ASSOCIATION BETWEEN HIV-1 SUBTYPES AND DRUG RESISTANCE TO FIRST-LINE THERAPY AMONG INDIVIDUALS WITH ADVANCED DISEASE IN HOMA BAY COUNTY, KENYA

BY

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DECLARATION

Declaration by the student

I declare that this thesis is my own original work and has not been submitted in whole or in part for any other degree at Maseno University or any other institution. I have conducted the research reported in this thesis in accordance with the University's policies and procedures, obtained all necessary ethical approvals, and I have acknowledged all sources of information used in this thesis by means of appropriate citations and references.

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DEDICATION

I dedicate this work to my children, Mark Finley and Jaelyn Moraa, who have taught me the essence of love and joy. Your presence in my life inspires and motivates me.

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ABSTRACT

Human Immunodeficiency Virus-1 remains a public health threat globally, and although antiretroviral therapy has greatly improved the lives of people living with HIV, challenges persist due to high HIV genetic diversity and drug resistance. Analysis of HIV-1 subtypes over time demonstrates high subtype diversity and dynamic changes with variations in drug resistance among different HIV-1 subtypes. Homa Bay County carries one of the highest HIV-1 burdens worldwide, yet up-to-date information on circulating subtypes and subtype-specific drug resistance is lacking. The general objective of this study was to determine the association between HIV-1 subtypes and drug resistance to first-line ART among individuals with advanced disease in Homa Bay County. The study specifically sought to characterize HIV-1 subtypes and recombinants, to determine HIV-1 subtype-specific drug-resistance mutations, and to determine HIV-1 subtype-specific polymorphisms associated with drug resistance among HIV-1 patients on first-line ART in Homa Bay County, Kenya. A facility-based, cross-sectional survey was conducted, enrolling individuals aged 15 years and above, with advanced HIV-1 disease, and who had been on first-line ART for at least 6 months. The sample size determined using Cochran's formula was 70 participants. Plasma samples were analyzed using CAP/CTM real-time PCR for HIV-1 viral load and genotyping was performed on dried blood spots for samples with a viral load ≥1000 copies/mL. A genetic analysis of a 1,084-bp fragment of the HIV-1 pol gene encoding amino acids 6-99 of the protease (PR) and 1-251 of the reverse transcriptase (RT) region from 65 participants was performed using an in-house assay. Drug resistance was determined using the Stanford University HIV Database. Subtypes were identified using phylogenetic analysis and REGA subtyping tool. Fisher's exact test was used to assess the association between subtypes and drug-resistance mutations. Samples with a significant association were subjected to logistic regression analysis, controlling for regimen. The findings revealed that subtype A1 was most prevalent in 46 patients (70.8%), with lower prevalence rates for subtype D (n=9, 13.8%), recombinants (n=6, 9.2%) and A2 (n=4, 6.2%). No statistically significant association was observed between subtypes and nucleoside reverse transcriptase inhibitor (NRTI) mutations or polymorphisms. However, a notable finding was the lower likelihood of observing NNRTI mutations K101E/H and Y181C/I/V, associated with high-level resistance to Nevirapine (NVP) and Efavirenz (EFV), in subtype A1 than in other subtypes (OR=0.14, 95% CI=0.03-0.60 and OR=0.21, 95% CI=0.06-0.72, respectively). This analysis found that HIV-1 subtypes are associated with resistance mutations, with K101 and Y181 less likely in subtype A. This suggests that areas with a high prevalence of subtype A1 may experience reduced compromised efficacy of NVP and EFV as first-line ART options. This implies that ART choice may need to be tailored to the HIV-1 subtypes. However, further investigations with large sample sizes and longitudinal designs are warranted to confirm this and assess the clinical impact of these subtype-specific differences to inform ART regimen policies effectively.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
APOBEC	Apolipoprotein B mRNA-editing Enzyme, Catalytic Polypeptide
ART	AntiRetroviral Therapy
AZT	Zidovudine
CAP	Cobas Ampliprep
CD4	Cluster of Differentiation 4
cDNA	Complimentary Deoxyribonucleic Acid
CRFs	Circulating Recombinant Forms
CTM	Cobas Taqman
DBS	Dried Blood Spot
DEPC	Diethyl Pyrocarbonate
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide Triphosphate
DTG	Dolutegravir
EDTA	Ethylene diamine Tetra acetic Acid
EFV	Efavirenz
FTC	Emtricitabine
HIV	Human Immunodeficiency virus
HIVdb	Human Immunodeficiency virus database
IASUSA	International AIDS Standards
KEMRI	Kenya Medical Research Institute
LTR	Long Terminal Repeats
MEGA	Molecular Evolutionary Genetic Analysis
MSF	Médecins sans Frontières
NAM	Nucleoside/nucleotide-associated mutations
NASCOP	National AIDS and STI Control Programme
NAT	Nucleic Acid Testing
NNRTI	Nonnucleoside reverse transcriptase inhibitors
NRTI	Nucleotide/Nucleoside Transcriptase inhibitors
NVP	Nevirapine

Pol	Polymerase
PR	Protease
QS	Quantitation standard
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
RTPCR	Reverse Transcription Polymerase Chain Reaction
SDRMs	Surveillance drug-resistance mutations
SPSS	Statistical Package for Social Sciences
TAMs	Thymidine analog mutations
TDF	Tenofovir disoproxil fumarate
UNAIDS	United Nations Programme on HIV/AIDS
URFs	Unique Recombinant Forms
VL	Viral Load
WHO	World Health Organization
µL	Microliter
3TC	Lamivudine

DEFINITION OF OPERATIONAL TERMS

Antiretroviral therapy (ART): A combination of drugs that suppress the replication of HIV in the body.

First line ART: regimen consisting of two nucleoside reverse-transcriptase inhibitors (NRTIs) plus a non-nucleoside reverse-transcriptase inhibitor (NNRTI).

HIV/AIDS-related deaths/morbidity: Deaths and illnesses caused by HIV infection and its complications, such as opportunistic infections and cancers.

Acute HIV infection: The early stage of HIV infection, which occurs within a few weeks of exposure to the virus.

Chronic HIV infection: The long-term stage of HIV infection, which occurs after the acute phase.

Advanced HIV disease: The most severe stage of HIV infection, defined as current CD4 counts less than 350 cells /μL

CD4+ T cell count: The number of CD4+ T cells in a cubic millimeter of blood

Drug resistance: The ability of HIV to develop mutations that make it less susceptible to ART drugs. This can make it difficult to treat HIV infection and can lead to treatment failure.

Resistance mutations: Specific mutations in the HIV genome that make the virus less susceptible to ART drugs.

Major drug resistance mutation: A mutation that has been shown to reduce in-vitro drug susceptibility to the drug

Genotypic resistance: ART drug resistance caused by mutations in the HIV virus's genetic structure

Virologic failure: The inability to achieve or maintain suppression of viral load (defined as viral load >1000 copies/mL)

Genetic diversity/variation: The differences in the genetic sequences of different HIV strains.

HIV-1 subtypes: Different genetic variants of HIV-1. There several HIV-1 subtypes, each of which is most common in different parts of the world.

Subtype-specific mutations: Mutations that are more common in certain HIV-1 subtypes than others are.

Polymorphic mutations/ polymorphism: Naturally occurring genetic variations at a specific genomic position within a population without necessarily being triggered by drug pressure

Subtype-specific polymorphisms: Polymorphisms that are more common in certain HIV-1 subtypes than others are.

Treatment failure: The failure of ART to prevent the progression of HIV disease to AIDS or to improve the quality of life and life expectancy of PLHIV.

Antigenic variability: The ability of HIV to change its surface proteins, which can make it more difficult for the immune system to recognize and fight the virus.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Human Immunodeficiency virus remains a persistent threat to public health with an estimated population of over 38 million people living with the virus globally, and Africa bears a large burden of 25.7 million (World Health Organization, 2022). While HIV prevalence has declined over the years, Kenya is ranked thirteenth highest HIV-burdened country globally with 1.4 million persons infected with the disease, 42, 000 new infections and 22,000 HIV/AIDS-related deaths (Kenya|UNAIDS, 2022). Homa Bay County is among the highest burdened counties in Kenya with HIV prevalence, 4 times higher (20.7%) than the national prevalence (4.9%) (NASCOP, 2018). Although the ART coverage in Homa Bay County is wide, 79% as of 2017, the county recorded the highest number (4,558) of HIV/AIDS-related deaths (NASCOP, 2018). A study conducted by Ousley et al., (2018) in Homa Bay found out that 83.7% of the patients admitted in Homa Bay county Hospital had advanced HIV disease. Within the cohort of patients who had undergone antiretroviral therapy (ART) for a period exceeding six months, it was observed that 45.8% of them were hospitalized while exhibiting immunological failure;80.7% of these had virological failure (VL ≥1000 cp/mL), and mortality was 17.2% (Ousley et al., 2018).

HIV infection manifests in several clinical stages; the first stage is acute HIV infection, which occurs shortly after exposure to the virus, marked by rapid viral replication, making the person highly infectious (HIVinfo.NIH.gov, 2021). This is followed by the chronic HIV infection/clinical latency, which mainly shows no symptoms but the virus keeps replicating (HIVinfo.NIH.gov, 2021) . The final stage is advanced disease. An individual is classified as

having advanced HIV disease if their CD4+ T cell count falls below 350 cells/ul or if they exhibit stage 3/4 clinical events (WHO, 2016) . People with advanced HIV disease are the most likely group of people living with HIV (PLHIV) at the highest risk of HIV-related mortality due to their high immune system deficiencies (Tenforde et al., 2017) and therefore the need for a close monitoring of this group.

In the recent past, the quality of life of individuals living with HIV has greatly improved due to the rising availability and wide utilization of ART (Ghiasvand et al., 2019). Between 2014 and 2017, the use of ART saved over 635, 500 lives in Kenya by preventing HIV/AIDS-related deaths which, has been attributed to the wide access to treatment through the roll-out of free ART in 2003 (NASCOP, 2018). Antiretroviral therapy for HIV consists of a combination of drugs targeting various stages of the HIV life cycle. ART includes nucleoside reverse transcriptase inhibitors (NRTIs) (like tenofovir, emtricitabine, and abacavir), non-nucleoside reverse transcriptase inhibitors (NNRTIs) (such as efavirenz, rilpivirine, and nevirapine) integrase strand transfer inhibitors (INSTIs) (like dolutegravir, raltegravir, and elvitegravir), and protease inhibitors (PIs) (such as darunavir, atazanavir, and lopinavir/ritonavir) (HIVinfo.NIH.gov, 2021). The World Health Organization (WHO) recommends using at least three drugs from two different classes, with the first-line treatment typically consisting of two NRTIs and one NNRTI or integrase inhibitor (WHO, 2016).

However, the wide use of ART has faced the danger of increased drug resistance (Hamers et al., 2011). Whereas drug resistance can result from exposure to antiretroviral drugs (acquired drug resistance), even drug naïve patients can be infected with the virus that is already resistant (transmitted drug resistance) (Kuritzkes, 2011). Drug resistance remains a great concern as it

hampers viral load suppression, the main goal for treatment, and can result in increased HIV infections and spread of resistant variants, morbidity and mortality if not managed in time. Mutations can develop in various regions of the HIV-1 virus, including the reverse transcriptase, integrase, protease, and the gp41 protein, all of which are targets for various ARTs (WHO, 2018). Because of these mutations, the virus develops resistance to drugs that specifically target the corresponding enzymes, thus enabling the virus to evade the effects of the drugs and continue to multiply even in the presence of medication.

HIV-1 is well known for its extensive genetic variation and recombination, which has been associated with a number of clinical implications challenging efforts to combat the disease. The genetic diversity of HIV-1 ranges from 25% to 35% variations between subtypes and 15% to 20% within subtypes (Santos & Soares, 2010). HIV is broadly categorized into two types: HIV-1 and HIV-2; HIV-1 demonstrates the highest level of genetic diversity, thus further subdivided into distinct groups, namely M, O, N, and P (Hemelaar et al., 2019). Among these groups, M is the most common, subdivided into various subtypes including A, B, C, D, F, G, H, J, K, CRFs and URFs (Hemelaar et al., 2019). A global analysis of the distribution of HIV-1 shows that the virus is spread unevenly across the world, with different regions exhibiting distinct patterns of subtype prevalence (Giovanetti et al., 2020). These distribution patterns could be due to inadvertent trafficking (viral migration), leading to the "founder effect," or a predominant subtype transmitted in that geographical population (Buonaguro et al., 2007). Globally, the most predominant HIV-1 subtypes are CRFs (all combined), subtype C, subtype B, and subtype A; notably, subtype C accounts for about 50% of the HIV-1 infections (Hemelaar et al., 2019). Consequently, up-to-date knowledge of the varied HIV-1 subtypes in an HIV high burdened county such as Homa Bay County is needed.

The diversity of HIV has notable implications on HIV diagnosis and viral load quantification, potentially influencing the response to antiretroviral treatment and the development of drug resistance (Koning et al., 2013). Studies have suggested that there are varied pathways across the diverse HIV subtypes in drug resistance emergence, response to therapy, disease progression, and viral transmission (Santos & Soares, 2010, Santoro & Perno, 2013). Several reports have highlighted variations in the development of drug resistance among different HIV subtypes (Hosseinipour et al., 2009, Sui et al., 2014). This can be attributed to subtype differences in the usage of nucleotide, subtype-specific amino acid variances at drug target sites and specific differences in sequence motifs (Santoro & Perno, 2013). Moreover, there are indications that specific protein backbones associated with different HIV subtypes can result in varying levels of drug resistance to certain drugs (Gupta et al., 2011). Data on the sub-type specific mutations is missing in Homa Bay County. Further, whereas the prevalent subtype in Kenya is A, most of the studies examining the association of subtypes to drug resistance conducted elsewhere, have focused on the characteristics of Subtype C or G in comparison to other subtypes and some have yielded divergent results (Chaplin et al., 2019, Lessells et al., 2012, Hosseinipour et al., 2009, Lihana et al., 2009).

Resistance to ARTs can further be exacerbated by the occurrence of some polymorphisms that occur naturally among varied HIV-1 subtypes, which may render HIV-1 less susceptible to certain ARTs. Like drug resistance-associated mutations, natural polymorphisms vary across subtypes and hence discreetly distributed across varied geographical regions. These polymorphisms may increase the degree of resistance as a result of particular key resistance mutations and the tendency of some HIV-1 gene regions to develop particular mutations that cause HIV-1 drug resistance (Wainberg & Brenner, 2012). These natural polymorphisms can be

subtype-specific and thus influencing the differences observed in the occurrence of drug resistance mutations across subtypes (Santoro & Perno, 2013). Nonetheless, HIV-1 Subtype-specific polymorphisms linked to drug-resistance mutations in Homa Bay County remain yet to be studied.

1.2 Problem Statement

Homa Bay County is one of the highest HIV-burdened counties in Kenya with an extremely high prevalence of persons on ART with advanced HIV disease, virological failure, suspected first-line treatment failure and high mortality rates associated with HIV/AIDS (Ousley et al., 2018). This can be attributed to the emergency of drug resistance and high genetic variation of the HIV-1 virus, due to the use of ART for a long period and wide ART coverage in Homa Bay County. However, there is a lack of up-to-date data on the prevailing HIV-1 subtypes in this county, as well as the association between these subtypes and drug resistance mutations and polymorphic mutations. This information is crucial in guiding patient management and the selection of appropriate treatment regimens.

1.2 Objectives

1.3.1 General Objective

To determine the association between HIV-1 subtypes and drug resistance to first-line ART among individuals hospitalized with advanced HIV-1 disease in Homa Bay County

1.3.1 Specific Objectives

i. To characterize HIV-1 subtypes and recombinants among HIV-1 patients on first-line ART with advanced HIV-1 disease in Homa Bay County, Kenya
ii. To determine HIV-1 subtype-specific drug-resistance mutations among HIV-1 patients on first-line ART with advanced HIV-1 disease in Homa Bay County, Kenya.
iii. To determine HIV-1 subtype-specific polymorphisms associated with drug resistance among HIV-1 patients on first-line ART in Homa Bay County, Kenya.

1.4 Research questions

i. What are the current HIV-1 subtypes and recombinants circulating among patients on first-line ART with advanced disease in Homa Bay County?
ii. What are the HIV-1 subtype-specific drug-resistance mutations among patients on first-line ART with advanced disease in Homa Bay County?
iii. What are the HIV-1 subtype-specific polymorphic mutations associated with drug resistance among patients on first-line ART with advanced disease in Homa Bay County?

1.5 Justification

HIV-1 genetic variability may confer biological differences that could affect treatment response and outcomes. Therefore, appreciating the genetic distribution of HIV-1 subtypes in high-burdened regions like Homa Bay County is an important step towards improving HIV-1 management. Due to the extensive variation of HIV-1 subtypes across different geographical locations and in localized populations, it is essential to design targeted interventions tailored to the specific genetic variants prevalent in each area. To implement such interventions effectively,

it is imperative to determine the specific HIV-1 subtypes and recombinants circulating in such localities and their associated drug-resistance mutations and polymorphisms. Constant monitoring and updating of the molecular trends and patterns of HIV subtypes in localities are necessary to develop and implement successful intervention strategies.

It is crucial to define the HIV-1 subtypes-mutation association in Kenya, where subtypes A is prevalent, to enhance knowledge of the subtype-drug resistance relationship. Monitoring clinical isolates for resistance-associated mutations and polymorphisms is not only vital for directing initial therapy but also for guiding ART drug choice after treatment failure.

1.6 Significance

The findings of this study will enhance our understanding of the HIV-1 genetic diversity and distribution in Homa Bay County as well as the association between these subtypes and drug resistance. These findings can inform policy decisions on the selection of optimal ART regimens in the region, thereby improving treatment efficacy and enhancing the quality of life of patients.

Additionally, as HIV vaccine development remains a challenge, the knowledge gained from exploring the association between HIV subtypes and drug-resistance mutations will contribute to enriching vaccine development efforts. Understanding how different subtypes interact with drug-resistance mutations can provide valuable insights into designing effective vaccines.

Moreover, the study results will also advance our understanding of HIV-1 transmission chains and evolutionary phylogenetics specific to this region. This knowledge will contribute to an in-depth comprehension of HIV-1 dynamics and aid in the development of targeted prevention and control strategies.

CHAPTER TWO

LITERATUREREVIEW

2.1 HIV-1 Genetic Variability

HIV-1 is known for its high genetic and antigenic variability and rapid evolution within and between infected people. Africa harbors the greatest diversity of the HIV-1 virus (Tatem et al., 2012). HIV-1 derives its diversity from varied sources; through its high replication rate, lack of proofreading and high recombinogenic power by its Reverse Transcriptase enzyme as well as through multiple introduction of the virus into the human genome (Castro-Nallar et al., 2012), (Maldarelli et al., 2013). Further, HIV-1 diversity can result from high positive selection pressure due to drugs or the host immune system (Ariën et al., 2005). Host APOBEC-mediated substitutions is also a factor that plays a role in the high variability of the HIV-1 virus (Bruner et al., 2017). However, the leading cause of HIV-1 diversity is as a result of mutations during retroviral replication (Abram et al., 2010).

The high replication rate results in high HIV-1 mutation rates of between 0.1 to 0.3 mutations per genome in one replication cycle (Abram et al., 2010). Reverse transcriptase undergoes an exceedingly high error-prone template switch between its double RNA stands, a process that permits recombinations at the rate of 0.14 recombinations per genome in a single replication cycle (Skar et al., 2011). HIV-1 infection in each infected individual evolves after a period to give rise to complex quiesces of viral genomes. These quasi species undergo persistent genetic variation, competition, and selection in an individual (Shafer et al., 2007).

Phylogenetic analysis of HIV-1 characterizes it into four groups; Main group (M) Outlier group (O) , Non-M or New group (N) and the Putative (P) group (Hemelaar et al., 2019). Group M is the major group of HIV-1 characterized by nine different subtypes and more than 100 circulating recombinant forms (CRFs) and other unique recombinant forms (URFs) (*Los Alamos HIV Database*, n.d.)Group M is the most common group globally and is responsible for the majority of HIV infections in Africa (Hemelaar et al., 2019). Figure 2.1 depicts the evolutionary relationships of HIV-1 groups, subtypes, sub-subtypes, and recombinant forms. Individuals infected from dual sources ordinarily have inter-subtype recombinant genomes, whereas HIV-1 recombinant genes infecting three or more epidemiologically unrelated individuals result in the formation of CRFs (Adungo et al., 2014). The name of the CRF is derived from the order in which it was reported and the subtypes that recombine to make up its genomic structure (*Los Alamos HIV Database*, n.d.). CRF01_AE and CRF02_AG are the most predominant CRFs globally(*Los Alamos HIV Database*, n.d.). Further, Subtypes A and F can be classified into additional sub-subtypes; A1, A2, A3, A4, A5, A6, F1 and F2 respectively (Lihana et al., 2012, (Hemelaar et al., 2019) (Figure 2.1).

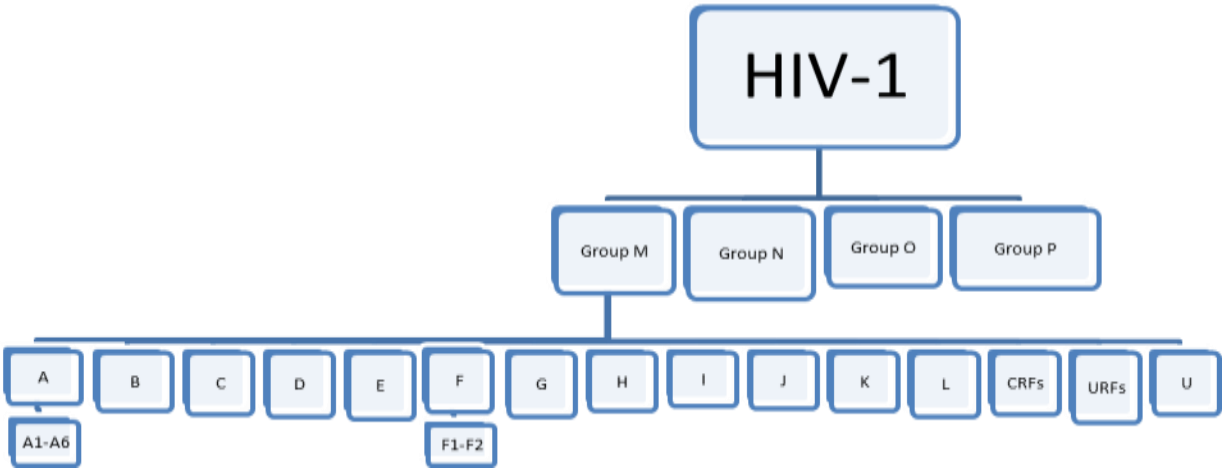

Figure 0: Evolutionary relationships of HIV-1 groups, subtypes, sub-subtypes, and recombinant forms. U stands for un-typed

Dual infection also increases the diversity of HIV-1 subtypes. Between 10% to 20% of HIV-1 infected persons are dually infected in various regions of Africa (Ndembi et al., 2011). Dual infection can occur as a result of co-infection, defined as concurrent infection with two heterologous strains, or superinfection which, refers to the occurrence of sequential infection with a different strain after seroconversion to the first infection (Hemelaar, 2012). Superinfection with a virus from the same group M subtype is known as intra-subtype superinfection and superinfection from different subtypes is known as inter-subtype superinfection; superinfection can take place in varied stages of the infection course (Hemelaar, 2012) and this may result in recombinant viruses. The existence of HIV-1 strains with sequences from different subtypes and different lineages in the same subtype is evident. For instance, broad intra-subtype recombination has been observed in subtype C of group M (Rousseau et al., 2007). Apparently, recombinant forms have become prevalent and are on the rise; it is estimated that these recombinant forms account for over 20% of the global HIV-1 infections (Hemelaar, 2012).

2.2 HIV-1 Subtype Distribution

The HIV-1 subtypes and recombinants are variedly distributed across different geographical regions and localities. In a global meta-analysis of the epidemiology of HIV-1 by Bbosa et al. (2019), subtype A was the most predominant in East Africa, Russia, and in the countries of the former Soviet Union. Subtype B is the principal subtype in Europe, North and South America, and in regions of the Pacific islands and their surroundings while in the Southern Africa and India, subtype C is the most prevalent subtype (Bbosa et al., 2019, Hemelaar, 2012). Asia is dominated by CRF01_AE whereas in Western Africa, CRF02_AG is predominant (Bbosa et al., 2019), (Hemelaar, 2012). Subtype A has been identified to be the most common subtype in

Kenya, subtypes D, C, G, and CRFs have also been reported in low prevalence (Adungo et al., 2014; Gounder et al., 2017; Kageha et al., 2012; Khoja et al., 2008). Table 2.1 shows regional differences in subtype distribution in different regions in Kenya.

Table 2.1. HIV-1 subtype distribution per region in Kenya

Author	Region		A	C	D	G	Others	Recombinants
Hassan et al., 2018	Coastal Strip		74	9	16	1	-	-
Adungo et al., 2014	Western		52	6.7	28	6.7	1.3(B) 2.7 (A2)	A1/C=1.3 A1/D= 1.3
Nyagaka et al., 2012	North Rift Valley		70	7	11	1	3 (A2)	A1/D - 4 A1/C - 3 A2/C - 1
Kageha et al., 2012	Central		69.8	11.5	18.7		-	-
Hué et al., 2012	Kilifi		59	8	10	1	-	combined-22
Arroyo et al., 2009	Kericho		56	5	10		-	combined-29
Khoja et al., 2008	Nairobi		56.5	10.1	18.8	2.9	-	AD; 4.3; CRF01_AE- 4.3; AC- 1.5; AD -1.5
Khamadi et al., 2005	Northern		50	39	11	-	-	-
Yang et al., 2004	Kisumu	gag-p24	69.4	6.1	20.4	1.7	2.4(unclassifiable)	-
		env-gp41	72.9	5.7	18.5	2	0.9(unclassifiable)	CRF02_AG -0.2

Analysis over a period reveals that regions exhibit a high degree of subtype diversity and undergo dynamic changes in terms of HIV-1 subtypes and recombinant forms. This can be because of the fast-spreading subtypes due to inter-regional interactions, high evolution rate as well as recombination of subtypes as a result of co-infection (Bbosa et al., 2019). For instance, in areas where multiple subtypes co-circulate in Asia and Africa, Bbosa et al., 2019 observed an increasing frequency of recombinant viruses. Whereas when the HIV pandemic initially invaded

Malaysia, subtypes B and CRF01_AE were the predominant subtypes, recombinant CRF33_01B was observed to be the major HIV subtype in a study conducted in Kelantan, Malaysia (Mohamad et al., 2012). A remarkable increase in CRF02_AG has recently been described in Northern Africa where the most prevalent subtype is B (Giovanetti et al., 2020). Figure 2.2 shows a map of the global distribution of HIV-1 group M subtypes.

The regional heterogeneity of HIV-1 in Kenya, like in other parts of the world, is evident. Gounder et al., (2017) observed increased widespread HIV-1 inter-subtype recombination among drug users in major cities in Kenya while subtype D prevalence was observed to be on the decline. Although it is apparent that over time, the regional distribution of HIV- subtypes may substantially vary, up-to-date knowledge of the HIV-1 distribution in a high-burden county such as Homa Bay County is not available.

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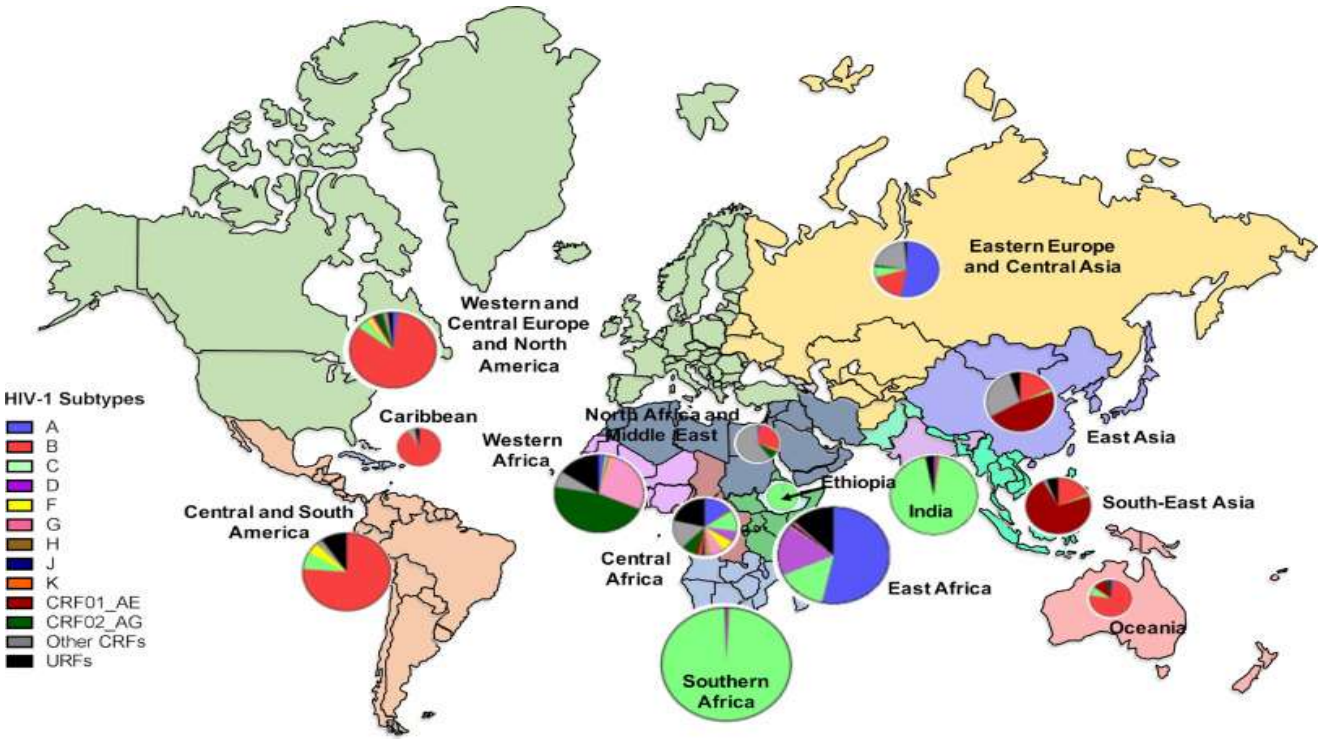

Figure 2.2: A map from Gartner et al., 2020 showing the distribution of HIV-1 group M subtype globally.

Key: Pie charts indicate the percentage of major subtypes in a region.

2.3 Antiretroviral Drugs and Resistance

The HIV genome contains three structural genes, namely gag, pol, and env (Figure 2.3), which are responsible for encoding the structural proteins of the virus. The pol gene codes for three enzymes; protease (PR), Reverse Transcriptase (RT), and integrase (IN) (Figure 2.3), all of which are major targets of antiretroviral drugs. The RT gene is the target for both NRTIs and NNRTIs and most of the resistance mutations to these drugs are in the 5' polymerase coding region (Shafer et al., 2001).

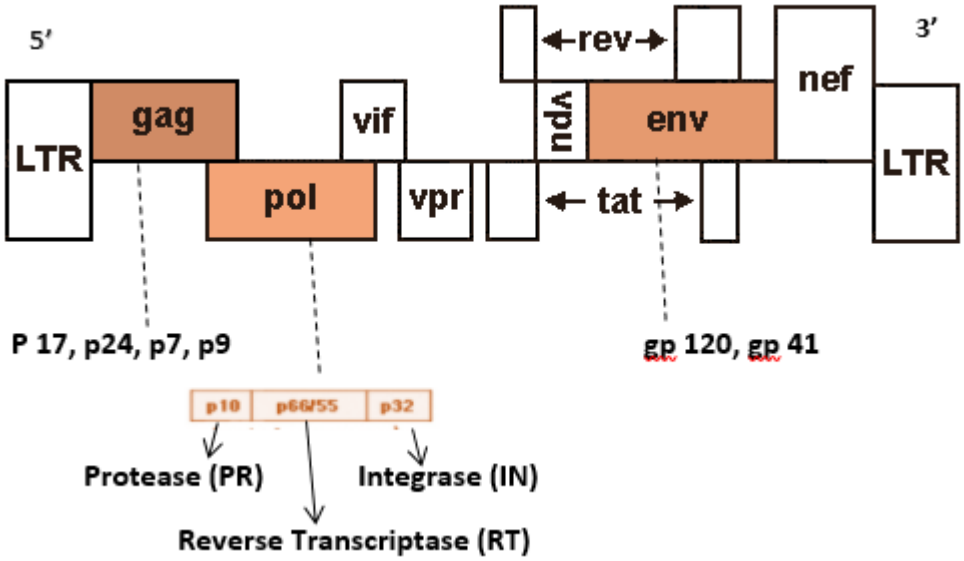

Figure 2.3: Schematic diagram of HIV-1 genome adapted from (Shafer et al., 2001).

Key:

LTR - Long-Terminal Repeat
Gag - Group-Specific Antigen
Pol – Polymerases
Vif - Viral Infectivity Factor
Vpr - Viral Protein R
Vpu - Viral Protein U
Nef - Negative Regulatory Factor
Rev - Regulator of Expression of Viral Proteins
Tat - Trans-Activator of Transcription

Approved antiretroviral drugs for the treatment of HIV encompass several main classes, which consist of nucleoside/nucleotide analog reverse transcriptase inhibitors (NRTIs), non-nucleoside analog reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors, and fusion or entry inhibitors (Warnke et al., 2007). To have an effective ART, a minimum of 3 agents from at least 2 dissimilar classes of ARVs are used in combination; WHO recommends two NRTIs and one NNRTI or integrase inhibitor for first-line treatment (WHO, 2016). The recommended first line ART regimen for adults and adolescents in Kenya consists of a combination of either lamivudine (3TC) or emtricitabine (FTC) with tenofovir disoproxil fumarate (TDF), along with a 600 mg dose of efavirenz (EFV) (WHO, 2016).

In 2018, first-line regimen was was revised as follows: for infants up to four weeks, AZT + 3TC + NVP; from 4 weeks-3 years, ABC + 3TC + LPV/r; 3-14years (<35 kg), ABC + 3TC + EFV; and for those aged >15yeas; TDF + 3TC + DTG (National AIDS and STI Control Programme, 2018). First line ART was further revised in 2022 as follows: infants (4 weeks), AZT + 3TC + NVP, > 4 weeks <15 years, ABC + 3TC + DTG, for > 15 years, TDF + 3TC + DTG is recommended, but if DTG is not tolerated, EFV should be used (Nationa AIDS and STI Control Program, 2022). Second-line consists of ritonavir boosted lopinavir with two NRTIs (Nationa AIDS and STI Control Program, 2022).

The emergence of HIV antiretroviral drug resistance is a key impediment in achieving viral suppression. The increasing occurrence of new infections with drug-resistant viruses, coupled with the accumulation of mutations associated with drug resistance, contributes to elevated cases of AIDS-related morbidity and mortality (WHO, 2018). The 2019 WHO report revealed high HIV drug resistance in six African countries in children and adults (World Health Organization, 2019). This resistance is partly due to the frequent use of NNRTI-based regimens in low-income countries due to their longer half-life and durability, and because NNRTI regimens have a low genetic barrier to resistance (Melku et al., 2022).

Resistant patterns in low-income areas are on the rise despite the resistance being progressively well managed in high-income areas. Particularly in sub-Saharan Africa, 10% of adults starting ART and 30% of those restarting first-line ART after exposure, harbor a resistant virus (WHO, 2018a). The prevalence of NNRTI resistance among ART-experienced individuals is in the scale of between 5% and 28%, and the resistance in people failing ART is estimated to range from 50% to over 90% (World Health Organization, 2019). In Ndhiwa of Homa Bay County, Onywera et al., (2017) observed high transmitted drug resistance prevalence compared to most

regions within East Africa. High prevalence of NRTI and NNRTI drug resistance has also been described recently mong ART-experienced inpatients with advanced disease in Homabay County (Bossard et al., 2021). HIV drug resistance can be assessed through two primary methods: phenotypically, which involves cell culture-based assays, or genotypically, which involves DNA sequence analysis. The most broadly used method is the genotypic drug resistance testing and is the method recommended by WHO as the most accurate (WHO, 2010). The recommended classes ART drugs are described below:

2.3.1 Nucleoside Reverse Transcriptase Inhibitors

The drugs are referred to as nucleoside analog owing to their similarity in structure to the blocks that make up nucleic acids. However, in place of a hydroxy (-OH) group that is naturally found in the 3′ position, NRTIs have a slightly different group that is unable to create a 5′ to 3′ phosphodiester bond which is needed for elongation of DNA (Arts & Hazuda, 2012). Therefore, NRTIs impede the reverse transcriptase activity by integrating themselves onto the viral DNA in place of the natural building blocks thus terminating the proviral DNA synthesis chain (Warnke et al., 2007). Chain termination can occur during RNA-dependent or DNA-dependent DNA synthesis, which inhibits the production of either the negative (-) or Positive (+) strands of HIV-1 proviral DNA (Arts & Hazuda, 2012). NRTIs are taken in their inactive form (prodrugs), which are then activated once they enter the host cell and undergo phosphorylation by cellular kinases (Arts & Hazuda, 2012). Some of the examples of NRTI include tenofovir disoproxil fumarate, emtricitabine, and abacavir (HIVinfo.NIH.gov, 2021).

NRTI resistance occurs when mutations in the RT gene allow the RT enzyme to evade NRTI blocks during synthesis or increase the rate at which the NRTI is hydrolytically removed (Shafer

et al., 2007). Resistance to NRTIs can occur through two primary mechanisms. The first mechanism, known as ATP-dependent pyrophosphorolysis, is characterized by the elimination of NRTIs from the 3' end of the developing chain, effectively reversing cessation of chain elongation and enhancing the ability of the RT enzyme to differentiate the native dNTP substrate and the inhibitory compound (Arts & Hazuda, 2012). Mutations in the RT gene that confer resistance to NRTIs can be categorized into two groups: nucleoside/nucleotide associated mutations (NAMs) and thymidine analog mutations (TAMs) (Arts & Hazuda, 2012). The HIV-1 RT gene exhibits two distinct pathways for TAM. The first pathway, known as TAM1, involves mutations such as M41L, L210W, T215Y, and occasionally D67N and the second pathway, referred to as TAM2, is characterized by mutations including D67N, K70R, T215F, and 219E/Q (Arion et al., 1998). The second mechanism of NRTI resistance involves impeding the integration of NRTIs into the developing chain and it is associated with mutations like M184V/I and K65R (Arts & Hazuda, 2012). The functional capacity of RT gene and the replicative fitness of the HIV-1 virus are diminished by numerous primary and secondary NRTI mutations or various combinations thereof (Arts & Hazuda, 2012).

2.3.2 Nonnucleoside Reverse Transcriptase Inhibitors

NNRTIs exert their inhibitory effect by binding directly and in a non-competitive manner to the RT enzyme at a site separate from that of the dNTP substrate, effectively obstructing the DNA polymerase activity (Warnke et al., 2007). Binding of NNRTIs to the RT induces the creation of a hydrophobic pocket in close proximity to, but not directly overlapping, the active site thus modifying the substrate site spatial conformation and consequently reduces the activity of the

polymerase enzyme (Sluis-Cremer et al., 2005). NNRTIs include efavirenz, rilpivirine, and nevirapine (HIVinfo.NIH.gov, 2021).

Mutations for NNRTI resistance occur in the hydrophobic pocket, which binds the inhibitors (Shafer et al., 2007). Amino acid substitutions within the NNRTI-binding pocket of the RT enzyme, such as L100, K101, K103, E138, V179, Y181, and Y188, commonly contribute to the development of NNRTI resistance with K103N and Y181C being the most frequently observed mutations (Arts & Hazuda, 2012).

The majority of NNRTI mutations result in varying degrees of cross-resistance across different NNRTIs, particularly when additional secondary mutations are present (Antinori et al., 2002). Minor alterations in single nucleotides can result in a significant increase in resistance to NNRTIs with just a slight loss of replicative (Arts & Hazuda, 2012). The transmission and persistence of NNRTI-resistant HIV-1 primarily occur due to a lower genetic barrier, a modest impact on replicative fitness, and the gradual reversal of these mutations in the absence of drug pressure (Warnke et al., 2007).

2.3.3 Protease Inhibitors

The protease enzyme plays a crucial role in the HIV life cycle by cleaving the large precursor polypeptide chains into tiny, functional proteins. This cleavage process facilitates the process of viral particle maturation which occurs in the very last stages of the HIV life cycle (Warnke et al., 2007). Protease inhibitors are developed to inhibit the activity of the protease enzyme. Blocking the activity of the protease enzyme leads to the production of virions that lack structural integrity and are incapable of causing infection. (Arts & Hazuda, 2012). Protease inhibitors include darunavir, atazanavir, and lopinavir/ritonavir (HIVinfo.NIH.gov, 2021).

Considering the critical function of the protease enzyme in the HIV-1 life cycle and its compact size (11 kDa), initial expectations were that protease resistance would be uncommon (Arts & Hazuda, 2012). Nonetheless, extensive variability has been detected in the protease gene, exhibiting polymorphisms in 49 out of its 99 codons; moreover, over 20 substitutions have been linked to resistance in this gene (Arts & Hazuda, 2012; Shafer et al., 2007). Protease inhibitor resistance is mediated by changes in structure that decrease the binding affinity between the inhibitor and protease molecule (Shafer et al., 2007). In contrast to NNRTIs, primary drug-resistant PI mutations are seldom detected within the viral populations of individuals who have not been exposed to protease inhibitors (Warnke et al., 2007). For most PIs, primary resistance-associated mutations tend to cluster in proximity to the protease active site. These mutations occur at positions within the substrate/inhibitor binding site; they include D30N, G48V, I50V, V82A, or I84V, among others, consequently, these amino acid changes adversely impact the replicative fitness of the virus (Shafer et al., 2007). In addition to mutations in the PR gene, alterations occurring within eight prominent cleavage sites of the protease gene have been associated with resistance to protease inhibitors (Warnke et al., 2007).

2.3.4 Integrase Inhibitors

The integrase enzyme plays a critical role in the processing of the 3' end of viral DNA and facilitates the strand transfer process (Arts & Hazuda, 2012). Integrase inhibitors specifically target the integration step of the viral DNA, which involves the insertion of viral DNA into the host genome (Hazuda et al., 2004). The specific impact on strand transfer arises from the mechanism of action, wherein the inhibitor selectively binds either to the complex formed between integrase and viral DNA or interacts with the essential magnesium metal ion cofactors

present in the active site of integrase enzyme and the DNA (Arts & Hazuda, 2012). Integrase strand transfer inhibitors (INSTIs) are dolutegravir, raltegravir, and elvitegravir (HIVinfo.NIH.gov, 2021).

Mutations that confer resistance to integrase inhibitors are typically found in the integrase active site, particularly in close proximity to the amino acid residues responsible for coordinating the essential magnesium cofactors (Warnke et al., 2007). As a result, these mutations exert detrimental effects on both enzymatic function and the capacity of the virus to replicate (Arts & Hazuda, 2012). Some of the common integrase inhibitor mutations that have been observed are E92Q, V151L, T97A, G163R, and L74M, Q148 (K/R/H), G140S/A, and E138K (Fransen et al., 2009). Integrase inhibitors exhibit substantial cross-resistance across different primary and secondary mutation sets, regardless of the specific combinations of mutations (Hazuda et al., 2004). While cross-resistance is commonly observed, it is notable that distinct agents tend to selectively induce distinctive mutation patterns (Hazuda et al., 2004).

2.3.5 Fusion/Entry Inhibitors

Another class of antiretroviral drugs, uncommonly used, are Fusion inhibitors, which act outside of the cells to impede the fusion of HIV to the CD4 or other target cells. Fusion inhibitors disrupt the binding, fusion, as well as the entry of an HIV particle into the human cell (Arts & Hazuda, 2012). Through impeding this crucial stage in the replication cycle of HIV; fusion inhibitors decelerate the HIV infection progression to AIDS (Briz et al., 2006).

2.4 HIV-1 subtypes and Drug-Resistance mutations

HIV-1 subtypes exhibit considerable sequence discrepancies in their structural and regulatory genes, pointing to subtype-specific functional biological differences. Variances in the HIV-1 transmission rate, disease progression, treatment failure, accuracy of viral load measurements, and development of mutations that are associated with drug resistance can be observed in different subtypes (Siemieniuk et al., 2013). While a combination of antiretroviral regimens shows effectiveness in combating all subtypes of HIV-1 group M, there is increasing evidence indicating variations in drug resistance among subtypes in different regions worldwide (Santoro & Perno, 2013). Differences in the emergence of drug resistance occur among various HIV-1 subtypes due to the inherent characteristics of the virus and the distinct drug selection pressures they encounter (Santoro & Perno, 2013). This phenomenon comes about because of three main factors. First, the resistance genetic barrier can be affected by subtype differences in the usage of nucleotide and mutational motifs, that is, the number of transitions/transversions necessary to develop drug resistance (Santoro & Perno, 2013). For instance, mutations at codon 106 of RT in subtypes C, have been linked to NNRTIs resistance when exposed to efavirenz (Brenner et al., 2003). Secondly, when exposed to the same drug pressure, various mutations can emerge due to subtype-specific dissimilarities in amino acids, which are involved in minor structural modifications in the gene segments targeted by the drug. Thirdly, subtypes exhibit distinct dissimilarities in sequence motifs that can favor nucleotide substitutions that contribute to the development of drug resistance (Santoro & Perno, 2013).

Research shows differences in drug resistance patterns among different HIV-1 subtypes. For example, the pathway to the development of resistance to Stavudine is different in persons with

subtype B in comparison to persons with subtype C (Hosseinipour et al., 2009), as well as in persons with subtype G compared to CRF02_AG (Hawkins et al., 2009). A comprehensive study conducted to examine the prevalence of drug-resistant mutations and the distribution of amino acids at these sites in the most prevalent HIV-1 subtypes found in China, namely subtype B, CRF01_AE, CRF07_BC, and CRF08_BC revealed diverse patterns and frequencies of mutations associated with drug resistance across these different HIV-1 subtypes (Sui et al., 2014). A notable disparity in surveillance drug-resistance mutations (SDRMs) has been observed among treatment-naïve children in Nigeria, specifically in the GWA-II and GCA subclades when compared to CRF02_AG and GWA-I (Chaplin et al., 2019). Regarding the development of NFV resistance, persons with HIV-1 subtype B have been reported to have a tendency of developing D30N mutation preferentially while those with other subtypes tend to preferentially develop L90M mutation (Grossman et al., 2004). Another study by Smit et al., 2017 observed a double frequency of K65R mutation in patients with subtype C in comparison to the other subtypes. Koning et al., 2013 observed distinct variations in the progression and impact of accessory mutations associated with high-level resistance to reverse transcriptase (RT) inhibitors between subtypes B and C.

Whereas it may be challenging to compare subtypes since their prevalence is linked to geographical locality, race, and standard of care, considerable evidence has pointed to variances that are specific to subtypes in drug resistance development (Chaplin et al., 2019; Grossman et al., 2004; Koning et al., 2013), underlining the importance of the knowledge on subtype and drug resistance association. Yet, such critical information on the sub-type specific mutations in Homa Bay County, which bears very high HIV-1 prevalence, is lacking.

2.5 HIV-1 subtype-specific polymorphisms and drug resistance

Specific natural polymorphisms in viral sequences exist before drug exposure and these polymorphisms vary across different subtypes. Whereas the impact of natural polymorphisms on ART response and drug resistance development is not fully understood, genotypic diversity in the HIV-1 subtype-specific polymorphisms may lead to emergency of drug resistance associated mutations and varied response to drugs (Santoro & Perno, 2013). The variance in amino acid background across subtypes influences the degree of resistance conferred by HIV-1-associated mutations, contributing to the observed variability in resistance levels between subtypes (Martínez-Cajas et al., 2008; Wainberg & Brenner, 2012) or compensate for fitness loss (Santos et al., 2012). For instance, NVP and EFV drug pressure results in a V106M mutation common in HIV-1 subtype C infected patients and a V106A mutation in subtype B patients; this difference is a result of a natural polymorphism (GTG in clade C and GTA in clade B at codon 106 in the RT gene (Brenner et al., 2003). The amino acid that is located at position 36 of the HIV-1 protease gene varies across different subtypes; methionine is common in clade B while isoleucine is in other subtypes, and this polymorphism is associated with high protease inhibitor treatment failure in non-subtype B infected patients (Lisovsky et al., 2010).

Sometimes, exposure to drug may lead to the intensification of natural polymorphisms leading to potential drug resistance, as is the case in A98G/S in the RT gene, M36I, K20I, and L89M in the protease gene (Santoro & Perno, 2013). Similar to how natural polymorphisms can augment resistance or compensate for fitness decline, studies have shown that subtype-specific polymorphisms can elevate viral susceptibility to certain protease inhibitors (Santos et al., 2012).

Contrastingly, a study by Kantor et al., 2005 that investigated the impact of subtype on polymorphism and development of drug resistance did not find a significant difference in in drug resistance-associated positions for the subtype B compared to other subtypes; however, polymorphic mutations in non-B subtype HIV-1 strains was observed to be higher. The lack of information linking drug resistance-associated polymorphisms with subtypes in regions with high HIV-1 prevalence including Homa Bay County, presents a gap that this study endeavors to bridge.

CHAPTER THREE

METHODOLOGY

3.1 Study design

This study was nested within a larger facility-based, cross-sectional survey conducted at Homa Bay County Referral Hospital, in Western Kenya by Médecins Sans Frontières (MSF) International. The study was conducted from February to July 2018 to assess virological failure and drug resistance among patients that were admitted to the hospital with advanced HIV-1 disease (Bossard et al., 2021). The study enrolled 187 individuals living with HIV-1, aged 15 years and above, hospitalized in the medical ward within the hospital during the study period, and initiated first-line ART at least 6 months prior to the time of admission. Among them, 69 had viral failure (defined as HIV-1 viral load (VL) of 1000 copies/ml and above) and were subjected to drug resistance testing. The present nested study assessed the association of individual DRMs and HIV-1 subtypes among 65 participants with viral failure from available sequencing results.

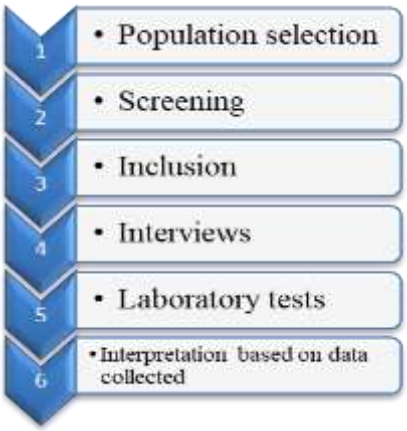

Figure 3.1: General Study Design

3.2 Study site

The study location for this survey was Homa Bay County Hospital in Homa Bay County. Homa Bay County is located at latitude 0.6221° S and longitude, 34.3310° E in the western part of Kenya. Homa Bay County Teaching and Referral Hospital is a tertiary referral hospital for Homa Bay County, with four main departments: Medical, Surgery, Gynecology/obstetrics, and Pediatrics. The medical department has approximately 75 beds with nearly 100% of bed occupancy. The number of admissions per day is approximately 10 patients. Homa bay county referral hospital provides comprehensive care and support to people living with HIV. Among all the patients admitted in the adult medical wards of the hospital in 2015, 49.1% were HIV positive as per the records in the hospital.

3.3 Study population

The study population is made up of HIV-1 infected patients aged 15 years and above, hospitalized in the medical ward in Homa Bay County Teaching and Referral Hospital during the study inclusion period, who have initiated first-line ART at least 6 months prior, classified as having advanced disease (CD4 <350 cells/uL) and fulfilling the eligibility criteria.

3.3.1 Sample size

This is a cross-sectional survey and the sample size was arrived at using Cochran's formula (Cochran, 1977):

$n = [z\alpha^2 * p * q]/d^2$

Where: n is the sample size, zα is the Z score for α of 0.05, P is the anticipated drug resistance prevalence (Beck et al., 2020), q is 1-p and d is the level of precision at 95% confidence interval (5%).

$n= [1.96^2*0.049*0.951]/0.05^2$ = 68 rounded up to 70 samples for drug resistance testing

Since samples with Viral load above the genotyping threshold (1000 cps/mL) would be genotyped, assuming 50% of patients with advanced disease would have VL above the threshold, (World Health Organization, 2019) the sample size was calculated by doubling samples for DRT (70), hence 140 samples of patients with advanced disease.

3.3.2 Inclusion criteria

The study included all HIV-positive in-patients, 15 years or older, initiated first-line ART for the first time more than 6 months previously, initiated first-line ART for the first time more than 6 months previously, on first-line ARV, classified as having advanced HIV disease (CD4 <350 cells/uL), and residing in Homa Bay County,

3.3.3 Exclusion criteria

The study excluded patients with severe comorbidities/medical conditions that would make participation in the study risky or impossible, and patients with incomplete medical records or missing data,

3.3.4 Sampling Technique

This study utilized a convenience sampling technique where all individuals that met the inclusion criteria were recruited over the study period until the sample size was reached.

3.3.5 Laboratory and Analytical Procedures

Four milliliters of venous blood was obtained from eligible patients. The blood sample was collected in EDTA tubes, via venipuncture by trained, competent staff. 100µl of whole blood was used to spot DBS on each circle of 903 Whatman filter paper (Whatman Inc, Piscataway, NJ). The DBS were used for genotyping. The rest of the EDTA blood sample was span at 2000g for 15 minutes to isolate plasma for viral load testing. The samples were then transported to KEMRI/HIV-R laboratory for the testing. The plasma samples were frozen at -80 ^{0}C until shipment when they would be transported in frozen state. The DBS samples were transported at room temperature.

3.3.6 HIV-1 Viral Load Quantification

The frozen plasma received at HIV-R laboratory were stored at -80^{0}C till testing. HIV-1 viral load RNA quantification was done by COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 (an automated real-time PCR), as per manufacturer's instructions and reagents (Roche Diagnostic System Branchburg, NL, USA) using plasma samples. The detectable range of this real-time PCR is 20 copies/mL to 10,000,000 copies/mL. Briefly, 1100 µL of each plasma sample was aliquoted into the corresponding S-tubes for further processing. The S-tubes were then loaded onto the CAP machine to extract RNA and subsequently amplification using RT-PCR tech nology for quantification in CTM. The assay permits automatic extraction of nucleic acid from 1000µl of plasma using magnetic bead technology, followed by automated reverse transcription and PCR amplification targeting dual regions of the HIV-1 genome, the gag and LTR. The HIV-1 RNA is then detected and quantified using a quantitation standard (QS).

3.3.7 HIV-1 Genotyping

The DBS samples for patients whose plasma samples had viral load 1000 copies/mL and above, which is defined as virological failure, were tested for genotypic resistance. This is because drug resistance testing is recommended for patients with viral load above 1000 cp/mL, which is the amplification sensitivity threshold for genotyping (Mackie et al., 2010).

Amplification of a 1,084-bp HIV-1 pol gene fragment, (amino acids 6 to 99 of the protease region and codons 1 to 251 of the RT region), was performed using an in-house assay validated to be performing optimally and approved by WHO (Inzaule et al., 2013) by use of primers covering the target pol region. All PCR was done using GeneAmp® PCR System 9700 as per the instructions of the manufacturer (Applied Biosystems, Foster City, CA, USA). This in-house assay is low-cost, broadly sensitive, and with high amplification rates compared to commercial assays (Inzaule et al., 2013).

3.3.8 Extraction of HIV-1 Viral RNA

Nucleic acid was extracted from dried blood spots (DBS) by use of NucliSENS silica-based boom method, following the manufacturer's instructions (bioMérieux, Inc., Durham, NC). Briefly, 2 DBS were lysed in 2mL lysis buffer by centrifuging at 1500xg for 15 seconds, then 50 µL of pre-vortexed magnetic silica suspension was added and mixed briefly, followed by incubation at room temperature for 10 minutes and a final centrifugation at at 1500g for 2 minutes. After aspiration of the supernatant, the pellet was re-suspended in 400 µL of Wash Buffer 1. The 1.5ml tubes were transferred into the tube holders on the Nuclisens miniMAG with the magnetic assembly rack backward for 30 seconds, the supernatant was discarded and 400 µL of Wash Buffer1 was added. The same steps were repeated with wash buffers 2 and 3 after

which 25 µL of elution buffer was used to elute RNA from the magnetic beads after incubation at 60°C for 5 minutes with gentle agitation in a thermomixer at 1400rpm. The magnet assembly rack was placed in an upright position and the beads were allowed to separate. The elute (RNA extract) was then aspirated for use in RT-PCR.

3.3.9 RT-PCR

Two outer primers, PrtM-F, and RT-R1 (Appendix I) were used to generate multiple copies of HIV-1 cDNA. RT-PCR was performed utilizing the Invitrogen Superscript III RT kit in a 50-microlitre master mix prepared as shown in Table 3.1.

Table 3.1: RT-PCR master mix utilizing Invitrogen Superscript III RT kit

Reagents	Amount for One RT-PCR Reaction
2 X Reaction Mix	25 µL
PrtM-F1 (8µM)	1 µL
RT-R1 (8 µM)	1 µL
DEPC-treated Water	7 µL
Super Script III one-step RT-PCR High Fidelity	1 µL
Template	15 µL
Total	50 µL

To obtain cDNA, the PCR mixture was subjected to thermocycling conditions in Table 3.2.

Table 3.2: RT-PCR Thermocycling conditions

Temperature	Time	Cycles
65^0 C	10 minutes	1
50^0c	45 minutes	1
94º C	2 minutes	40
94º C	15 seconds	40
50º C	15 seconds	40
72 º C	2 minutes	40
72 º C	10 minutes	1
4º C	∞ hold	

3.3.9 Nested PCR

Two sets of inner primers, Prt-F2 and Prt-R2 (appendix I) and a commercial enzyme, Taq polymerase (Applied Biosystems (ABI) California USA) were used to amplify the RT-PCR product. A 50-µL PCR reaction mixture with reagent amounts shown in Table 3.3.

Table 3.3: Nested PCR master Mix utilizing the ABI recipe

PCR Mixes	Reaction
DEPC Treated Water	34.5 µL
10x PCR Buffer	5.0 µL
$MgCl_2$ (25mM)	4.0 µL
dNTPs (10mM)	1.0 µL
Prt-F2 (4µM)	1.5 µL
RT-R2 (4µM)	1.5 µL
Taq polymerase	0.5 µL
Template	2.0 µL
TOTAL	**50.0** µL

The master mix was subjected to the following thermocycling conditions as indicated in Table 3.4.

Table 3.4: Nested PCR Thermocycling Conditions

Temperature	Time	Number of Cycles
94^0c	4 minutes	1
94^0c	15 Seconds	40
55^0c	20 seconds	40
72^0c	2 minutes	40
72º C	10 minutes	1
4º C	∞ Hold	

The presence and quality of the amplified product were assessed through electrophoresis that was done on 1 % agarose gel stained by SYBR-safe dye and visualized under ultraviolet light. The product was then purified in preparation for sequencing.

3.3.10 DNA Sequencing

Big-Dye Terminator sequencing was performed on the purified nested PCR product using six overlapping in-house sequencing primers covering the protease (amino acid 6-99) and RT (amino acids 1-251) regions (Appendix I).

A 20µl sequencing reaction mix utilizing ABI reagent was prepared as shown in Table 3.5.

Table 3.5: Cycle sequencing Recipe utilizing ABI Reagent

Reagents	1 primer reaction (μL)
Big dye terminator	1.0
Big dye 5x Buffer	4.0
Water	11.0
Primer (10 μM)	2.0
Template	2.0
TOTAL	**20.0**

The master mix was subjected to the thermocycling conditions shown in Table 3.6

Table 3.6: Cycle sequencing thermocycling conditions

Temperature	Time	Cycles
96º C	10 seconds	x 25 cycles
50º C	5 seconds	
60º C	4 minutes	
4º C		∞ Hold

The products were then purified by running the sequencing product through a gel using centrisep column purification method.

The purified big dye sequence product was analyzed using ABI 3130xl Genetic Analyzer, as per the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).

3.3.11 Sequence Assembly

The resulting sequence data were assembled using RECall v.3.1 software (Woods et al., 2018). The quality was assessed following WHO recommendations using RECall, WHO HIVDR QC Tool, and Stanford University HIVdb tools.

Figure 3.2 shows the general process of Genotyping that was used in this study

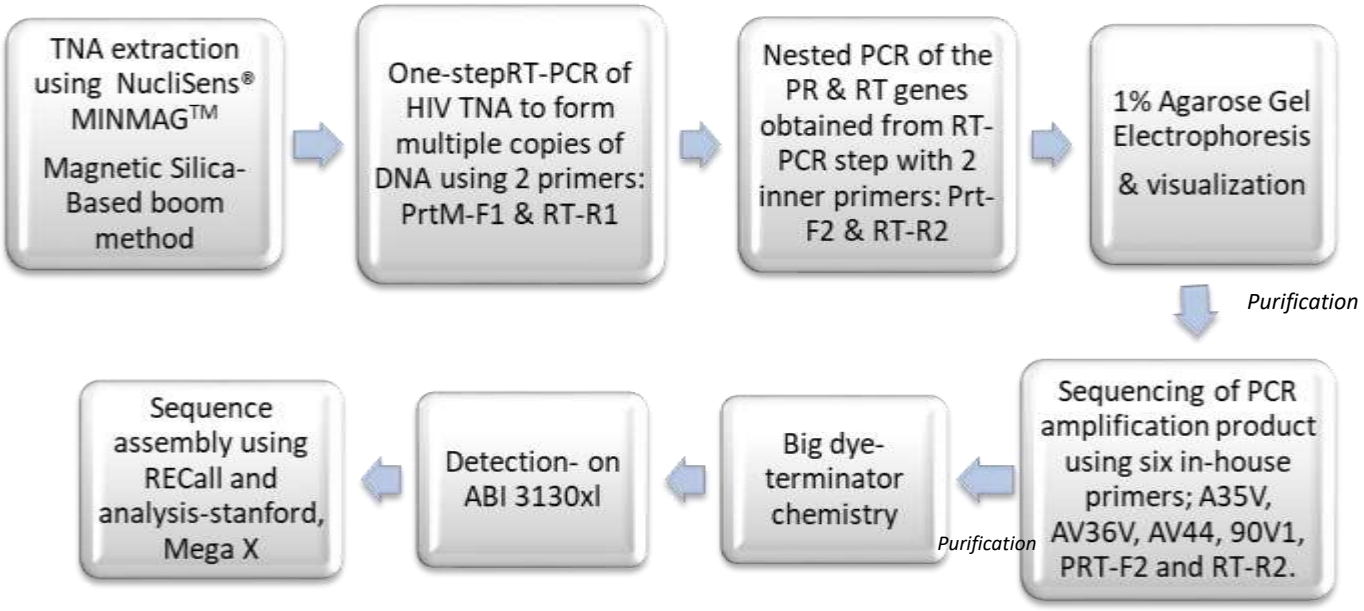

Figure 3.2 Genotyping process

Subtypes were identified by use of REGA Version 3.0 HIV subtyping software (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/) that is impeded

into the Stanford database and confirmed by phylogenetic analysis using MEGA X (Kumar et al., 2018).

To understand the genetic relatedness of the samples sequenced and their genetic distances, a phylogenetic tree of the PR and RT region of the sample sequences and reference sequences was constructed using the neighbor-joining method using MEGA X software (Kumar et al., 2018). Kimura 2 parameter model was used to define the distance matrix for the nucleotides and Bootstrap resampling at 1000 replicates was used to assess the statistical power of the neighbor-joining methodology. Representative subtypes/CRFs obtained from the Los Alamos Sequence Database were aligned with the study samples using ClustalW on Mega X software before a phylogenetic tree was constructed.

3.3.13 Genotypic Mutation and Polymorphism Analysis

Drug resistance-associated mutations and polymorphisms were determined with the help of the Stanford University HIV drug resistance database (available at https://hivdb.stanford.edu/) and subsequently interpreted using the Stanford HIV database algorithm (available at https://hivdb.stanford.edu/hivdb/by-mutations/) together with the IAS-USA drug resistance list (Wensing et al., 2019).

3.3.14 Statistical Analysis

To summarize the baseline demographics, clinical characteristics, and DRMs among the participants, descriptive statistics was used. The association between HIV-1 subtypes and drug-resistance mutations and polymorphisms was determined using Fisher's exact test. For computation in a contingency table, the absence or presence of a subtype, mutation, and

polymorphism were converted to binary values (1=present, 0=absent). Logistic regression analysis was further performed where associations indicated significance using Fisher's exact test to determine the direction and strength of the association, this logistic analysis was done again when controlling for regimen. For interpretation of significance, the alpha significance level, $p < 0.05$, was used in all analyses. All data analyses were performed using the IBM SPSS Statistics for Windows, Version 20.0. (Armonk, NY: IBM Corp).

3.4 Ethical Considerations

The study was approved by the MSF Ethics Review Board as well as KEMRI's Ethics Committee (Appendix II). Potential participants who fulfilled the eligibility criteria and expressed interest in enrolling in the study were provided with comprehensive oral and written information regarding the objectives of the study, procedures, as well as the associated risks and benefits. The patients who expressed their willingness to participate in the study provided their consent by signing an informed consent document. For individuals below 18 years of age but above 15 years of age, the details of the study were explained to at least one parent or guardian to obtain written permission, followed by a discussion with the minor to acquire their assent through signing the assent form.

CHAPTER FOUR

RESULTS

4.1 Patient Characteristics

In this study, 187 patients were included in the study and subjected to viral load testing to ascertain eligibility for genotyping (Figure 4.1). From the quantitative real-time RT-PCR results, 69 (36.9%) of the participants had plasma HIV-1 viral load of 1,000 copies/mL and above, 118 (63.1%) of the patients included in the study had suppressed VL. Four of the 69 samples failed amplification and could not be sequenced. Whereas the study targeted a sample size of 70 participants, successful amplification and sequencing of the PR and RT genes was achieved in 65 samples for the analysis of the results presented herein. By gender, 31 (47.78%) of the patients were females, 34 (52.3%) were males (Table 4.1).

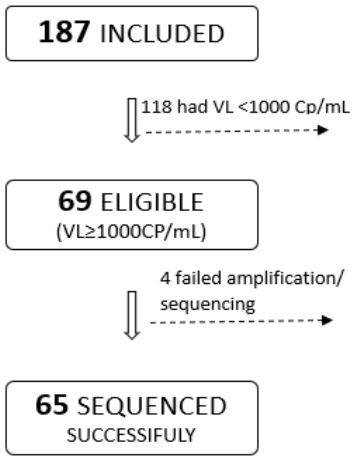

Figure 4.1: Study flow diagram.

The median age of participants was 33 years, with 50% lying between 28 and 39 years. The participants were on six (6) different first-line ART regimens, with most patients (70.8%) on a TDF/3TC/EFV. Table 4.1 gives a summary of the participant characteristics.

Table 4.1: Descriptive characteristics of the study participants

Characteristic	**N= 65**
Females, n (%)	31 (47.7)
Males, n (%)	34 (52.3)
Age in years, median [IQR]	33 (28-39)
Current first-line ART,	**n (%)**
TDF/3TC/EFV	46 (70.8)
AZT/3TC/NVP	9 (13.8)
TDF/3TC/NVP	2 (3.1)
ABC/3TC/EFV	1 (1.5)
AZT/3TC/EFV	6 (9.2)
TDF/3TC/DTG	1 (1.5)

KEY: *TDF- Tenofovir, 3TC- Lamivudine, AZT- Zidovudine and ABC- Abacavir (NRTIs), EFV- Efavirenz and NVP-Nevirapine (NNRTI), DTG-Dolutegravir (INTSI)*

4.2 Subtype characterization

Subtype determination on REGA subtyping tool revealed the following as circulating subtypes in the study population: subtype A1 was the most prevalent, detected in 46 patients (70.8%), 9 patients (13.8%) had subtype D, 6 patients (9.2%) had recombinant subtypes (5 had A1D and 1 had CD) and 4 patients (6.2%) had subtype A2 (Table 4.2). This subtype distribution was confirmed by phylogenetic analysis. The amplified sequences of the PR and RT genes were aligned with HIV-1 reference sequences obtained from the Los Alamos Sequence Database, representing different subtypes and circulating recombinant forms (CRFs) using ClustalW on

Mega X software. The results of a phylogenetic tree constructed from the amplified sequences and the reference sequences are shown in Figure 4.2. The phylogenetic analysis of the samples indicated five distinct clusters. One subtype identified as recombinant A1D by REGA sat between A1D cluster and subtype A1, this was assigned recombinant A1D. Another sample identified as recombinant CD by REGA formed a cluster singly, next to reference subtypes C and CRF07BC, other samples were in clusters with reference subtypes A1, A2, A1D, and D were all in agreement with the REGA subtyping tool outcome (figure 4.2). By consensus with REGA subtyping tool, the subtypes were grouped into four (4) subtypes (Table 4.2).

Table 4.2: Descriptive analysis of HIV-1 subtypes for patients with advanced disease in Homabay County

Subtypes	Frequency (n)	Percentage (%)
D	9	13.9
Recombinants (A1D & CD)	6	9.2
A2	4	6.2
A1	46	70.8

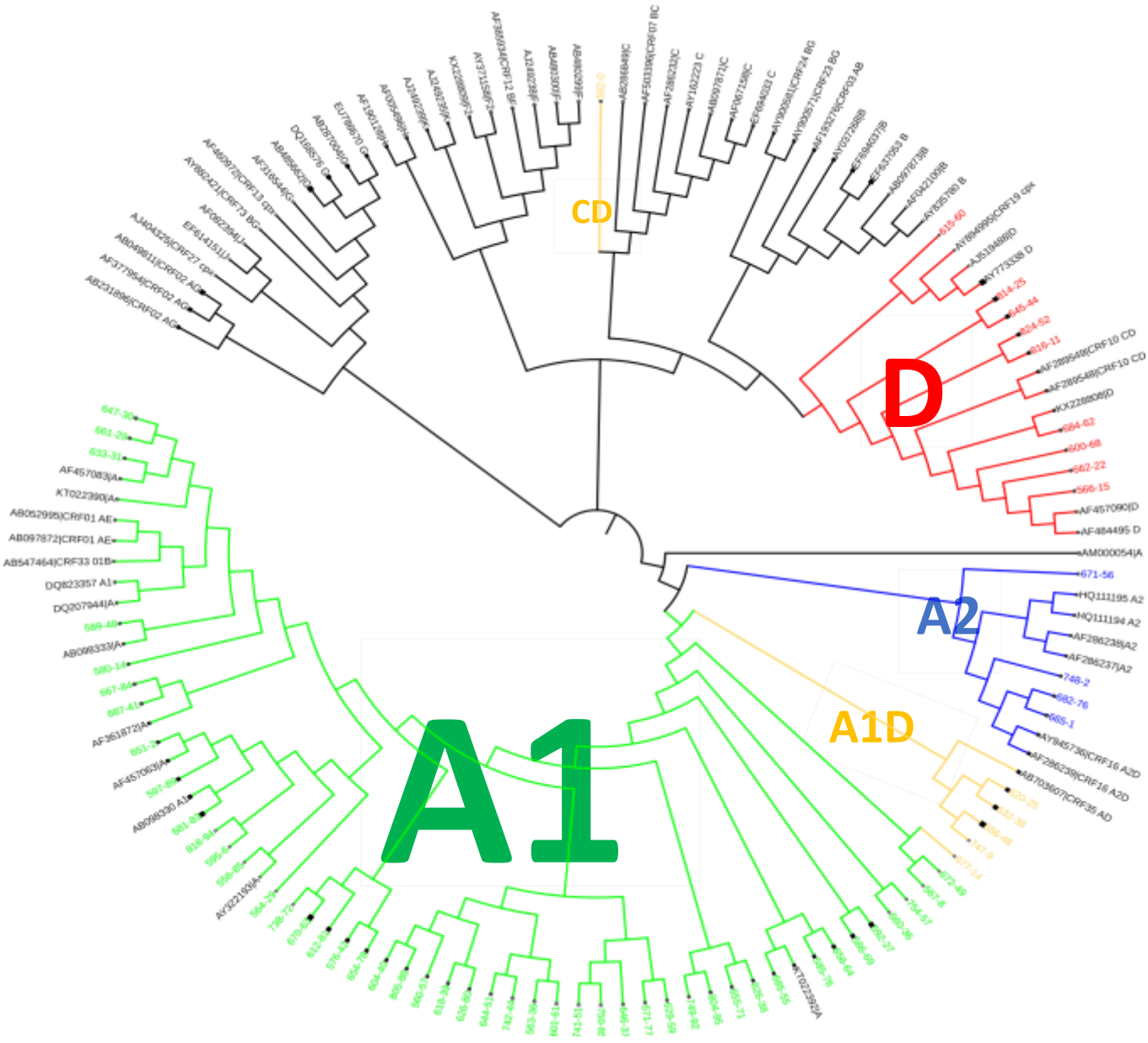

Figure 4.2: A phylogenetic tree showing the clusters of subtypes as identified by MEGA 11

Key: Green- Subtype A1, Blue- Subtype A2, Red- Subtype D, Orange- Recombinants A1D and CD and black- Reference sequences obtained from Los Alamos database (*Los Alamos HIV Database*, n.d.)

4.3 Drug Resistance-Associated Mutations and Polymorphisms

HIV-1drug-resistance mutations were detected in 54 (83.5%) participants, and 47 (72.3%) had dual mutations (NRTI and NNRTI). M181V was the most common NRTI mutation identified in

42 patients (64.6%) followed by TAM1, TAM2, and K70E/R/G/T mutations (18%, 17% and 17% respectively). K103N/S mutation was the most common NNRTI mutation identified in 39 patients (60%); G190A/S was identified in 23 patients (35.4%), and Y181C/I/V (26.2%). Most patients had more than four (4) NRTI and NNRTI mutations (32.3% and 26.2% respectively). Table 4.3 presents the descriptive analysis of NRTI mutations; Table 4.4 displays the descriptive analysis of NNRTI and polymorphic mutations. Although the study participants were all on first-line treatment and had not taken any protease, inhibitor (PI)-containing regimens, 3 patients had a L33F accessory PI mutation and one had a V82VA PI mutation.

Polymorphic mutations identified in the RT region included V179D/I (30.8%), followed by S68G (18.5%) (Table 4.4). PI resistance-associated polymorphic mutations, K20R and L101I/V (33.8% and 29.2% respectively), were also identified on the protease region. Table 4.4 has a list of the drug resistance-associated polymorphisms that were identified in the present study.

This analysis exclusively focused on mutations and polymorphisms associated with drug resistance,

Table 4.3: Descriptive Analysis of NRTI Mutations in first line ART experienced Individuals in Homa Bay County

Mutations	Number (n)	Percentage (%)
NRTI numbers		
0	17	26.2
1	4	6.2
2	10	15.4
3	13	20
>=4	21	32.3
NRTI specific mutations		
E44A/D	8	12.3
K65R	15	23.1
K70E/G/Q/T/R	17	26.2
L741/V	13	20
M184V	42	64.6
TAM1	18	27.7
TAM2	17	26.2
V75M	5	7.7
Y115F	5	7.7

4.4 Mutation-Subtype Association and Polymorphism- Subtype Association

The association between each NRTI mutation and each of the 4 subtypes identified in this study were tested; although no statistically significant evidence of association was found for specific NRTI mutation and any of the subtypes by Fishers' exact test, 5 of the 6 Y115F (83.3%) mutations and 4 of the 5 V75M mutations (80%) were observed in subtype A1. Additionally, the association between the HIV-1 subtypes and the number of NRTI mutations was tested using Fisher's exact test but it did not yield a significant association (Table 4.5).

A significant variation was noted in the NNRTI mutations (Table 4.6) where a significant association (p-value= 0.011) was observed between NNRTI mutation K101E/H and subtype A1. The K101E/H mutation is considered a non-polymorphic accessory mutation that primarily confers resistance to NVP and RPV, along with limited resistance to EFV. When combined with

other NNRTI-resistance mutations, the presence of K101H further decreases the effectiveness of the two NNRTIs. Mutation Y181C/I/V also indicated a statistically significant association with subtype A1 (p- Value= 0.027) (Table 4.6). The Y181C/I/V mutation is a non-polymorphic mutation that develops in patients on NVP, ETR, and RPV treatment. This mutation leads to high-level resistance against NVP, ETR, RPV, and EFV.

Table 4.4: Descriptive Analysis of NNRTI and Polymorphic Mutations in first line ART experienced Individuals in Homa Bay County

Mutations	Number (n)	Percentage (%)
NNRTI numbers		
0	12	18.5
1	5	7.7
2	14	21.5
3	17	26.2
>=4	17	26.2
NNRTI specific mutations		
A98G	10	15.4
E138Q	6	9.2
G190A/S	23	35.4
H221Y	6	9.2
K101E/H	10	15.4
K103N/S	39	60.0
L100I	11	16.9
P225H	8	12.3
V108I	12	18.5
V179T	6	9.2
Y181C/I/V	17	26.2
Y188L	4	6.2
Dual mutation (NRTI and NNRTI	47	72.8
Single mutations (NRTI or NNRTI)	7	10.2
Any mutation	54	83.5
No mutation	11	16.9
Polymorphisms		
K20R	22	33.8
L10I/V	19	29.2
K101A	5	7.7
S68G	12	18.5
T69I/N	7	10.8
V118I	4	6.2
V179D/I	20	30.8
V90I	7	10.8

Whereas the rest of the other NNRTI mutations did not show statistically significant association with subtypes, it was observed that 100% of the P225H and V/179I/T mutations were detected in subtype A1, and 9 of the 10 A98G (90%) mutations observed were in subtype A1 (one in subtype A2, which is closely related to A1).

No statistically significant association was observed between having both NNRTI and NRTI mutations and subtype (Table 4.6). Equally, the fishers' exact test of association between having any mutation and subtype did not yield statistical significance (Table 4.6).

The association between resistance-associated polymorphic mutations on the PI and RT regions and the HIV-1 subtypes identified was tested using Fisher's exact test; the results revealed a significant association between V179D/I polymorphic mutation and subtype D (p-value = 0.048) (Table 4.6). **V179D/I** is a polymorphic accessory mutation selected under NNRTI pressure that plays a role in reducing susceptibility to each NNRTIs. For the mutations or polymorphisms that showed a statistically significant association with subtypes using Fisher's exact test, logistic regression analysis was used to assess the magnitude and direction of the association as shown in table 4.7.

Table 4.5: Association between Subtype and NRTI Mutations in first line ART-experienced Individuals in Homa Bay County

	A1		A2		Recombinants		D	
NRTI specific mutations	n (%)	p-value	n (%)	p-value	n (%)	p-value	n (%)	p-value
E44A/D	6 (75.0)	1.000	1 (12.5)	0.417	0 (0.0)	0.594	1 (12.5)	1.000
K65R	11 (73.3)	1.000	1 (6.7	1.000	2 (13.3)	0.615	1 (6.7)	0.672
K70E/G/Q/T/R	11 (64.7)	0.547	2 (11.8)	0.278	2 (11.8)	0.648	2 (11.8)	1.000
L74I/V	9 (69.2)	1.000	1 (7.7)	1.000	1 (7.7)	1.000	2 (15.4)	1.000
M184V	29 (69.0)	0.780	3 (7.1)	1.000	4 (9.5)	1.000	6 (14.3)	1.000
TAM1	14 (77.8)	0.550	1 (5.6)	1.000	1 (5.6)	1.000	2 (11.2)	1.000
TAM2	12 (70.6)	1.000	1 (6.2)	1.000	2 (11.8)	0.648	2 (11.8)	1.000
V75M	4 (80.0)	1.000	0 (0.0)	1.000	0 (0.0)	1.000	1 (20.0)	0.538
Y115F	5 (83.3)	0.662	0 (0.0)	1.000	0 (0.0)	1.000	1 (16.7)	1.000
No. of NRTI Mutations								
0	13 (76.5)	0.758	1 (5.9)	1.000	0 (0.0)	0.327	3 (17.6)	0.687
1	2 (50)	0.574	0 (0.0)	1.000	1 (25)	0.328	1 (25)	0.458
2	7 (70)	1.000	1 (10.0)	0.496	1 (10.0)	1.000	1 (10.0)	1.000
3	10 (76.9)	0.740	0 (0.0)	0.576	2 (15.4)	0.591	1 (7.7)	0.674
>=4	7 (33.3)	0.771	2 (9.5)	0.589	2 (9.5)	1.000	3 (14.3)	1.000

Fisher's exact test

The results from the logistics regression model indicated that mutation K101E/H and Y181C/I/V were less likely to be observed in HIV-1 subtype A1 ((OR=0.16; (95% C.I = 0.04 – 0.65) and OR = 0.23; (95% C.I = 0.07 – 0.76) respectively) compared to other subtypes (Table 4.7). When adjusted for TDF/3TC/EFV, AZT/3TC/NVP, AZT/3TC/EFV and other regimens, the logistic regression model still indicated that the K101E/H and Y181C/I/V were less likely to be observed in subtype A1 ((OR=0.14; (95% C.I = 0.03 – 0.60) and OR = 0.21; (95% C.I = 0.06 – 0.72) respectively) compared to other subtypes (Table 4.8).

Table 4.6: Association between Subtypes and NRTI and Polymorphic mutations in first line ART-experienced Individuals in Homa Bay County

Mutations	A1		A2		Recombinants		D	
NNRTI specific mutations	n (%)	p-value	n (%)	p-value	n (%)	p-value	n (%)	p-value
A98G	9 (90.0)	0.258	1 (10.0)	0.496	0 (0.0)	0.579	0 (0.0)	0.333
E138Q	3 (50.0)	0.347	0 (0.0)	1.000	2 (33.3)	0.091	1 916.7)	1.000
G190A/S	14 (60.9)	0.256	2 (8.7)	0.610	2 (8.7)	1.000	5 (21.7)	0.260
H221Y	4 (66.7)	1.000	1 (16.7)	0.328	1 (16.7)	0.455	0 (0.0)	0.584
K101E/H	4 (36.4)	**0.011**	2 (18.2)	0.130	3 (27.3)	0.056	2 (18.2)	0.642
K103N/S	28 (71.8)	1.000	2 (5.1)	1.000	4 (10.3)	1.000	5 (12.8)	1.000
L100I	8 (72.7)	1.000	1 (9.1)	0.533	2 (18.2)	0.266	0 (0.0)	0.337
P225H	8 (100.0)	0.093	0 (0.0)	1.000	0 (0.0)	1.000	0 (0.0)	0.586
V108I	9 (75.0)	1.000	1 (8.3)	0.567	0 (0.0)	0.583	2 (16.7)	0.667
V179I/T	6 (100.0)	0.169	0 (0.0)	1.000	0 (0.0)	1.000	0 (0.0)	0.584
Y181C/I/V	8 (47.1)	**0.027**	3 (17.6)	0.052	3 (17.6)	0.179	3 (17.6)	0.687
Y188L	3 (75.0)	1.000	0 (0.0)	1.000	1 (25.0)	0.328	0 (0.0)	1.000
No. of NNRTI Mutations								
0	8 (66.7)	0.735	1 (8.3)	0.567	0 (0.0)	0.583	3 (25)	0.349
1	4 (80)	1.000	0 (0.0)	1.000	1 (20)	0.394	0 (0.0)	1.000
2	10 (71.4)	1.000	0 (0.0)	0.569	2 (14.3)	0.602	2 (14.3)	1.000
3	14 (82.4)	0.353	0 (0.0)	0.566	1 (5.9)	1.000	2 (11.8)	1.000
>=4	10 (58.8)	0.228	3 (17.6)	0.052	2 (11.8)	0.648	2 (11.8)	1.000
Polymorphisms								
K20R	16 (72.7)	1.000	3 (13.6)	0.109	2 (9.1)	1.000	1 (4.5)	0.152
L10I/V	16 (84.2)	0.148	0 (0.0)	0.313	1 (5.3)	0.662	2 (10.5)	1.000
K101A	4 (80.0)	1.000	0 (0.0)	1.000	0 (0.0)	1.000	1 (20.0)	0.538
S68G	36 (67.9)	0.484	0 (0.0)	1.000	1 (8.3)	1.000	1 (8.3)	1.000
T69I/N	5 (71.4)	1.000	0 (0.0)	1.000	1 (14.3)	1.000	1 (14.3)	1.000
V118/I	2 (50.0)	0.574	0 (0.0)	1.000	1 (25.0)	0.328	1 (25.0)	0.458
V179D/I	17 (85.0)	0.140	2 (10.0)	0.581	1 (5.0)	0.657	0 (0.0)	**0.048**
V90I	5 (71.4)	1.000	0 (0.0)	1.000	0 (0.0)	1.000	2 (28.6)	0.247
Dual Class Mutation	33 (68.8)	0.758	3 (6.2)	1.000	6 (12.5)	0.327	6 (12.5)	0.687
Presence of any mutation	39 (72.2)	0.406	3 (5.6)	0.533	6 (11.1)	0.313	6 (11.1)	0.171

Fisher's Exact Test

Although Fisher's exact test indicated a significant association between V179D/I polymorphism and subtype D, logistic regression did not support this association (Table 4.7)

Table 4.7: Association between subtypes and mutations in First line ART-experienced Individuals in Homa Bay County

	A1			A2			D		
	OR	95% CI.	p-value	OR	95% CI.	p-value	OR	95% CI.	p-value
K101E/H	0.16	0.04 - 0.65	0.010	5.78	0.72 - 46.42	0.099	1.49	0.27 - 8.38	0.649
Y181C/I/V	0.23	0.07 - 0.76	0.016	10.07	0.97 - 104.6	0.053	1.50	0.33 - 6.80	0.599
V179D/I	3.13	0.79 - 12.3	0.103	2.39	0.31 - 18.3	0.402	0.00	-	0.999
>=4 NNRTI Mutations	0.48	0.15-1.52	0.212	10.07	0.97-104.6	0.053	0.78	0.15-4.19	0.773

Logistic regression

Table 4.8: Association between subtypes and mutations in First line ART-experienced Individuals in Homa Bay County (controlling for Regimen)

	A1			A2			D		
	Adj. OR	95% CI.	p-value	Adj. OR	95% CI.	p-value	Adj. OR	95% CI.	p-value
K101E/H	0.14	0.03 - 0.60	0.008	5.96	0.69 – 51.50	0.105	1.72	0.28 – 10.6	0.560
Y181C/I/V	0.21	0.06 - 0.72	0.013	10.44	0.96 - 113.6	0.054	1.63	0.34 – 7.89	0.543
V179D	3.51	0.83 – 14.8	0.087	3.16	0.03 – 5.06	0.297	1	-	-
TDF/3TC/EFV									
AZT/3TC/NVP									
AZT/3TC/EFV									
OTHER									

Logistic regression, Controlling for Regimen

CHAPTER FIVE

DISCUSSION

5.1 Introduction

Homa Bay County is one of the highest HIV-burdened counties in Kenya and thus prone to high subtype diversity and dynamic changes due to inter-regional interactions, recombination of subtypes, and prolonged use of ART (Bbosa et al., 2019); it is therefore necessary to keep an updated list of the circulating HIV-1 subtypes. This study determined that subtype A1 is the most prevalent HIV-1 subtype in Homabay County, with subtype D following as the second most common. This is in agreement with studies that have previously been conducted in Kenya (Adungo et al., 2014; Arroyo et al., 2009; Hassan et al., 2018; Hué et al., 2012; Khamadi et al., 2005; Khoja et al., 2008; Nyagaka et al., 2012; Yang et al., 2004; Onywera et al., 2017). This confirms that these two subtypes remain the predominant subtypes that are consistently being spread in Kenya. A marginal frequency of Subtype A2 which has been reported in western Kenya and the Rift Valley (Adungo et al., 2014; Nyagaka et al., 2012) was also observed in this study.

In contrast to this study, research by Adungo et al., 2014, conducted in western Kenya found occurrences of HIV-1 subtypes C and G. A separate investigation carried out in 2012 in the Ndhiwa sub-county of Homa Bay reported a marginal occurrence of these subtypes (Onywera et al., 2017). The current study, however, did not observe any occurrence of these two subtypes; instead, a rare recombinant subtype CD was observed. Despite the differences in the populations studied in the mentioned studies, this may be suggestive of the diminishing frequency of pure subtype C and G, with their residuals inform of recombinants, this study also reports a decline in

subtype D compared to the findings of Onywera et al., 2017. However, further studies are required to confirm this.

This study further found occurrence of recombinant subtypes, predominantly A1D, consistent with other studies conducted in Kenya (Adungo et al., 2014; Khoja et al., 2008; Nyagaka et al., 2012) and understandably so given that the dominant subtypes in the locality are A1 and D. This indicates that the dominant subtypes A and D could slowly evolve and diverge into new strains. Recombinants have been observed in increasing prevalence, particularly in regions with multiple subtypes, as is the case in Homa Bay, and in major cities (Bbosa et al., 2019; Gounder et al., 2017). In Kelantan, Malaysia, the predominant HIV subtype identified was the recombinant CRF33_01B, overtaking pure subtype B that was prevalent previously (Mohamad et al., 2012). The presence of recombinants can be largely indicative of recombination between pure subtypes of dually infected patients but can also be due to independent infections of already recombined strains (Bello et al., 2010; Templeton et al., 2009). Recombinants, mainly those relating to subtype D as is the case in this study, have been reported to increase mother-to-child HIV transmission and hasten disease progression (Lihana, Khamadi, Lwembe, et al., 2009). This may partly explain the high number of people with advanced disease in Homa Bay County. However, all the recombinants may not have been detected since some may have parental sequences that are genetically too similar to distinguish (Rousseau et al., 2007) and whereas a common subtyping methodology was used, there may be recombination in the other regions of the genome that may have been missed, given that this study targeted only the pol gene. It is important to establish the prevalence of dual infections to define fully the major reason driving the epidemiology of recombinants, especially in high HIV-burdened areas like Homa Bay

County, as this will guide policies to curb the increase in recombinants that can pose difficulties in the management of HIV-1.

The presence of drug resistance in HIV continues to be a significant impediment to attaining and sustaining effective viral suppression. This study sought to establish the association between HIV-1 subtypes and drug resistance-associated mutations and polymorphisms. As already reported elsewhere (Bossard et al., 2021), there is a high prevalence of HIV-1 resistance to NRTI and NNRTI among individuals with advanced disease in Homa Bay County. Overall, NRTI drug resistance-associated mutations had no significant association with the HIV-1 subtypes in this locality, and whereas mutation Y115F and V75M were observed in high frequencies in subtype A1 compared to other subtypes, this did not overtly indicate any association. This is unlike the findings of Chaplin et al., 2019, which, though considering different subtypes among infants in Nigeria, found a significant association with NRTI mutations 67N, 70R, >=1TAM-1, >=1 TAM-2 or >=1NRTI mutation and M184I/V more likely to appear in subtype GWA-II/GCA compared to subtype CRF02_AG.

With regard to NNRTI mutations, this study determined a negative association between HIV-1 subtype A1 and K101E/H and Y181C/I/V mutations. The K101E mutation is a non-polymorphic mutation that is specifically observed in patients undergoing treatment with any of the NNRTIs, , normally occurring together with other NNRTI-resistance mutations (Reuman et al., 2010; Rimsky et al., 2012; Tambuyzer et al., 2010). The presence of the K101E mutation alone leads to a 3 to 10 fold decrease in susceptibility to NVP and a 1 to 5-fold decrease in susceptibility to EFV (Melikian et al., 2014; Rimsky et al., 2012). K101H is selected in patients taking NVP, EFV, and ETR (Reuman et al., 2010; Tambuyzer et al., 2010), when occurring with other NNRTI-resistance mutations, the presence of K101E is associated with diminished susceptibility

to both NVP and EFV (Melikian et al., 2014). The non-polymorphic Y181C mutation is selected primarily by NVP usage. (Reuman et al., 2010), ETR (Tambuyzer et al., 2010), and RPV (Rimsky et al., 2012) resulting to a reduction in NVP susceptibility of over 50-fold and approximately a 2-fold decrease in EFV susceptibility (Rhee et al., 2004). Y181I/V is selected by NVP and ETR (Tambuyzer et al., 2010) and confers high-level resistance to NVP (Azijn et al., 2010).

This study reports that mutation K101E/H and Y181C/I/V were less likely to be observed in HIV-1 subtype A1 than in the other subtypes. This agrees with an investigation carried out in sub-Saharan African countries in programs without routine viral load (VL) monitoring where mutations E138A, V106M, K101E and Y181C were more common in subtypes C and D than in subtype A1 (Kityo et al., 2017). The findings of (Chaplin et al., 2019) found no association between NNRTI mutations and HIV-1 subtypes among infants in Nigeria but noted an association with NRTI. In a facility-based study on patients with virological failure, Ayitewala et al., (2020) did not find any significant association between drug resistance mutation profiles with HIV-1 subtypes and submits that accumulation of resistance mutations is possibly due to treatment regimen and not subtypes. However, when adjusted for regimen, this study still demonstrated an association between subtype A1 and mutations K101E/H and Y181C/I/V. The difference may be attributed to the fact that Ayitewala et al., (2020) tested the association between overall drug resistance mutation profiles and subtypes as opposed to individual resistance mutations as tested in this study. The findings of this study agree with Hamers et al., 2012 and Venner et al., 2016 which argues that there is an association between HIV-1 subtypes and resistance mutations and polymorphisms.

As per the WHO guidelines (World Health Organization, 2019) countries in which resistance to ART is above 10%, an alternative first-line therapy that does not contain EFV and NVP, preferably DTG should be used, for this reason, therefore Kenya incorporated the use of DTG based first-line therapy in place of NVP and EFV (National AIDS and STI Control Programme, 2018). EFV, considered superior to NVP in effectiveness (Bock et al., 2013) EFV is still recommended in Kenya as an alternative first-line drug for those who cannot tolerate Dolutegravir (DTG), the anchor drug of the current preferred first-line regimen. NVP is also an alternative first-line drug for infants (Nationa AIDS and STI Control Program, 2022). The findings of this study that K101E/H and Y181C/I/V which hamper EFV and NVP susceptibility were less likely to be observed in HIV-1 subtype A1, which is the most prevalent subtype in Kenya, would imply that in areas where subtype A is common, the use of NVP and EFV might have a lower risk of being compromised. However, more studies on subtype-resistance mutation association would further elucidate treatment response to specific antiretroviral medications based on HIV subtype and define the clinical implications of the findings.

The presence of certain natural polymorphisms can exacerbate HIV-1 drug resistance by affecting the virus's susceptibility to ART, the level of resistance conferred by key mutations, and the likelihood of acquiring resistance mutations. In this study, although a significant association was observed between subtype D and polymorphism V179D/I using Fishers' exact test, the logistic regression test did not support this association and therefore this study does not overtly indicate an association between drug resistance-associated polymorphisms and HIV-1 subtypes that were considered in this analysis. A study conducted by Kantor et al., 2005 that focussed on subtype B in comparison to other subtypes did not equally determine an association between HIV-1 subtypes and polymorphisms associated with drug resistance, despite observing a

high frequency of polymorphisms in non-subtype B subtypes than in B subtypes. Nevertheless, Santoro & Perno, 2013 hold that genotypic diversity in the HIV-1 subtype-specific polymorphisms may lead to both emergency of drug-resistance mutations and varied responses to drugs, pointing to an association between polymorphisms and HIV-1 subtypes. There is a need to explore further the possibility of this association with a larger data set.

5.2 Limitations

The sample size was relatively small, especially for non-subtype A1, which limits statistical power. However, the sample is considered representative and useful to shed more light on subtype-specific resistance mutations

Our study targeted only the Protease and Reverse transcriptase regions within the HIV-1 pol gene; it is possible that recombinants in other regions that were not targeted will have been missed.

The study was conducted in a single location, so the findings may not be overtly generalizable to other populations.

Further, the study was cross-sectional, so it cannot establish cause-and-effect relationships.

CHAPTER SIX

CONCLUSION

6.1 Introduction

HIV-1 subtype A1 is still the most prevalent subtype in Homa Bay County followed by subtype D, all the recombinant subtypes had recombination with subtype D, which could pose a challenge to treatment as recombinants with subtype D have been said to lead to increased mother-to-child transmission and disease progression.

There was no statistically significant association observed between HIV-1 first-line resistance-associated polymorphisms, NRTI resistance mutations and HIV-1 subtypes

The study demonstrates a negative association between HIV-1 subtype A1 and NNRTI resistance-associated mutations K101E/H and Y181C/I/V; this would imply that in areas where subtype A is common, the use of NVP and EFV might have a lower risk of being compromised.

This study validates the existence of variations in treatment response to specific antiretroviral therapies based on HIV-1 subtypes, which underscores the implication of subtype-specific causes of susceptibility to antiretroviral treatment and immune response.

6.2 Recommendations for the Study

Considering the high risk of mother-to-child transmission and disease progression associated with recombinants involving subtype D; it is important to implement tailored prevention strategies. This may involve intensified efforts to prevent vertical transmission of HIV, such as promoting antenatal care, offering HIV testing and counselling to pregnant women, and ensuring access to antiretroviral therapy for HIV-positive pregnant women.

The present study adds to the growing evidence affirming an association between HIV-1 subtypes and drug-resistance mutations. This implies that there may be a need to tailor the choice of antiretroviral therapy to the HIV-1 subtype.

Continued surveillance and monitoring of HIV-1 subtypes and drug-resistance mutations are essential. This information can help healthcare providers and public health officials to understand better the prevalence and distribution of specific subtypes as well as the associated resistance mutations in different geographic regions or populations. Regular monitoring can aid in detecting emerging patterns of drug resistance and guide treatment decisions.

The study findings suggest that NVP and EFV, which belong to the NNRTI class, may be a preferred choice in regions where subtype A1 is common due to the lower prevalence of the K101E/H and Y181C/I/V mutations.

6.3 Recommendations for future studies

The significant persistence of recombinant presence calls for studies to define the main cause of HIV-1 recombinants in Homa Bay County; whether it is due to infection with recombined variants or dual infections or co-infections.

Future studies with larger sample sizes and longitudinal designs are warranted to confirm the study findings and to further investigate the clinical impact of these subtype-specific differences. These investigations will provide valuable insights for optimizing treatment strategies and improving patient outcomes in the management of HIV-1 infections.

More studies should be conducted to understand the underlying mechanisms driving the association, including factors such as viral replication dynamics, host immune response, and treatment history. Such research can inform the development of more effective prevention and treatment strategies to tackle drug resistance.

REFERENCE

Abram, M. E., Ferris, A. L., Shao, W., Alvord, W. G., & Hughes, S. H. (2010). Nature, Position, and Frequency of Mutations Made in a Single Cycle of HIV-1 Replication. *Journal of Virology*, *84*(19), 9864–9878. https://doi.org/10.1128/jvi.00915-10

Adungo, F. O., Gicheru, M. M., Adungo, N. I., Matilu, M. M., Lihana, R. W., & Khamadi, S. A. (2014). Diversity of Human Immunodeficiency Virus Type-1 Subtypes in Western Kenya. *World Journal of AIDS*, *04*(04), 365–372. https://doi.org/10.4236/wja.2014.44043

Antinori, A., Zaccarell, M., Cingolani, A., Forbici, F., Rizzo, M. G., Trotta, M. P., Giambenedetto, S. Di, Narciso, P., Ammassari, A., Girardi, E., De Luca, A., & Perno, C. F. (2002). Cross-resistance among nonnucleoside reverse transcriptase inhibitors limits recycling efavirenz after nevirapine failure. *AIDS Research and Human Retroviruses*, *18*(12), 835–838. https://doi.org/10.1089/08892220260190308

Ariën, K. K., Abraha, A., Quiñones-Mateu, M. E., Kestens, L., Vanham, G., & Arts, E. J. (2005). The Replicative Fitness of Primary Human Immunodeficiency Virus Type 1 (HIV-1) Group M, HIV-1 Group O, and HIV-2 Isolates. *Journal of Virology*, *79*(14), 8979–8990. https://doi.org/10.1128/jvi.79.14.8979-8990.2005

Arion, D., Kaushik, N., McCormick, S., Borkow, G., & Parniak, M. A. (1998). Phenotypic mechanism of HIV-1 resistance to 3'-azido-3'-deoxythymidine (AZT): Increased polymerization processivity and enhanced sensitivity to pyrophosphate of the mutant viral reverse transcriptase. *Biochemistry*, *37*(45), 15908–15917. https://doi.org/10.1021/bi981200e

Arroyo, M. A., Sateren, W. B., Foglia, G., Kibaya, R., Langat, L., Wasunna, M., Bautista, C. T., Scott, P. T., Shaffer, D. N., Robb, M. L., Michael, N. L., Birx, D. L., & McCutchan, F. E. (2009). Short communication: HIV type 1 genetic diversity among tea plantation workers in Kericho, Kenya. *AIDS Research and Human Retroviruses*, *25*(11), 1061–1064. https://doi.org/10.1089/aid.2009.0092

Arts, E. J., & Hazuda, D. J. (2012). HIV-1 antiretroviral drug therapy. *Cold Spring Harbor Perspectives in Medicine*, *2*(4). https://doi.org/10.1101/cshperspect.a007161

Ayitewala, A., Kyeyune, F., Ainembabazi, P., Nabulime, E., Kato, C. D., & Nankya, I. (2020). Comparison of HIV drug resistance profiles across HIV-1 subtypes A and D for patients receiving a tenofovir-based and zidovudine-based first line regimens in Uganda. *AIDS Research and Therapy*, *17*(1), 1–9. https://doi.org/10.1186/s12981-020-0258-7

Azijn, H., Tirry, I., Vingerhoets, J., De Béthune, M. P., Kraus, G., Boven, K., Jochmans, D., Van Craenenbroeck, E., Picchio, G., & Rimsky, L. T. (2010). TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. *Antimicrobial Agents and Chemotherapy*, *54*(2), 718–727. https://doi.org/10.1128/AAC.00986-09

Bbosa, N., Kaleebu, P., & Ssemwanga, D. (2019). HIV subtype diversity worldwide. *Current Opinion in HIV and AIDS*, *14*(3), 153–160. https://doi.org/10.1097/COH.0000000000000534

Beck, I. A., Levine, M., McGrath, C. J., Bii, S., Milne, R. S., Kingoo, J. M., So, I., Andersen, N., Dross, S., Coombs, R. W., Kiarie, J., Chohan, B., Sakr, S. R., Chung, M. H., & Frenkel, L. M. (2020). Pre-treatment HIV-drug resistance associated with virologic outcome of first-line NNRTI-antiretroviral therapy: A cohort study in Kenya. *EClinicalMedicine*, *18*. https://doi.org/10.1016/j.eclinm.2019.100239

Bello, G., Aulicino, P. C., Ruchansky, D., Guimarães, M. L., Lopez-Galindez, C., Casado, C., Chiparelli, H., Rocco, C., Mangano, A., Sen, L., & Morgado, M. G. (2010). Phylodynamics of HIV-1 Circulating Recombinant Forms 12_BF and 38_BF in Argentina and Uruguay. *Retrovirology*, *7*, 1–9. https://doi.org/10.1186/1742-4690-7-22

Bock, P., Fatti, G., & Grimwood, A. (2013). Comparing the effectiveness of efavirenz and nevirapine for first-line antiretroviral therapy in a South African multicentre cohort. *International Health*, *5*(2), 132–138. https://doi.org/10.1093/inthealth/iht002

Bossard, C., Schramm, B., Wanjala, S., Jain, L., Mucinya, G., Opollo, V., Wiesner, L., van Cutsem, G., Poulet, E., Szumilin, E., Ellman, T., & Maman, D. (2021). High Prevalence of NRTI and NNRTI Drug Resistance Among ART-Experienced, Hospitalized Inpatients. *Journal of Acquired Immune Deficiency Syndromes (1999)*, *87*(3), 883–888. https://doi.org/10.1097/QAI.0000000000002689

Brenner, B., Turner, D., Oliveira, M., Moisi, D., Detorio, M., Carobene, M., Marlink, R. G., Schapiro, J., Roger, M., & Wainberg, M. A. (2003). A V106M mutation in HIV-1 clade C viruses exposed to efavirenz confers cross-resistance to non-nucleoside reverse transcriptase inhibitors. *Aids*, *17*(1). https://doi.org/10.1097/00002030-200301030-00001

Briz, V., Poveda, E., & Soriano, V. (2006). HIV entry inhibitors: Mechanisms of action and resistance pathways. *Journal of Antimicrobial Chemotherapy*, *57*(4), 619–627. https://doi.org/10.1093/jac/dkl027

Bruner, K. M., Murray, A. J., Pollack, R. A., Soliman, M. G., Sarah, B., Capoferri, A. A., Lai, J., Strain, M. C., Lada, S. M., Ho, Y., Richman, D. D., Deeks, S. G., Siliciano, J. D., & Siliciano, R. F. (2017). Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med.*, *22*(9), 1043–1049. https://doi.org/10.1038/nm.4156

Buonaguro, L., Tornesello, M. L., & Buonaguro, F. M. (2007). Human Immunodeficiency Virus Type 1 Subtype Distribution in the Worldwide Epidemic: Pathogenetic and Therapeutic Implications. *Journal of Virology*, *81*(19), 10209–10219. https://doi.org/10.1128/jvi.00872-07

Castro-Nallar, E., Pérez-Losada, M., Burton, G. F., & Crandall, K. A. (2012). The evolution of HIV: Inferences using phylogenetics. *Molecular Phylogenetics and Evolution*, *62*(2), 777–792. https://doi.org/10.1016/j.ympev.2011.11.019

Chaplin, B., Akanmu, A. S., Inzaule, S. C., Samuels, J. O., Okonkwo, P., Ilesanmi, O., Adewole, I. F. A., Asadu, C., Khamofu, H., Mpazanje, R., Ndembi, N., Odafe, S., Sigaloff, K. C. E., Ngige, E. N., Abatta, E. O., Akinbiyi, G., Dakum, P., Rinke De Wit, T. F., & Kanki, P. (2019). Association between HIV-1 subtype and drug resistance in Nigerian infants. *Journal of Antimicrobial Chemotherapy*, *74*(1), 172–176. https://doi.org/10.1093/jac/dky380

Cochran. (1977). *Cochran_1977_Sampling_Techniques__Third_Edition.pdf*.

Fransen, S., Gupta, S., Danovich, R., Hazuda, D., Miller, M., Witmer, M., Petropoulos, C. J., & Huang, W. (2009). Loss of Raltegravir Susceptibility by Human Immunodeficiency Virus Type 1 Is Conferred via Multiple Nonoverlapping Genetic Pathways. *Journal of Virology*, *83*(22), 11440–11446. https://doi.org/10.1128/jvi.01168-09

Ghiasvand, H., Waye, K. M., Noroozi, M., Harouni, G. G., Armoon, B., & Bayani, A. (2019). Clinical determinants associated with quality of life for people who live with HIV/AIDS: A Meta-analysis. *BMC Health Services Research*, *19*(1), 1–11. https://doi.org/10.1186/s12913-019-4659-z

Giovanetti, M., Ciccozzi, M., Parolin, C., & Borsetti, A. (2020). Molecular epidemiology of hiv-1 in african countries: A comprehensive overview. *Pathogens*, *9*(12), 1–10. https://doi.org/10.3390/pathogens9121072

Gounder, K., Oyaro, M., Padayachi, N., Zulu, T. M., De Oliveira, T., Wylie, J., & Ndung'u, T. (2017). Complex subtype diversity of HIV-1 among drug users in Major Kenyan cities. *AIDS Research and Human Retroviruses*, *33*(5), 500–510. https://doi.org/10.1089/aid.2016.0321

Grossman, Z., Paxinos, E. E., Averbuch, D., Maayan, S., Parkin, N. T., Engelhard, D., Lorber, M., Istomin, V., Shaked, Y., Mendelson, E., Ram, D., Petropoulos, C. J., & Schapiro, J. M. (2004). Mutation D30N is not preferentially selected by human immunodeficiency virus type 1 subtype C in the development of resistance to nelfinavir. *Antimicrobial Agents and Chemotherapy*, *48*(6), 2159–2165. https://doi.org/10.1128/AAC.48.6.2159-2165.2004

Gupta, R. K., Kohli, A., Mccormick, A. L., & Towers, G. J. (2011). Full length HIV-1 Gag determines protease inhibitor susceptibility within in vitro assays. *AIDS*, *24*(11), 1651–1655.

Hamers, R. L., Oyomopito, R., Kityo, C., Phanuphak, P., Siwale, M., Sungkanuparph, S., Conradie, F., Kumarasamy, N., Botes, M. E., Sirisanthana, T., Abdallah, S., Li, P. C. K., Ngorima, N., Kantipong, P., Osibogun, A., Lee, C. K. C., Stevens, W. S., Kamarulzaman, A., Derdelinckx, I., … Zhou, J. (2012). Cohort profile: The PharmAccess African (PASER-M) and the TREAT Asia (TASER-M) monitoring studies to evaluate resistance-HIV drug resistance in sub-Saharan Africa and the Asia-Pacific. *International Journal of Epidemiology*, *41*(1), 43–54. https://doi.org/10.1093/ije/dyq192

Hamers, R. L., Wallis, C. L., Kityo, C., Siwale, M., Mandaliya, K., Conradie, F., Botes, M. E., Wellington, M., Osibogun, A., Sigaloff, K. C. E., Nankya, I., Schuurman, R., Wit, F. W., Stevens, W. S., van Vugt, M., & de Wit, T. F. R. (2011). HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: A multicentre observational study. *The Lancet Infectious Diseases*, *11*(10), 750–759. https://doi.org/10.1016/S1473-3099(11)70149-9

Hassan, A. S., Esbjörnsson, J., Wahome, E., Thiong'o, A., Makau, G. N., Price, M. A., & Sanders, E. J. (2018). HIV-1 subtype diversity, transmission networks and transmitted drug resistance amongst acute and early infected MSM populations from Coastal Kenya. *PLoS ONE*, *13*(12), 4–16. https://doi.org/10.1371/journal.pone.0206177

Hawkins, C. A., Chaplin, B., Idoko, J., Ekong, E., Adewole, I., Gashau, W., Murphy, R. L., & Kanki, P. (2009). Clinical and genotypic findings in HIV-infected patients with the k65r mutation failing first-line antiretroviral therapy in Nigeria. *Journal of Acquired Immune Deficiency Syndromes*, *52*(2), 228–234. https://doi.org/10.1097/QAI.0b013e3181b06125

Hazuda, D. J., Anthony, N. J., Gomez, R. P., Jolly, S. M., Wai, J. S., Zhuang, L., Fisher, T. E., Embrey, M., Guare, J. P., Egbertson, M. S., Vacca, J. P., Huff, J. R., Felock, P. J., Witmer, M. V., Stillmock, K. A., Danovich, R., Grobler, J., Miller, M. D., Espeseth, A. S., … Young, S. D. (2004). A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(31), 11233–11238. https://doi.org/10.1073/pnas.0402357101

Hemelaar, J. (2012). The origin and diversity of the HIV-1 pandemic. *Trends in Molecular Medicine*, *18*(3), 182–192. https://doi.org/10.1016/j.molmed.2011.12.001

Hemelaar, J., Elangovan, R., Yun, J., Dickson-Tetteh, L., Fleminger, I., Kirtley, S., Williams, B., Gouws-Williams, E., Ghys, P. D., Abimiku, A. G., Agwale, S., Archibald, C., Avidor, B., Barbás, M. G., Barre-Sinoussi, F., Barugahare, B., Belabbes, E. H., Bertagnolio, S., Birx, D., … Zhang, R. (2019). Global and regional molecular epidemiology of HIV-1, 1990–2015: a systematic review, global survey, and trend analysis. *The Lancet Infectious Diseases*, *19*(2), 143–155. https://doi.org/10.1016/S1473-3099(18)30647-9

HIVinfo.NIH.gov. (2021). *Stages of HIV*. https://hivinfo.nih.gov/understanding-hiv/fact-sheets/stages-hiv-infection#:~:text=The three stages of HIV,one stage to the next.

Hosseinipour, M. C., Van Oosterhout, J. J. G., Weigel, R., Phiri, S., Kamwendo, D., Parkin, N., Fiscus, S. A., Nelson, J. A. E., Eron, J. J., & Kumwenda, J. (2009). The public health approach to identify antiretroviral therapy failure: High-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy. *Aids*, *23*(9), 1127–1134. https://doi.org/10.1097/QAD.0b013e32832ac34e

Hué, S., Hassan, A. S., Nabwera, H., Sanders, E. J., Pillay, D., Berkley, J. A., & Cane, P. A. (2012). HIV type 1 in a rural coastal town in Kenya shows multiple introductions with many subtypes and much recombination. *AIDS Research and Human Retroviruses*, *28*(2), 220–224. https://doi.org/10.1089/aid.2011.0048

Inzaule, S., Yang, C., Kasembeli, A., Nafisa, L., Okonji, J., Oyaro, B., Lando, R., Mills, L. A., Laserson, K., Thomas, T., Nkengasong, J., & Zeh, C. (2013). Field evaluation of a broadly sensitive HIV-1 In-house genotyping assay for use with both plasma and dried blood spot specimens in a resource-limited country. *Journal of Clinical Microbiology*, *51*(2), 529–539. https://doi.org/10.1128/JCM.02347-12

Kageha, S., Lihana, R. W., Okoth, V., Mwau, M., Okoth, F. A., Songok, E. M., Ngaira, J. M., & Khamadi, S. A. (2012). HIV type 1 subtype surveillance in central Kenya. *AIDS Research and Human Retroviruses*, *28*(2), 228–231. https://doi.org/10.1089/aid.2011.0089

Kantor, R., Katzenstein, D. A., Efron, B., Carvalho, A. P., Wynhoven, B., Cane, P., Clarke, J., Sirivichayakul, S., Soares, M. A., Snoeck, J., Pillay, C., Rudich, H., Rodrigues, R., Holguin, A., Ariyoshi, K., Bouzas, M. B., Cahn, P., Sugiura, W., Soriano, V., … Shafer, R. W. (2005). Impact of HIV-1 subtype and antiretroviral therapy on protease and reverse transcriptase genotype: Results of a global collaboration. *PLoS Medicine*, *2*(4), 0325–0337. https://doi.org/10.1371/journal.pmed.0020112

Kenya|UNAIDS. (2022). *Kenya | UNAIDS*. https://www.unaids.org/en/regionscountries/countries/kenya

Khamadi, S. A., Ochieng, W., Lihana, R. W., Kinyua, J., Muriuki, J., Mwangi, J., Lwembe, R., Kiptoo, M., Osman, S., Lagat, N., Pelle, R., Muigai, A., Carter, J. Y., Oishi, I., Ichimura, H., Mwaniki, D. L., Okoth, F. A., Mpoke, S., & Songok, E. M. (2005). HIV type 1 subtypes in circulation in Northern Kenya. *AIDS Research and Human Retroviruses*, *21*(9), 810–814. https://doi.org/10.1089/aid.2005.21.810

Khoja, S., Ojwang, P., Khan, S., Okinda, N., Harania, R., & Ali, S. (2008). Genetic analysis of HIV-1 subtypes in Nairobi, Kenya. *PLoS ONE*, *3*(9). https://doi.org/10.1371/journal.pone.0003191

Kityo, C., Thompson, J., Nankya, I., Hoppe, A., Ndashimye, E., Warambwa, C., Mambule, I., Van Oosterhout, J. J., Kara Wools-Kaloustian, ¶, Bertagnolio, S., Easterbrook, P. J., Mugyenyi, P., Walker, A. S., Paton, N. I., Paton, N., & Lin, Y. L. (2017). HIV Drug Resistance Mutations in Non-B Subtypes After Prolonged Virological Failure on NNRTI-Based First-Line Regimens in Sub-Saharan Africa for the Europe Africa Research Network for Evaluation of Second-line Therapy (EARNEST) Trial Team. In *J Acquir Immune Defic Syndr* (Vol. 75). www.jaids.com%7Ce45

Koning, F. A., Castro, H., Dunn, D., Tilston, P., Cane, P. A., & Mbisa, J. L. (2013). Subtype-specific differences in the development of accessory mutations associated with high-level resistance to hiv-1 nucleoside reverse transcriptase inhibitors. *Journal of Antimicrobial Chemotherapy*, *68*(6), 1220–1236. https://doi.org/10.1093/jac/dkt012

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Kuritzkes, D. R. (2011). Drug resistance in HIV-1. *Current Opinion in Virology*, *1*(6), 582–589. https://doi.org/10.1016/j.coviro.2011.10.020

Lessells, R. J., Katzenstein, D. K., & Oliveira, T. De. (2012). Europe PMC Funders Group Are subtype differences important in HIV drug resistance? *Current Opinion in Virology*, *2*(5), 636–643. https://doi.org/10.1016/j.coviro.2012.08.006.Are

Lihana, R. W., Khamadi, S. A., Lubano, K., Lwembe, R., Kiptoo, M. K., Lagat, N., Kinyua, J. G., Okoth, F. A., Songok, E. M., Makokha, E. P., & Ichimura, H. (2009). HIV type 1 subtype diversity and drug resistance among HIV Type 1-infected kenyan patients initiating antiretroviral therapy. *AIDS Research and Human Retroviruses*, *25*(12), 1211–1217. https://doi.org/10.1089/aid.2009.0007

Lihana, R. W., Khamadi, S. A., Lwembe, R. M., Ochleng, W., Kinyua, J. G., Kiptoo, M. K., Muriuki, J. K., Lagat, N., Osman, S., Mwangi, J. M., Okoth, F. A., & Songok, E. M. (2009). The changing trend of HIV type 1 subtypes in Nairobi. *AIDS Research and Human Retroviruses*, *25*(3), 337–342. https://doi.org/10.1089/aid.2008.0228

Lihana, R. W., Ssemwanga, D., Abimiku, A., & Ndembi, N. (2012). Update on HIV-1 diversity in Africa: A decade in review. *AIDS Reviews*, *14*(2), 83–100.

Lisovsky, I., Schader, S. M., Martinez-Cajas, J. L., Oliveira, M., Moisi, D., & Wainberg, M. A. (2010). HIV-1 protease codon 36 polymorphisms and differential development of resistance to nelfinavir, lopinavir, and atazanavir in different HIV-1 subtypes. *Antimicrobial Agents and Chemotherapy*, *54*(7), 2878–2885. https://doi.org/10.1128/AAC.01828-09

Los Alamos HIV Database. (n.d.). Retrieved July 14, 2023, from https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html

Mackie, N. E., Phillips, A. N., Kaye, S., Booth, C., & Geretti, A. M. (2010). Antiretroviral drug resistance in HIV-1-infected patients with low-level viremia. In *Journal of Infectious Diseases* (Vol. 201, Issue 9, pp. 1303–1307). https://doi.org/10.1086/651618

Maldarelli, F., Kearney, M., Palmer, S., Stephens, R., Mican, J., Polis, M. A., Davey, R. T., Kovacs, J., Shao, W., Rock-Kress, D., Metcalf, J. A., Rehm, C., Greer, S. E., Lucey, D. L., Danley, K., Alter, H., Mellors, J. W., & Coffin, J. M. (2013). HIV Populations Are Large and Accumulate High Genetic Diversity in a Nonlinear Fashion. *Journal of Virology*, *87*(18), 10313–10323. https://doi.org/10.1128/jvi.01225-12

Martínez-Cajas, J. L., Pant-Pai, N., Klein, M. B., & Wainberg, M. A. (2008). Role of genetic diversity amongst HIV-1 non-B subtypes in drug resistance: A systematic review of virologic and biochemical evidence. *AIDS Reviews*, *10*(4), 212–223.

Melikian, G. L., Rhee, S. Y., Varghese, V., Porter, D., White, K., Taylor, J., Towner, W., Troia, P., Burack, J., DeJesus, E., Robbins, G. K., Razzeca, K., Kagan, R., Liu, T. F., Jeffrey Fessel, W., Israelski, D., & Shafer, R. W. (2014). Non-nucleoside reverse transcriptase inhibitor (NNRTI) cross-resistance: Implications for preclinical evaluation of novel NNRTIs and clinical genotypic resistance testing. *Journal of Antimicrobial Chemotherapy*, *69*(1), 12–20. https://doi.org/10.1093/jac/dkt316

Melku, M., Gesesew, H. A., & Ward, P. R. (2022). Magnitude and predictors of HIV-Drug resistance in Africa: A protocol for systematic review and meta-analysis. *PLoS ONE*, *17*(4 April), 1–10. https://doi.org/10.1371/journal.pone.0267159

Mohamad, S., Deris, Z. Z., Yusoff, N. K., Ariffin, T. A. A. T. M., & Shueb, R. H. (2012). Assessing subtypes and drug resistance mutations among HIV-1 infected children who failed antiretroviral therapy in Kelantan, Malaysia. *Brazilian Journal of Infectious Diseases*, *16*(3), 284–288. https://doi.org/10.1590/s1413-86702012000300012

Nationa AIDS and STI Control Program. (2022). *Kenya HIV Prevention and Treatment Guidelines, 2022*.

National AIDS and STI Control Programme. (2018). Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya 2018 Edition. *Nascop*.

National AIDS and STI Control Programme (NASCOP). (2018). Kenphia 2018 Preliminary Report. *Nascop*, 1–26.

Ndembi, N., Iwamoto, S., Ngansop, C., Lemey, P., Abimiku, A., Mbanya, D., Kaptue, L. N., & Ido, E. (2011). High frequency of HIV-1 dual infections in cameroon, West Central Africa. *Journal of Acquired Immune Deficiency Syndromes*, *57*(2), 84–92. https://doi.org/10.1097/QAI.0b013e3182142082

Nyagaka, B., Kiptoo, M. K., Lihana, R. W., Khamadi, S. A., Makokha, E. P., Kinyua, J. G., Mwangi, J., Osman, S., Lagat, N. J., Muriuki, J., Okoth, V., Gicheru, M., Ng'ang'a, Z., & Songok, E. M. (2012). HIV type 1 gag genetic diversity among antenatal clinic attendees in North Rift Valley, Kenya. *AIDS Research and Human Retroviruses*, *28*(5), 523–526. https://doi.org/10.1089/aid.2011.0223

Onywera, H., Maman, D., Inzaule, S., Auma, E., Were, K., Fredrick, H., Owiti, P., Opollo, V., Etard, J. F., Mukui, I., Kim, A. A., & Zeh, C. (2017). Surveillance of HIV-1 pol transmitted drug resistance in acutely and recently infected antiretroviral drug-naïve persons in rural western Kenya. *PLoS ONE*, *12*(2). https://doi.org/10.1371/journal.pone.0171124

Ousley, J., Niyibizi, A. A., Wanjala, S., Vandenbulcke, A., Kirubi, B., Omwoyo, W., Price, J., Salumu, L., Szumilin, E., Spiers, S., Van Cutsem, G., Mashako, M., Mangana, F., Moudarichirou, R., Harrison, R., Kalwangila, T., Lumowo, G., Lambert, V., & Maman, D. (2018). High Proportions of Patients with Advanced HIV Are Antiretroviral Therapy Experienced: Hospitalization Outcomes from 2 Sub-Saharan African Sites. *Clinical Infectious Diseases*, *66*(Suppl 2), S126–S132. https://doi.org/10.1093/cid/ciy103

Reuman, E. C., Rhee, S. Y., Holmes, S. P., & Shafer, R. W. (2010). Constrained patterns of covariation and clustering of HIV-1 non-nucleoside reverse transcriptase inhibitor resistance mutations. *Journal of Antimicrobial Chemotherapy*, *65*(7), 1477–1485. https://doi.org/10.1093/jac/dkq140

Rhee, S. Y., Liu, T., Ravela, J., Gonzales, M. J., & Shafer, R. W. (2004). Distribution of human immunodeficiency virus type 1 protease and reverse transcriptase mutation patterns in 4,183 persons undergoing genotypic resistance testing. *Antimicrobial Agents and Chemotherapy*, *48*(8), 3122–3126. https://doi.org/10.1128/AAC.48.8.3122-3126.2004

Rimsky, L., Vingerhoets, J., Van Eygen, V., Eron, J., Clotet, B., Hoogstoel, A., Boven, K., & Picchio, G. (2012). Genotypic and phenotypic characterization of HIV-1 isolates obtained from patients on rilpivirine therapy experiencing virologic failure in the phase 3 ECHO and THRIVE studies: 48-week analysis. *Journal of Acquired Immune Deficiency Syndromes*, *59*(1), 39–46. https://doi.org/10.1097/QAI.0b013e31823df4da

Rousseau, C. M., Learn, G. H., Bhattacharya, T., Nickle, D. C., Heckerman, D., Chetty, S., Brander, C., Goulder, P. J. R., Walker, B. D., Kiepiela, P., Korber, B. T., & Mullins, J. I. (2007). Extensive Intrasubtype Recombination in South African Human Immunodeficiency Virus Type 1 Subtype C Infections. *Journal of Virology*, *81*(9), 4492–4500. https://doi.org/10.1128/jvi.02050-06

Santoro, M. M., & Perno, C. F. (2013). HIV-1 Genetic Variability and Clinical Implications. *ISRN Microbiology*, *2013*, 1–20. https://doi.org/10.1155/2013/481314

Santos, A. F. A., Tebit, D. M., Lalonde, M. S., Abecasis, A. B., Ratcliff, A., Camacho, R. J., Diaz, R. S., Herchenröder, O., Soares, M. A., & Arts, E. J. (2012). Effect of natural polymorphisms in the HIV-1 CRF02-AG protease on protease inhibitor hypersusceptibility. *Antimicrobial Agents and Chemotherapy*, *56*(5), 2719–2725. https://doi.org/10.1128/AAC.06079-11

Santos, A. F., & Soares, M. A. (2010). HIV genetic diversity and drug resistance. *Viruses*, *2*(2), 503–531. https://doi.org/10.3390/v2020503

Shafer, R. W., Dupnik, K., Winters, M. A., & Eshleman, S. H. (2001). A Guide to HIV-1 Reverse Transcriptase and Protease Sequencing for Drug Resistance Studies. *HIV Sequence Compendium*, *2001*, 1–51. http://www.ncbi.nlm.nih.gov/pubmed/22324021%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3274565

Shafer, R. W., Rhee, S. Y., Pillay, D., Miller, V., Sandstrom, P., Schapiro, J. M., Kuritzkes, D. R., & Bennett, D. (2007). HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *Aids*, *21*(2), 215–223. https://doi.org/10.1097/QAD.0b013e328011e691

Siemieniuk, R. A. C., Beckthold, B., & John, G. M. (2013). Increasing HIV subtype diversity and its clinical implications in a sentinel North American population. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *24*(2), 69–73. https://doi.org/10.1155/2013/230380

Skar, H., Hedskog, C., & Albert, J. (2011). HIV-1 evolution in relation to molecular epidemiology and antiretroviral resistance. *Annals of the New York Academy of Sciences*, *1230*(1), 108–118. https://doi.org/10.1111/j.1749-6632.2011.06128.x

Sluis-Cremer, N., Temiz, N., & Bahar, I. (2005). Conformational Changes in HIV-1 Reverse Transcriptase Induced by Nonnucleoside Reverse Transcriptase Inhibitor Binding. *Current HIV Research*, *2*(4), 323–332. https://doi.org/10.2174/1570162043351093

Smit, E., White, E., Clark, D., Churchill, D., Zhang, H., Collins, S., Pillay, D., Sabin, C., Nelson, M., Winston, A., Jose, S., Tostevin, A., Dunn, D. T., Sachikonye, M., Glabay, A., Hill, T., Phillips, A., Thornton, A., Orkin, C., … Atkinson, C. (2017). An association between K65R and HIV-1 subtype C viruses in patients treated with multiple NRTIs. *Journal of Antimicrobial Chemotherapy*, *72*(7), 2075–2082. https://doi.org/10.1093/jac/dkx091

Sui, H., Gui, T., Jia, L., Guo, W., Han, J., Liu, Y., Bao, Z., Li, H., Li, J., & Li, L. (2014). Different frequencies of drug resistance mutations among HIV-1 subtypes circulating in China: A comprehensive study. *PLoS ONE*, *9*(3). https://doi.org/10.1371/journal.pone.0091803

Tambuyzer, L., Vingerhoets, J., Azijn, H., Daems, B., Nijs, S., Béthune, M. P. De, & Picchio, G. (2010). Characterization of genotypic and phenotypic changes in HIV-1-infected patients with virologic failure on an etravirine-containing regimen in the DUET-1 and DUET-2 clinical studies. *AIDS Research and Human Retroviruses*, *26*(11), 1197–1205. https://doi.org/10.1089/aid.2009.0302

Tatem, A. J., Hemelaar, J., Gray, R. R., & Salemi, M. (2012). Spatial accessibility and the spread of HIV-1 subtypes and recombinants. *Aids*, *26*(18), 2351–2360. https://doi.org/10.1097/QAD.0b013e328359a904

Templeton, A. R., Kramer, M. G., Jarvis, J., Kowalski, J., Gange, S., Schneider, M. F., Shao, Q., Zhang, G. W., Yeh, M. F., Tsai, H. L., Zhang, H., & Markham, R. B. (2009). Multiple-infection and recombination in HIV-1 within a longitudinal cohort of women. *Retrovirology*, *6*. https://doi.org/10.1186/1742-4690-6-54

Tenforde, M. W., Mokomane, M., Leeme, T., Patel, R. K. K., Lekwape, N., Ramodimoosi, C., Dube, B., Williams, E. A., Mokobela, K. O., Tawanana, E., Pilatwe, T., Hurt, W. J., Mitchell, H., Banda, D. L., Stone, H., Molefi, M., Mokgacha, K., Phillips, H., Mullan, P. C., … Jarvis, J. N. (2017). Advanced human immunodeficiency virus disease in Botswana following successful antiretroviral therapy rollout: Incidence of and temporal trends in cryptococcal meningitis. *Clinical Infectious Diseases*, *65*(5), 779–786. https://doi.org/10.1093/cid/cix430

Venner, C. M., Nankya, I., Kyeyune, F., Demers, K., Kwok, C., Chen, P. L., Rwambuya, S., Munjoma, M., Chipato, T., Byamugisha, J., Van Der Pol, B., Mugyenyi, P., Salata, R. A., Morrison, C. S., & Arts, E. J. (2016). Infecting HIV-1 Subtype Predicts Disease Progression in Women of Sub-Saharan Africa. *EBioMedicine*, *13*, 305–314. https://doi.org/10.1016/j.ebiom.2016.10.014

Wainberg, M. A., & Brenner, B. G. (2012). The Impact of HIV Genetic Polymorphisms and Subtype Differences on the Occurrence of Resistance to Antiretroviral Drugs. *Molecular Biology International*, *2012*, 1–10. https://doi.org/10.1155/2012/256982

Warnke, D., Barreto, J., & Temesgen, Z. (2007). Therapeutic review: Antiretroviral drugs. *Journal of Clinical Pharmacology*, *47*(12), 1570–1579. https://doi.org/10.1177/0091270007308034

Wensing, A. M., Calvez, V., Ceccherini-Silberstein, F., Charpentier, C., Günthard, H. F., Paredes, R., Shafer, R. W., & Richman, D. D. (2019). 2019 update of the drug resistance mutations in HIV-1. *Topics in Antiviral Medicine*, *27*(3), 111–121.

WHO. (2016). Clinical Guidelines: Antiretroviral Therapy. *Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach., Second Edition*, 129. http://www.who.int/hiv/pub/arv/chapter4.pdf?ua=1

WHO. (2018). *Global Action Plan on HIV Drug Resistance 2017-2021: 2018 Progress Report - Hiv Drug Resistance* (Issue July). http://apps.who.int/bookorders.%0Ahttps://clintonhealthaccess.org/content/uploads/2017/09/2017-

Woods, C. K., Brumme, C. J., Liu, T. F., Chui, C. K. S., Chu, A. L., Wynhoven, B., Hall, T. A., Trevino, C., Shafer, R. W., & Harrigan, P. R. (2018). Automating HIV drug resistance genotyping with RECall, a freely accessible sequence analysis tool. *Journal of Clinical Microbiology*, *50*(6), 1936–1942. https://doi.org/10.1128/JCM.06689-11

World Health Organization. (2010). *WHO/HIVResNet Global HIV Drug Resistance Strategy*. 86. http://www.who.int/hiv/pub/drugresistance/hiv_reslab_strategy.pdf

World Health Organization. (2019). HIV Drug Resistance Report 2019. In *Who*. http://www.who.int/hiv/pub/drugresistance/hivdr-report-2019/en/%0Ahttp://scholar.google.com/scholar/Who hiv drug resistance report 2012%0Ahttp://scholar.google.com/scholar/Who hiv drug resistance report 2012%0Ahttp://www.who.int/hiv/pub/drugresistance/hiv

World Health Organization. (2022). *HIV data and statistics*. https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information/hiv-data-and-statistics

Yang, C., Li, M., Shi, Y. P., Winter, J., Van Eijk, A. M., Ayisi, J., Hu, D. J., Steketee, R., Nahlen, B. L., & Lal, R. B. (2004). Genetic diversity and high proportion of intersubtype recombinants among HIV type 1-infected pregnant women in Kisumu, western Kenya. *AIDS Research and Human Retroviruses*, *20*(5), 565–574. https://doi.org/10.1089/088922204323087822

APPENDICES

Appendix I: Primers

Primer	Nucleotide range based on HXBII	Direction	Amino acids (5'-3')	Procedure
PrtM-F1	2253-2275	Forward	CCT CAA ATC ACT CTT TGG CAR CG	RT-PCR
RT-R1	3370-3348	Reverse	ATCCCT GCA TAA ATC TGA CTT GC	
Prt-F2	2265-2288	Forward	CTT TGG CAA CGA CCC CTY GTC WCA	Nested PCR& Sequencing
RT-R2	3326-3304	Reverse	CTT CTG TAT GTC ATT GAC AGTCC	
A35V	2556-2577	Forward	AGT CCT ATT GAR ACT GTR CCA G	Sequencing
AV36V	2869-2889	Forward	CAG TAC TGG ATG TGG GRG AYG	
AV44	2639-2619	Reverse	TTT YTC TTC TGT CAA TGG CCA	
90V1	29522931	Reverse	TAC TAG GTA TGG TAA ATG CAG T	

Appendix II: Ethical Approvals

Ethics Review Board
Instituted by *Médecins Sans Frontières*

Dr Petros Isaakidis
Medical Research Coordinator
Médecins Sans Frontières – Operational Centre Brussels

10 September 2017

Re: Ethics approval of "Prevalence of major genotypic resistance among first line ART experienced individuals hospitalized with advanced HIV disease", Version 4, dated August 2017, (ID: 1743)

Dear Petros,

Thank you for your reply to our review of the above-mentioned protocol. We are happy with the answers provided by the investigators. **We thus approve the protocol for a period of 12 months, until 9 September 2018**. Please ensure that all people associated with this particular research are duly informed about the changes introduced compared to the initially submitted version, and that they all receive a copy of the approved protocol.

If the study is not started within the next twelve months, you should submit a request for amendment of the study schedule one month before this approval expires. If the study is not completed within the next twelve months, you should request an extension of approval at least one month before this approval expires.

Any revisions you might wish to make to the protocol must be submitted to the Ethics Review Board through a request for amendment, for further review and approval. Anything that may occur during the research that may affect ethical acceptability of the project, including adverse effects on participants or unforeseen events, must be reported to the Ethics Review Board. We would also like to remind you that the Ethics Review Board will routinely check the reported and published outcome measure(s) against the outcome measure(s) listed in the approved protocol. If the outcome measures are changed during the course of the research, a request for amendment should be submitted to the Ethics Review Board.

Please notify us once the study is completed or if it is stopped. The ERB considers that a study is finished when there is no more contact with study participants and when data are collected, cleaned and analysed and, as applicable, samples have been analysed and coded/anonymised. We would appreciate receiving the final research report.

Please send us in due time the approval by the KEMRI Scientific and Ethics Review Committee (Kenya) and the *Comité d'Ethique à la Recherche Scientifique* (DRC).

Please notify us once this study is initiated. We wish you much success with the research.

Yours sincerely,

Raffaella Ravinetto
Chairperson, Ethics Review Board

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KEMRI/RES/7/3/1 **October 09, 2017**

TO: **DR. CLAIRE BOSSARD,**
EPICENTRE-MSF,
PRINCIPAL INVESTIGATOR

Dear Madam,

RE: NON-KEMRI 586 (*RESUBMITTED INITIAL SUBMISSION*): PREVALENCE OF MAJOR GENOTYPIC RESISTANCE AMONG FIRST LINE ART EXPERIENCED INDIVIDUALS HOSPITALIZED WITH ADVANCED HIV DISEASE.

Reference is made to your letter dated September 28, 2017. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on October 05, 2017.

This is to inform you that the issues raised during the 267th Ethics Review Committee (ERC) meeting of the KEMRI Scientific Ethics Review Unit (SERU) held on **September 19, 2017** have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day, **October 09, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **October 08, 2018.** If you plan to continue with data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **August 27, 2018**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

for **DR. MERCY KARIMI NJERU,**
ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT