceptibility assays employ recombinant viruses into which viral gene components, particularly RT, PR, integrase, or envelope proteins have been incorporated (Clutter et al., 2016). The clinical use of these tests is however limited due to their lengthy turnaround time, complexity, and high cost (Clutter et al., 2016). Genotypic drug resistance tests (GRTs) entail the application of sequencing techniques, especially the sanger sequencing approach to sequence RT and PR genes to identify clinically significant DRMs (Clutter et al., 2016; Inzaule et al., 2013; Zhou et al., 2011). A minimum sequence of about 10-99 codons of PR and codons 41-240 of RT is required (Zhou et al., 2011). Owing to the vast heterogeneity of the virus population within an individual, there is a need to distinguish nucleotide mixtures, hence software such as the Stanford HIV database has algorithms to identify mixtures and use the International Aids Society (IAS) mutations list for DRMs interpretations are of great importance (Clutter et al., 2016; Kuiken et al., 2002). Sanger sequencing however has the limitation of missing minority resistance variants (MRVs) that occur below its 20% detection threshold (Clutter et al., 2016). Next-generation sequencing techniques counter this sanger limitation, permits scaling-up, and is both time and cost-efficient owing to the ability to batch specimen for processing (Clutter et al., 2016). GRTs are however quite costly in LMICs (Chaturbhuj et al., 2014; Clutter et al., 2016; de Oliveira et al., 2005; Inzaule et al., 2016).

2.13 Key Populations (KPs)

Key populations (KPs) are people at a higher risk of acquiring HIV because of their high-risk engagements and or because of societal marginalization and are consequently hesitant to access HIV services (UNAIDS, 2019). They include SWs, MSM, transgender people, and IDUs, and they collectively account for 30% of new infections annually in Kenya (National AIDS Control Council, 2019; UNAIDS, 2019).

2.13.1 Female Sex Workers (FSWs)

Sex workers (SWs) are those who trade sex for money. Most sex workers are female (Shannon et al., 2015). FSWs are part of the KPs and are therefore considered high-risk individuals in the HIV epidemic (Mpondo et al., 2017; Okal et al., 2013; Vandenhoudt et al., 2013; Zalla et al., 2019). At least one-third of all new HIV infections occur among KPs (Nduva et al., 2020). HIV transmission is largely attributed to sexual intercourse. Some studies have shown FSWs are 14 times more likely to contract HIV while more recent studies have shown a 21-fold likelihood compared to the general population owing to their multiple sexual encounters besides being the receptive partners who bear a greater risk of HIV acquisition than their insertive counterparts especially when anal sex is at play during intercourse (Baral et al., 2012; Patel et al., 2014; UNAIDS, 2019). This is further magnified when ulcerative sexually transmitted infections are existent in the FSWs (Mahlen et al., 2012). Consequently, in the HIV transmission dynamics, FSWs top the list (Mpondo et al., 2017). An investigation described a 75% prevalence of HIV among SWs in Kisumu, Kenya (Morison et al., 2001). Fifteen percent of all HIV cases worldwide among women are attributable to FSWs, especially in the sub-Sahara where approximately 18% of all HIV cases among women are associated with female sex work (Wolf et al., 2013). Besides reducing HIV-related deaths and improving quality of life, ART also reduces the infectiousness of ART-treated persons (Lindman et al., 2020). The increase in ART uptake over the past decade has resulted in the development of resistance (Arts & Hazuda, 2012). Therefore, TDR and PDR are likely to occur within and be transmitted by FSWs at relatively higher rates than by the general population, posing a potential risk to the efficiency of ART amongst themselves and down the ensuing HIV transmission dynamics. A 22% prevalence of HIVDR among SWs in Kenya, and PDR was found to be most prevalent among treatment naïve young women (Sampathkumar et al., 2014; Silverman et al., 2018). Therefore, FSWs are a

key consideration where HIV mitigation strategies are concerned. (Mpondo et al., 2017). Despite this, the HIV care continuum and HIVDR within and between FSWs have not been well studied in Africa (Coetzee et al., 2017; Nduva et al., 2020).

CHAPTER THREE: METHODOLOGY

3.1 Study design

A descriptive cross-sectional study design was used to characterize the HIV ***pol***-RT and ***pol***-PR region of ART-naïve FSWs from different sites in Nairobi, Kenya. This study utilized frozen remnant plasma samples from a larger study on the genetic diversity and transmission dynamics of HIV-1 isolated from treatment-naive FSWs in Kenya. The collection of study samples for the larger study was done by the Sex Workers Outreach Program (SWOP), a collaboration coordinated by the University of Manitoba/ University of Nairobi collaborative HIV group. Treatment-naïve FSWs were defined as those who had no prior exposure to ART and this was confirmed by SWOP through the use of questionnaires.

3.2 Study area description

The study sites included were Donholm 16 (16%), City 21 (13%), Kawangware 37 (24%), Korogocho 24 (15%), Langata 7 (4.5%), Majengo 10 (6.4%) and Thika road 42 (27%). Study samples were drawn from FSWs in these areas between November 2020 and April 2021 and stored frozen at a temperature of about -70°C at the Molecular and Infectious Disease Reference (MIDR) Laboratory in the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID). FSWs from this population are ideal study participants owing to the dense population of these areas. All tests for this study were done within the MIDR molecular laboratory.

3.3 Study population

Treatment-naïve FSWs were recruited into the parent study between November 2020 and April 2021. These participants had been recruited into the parent study that was investigating the genetic diversity and transmission dynamics of HIV-1 isolated from treatment-naive FSWs in Kenya. They included FSWs from the major sites in Kenya's capital city; Nairobi. The designated areas of work from which study participants were recruited are densely populated hence making them strategic hotspots for high-risk sexual proclivities, and as such, the transmission of HIV and possibly PDR is considerably higher amongst the sex workers in the region. This, therefore, creates an ideal data source for studying the genetic relatedness of HIV strains isolated from FSWs. Study samples used for the parent study were stored frozen

at the Molecular and Infectious Diseases Research (MIDR) Laboratory. These frozen remnant plasma samples used for the were retrieved and used for this study.

3.3.1 Inclusion criteria

1. HIV-positive, treatment-naïve female sex workers.
2. FSWs with complete medical records for the desired variables of age, sex, time engaged in sex work, and work site.

3.3.2 Exclusion criteria

1. ART-naïve FSW sample from outside the designated study areas.

3.4 Sample size estimation

Fisher's method for sample size estimation at 95% confidence was employed for size estimation (Charan & Biswas, 2013). The proportion of FSWs with PDR in Kenya is 10% (Silverman et al., 2018). The rationale for the use of the proportion of FSWs with PDR is that this is our study population.

$$Sample\ size(n) = \frac{Z_{1-\alpha 2^2} P(1-p)}{d^2}$$

$$n = \frac{1.96^2 X 0.1(1-0.1)}{0.02^2}$$

$$n = 138$$

A total of 157 samples were collected for the larger study and all were used in our study. This accounted for an additional 14%. This helped mitigate sample count loss when samples failed to amplify.

3.5 Sampling procedure

Remnant participant FSWs' frozen plasma samples were selected from the MIDR Laboratory at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) archive using a convenient sampling approach. Only samples of treatment naïve FSWs from the larger study with a complete dataset of interest such as age, sex, CD4 count, and work site were picked for the study.

3.6 Variables

The independent variables for this study included HIV subtype and nucleotide sequence similarity. The dependent variables were pre-treatment drug resistance (PDR). Other variables of interest collected included age, CD4 count, and CD4 percentage.

3.7 Data collection procedures

3.7.1 Female Sex Workers' information

Study participants' information was extracted from electronic and manual data collection tools used by the larger study team to collect FSWs information. Information of interest obtained included age and work site. Baseline CD4 counts and percentages obtained by the larger study were also retrieved.

3.7.2 Laboratory procedures

3.7.2.1 Viral RNA extraction.

Extraction of HIV RNA was done as per the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Samples were frozen frozen at -70°C immediately after use by the larger study to ensure RNA integrity. A 500 µL aliquot of the thawed plasma was added to a 500 µL Lysis Buffer, the mixture vortexed for 15 seconds and then incubated at 56°C for 15 minutes. Ethanol (100%) was pipetted into the mixture, and vortexing was done for 15 seconds followed by a 5-minute incubation of the lysate at room temperature. The incubated lysate was added to a Viral Spin Column in a collection tube and then centrifuged at 6800 × g for 1 minute. The flow-through was jettisoned and washing of the column was done twice using 500 µL of Wash Buffer (WII), whilst centrifuging at 6800g in between washes for 1 minute and discarding the collection tube with its contents. A dry spin was done at maximum speed for 1 minute after the final wash. Lastly, the spin column was transferred to a recovery tube, and elution was done twice with 20µl sterile RNAase-free water allowing for a minute incubation before centrifuging at maximum speed for 1 minute.

3.7.2.2 Reverse- Transcriptase PCR (RT-PCR)

cDNA was generated using superscript^{TM} III one-step RT-PCR Kit. The kit contains two vials; one containing an RT-PCR master mix and the other containing the Superscript^{TM} III

one-step RT-PCR with Platinum™ *Taq* High Fidelity Enzyme (Invitrogen, Carlsbad, CA, USA). These two were mixed according to the number of samples as per the manufacturers' instructions. For a single reaction, the RT-PCR master mix was prepared as follows:

Table 3.1: RT-PCR Reaction mix.

Component	volume
RT-PCR master mix	39 µl
Superscript™ III one-step RT-PCR with Platinum™ *Taq* High Fidelity Enzyme	1 µl
Total volume	40 µl

First, the RNA templates and control were denatured for 10 minutes at 65ºC, then immediately cooled at 4ºc for 3 minutes in a Veriti Thermocycler before they were added to the other components of the RT-PCR reaction mix. The conditions for the denaturation process and RT- PCR were as follows:

Table 3.2: RNA denaturation conditions

Temperature (ºC)	Number of cycles	Time (minutes)
65	1	10
4	1	3

Table 3.3: RT- PCR conditions

Step	Temperature (ºC)	Number of cycles	Time
Reverse transcription	50	1	45 minutes
Enzyme inactivation	94	1	2 minutes

Denature	94	40	15 seconds
Anneal	50		20 seconds
Extend	72		2 minutes
Final extension	72	1	10 minutes
hold	4	Maximum of 18 hours	

The RT-PCR was performed using a Veriti Thermocycler. The reaction began with a 45-minute reverse transcription reaction at 50°C to generate cDNA. This was proceeded by a 2-minute reaction at 94°C to inactivate the enzyme and denature the cDNA- RNA hybrid, after which second strand synthesis and PCR amplification occurred across 40 cycles of 94°C for 15 seconds (denaturation), 50°C for 40 seconds (annealing), 72°C for 2 minutes (extension) and a single cycle final extension at 72°C for 10 minutes. Finally, the PCR products were allowed to cool at 4°C for 30 minutes before being used for downstream processes**.**

3.7.2.3 Nested PCR

Nested PCR was done to exclusively amplify the *pol* PR and RT regions. The nested PCR kit contains two vials; one contains the nested PCR master mix, while the other contains Amplitaq gold^{TM} LD DNA polymerase (Thermo Fischer Scientific Inc., Massachusetts, USA). These two were mixed according to the number of reactions as per the manufacturer's instructions. For a single reaction, the nested PCR master mix was prepared as follows:

Table 3.4: Nested PCR Reaction Mix

Component	volume
Nested PCR master mix	47.5 µl
AmpliTaq Gold^{TM} LD DNA Polymerase	0.5 µl
Total volume	48 µl

The above mixture was mixed thoroughly, then briefly centrifuged to collect contents at the bottom of the tube. 48 µL of the nested PCR reaction mix was put into each well in chilled, well-labeled reaction tubes/strips. 2 µL of RT-PCR products and controls were added to individual wells and then gently vortexed. The reaction mixture was then subjected to a nested PCR process in a Veriti Thermocycler. The reaction proceeded as follows:

Table 3.5: Nested PCR conditions

Step	Temperature (ºC)	Time	Number of cycles
Initial denaturation	94	4 minutes	1
Denature	94	15 seconds	40
Anneal	55	20 seconds	
Extend	72	2 minutes	
Final extension	72	10 minutes	1
hold	4	Maximum of 18 hours	

3.7.2.4 PCR product cleanup

For removal of residual primers and nucleotides, 10µl of nested PCR products was pipetted into 4µl of ExoSAP-IT™ reagent. The ExoSAP-IT™ reagent comprises two enzymes; Exonuclease I which digests single-stranded oligonucleotides and Shrimp Alkaline Phosphatase which dephosphorylates nucleotides to prevent ligation to the newly synthesized DNA strand. Incubation of the above mixture in a Veriti Thermocycler at 37ºc for 15 minutes ensued during which the activities of the enzymes occurred, after which a 15-minute incubation at 80ºc to inactivate the enzymes ensued. Lastly, the cleanup product was cooled for 5 minutes at 4ºC.

Table 3.6: EXOSAP cleanup conditions

Step	Temperature (ºC)	Time (minutes)

Digest	37	15
Heat deactivation	80	15
Hold	4	hold

3.7.2.5 Gel electrophoresis

The presence of nested PCR products was confirmed by gel electrophoresis on a 1% Agarose gel. Briefly, 1 gram of Agarose powder was dissolved in 100ml of Tris-acetate EDTA (TAE) buffer, and the mixture dissolved by heating in a microwave for about 2 minutes. 4.5µl SYBR Safe, a DNA stain, was added to the mixture before pouring it into an electrophoresis tank. A comb was then added and the gel was allowed to cool and solidify. 2µlof the viral DNA was then loaded into the wells with the aid of a loading dye. Electrophoresis proceeded for 30 minutes after which the gel was visualized under the UV light of the UVITEC machine. Only samples giving bands proceeded subjected to cycle sequencing.

3.7.2.6 Cycle sequencing

The Big Dye™ Terminator v3.1 Cycle sequencing kit with six overlapping primers was used for cycle sequencing. The six primers, F1, F2, F3, R1, R2, and R3 are separately contained in six different sequencing mixes. 18 µl of each sequencing mix was added separately to appropriate wells of a chilled 96-well reaction plate. 2 µL of ExoSAP-IT^{TM} cleaned nested PCR products was added accordingly to each well such that each sample was run with each of the six sequencing primers. 20 µL of pGEM Sequencing Control was added to at least one well per run. The sequencing reaction was set to run in a Veriti Thermocycler as follows:

Table 3.7: Cycle sequencing conditions

Step	Temperature (ºC)	Time	Number of cycles
Denature	96	10 seconds	40
Anneal	50	5 seconds	
Extend	60	4 minutes	

Hold	4	Maximum of 18 hours

The cycle sequencing reaction occurred in 40 cycles, with each cycle beginning at a denaturation step at 96 ºC for 10 seconds, then annealing at 50 ºC for 5 seconds and extension at 60 ºC for 4 minutes. The end product of the reaction was held at 4 ºC before proceeding to other downstream processes.

3.7.2.7 Purification of sequencing products

The cycle sequencing reaction products were cleaned with Big Dye X Terminator purification kit. The kit has two components; the SAM™ solution and the Big Dye X Terminator™ solution. These two were mixed properly according to the number of reactions as per the manufacturer's instructions. For a single reaction, the purification mix was prepared as follows:

Table 3.8: Sequencing Purification mix

Component	volume
SAM™ solution	90 µl
X Terminator™ solution	20 µl
Total volume	40 µl

To each of the sequencing products, 110 µl of the working bead solution was added, and the mixture was properly mixed by pipetting. The plate was sealed and vortexed for 30 minutes at 1800 rpm, and finally centrifuged at 1000 × g for 2 minutes at room temperature before transferring 30µl of the purified sequenced products to a reaction plate and loading it into the ABI 3730 genetic analyzer for capillary electrophoresis.

3.8 Ethical considerations

The Kenyatta National Hospital - University of Nairobi Ethics and Research Committee (KNH-UoN ERC) approved this study before it commenced. The ethics approval reference number is P630/07/2021.

The investigator requested for a waiver for individual informed consent for this study since this study involved no more than minimal risk to the participating FSWs. We used remnant FSWs plasma samples on which other molecular tests had been done. At the time of collection, participants had consented to have their samples stored for future research. It would have been impracticable to conduct this study without the waiver owing to: i) The prevailing Universal Test and Treat (UTT) program made treatment-naïve samples hard to come by. ii) The COVID-19 pandemic that had changed health-seeking patterns and restrictions on interactions with patients thereby limiting our ability to obtain individual informed consent from random FSWs. The waiver did not adversely affect the rights and welfare of the participants. All information required was obtained from the records provided by the larger study. Any patient identifiers were removed to preserve participants' confidentiality.

The study subject's plasma samples were each accorded specific identification numbers to replace their personal identifying information. All records were protected under lock and key, and by passwords depending on the nature of the records. Only the principal investigator and co-investigators had access to the retrieved identifiers. Participants' identities will not be disclosed in the final reports and publications of this work.

3.9 Data management

3.9.1 Data entry, storage, and disposition

Participants' information was provided by the SWOP team. The obtained data was put into a password-protected R statistical package (R version 4.0.3), and the database and data were subjected to validation as a quality assurance/ quality control (QA/QC) measure using a small random sample (5%) of all the input records. Corrections to any errors that emerged during the validation process were corrected in line with the ICH GCP guidelines. Participants were accorded unique study identification numbers. A separate file containing participants' names against their assigned unique identification numbers was kept in a password-protected

computer file. All study files were protected using a password and were only accessible to authorized individuals.

3.10 Data analysis plan

3.10.1 Bioinformatics Analysis

An ABI 3730 genetic analyzer (Applied Biosystems, CA, USA) was used for capillary electrophoresis of the purified sequenced products. Consensus sequences of the raw AB1 files from the genetic analyzer were generated using RECall software and uploaded onto the Stanford HIV database for interpretation of DRMs.

3.10.2 Phylogenetics

The online COMET HIV-1 Subtyping platform was employed to classify study sequences into HIV-1 subtypes using bootscanning methods. Study HIV-1 *pol*-RT and *pol*-PR sequences, and Kenyan HIV *pol*-RT and *pol*-PR sequences archived in the Los Alamos National Laboratory (LANL) HIV database were loaded onto the MEGA11 software and aligned by multiple sequence alignment using an in-built tool; Muscle. The neighbor-joining technique was employed for phylogenetic analysis and phylogenetic tree construction. The phylogenetic tree was rooted to the HXB2 HIV reference strain and tree topology was confirmed by a bootstrapping analysis and evolutionary distances were computed using the maximum composite likelihood (MCL) method to establish any genetic relatedness between the two sets of sequences.

3.10.3 Statistical analyses

MS Office Excel 2010 spreadsheet was used to manage all data collected from the study. The sum of HIVDRM counts (NRTIs, NNRTI, and PI) was the primary outcome of concern. Age, HIV subtype, enrolment site, CD4%, and CD4 count were predictor variables. Categorical variables were compared using Fischer’s exact test while continuous variables were compared using the Wilcoxon rank sum test, and the P-values were recorded. Spearman's correlation was used to investigate the relationship between continuous predictor variables and the outcome variable. R statistical package (R version 4.0.3) was used to perform all the statistical analyses.

3.11 Study results dissemination plan

Study findings will be disseminated to the public, beginning with the crucial teams that will be involved in the study. The findings have already been presented to the University of Nairobi team in the postgraduate journal club. On a larger scale, study-generated ***pol*** sequences have been uploaded onto the DNA Data Bank of Japan (DDBJ) database; accession no *LC723952 – LC724015*, and a manuscript is underway for publication in the journal *Nature*. Clinicians at the Kenyatta National Hospital will be made privy to study results via seminars and Continuous Medical Education (CME). Study results will also be shared with key policymakers including the Ministry of Health (MOH), the National Aids Control Council (NACC), and the National AIDS and STIs Control Program (NASCOP). The study findings will also be presented at local conferences such as the Infectious Diseases Society of Kenya meeting and international conferences.

CHAPTER FOUR: RESULTS

4.1 Study population

Only 64 samples of the 158 collected were successfully sequenced. FSWs ages ranged from 22 to 58 years old (Median =35.5, IQR =10.0). Baseline CD4 counts ranged from 23 - 1,113 cells/mm^3. CD4 counts for 18 (28%) of the subjects were below 200 cells/mm^3.

Table 2.1: Sociodemographic characteristics of the study participants

Characteristics	Total (N=64)	Major DRM	
		No (N=42)	Yes (N=22)
AGE (years)			
Median (IQR)	35.5 (10.0)	38.0 (11.0)	30.0 (10.0)
AGE group			
20-29	5 (8 %)	5 (12 %)	s0 (0 %)
30-39	14 (22 %)	5 (12 %)	9 (41 %)
40-49	26 (41 %)	19 (45 %)	7 (32 %)
>50	13 (20 %)	10 (24 %)	3 (14 %)
CD4 PERCENT			
Median (IQR)	19.0 (15.3)	21.0 (14.8)	17.5 (15.3)
CD4 count (cells/mm3)			
Median (IQR)	339 (355)	385 (361)	287 (321)
CD4 group			
<200	18 (28 %)	11 (26 %)	7 (32 %)
200-349	18 (28 %)	13 (31 %)	5 (23 %)
350-500	16 (25 %)	9 (21 %)	7 (32 %)
>500	12 (19 %)	9 (21 %)	3 (14 %)
SITE			
City	10 (16 %)	6 (14 %)	4 (18 %)
Donholm	2 (3 %)	1 (2 %)	1 (5 %)
Kawangware	19 (30 %)	14 (33 %)	5 (23 %)
Korogocho	9 (14 %)	2 (5 %)	7 (32 %)
Lang'ata	3 (5 %)	3 (7 %)	0 (0 %)
Majengo	5 (8 %)	3 (7 %)	2 (9 %)
Thika road	16 (25 %)	13 (31 %)	3 (14 %)

Table 2 2: Baseline CD4 data for study participants

Characteristic	City, N = 21	Donholm, N = 16	Kawangware, N = 37	Korogocho, N = 24	Langata, N = 7	Majengo, N = 10	TKA road, N = 42	p-value[†]
CD4_COUNT								0.75
N	21	16	37	24	6	10	42	
Median (IQR)	486 (250, 682)	464 (331, 580)	453 (283, 587)	459 (248, 824)	615 (376, 993)	486 (251, 670)	578 (317, 737)	
Range	116, 824	192, 775	49, 1,783	23, 1,812	197, 1,304	57, 1,420	34, 1,172	
CD4_PERCENT								0.77
N	20	16	37	24	6	10	42	
Median (IQR)	24 (18, 28)	24 (21, 30)	23 (14, 29)	30 (15, 36)	25 (17, 39)	32 (17, 39)	26 (16, 34)	
Range	9, 35	9, 36	4, 58	3, 52	13, 44	4, 45	2, 46	

[†] One-way ANOVA

FSWs from Lang'ata had the highest baseline median CD4 counts (615, 376-993), while those from Kawangware had the lowest (453, 283-587).

4.2 HIV subtypes

The genetic diversity of sequenced samples showed that subtype A1 (43/64, 67.1%) was the most prevalent in circulation in this cohort of FSWs. Other subtypes were: D 15.6% (10/64), C 1. 6% (1/64), B 1.6% (1/64), A2 1.6% (1/64), and recombinants 12.5% (8/64). CRF A1D (4/8, 50%) was the most common recombinant. The recombinants were present only in Majengo (37.5%), Kawangware (37.5%), and Thika road (25%). There was no significant statistical association between the occurrence of DRMs and the HIV-1 subtype (P=0.4).

Table 2 3: HIV subtypes

	A1 (N=43)	A2 (N=1)	B (N=1)	C (N=1)	CRF (N=8)	D (N=10)	Total (N=64)
Site							
City	9 (20.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10.0%)	10 (15.6%)
Donholm	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (10.0%)	2 (3.1%)
Kawangware	13 (30.2%)	0 (0%)	0 (0%)	0 (0%)	3 (37.5%)	3 (30.0%)	19 (29.7%)
Korogocho	9 (20.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	9 (14.1%)
Lang'ata	2 (4.7%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (4.7%)
Majengo	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (37.5%)	2 (20.0%)	5 (7.8%)
Thika road	10 (23.3%)	0 (0%)	0 (0%)	1 (100%)	2 (25.0%)	3 (30.0%)	16 (25.0%)
Age Group							
20-29	3 (7.0%)	0 (0%)	0 (0%)	0 (0%)	2 (25.0%)	0 (0%)	5 (7.8%)
30-39	9 (20.9%)	0 (0%)	0 (0%)	0 (0%)	2 (25.0%)	3 (30.0%)	14 (21.9%)
40-49	19 (44.2%)	1 (100%)	1 (100%)	0 (0%)	1 (12.5%)	4 (40.0%)	26 (40.6%)
>50	9 (20.9%)	0 (0%)	0 (0%)	0 (0%)	2 (25.0%)	2 (20.0%)	13 (20.3%)
Major DRM							
No	27 (62.8%)	1 (100%)	0 (0%)	0 (0%)	6 (75.0%)	8 (80.0%)	42 (65.6%)

	A1 (N=43)	A2 (N=1)	B (N=1)	C (N=1)	CRF (N=8)	D (N=10)	Total (N=64)
Yes	16 (37.2%)	0 (0%)	1 (100%)	1 (100%)	2 (25.0%)	2 (20.0%)	22 (34.4%)

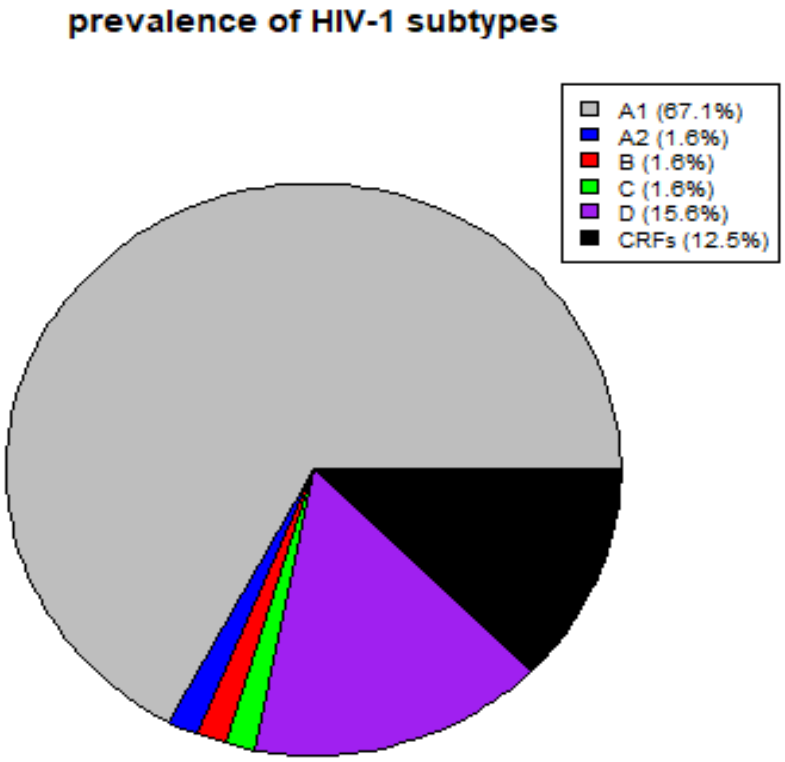

Figure 4.1: Prevalence of HIV-1 subtypes among treatment naïve FSWs

4.3 HIV, age, and CD4 count.

FSWs aged 40 – 50 years constituted 41% (26/64) of the study participants. The majority of the CRFs occurred in FSWs aged 30 years and above (5/8, 62.5%). The majority of the DRMs occurred within the age group 31 – 39 years (9/64, 41%). There was a significant statistical association between the occurrence of DRMs and the age (p= 0.004) and age group (p= 0.03) of FSWs. Spearman's rank correlation was computed to assess the relationship between age and HIVDRM counts. There was a negative correlation between the two, r(-2)= -0.39, p= .003. A majority (17/22,77.3%) of the FSWs who had DRMs had CD4 counts below 500 cells/mm^3. There was no significant statistical association between the occurrence of DRMs and CD4 strata (p= 0.7) and CD4 counts (p= 0.2).

4.4 Drug resistance profiles

We detected DRMs in 34.4% (22/64) of the amplifiable samples, several of which were found to have FTC/3TC /TDF and NVP/EFV resistance. Major RTI-associated-DRMs were present in 32.8% (21/64) of the FSWs. Of these, 6.3% (4/64) had NRTI-associated DRMs such as M184V, K70E, K65R, M41L, K219R, L74I, and Y115F. NNRTIs were present in 32.8% (21/64) FSWs, with K103N and E138A being detected in 13 and 8 FSWs respectively while other major NNRTIs detected were K238T, V179E, P225H, V179L, F227L, V106I, V108I, K101E, G190A, Y181C, and V179T. Four samples had both NRTI and NNRTI mutations. Out of 21 samples with RTIs associated mutations, 26.7% (17/64) were infected with subtype A1. Only 4.7% (3/64) had major protease inhibitors (PIs) associated mutations, 3.1% (2/64) of which were occurring together with RTIs. PI mutations observed were: K43T, L33F, I54M, and L90M. Several other minor mutations and polymorphisms were also observed in all the samples sequenced. Multiple drug resistance to both RTIs and PIs was observed in 2 (2/64, 3.1%) FSWs.

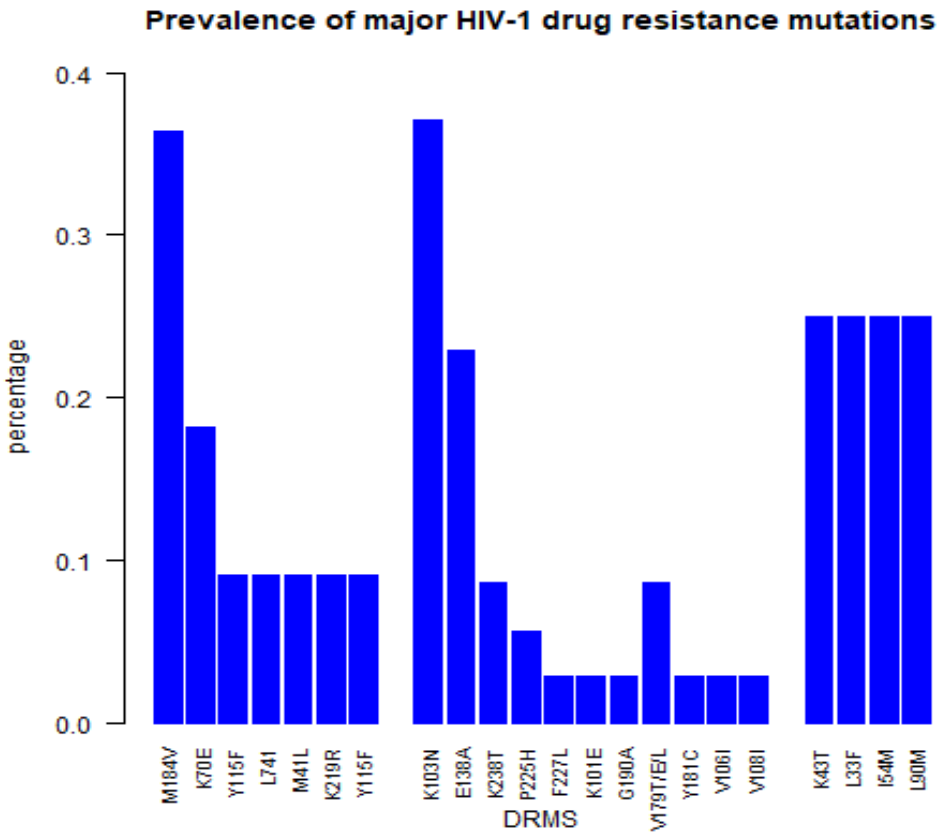

Figure 4.2: Prevalence of major RT and PI-associated DRMs among ART-naïve FSWs

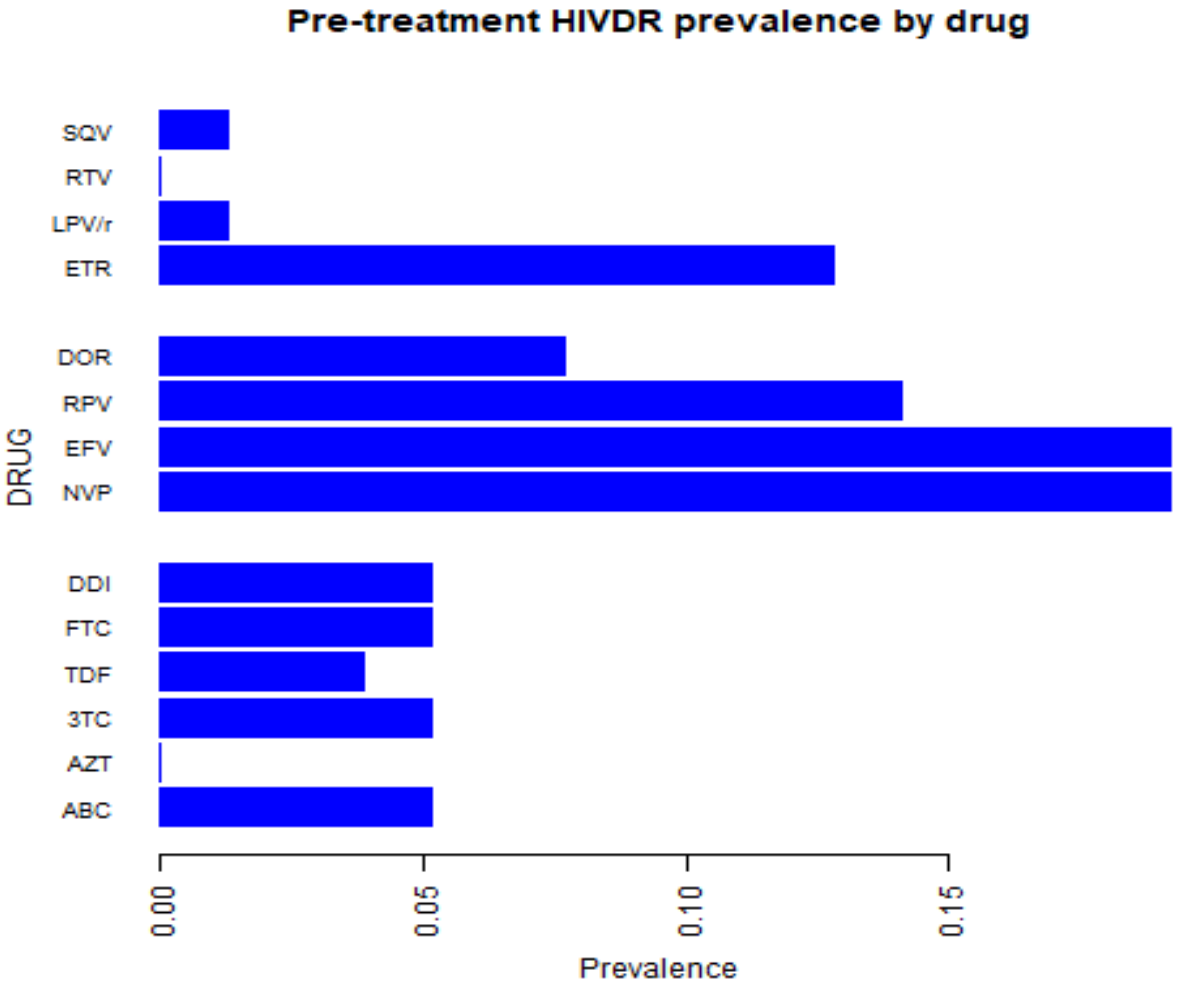

Figure 4.3: Prevalence of Pretreatment HIVDR by drug among treatment naïve FSWs. SQV, saquinavir; RTV, ritonavir; LPV/r, lopinavir; ETR, etravirine; DOR, Doravirine; RPV, rilpivirine; EFV, efavirenz; NVP, nevirapine; DDI, Didanosine; FTC, emtricitabine; TDF; tenofovir, 3TC, lamivudine; AZT, zidovudine; ABC, abacavir.

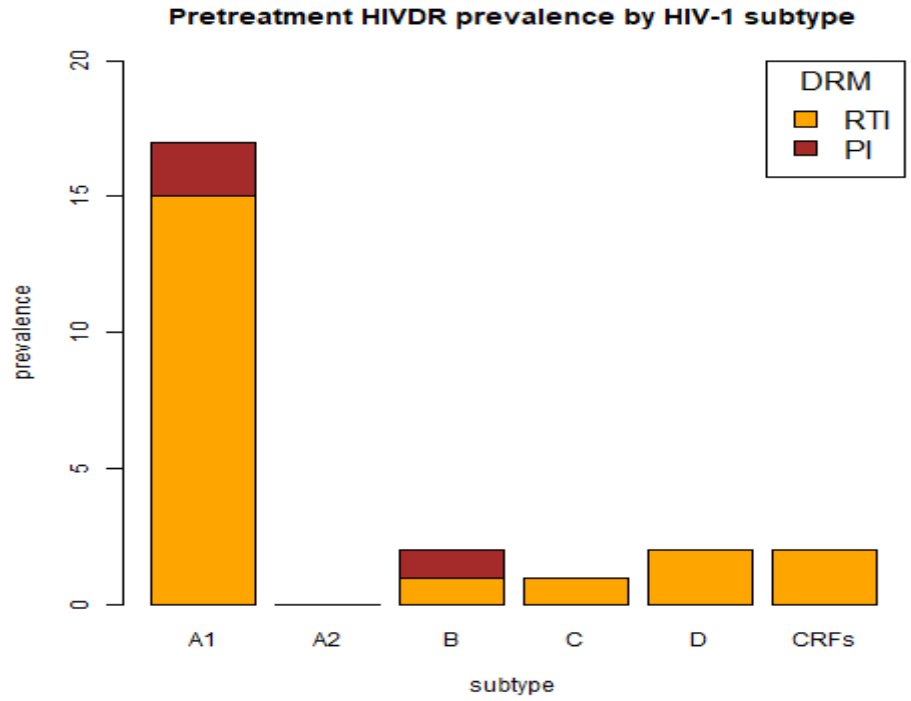

Figure 4.4: Prevalence of pretreatment HIVDR by subtype among treatment-naïve FSWs

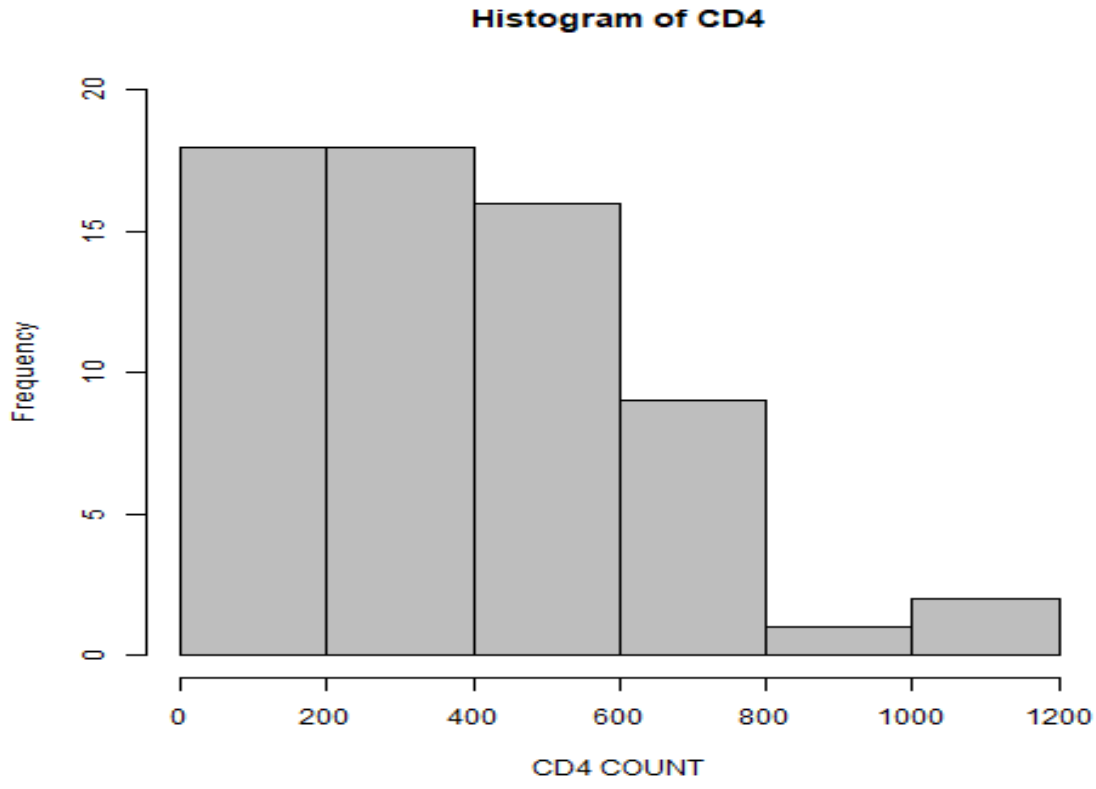

Figure 4.5: Prevalence of pretreatment HIVDR by CD4 count among ART-naïve FSWs

4.5 Phylogenetic analyses.

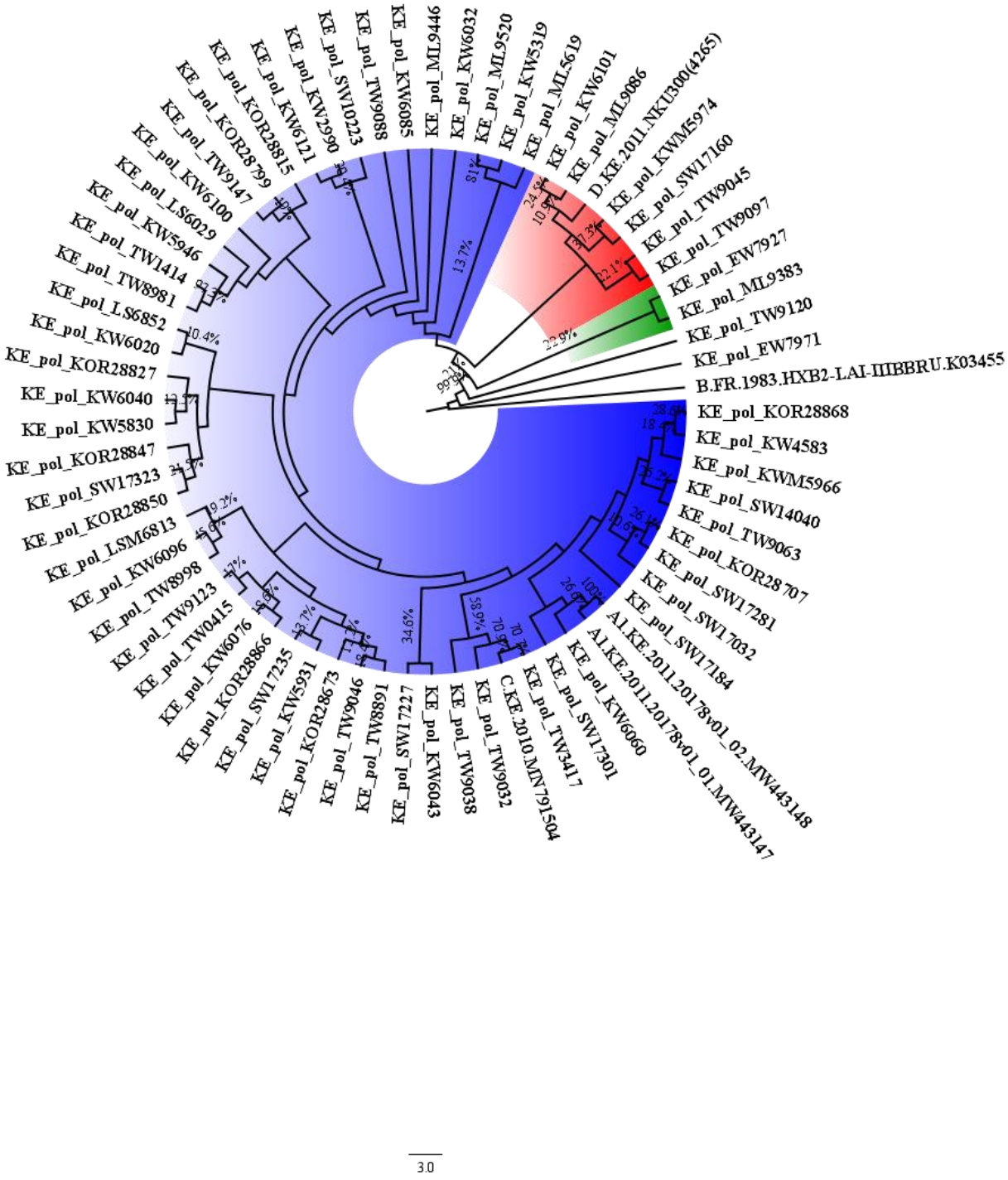

Figure 4.6: A polar phylogenetic tree of the study sequences. Colors were used to distinguish the clades. Reference sequences used are indicated. The scale represents the number of substitutions per site. The values at the nodes represent bootstrap support values.

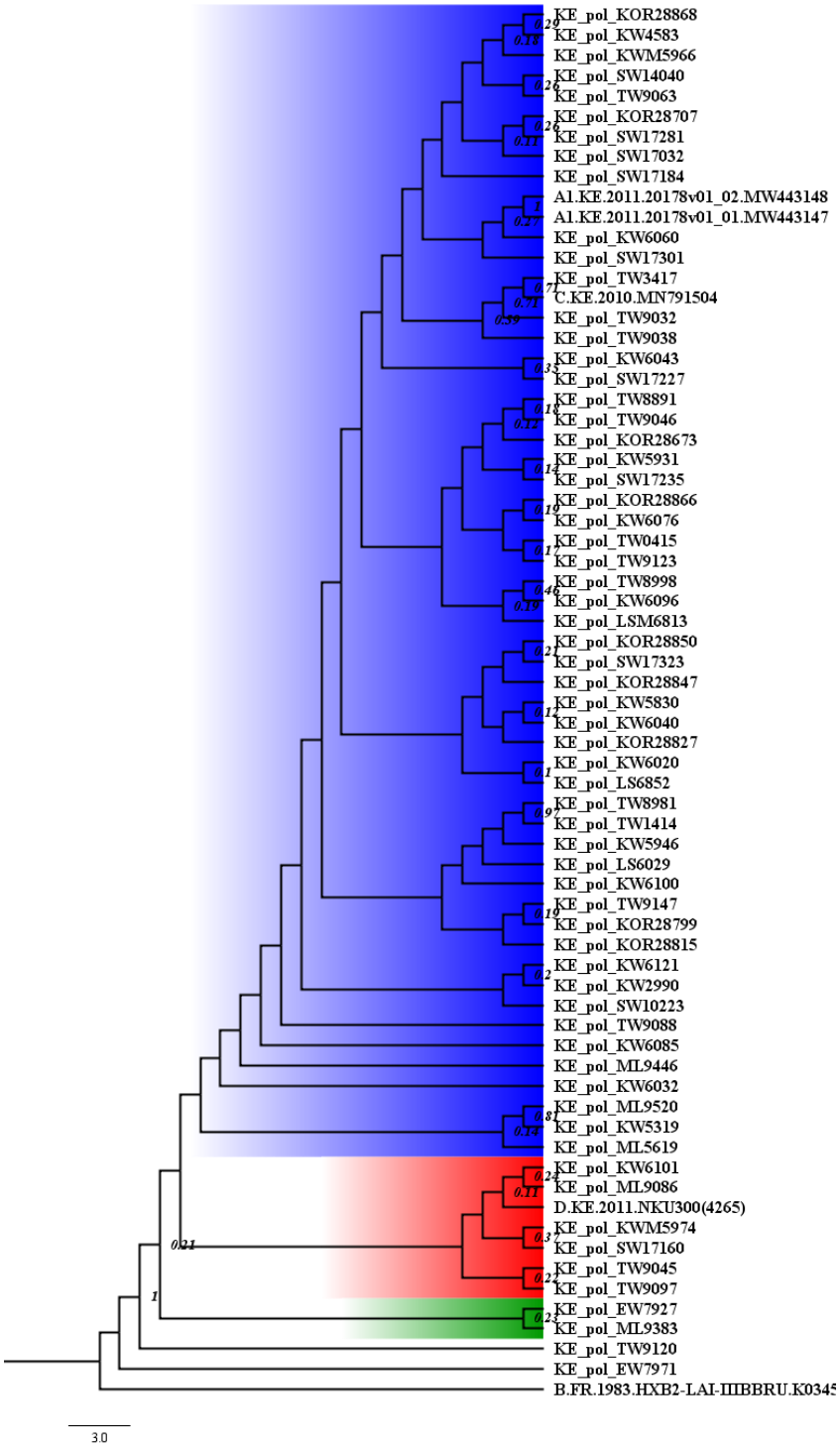

Figure 4.7: **A rectangular phylogenetic tree.** Colors were used to distinguish the clades. Reference sequences used are indicated. The scale represents the number of substitutions per site. The values at the nodes represent bootstrap support values.

The sequences clustered into three major paraphyletic clades, these are presented as the blue, red, and green clades. In addition, two other clades were each populated with single members and were basal to the green clade. These 2 clades included: KE**_pol_TW9120** and **KE_pol_EW7971.**

The green clade was the most basal major clade. It was populated with only two sequences; **KE_pol_Ew7927** and **KE_pol_ML9383**. The clade had weak bootstrap support of 22.9%.

The red clade was anterior to the green clade. The clustering of the red clade was supported with a bootstrap value lower than 1%. Within this clade, two sub-clades could be resolved. Within the same red clade, the reference sequence **D.KE. 2011.NKU300(4265)** formed a monophyletic clade with the sequences **KE_pol_KW6101** and **KE_pol_ML9086** with **D.KE. 2011.NKU300(4265)** being the basal sequence of the subclade. Of the total sequences in this analysis, only 7 were located within the red clade, this shows that there is low diversification within this clade compared to the blue clade.

The blue clade displayed greater diversification as compared to the red and green clades with 5 major paraphyletic clusters being observed. The most anterior clade had 19 sequences and reference sequences **A1.KE.2011.20178v01_02.MW443148**, **A1.KE.2011.20178v01_01.MW443147** and **C.KE. 2010.MN791504** were located within this clade. Phylogenetic analysis showed that the reference sequences **A1.KE.2011.20178v01_02.MW443148** and **A1.KE.2011.20178v01_01.MW443147** were more closely related to each other than with any other sequence since they formed a monophyletic clade with each other with bootstrap support of 100%. They were also close relatives of **KE_pol_SW17301 and KE_pol_KW6060** forming a monophyletic clade. **C.KE. 2010.MN791504** closest relative was **KE_pol_TW3417** with bootstrap support of 70.7%. The other subclades within the blue clade housed the remaining sequences with most of the nodes not supported with a bootstrap greater than 50%. This suggested that there has been rapid contemporaneous diversification within the blue clade.

CHAPTER FIVE: DISCUSSION

HIVDR is an important cause of treatment failure worldwide, and the prevalence of resistance is increasing in LMICs (WHO, 2019a). Owing to the absence of routine DRT as part of treatment monitoring in LMICs, failure of viral suppression can often be mistakenly linked to poor ART adherence (Beck et al., 2020). When HIVDR is not recognized as the cause of poor treatment response, the viral load could shoot exponentially to levels that further accentuate viral transmission and transmission of PDR.

This study evaluated patterns of pretreatment HIVDR among FSWs in Nairobi. Previous investigations in Kenya indicated a HIVDR prevalence of 22% among sex workers in Kenya (Sampathkumar et al., 2014). Our study demonstrated that one in three ART-naïve FSWs (34.4%) had HIVDR-associated mutations. This high prevalence emphasizes the susceptibility of FSWs to the acquisition of PDR and the ramifications of their response to future treatment.

Our findings demonstrate that majority of the circulating DRMs in this population are to NNRTIs. There is a relatively high occurrence of high-level resistance to NVP and EFV compared to previous studies which can be attributed to the previous long-term use of NVP/EFV-based regimen for the treatment of HIV in Kenya ((Antinori et al., 2002; Melikian et al., 2014; Sluis-Cremer et al., 2015; WHO, 2019c). Furthermore, NNRTI mutations are known to be more easily transmitted than NRTI and PI mutations (Little et al., 2008; Yanik et al., 2012). NRTI-associated mutations to TDF and 3TC were observed in 6.3% of the study subjects. These are part of the WHO-recommended ART regimen (WHO, 2019c). The majority in this category was M184V, a finding that concurs with previous studies (Lihana et al., 2009). This points to a potential switch from a predominantly NNRTI epidemic to a combined RTI epidemic and therefore highlights the possibility that first-line therapy may be ineffective in a third of FSWs for reasons unrelated to adherence. The recent inclusion of DTG as part of the first-line ART calls for research on mutations that may confer resistance to this drug too**.** A potential for poorer health outcomes may further arise if the SWs become non-adherent. As such, SWs must be engaged on the importance of adherence once they initiate ART. Furthermore, VL must be routinely monitored amongst these SWs once ART is

initiated to ascertain that treatment failure is countered appropriately, even if second-line therapy fails.

Our results further established the high potential for sexually transmitted HIVDR. The high prevalence of HIVDR in the study population calls for interventions targeting SWs and their clients to educate them on countermeasures against unsafe sex practices, encourage them to take preventive measures, and enlighten them on HIVDR and its effects on treatment efficacy. It also points to a need for research on the prevalence and transmission dynamics of transmitted HIVDR among the general population.

Phylogenetic analyses demonstrated that HIV-1 subtype A1 predominates in this population followed by subtype D, whereas subtypes A2, C, B, and the variants of intersubtype recombinant mosaics were also marginally present. Intersubtype AD was the most prevalent recombinant. This is consistent with previous studies on the prevalence of various subtypes in Kenya (Khoja et al., 2008; Lihana et al., 2009). The majority of participating FSWs were aged 40 – 49 years, which was largely dominated by subtype A1 (73.1%) and subtype D (15.4%). The majority of the CRFs also occurred within the age group 41 - 50 years, owing to a possibly longer duration of sex work and therefore more exposure to circulating subtypes. The prevalence of subtype A genome among the occurring CRFs (87.6%) not only further demonstrates the dominance of subtype A in Nairobi, but also the possibility that it may evolve into new strains over time. The existence of recombinants might be an indication of independent transmission networks or multiple infections with these subtypes, but deeper investigations may be required to answer this.

Policymakers must be enlightened on the high prevalence of PDR and the chances that first-line therapy will fail in a large proportion of FSWs. HIVDR surveillance programs, therefore, need to be initiated at facilities convenient to SWs. This will not only reduce unnecessary switches to the costly and burdensome regimen but also increase the data on HIVDR available to policymakers and healthcare personnel, thus informing interventions to counter this trend and drive us towards the realization of the WHO 95-95-95 target.

Study limitations and strengths.

Firstly, owing to financial constraints, the sample size was small and does not allow for the generalizability of findings. This was however partly mitigated by seeking funds from well-wishers. Secondly, data on sex workers regarding duration and geographical coverage of sex work by the participants and the average number of clients were unavailable to us, and as such, a geographical pattern of the origin and distribution of the circulating types could not be vividly established. Lastly, this study illustrated the prevalence of PDR but cannot conclusively inform on the cause of this PDR. The study's strength is that it is a pioneering study in Nairobi that highlights the existing DRMs within circulating strains among ART-naïve FSWs in Kenya.

Conclusions and recommendations.

The prevalence of PDR among ART-naive FSWs in Nairobi is increasing. Given this high prevalence, DRT surveillance and/or re-evaluation of first-line ARVs issued may need to be implemented. The observation of an evolving subtype divergence in this population may present a problem to vaccine strategies in the prevention of HIV, and therefore calls for a need to continually survey the subtype divergence among FSWs and their clients. We also recommend a nationwide PDR survey to investigate the causes of PDR, and effective measurements to be taken in the event of persistent PDR.

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APPENDICES

Appendix 1: Ethics approval

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3rd December 2021

KENYATTA NATIONAL HOSPITAL APPROVED 03 DEC 2021 KNH/UoN-ERC P.O. Box 20723 - 00202 NAIROBI

Opiyo Jeff Oluoch
Reg. No. H56/36226/2019
Dept. of Medical Microbiology
Faculty of Health Sciences
University of Nairobi

Dear Jeff

RESEARCH PROPOSAL: HIV DRUG RESISTANCE-ASSOCIATED MUTATIONS AMONG ART-NAÏVE FEMALE SEX WORKERS IN NAIROBI, KENYA (P630/07/2021)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P630/07/2021.** The approval period is 3rd December 2021 – 2nd December 2022.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Protect to discover

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Dean-Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
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Dr.Frank Gekara Onyambu,Dept. of Clinical Medicine & Therapeutics, UoN
Dr. Moses Masika, Dept.of Medical Microbiology, UoN

Appendix 2: Plagiarism report

Turnitin Originality Report

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