PROFILING NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR DRUG-RESISTANCE AND SUSCEPTIBILITY PATTERNS OF NAIVE HIV POSITIVE PATIENTS FROM MACHAKOS LEVEL 5 HOSPITAL

A Thesis Submitted to the Department of Biological Sciences and Agriculture,
School of Science and Technology

University of Eastern Africa, Baraton

In Partial Fulfillment of the Requirements
For the Degree of
Master of Biological Sciences: Biomedical Sciences

Stanley Kipkurui Kiprop

SSTAKI1811

July, 2022

APPROVAL SHEET

This thesis entitled "**Profiling nucleoside reverse transcriptase inhibitor drug-resistance and susceptibility patterns of naive HIV positive patients from Machakos level 5 Hospital"** is written and submitted by **Stanley Kipkurui Kiprop**, in partial fulfillment of the requirements for the degree of Master of Science: Biomedical Sciences is hereby accepted and approved.

1. Dr. Gracelyn Portia, PhD

   Signature: _____

   Date: _____

2. Dr. Mutemi Muthangya, PhD

   Signature: _____

   Date: _____

Accepted in partial fulfillment of the requirements for the degree of Master of Science: Biomedical Sciences.

Department Chair: _____

Signature: _____

Date: _____

School Dean: _____

Signature: _____

Date: _____

Director of Graduate Studies and Research: _____

Signature: _____

Date: _____

ABSTRACT

This study focused on Nucleoside Reverse Transcriptase drug-resistance profiling and the susceptibility patterns for the plasma samples obtained from HIV-positive naïve patients enrolled at Machakos Level 5 Hospital. The research's specific objectives were to profile resistance to Nucleoside Reverse Transcriptase Inhibitor drugs and then identify the markers for resistance to Nucleoside Reverse Transcriptase Inhibitor.

This study used an experimental research design; DNA was extracted from the plasma samples, and PCR was amplified using polymerase-gene specific primers and later Gel electrophoresis. Then finally, cycle sequencing of the polymerase (pol) gen. The amplified products were sequenced, and drug-resistant mutations were determined using Los Alamos HIV DR database. All amplified samples from the PCR had the gel cut/excised and cleaned using the QIA quick gel extraction kit protocol. Sequences with high relatedness were fetched in a FASTA format and aligned using the Mega Evolutionary Genetic Analysis (MEGA) software version 10 using the Neighbour Joining (NJ) algorithm and the 1000 Bootstrap resampling algorithm.

The main HIV strain detected in this study was the HIV A1 subtype, the major sub-subtype in Kenya. No other subtypes were noted in the study. Regarding NRTIs, the major mutation noted was D67E which indicated inadequate level, zidovudine resistance, and drug susceptibility to abacavir, emtricitabine, lamivudine, and tenofovir noted with no resistance to NNRTIs. However, there were minor mutations noted.

Drug resistance mutations were found in high numbers associated with viral load and treatment time. Importantly, patients with triple and dual-class drug resistance should immediately alter ART regimens to alter the possibility of transmitting multi-drug-resistant HIV-1 strains. This finding emphasizes the importance of targeted resistance monitoring as a tool for addressing the problem.

DECLARATION SHEET

This thesis is my own work, and to the best of my knowledge, it has never been published or submitted to any university for a degree.

_____ _____

Stanley Kipkurui Kiprop

SSTAKI1811 Date

As university supervisors, we have approved the submission of this thesis for examination.

_____ _____

Dr. Gracelyn Ramesh, PhD
Lecturer, University of Eastern Africa, Baraton Date

Supervisor

_____ _____

Dr. Mutemi Muthangya, PhD Date
Lecturer, South Eastern Kenya University.

Supervisor

ACKNOWLEDGEMENT

I would like to thank the Almighty God for His agape love during this process. I would also like to take this opportunity to thank my supervisors for their diligent efforts in seeing that this work is properly done by correcting flaws and inaccuracies in the document. I am really thankful to my parents for their financial and emotional support during the entire process. Finally, I want to express my gratitude to my siblings and friends that supported me throughout the tough times.

DEDICATION

I would like to dedicate this Thesis to my parents whose support, prayers and encouragement helped me throughout this process.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATED ENTRIES

ART - Antiretroviral Therapy

ARV - Antiretroviral

DNTP - Deoxynucleotide Triphosphate

DRMs - Drug Resistance Mutations

HIV - Human Immunodeficiency Virus

HIVDR - HIV Drug Resistance

INI - Integrase Inhibitor

NACC - National Aids Control Council

NASCOP - National Aids and STI Control Program

NNRTI - Non-Nucleoside Reverse Transcriptase Inhibitor

NRTIs - Nucleoside Reverse Transcriptase Inhibitors

PDR: - Pretreatment Drug Resistance

PI - Protein Inhibitor

PLWHA - People Living with HIV/AIDS

RT - Reverse Transcriptase

TAMs - Thymidine Analogue Mutations

VCT - Voluntary Counseling and Testing

WHO - World Health Organization

ZDV - Zidovudine

CHAPTER ONE

INTRODUCTION

Background of the Study

In 2017, the national adult HIV frequency rate was projected at 4.8 percent, with prevalence higher among women (5.1 percent) than men (4.6 percent) (NASCOP, 2018). Since the Kenya HIV estimate report from National Aids Control Council (NACC) in 2017, the provision of free antiretroviral therapy (ART) to eligible individuals has increased significantly, resulting in the second highest number of People Living with HIV/AIDS (PLWHA) in the population. In 2018, the Kenya National AIDS and STI Control Program (NASCOP) reported a 66 percent and 32 percent decline in newly detected HIV infections as compared to 2017. As the program matures, it is important to make an assessment of the effectiveness of ART by making estimates of virological suppression among persons started off on a first-line regimen. Worry over the advent and spread of HIV drug resistance (HIVDR) further calls for studies of resistance surveillance to keep track of treatment end results (NASCOP, 2018).

The extensive use of antiretroviral (ARV) medication has greatly lowered HIV mortality and morbidity (Carrico, Shoptaw, Cox, Stall, Li, Ostrow and Plankey 2014). Stavudine is one of the most often utilized ARVs in resource-constrained settings owing to its effectiveness, cheap cost, short-term acceptability, and availability in the formulation form (Carrico et al., 2014). Nonetheless, many nations are discontinuing Stavudine (d4T), due mostly to mitochondrial toxicity associated with long-term d4T use. The World Health Organization (WHO, 2011) recommended using d4T even in individuals who did not have established virological failure in 2011 (Carrico et al., 2014).

A recent cross-cutting study, however, indicates a sharp rise in HIV Drug Resistance (HIVDR) prevalence, from 7.5 percent to 13.2 percent (Sigaloff, et al., 2012), among newly-diagnosed ARV-naive people in four Mombasa Voluntary Counselling and Testing (VCT) clinics, highlighting the necessity for continued demographic sampling and opioid resilience trend monitoring. This research, therefore, focused on drug-resistant profiling and sensitivity trends for ingenuous HIV-positive Nucleoside Reverse Transcriptase of patients in hospitals at Machakos levels 5.

Standards for treating people infected with human immunodeficiency (HIV) viruses include three or more HIV therapies, most often two nucleoside reverse transcriptase inhibitors (NRTIs) combined with an NNRTI, a protease inhibitor (PI), or, more recently, an integrase inhibitor (INI). The present therapy consists of three or more HIV medicines (Panel on Antiretroviral Guidelines for Adults and Youngsters, 2011). Treatment seeks to optimally inhibit HIV replication and restore immune function during long-term therapy (Yeni et al., 2012). To optimize control, cross-resistance and side effects, maintain potential treatment choices and increase the time of viral removal, rational drug selection is necessary (Gallant et al., 2013). Such therapeutic options may require close attention on the possible influence of viral resistance on more treatment alternatives, even if many antiretroviral (ARV) combinations might result in powerful abolition of viral replication.

First antiretroviral therapy in Kenya at the beginning of the '90s, HIV patients were prescribed zidovudine, lamivudine, and indinavir (Harrigan et al., 2015). The government routinely tracks CD4 T cells of all HIV/AIDS patients in Kenya to test ART. Tests on drug resistance are restricted and, if necessary, carried out by a doctor (Harrigan et al., 2015). The advent of antiretroviral therapy has greatly enhanced HIV-1 patients' health status worldwide (Harrigan et al., 2015).

Morbidity and mortality in HIV-1 patients in both developed and developing nations have been dramatically decreased (Aghokeng, et al., 2013). Unfinished virus suppression, however, allows HIV-1 resistance to drugs to propagate, endangers human medicinal effects, and threatens the whole world population (Leng et al., 2014). Medicament-resistant strains of HIV-1 can be spread from person to person. There may also be a drug-resistant virus not introduced to ART in a newly infected patient (WHO, 2012).

Drug resistance to human immunodeficiency virus is a serious weakness in antiretroviral treatment (ART). The supply of improved treatments and better control of treatment deficiencies and drug-resistant viruses has declined in capital-rich regions due to a treatment failure (Scherrer, et.al 2016). However, in resource-rich countries, the prevalence of drug-resistant viruses remains at nearly 10% (WHO, 2017). The creation of ART has decreased morbidity and death in developed countries (WHO, 2017), but in some countries, within the last couple of years, a prevalence of 10% or higher of TDR has been attained (Gupta et al., 2018).

The absence of reverse transcriptase (RT) and its revision functional association is largely unavoidably immune to antiretrovirals (Roberts, et al., 2018). The high rate of RT-mediated recombination events in an infected individual, as well as the number of replication cycles, promote the accumulation of HIV drug-resistant mutant strains (Coffin, 2015). In addition, some tissue compartments tend to be able to pick resistance mutations owing to low concentrations of drugs (Kepler et al., 2018). These mutations lie in those genes which encode antiretroviral goals such as RT, leading to RT development, which differs in structure and function from its wild-type (wt) counterpart. Although the protein continues to play a crucial role in HIV duplication, it is not as efficiently suppressed by ARV medications in the wild type protein. Nucleoside reverse transcriptase (NRTIs) tolerance and cross-resistance are being better known. This is a complicated phenomenon involving mutations under selective

drug pressure and interactions among mutations (Kepler et al., 2018). Resistance studies have shown different trends of mutations and cross-resistance within NRTIs that can affect the choice of potential salvage therapy (Coffin, 2015). A strong understanding of NRTI pathways is a core element of HIV-1 treatment strategies as it anticipates viral growth (Coffin, 2015).

The number of mutations needed for resistance ranges between drugs use and medicines (Kepler and Perelson, 2018). Many factors influence the relative resistance rate for various drugs and drug formulations. These genetic resistance challenge that denotes to the number of mutations needed within the target to be immune to a specific medication (Kepler and Perelson, 2018). Interactions between mutations, ability of viral replication, individual resistance mutations, and viral fitness all affect the mutational mechanisms and the ultimate effect of viral phenotype resistance mutations (Kepler and Perelson, 2018).

Statement of the Problem

Globally, antiretroviral therapy coverage has grown to more than 21 million individuals (UNAIDS, 2017). The antiretroviral therapy coverage in Sub-Saharan Africa has improved greatly (UNAIDS, 2016). Many nations, irrespective of CD4 T-cell count, have taken the advice of the WHO for the initiation of ART in all persons infected with HIV (WHO, 2016). While HIV mortality and morbidity have been reduced dramatically by ART, a sustained global expansion of HIV-resistant strains can lead to emergence and dissemination (Beyrer and Pozniak, 2017). A rise in pretreatment drug resistance (PDR) in low-resource environments has successfully increased ART (Gupta, Jordan, Sultan, Hill, Davis and Gregson, 2012). The latest World Health Organization study (WHO) found that the prevalence of PDR to NNRTIs in 6 of the 11 countries surveyed was more than 10%. The WHO 10 percent drug resistance level could entail improvements in the country's first line of ART regimes. In 63 countries (Gupta et al., 2012), a new meta-analytic study showed a global rise in PDR to NNRTI (up to 23% in Southern Africa). HIV-resistant medication

strains restrict treatment opportunities and risk the successful escalation of ART to monitor HIV infection (UN, 2015). Consequently, controlling the population level of HIV drug resistance (HIVDR) is important because it helps to keep the viral load low and the CD4 cell count high. HIV medicine can make the viral load very low by preventing HIV multiplication.

According to National Guidelines on Use of Antiretroviral Drugs for Treating and Preventing (NGUADTP) HIV Infection in Kenya, the recommended first-line ART regimen for treatment-naive adults consists of two nucleoside reverse-transcriptase inhibitors (NRTIs) and an integrase inhibitor (INSTI) (NASCOP, 2018). As an alternative, the use of a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or boosted protease inhibitor (PI) is recommended. Despite the effectiveness of ART and considerable efforts to help control the HIV/AIDS epidemic by 2030, ART failure due to drug resistance mutations is proving a challenge for ART provision and HIV care (NASCOP, 2018). In 2017, the World Health Organization (WHO) published a report on HIV drug resistance addressing the alarming increase in the prevalence of DRMs in individuals initiating their first-line ART regimen, linking DRMs to treatment failure. According to the National AIDS and STIs Control Program (NASCOP) recommendations of use of ART drugs, HIV resistance testing is recommended for all individuals with HIV infection who are newly diagnosed, before they initiate ART and in People Living with HIV (PLWH) with ART failure. Genotyping DNA-based assays are the most widely used for HIV DRMs detection. In Machakos Level 5 hospital, HIV genotyping is not performed in PLWH failing their first-line regimens; it is not routinely performed for all PLWH who are treatment-naive and starting their first-line. Several studies have reported the prevalence of HIV DRMs in treatment-naive Kenyan PLWH. According to a nationally representative survey, in Kenya the prevalence of any antiretroviral (ARV) resistance drug in treatment-naive PLWH is greater than 10%. Also, this

report concluded that PWLH who initiated with NNRTI-based regimens achieved significantly lower levels of viral suppression compared to those who initiated with Protease inhibitor-based regimens. Also, Drug resistance mutations can directly confer resistance to PI, in the absence of detectable DRMs in the PR. A recent cross-cutting study, however, reveals a sharp increase in HIVDR prevalence, from 7.5 percent to 13.2 percent among newly-diagnosed ARV-naive grownups in five VCT centers of Mombasa (Kepler and Perelson, 2018). Therefore, with missing reports on the general HIV-1 drug resistance, especially in resource limited rural settings with a longer history of ARV drug use, this research focused on drug-resistant profiling and sensitivity trends for ingenuous HIV-positive patients Nucleoside Reverse Transcriptase at Machakos levels 5 Hospital.

Objectives

Broad Objective

The goal of this study was to profile nucleoside reverse transcriptase inhibitor drug-resistance and susceptibility patterns of naïve HIV patients from Machakos level 5 Hospital.

Specific Objectives

1. To profile resistance to Nucleoside Reverse Transcriptase Inhibitor drugs.
2. To establish the markers for resistance to Nucleoside Reverse Transcriptase Inhibitor drugs.
3. To determine the HIV sub types circulating in Machakos Level 5 Hospital.

Significance of the Study

The determination of HIV subtypes circulating in Machakos Level 5 hospital guides molecular epidemiological surveys and vaccine trial studies. This information is important to clinical trials and pharmaceutical companies on the choice of subtype immunogenic component for use in clinical trials in Kenya. This study will aid the stakeholders in the health ministry develop a better plan to see how to make ARVs work best for the patients.

The findings of this study will aid realize the situation in real time and develop a better strategy for managing HIV drug resistance. It will also benefit the health ministry to remain outstanding in managing drug resistance to HIV. This research will also add to the literature on HIV drug resistance.

Justification of the Study

According to WHO (2018), surveillance of HIV drug resistance provides countries with evidence that can be used to optimize patient and population-level treatment outcomes. WHO recommends that countries routinely implement nationally representative HIV drug resistance surveys in different populations, including adults, children and adolescents. A well-known and significant topic in clinical bacteriology and virology has been the idea of drug resistance. Antiviral drug resistance cannot be prevented from impairing the antiviral effect in individuals infected with HIV-1. Statistics suggest that more than 50,000 new HIV infections occur yearly (De Cock, Rutherford, and Akhwale, 2014).

In developed countries, HIV-1 genotypic drug testing is done prior to initiation of ART to guide physicians to select the most efficacious class of ART and effectively reduce the likelihood of virologic failure (WHO, 2018). However, pretreatment resistance testing is not performed in Kenyan HIV patients prior to the initiation of ART. It is therefore, possible that this gap in test significantly contributes to the reported cases of treatment failure. In order to sustain ART gains in Kenya it is important to conduct drug resistance surveys to inform ARV choices in the clinical management of HIV/AIDS. HIV co-receptor studies explain the use of circulating HIV-1 strains among people living with HIV.

The principal role of this study is to detect the presence of nucleoside reverse transcriptase inhibitor drug-resistance and susceptibility patterns of naive HIV positive being considered for treatment with a CCR5 antagonist, which has been introduced and approved for use in developed countries. This study is relevant to highlight HIV-1 nucleoside reverse

transcriptase inhibitor which is necessary to decide on the use of CCR5 ART in the Kenyan HIV populace. ARVT is critical as it helps save lives that could be lost due to the HIV pandemic. ARVT is voluntary to develop medical safety to reduce death in HIV patients. The use of these tests in patients with a prescription infection, particularly in places where the local prevalence of the primary resistance to drugs can be understood, should be discussed before initiating care according to the latest guidelines (Gaolathe, et al., 2016). Awareness of the drug resistance profiles and sensitivity trends of naïve HIV positive patients in developing nations, for instance Kenya, is expected to enhance HIV/AIDS-related services delivery.

Scope of the Study

This study was conducted at Kenya Medical Research Institute (KEMRI) for the samples collected from Machakos Level 5 hospital, with the population target being both male and female HIV patients. KEMRI currently ranks as one of the leading Centres of excellence in health research both in Africa as well as globally. According to their official website, their mission is to improve human health and quality of life through research, capacity building, innovation and service delivery. Machakos Level 5 hospital is the most advanced facility in Machakos County. The study obtained blood samples of 18 patients, then used them for the DNA extraction. The time scope ran from Dec 2019 to May 2021.

Limitations

Due to the cost related charges, only fewer samples were used for sequencing during the time of the study. Though amplifications for most samples occurred, good and reliable sequence results could only be obtained from five samples out of eighteen that were used in the study. More information on circulating subtypes plus resistance patterns would be obtained if more samples could be amplified.

No patient information regarding gender, age, and treatment regimen was provided. This was because of ethical concerns at the health facility.

Operational Definition of Terms

Nucleoside Reverse Transcriptase Inhibitor	An Antiretroviral HIV drug class which blocks reverse transcriptase (an enzyme which HIV use to convert its RNA into DNA (reverse transcription)).
Drug Resistance	This is when a bacterium, virus, or other microorganism changes form and becomes insensitive to a drug that was previously effective.
Naive patients	These are patients with no previous ARV therapeutic exposure.

CHAPTER TWO

REVIEW OF RELATED LITERATURE AND STUDIES

HIV Epidemiology

HIV belong to the *retroviridae* family with two major types, HIV-1 and HIV-2 (Adhiambo et al., 2021). Human immunodeficiency virus type 1 is further classified into three genetic groups: M (major or primary), O (outlier), and N (new or non-M or non-O) (Adungo et al., 2014; Adhiambo et al., 2021). The HIV pandemic viruses are mostly caused by HIV group M, which is further fragmented into several subtypes. These subtypes include A, B, C, D, F. G, H, J, and K (Adungo et al., 2014). These subtypes are further divided by sub-sub types that include A1, A2, A3, F1, and F2, which are geographically distributed (Adungo et al., 2014). When two or more subtypes combine, they form HIV 'Circulating Recombinant Form (CFR) or Unique Recombinant Form (URF), which are hybrid inter-subtype viral sequences that do not show evidence of onward transmission (Akhome, 2021).

HIV primarily infects CD4-positive T lymphocytes and macrophages in some cases, by utilizing the CD4 protein which is the main receptor for entry and two identified co-receptors; CXCR4 and CCR5 co-receptors (Nael, Walavalkar, Wu, Nael, Kim, Rezk and Zhao, 2016). Productive HIV infection begins with attachment of the viral particle to a host cell following interactions between viral envelope proteins gp120/gp41 and the CD4 protein (Nael et al., 2016). Viral membrane fusion, a key step for enveloped viruses to enter host cells then follows. Membrane fusion is an energetically favorable process for HIV, using its envelope (Env) glycoprotein (Nael et al., 2016). The env polypeptide chain is produced as a precursor, gp160 and then undergoes cleavage to form two fragments gp120 and gp41. Gp120 is associated with HIV binding and attachment while gp41 facilitates fusion (Chen

and James, 2019). Viral entry is often described as an explosion of the HIV capsid into the host cytosol, with its two encapsulated RNA. The RNA strands undergo reverse transcription to synthesize DNA using HIV reverse transcriptase (Chen and James, 2019). The DNA is then translated into the host nucleus through the nuclear pore and then integrated by the unique viral enzyme integrase. The virus then sheds its protein capsid to allow for the synthesized DNA to be spliced together with that of the host. Subsequently, viral proteins are synthesized from host cytosol to allow formation of a new viral protein which buds out and infects new cells (Chen and James, 2019).

The emergence of treatment resistance in the heinous HIV infection has far-reaching consequences. Aside from the limits of inadequate treatment regimens, there are additional costs associated with switching to second- or third-line therapy and increased demands on laboratory care for patients (Zhao, Feng, Hu, Li, Zuo, Yan and Xing, 2018). Pretreatment or transmission HIV medication resistance has a considerable impact on antiretroviral rehabilitation effectiveness (Zhao et al., 2018). It leads in fewer options for successful therapy, a longer time to achieve viral suppression, and a faster time to virologic failure than infection with a non-drug-resistant virus strain (Zhao et al., 2018). Although several studies have demonstrated that ADR is a significant determinant of virologic failure in both treated and untreated HIV patients, the characteristics that predict ADR are unclear (Zhao et al., 2018). Numerous studies predict a 5% to 15% prevalence of HIV drug resistance in ART-naive people (Zhao et al., 2018). The high potency of the ARV regimen, low viremia at the start of ART, great compliance to the treatment plan, higher CD4 count at the start of ART (> 200 cells/mm3), and quick reduction of viral load in response to therapy all predict virologic success (Zhao et al., 2018). Understanding the combination of variables associated to HIV medication resistance is critical, especially in low to middle-income countries where viral load monitoring is limited.

Antiretroviral medication resistance variants acquired and propagated among people living with HIV (PLWH) are a serious public health issue because they may restrict the effectiveness of existing HIV therapies. Numerous scientists have identified resistance to antiretroviral medicines and subsequently growing quantities of the transmitted, resistant virus as potentially reversing the major advancements made possible by powerful ART (Adhiambo et al., 2021). TDR and ADR both reflect the frequency of different ARV medicines in the community and the natural genetic barrier to acquiring resistance to certain treatments (Marrazzo, Ramjee, Richardson, Gomez, Mgodi, Nair and Chirenje, 2015). At the end of 2017, an estimated 1.5 million persons were infected with HIV in Kenya (Musyoki, 2017). In the late 1990s, after introducing a test and treatment scheme, Kenya launched a national ART program (Musyoki, 2017). Kenya's present ART coverage for adults and children is estimated at 69% and 61% respectively. (Shade, et al., 2018). In Kenya, there are low levels of HIVDR. 4/53 (7.5 percent) of new clients had HIVDR in a 2005 experimental studio in Nairobi (Lihana, et al., 2009).

HIV infection reduces the immune abilities of the host, rendering the host susceptible to many opportunistic infections including tuberculosis (Lihana et al., 2010). HIV replicates very fast, with an estimated 1010 -1011 virions being produced daily in treatment naïve infected persons (Lihana et al., 2010). However, the replication machinery is highly error-prone, resulting in the rapid evolution of variants, some of which become drug resistant (Lihana et al., 2010). It has also been demonstrated that HIV-1 drug resistance mutations are mainly caused by selective pressure of drugs, poor drug compliance by patients on treatment and drug-drug interactions among others (Deeks, Overbaugh, Phillips and Buchbinder, 2015). These factors have varying impact on HIV patient populations from different socio-demographic backgrounds (Kiptoo et al., 2009).

HIV Drug Classes

A 'backbone' of two nucleoside analog reverse transcriptase inhibitors (NRTI) and a third drug-either a non-nucleoside reverse transcriptase inhibitor (NNRTI), a protease inhibitor (PI), or an integrase strand-transfer inhibitor-are typically included in combination antiretroviral therapy (ART) for HIV-1 infection (INSTI) (Stella-Ascariz, Arribas, Paredes and Li, 2017). In resource-rich settings, WHO processes serve as a critical foundation for HIV public policy. Since around February 2017, a NRTI backbone of tenofovir-emtricitabine/lamivudine with either efavirenz (NNRTI) or raltegravir (INSTI), or ritonavir-boosted atazanavir or darunavir (PI) was regarded 'Preferred' as first-line therapy (Stella-Ascariz et al., 2017). The clinical phase and CD4 lymphocyte count continue to guide when to start ART; pretreatment plasma HIV viral load was dissociated as an indicator of commencing ART in 2007. Although such recommendations are based on professional bodies' successive evaluations of individual studies, a systematic review of results from multiple studies may reveal features associated with success/failure and inform drug evolution, future study design, and, ultimately, patient care and treatment guidelines (Stella-Ascariz et al., 2017). Of the existing meta-analyses of initial ART, most focus on specific assessments or earlier studies. None have evaluated outcomes beyond 46 weeks or by regimen type ('Preferred' vs. 'Alternative') (Stella-Ascariz et al., 2017). Since the last comprehensive investigation of first ART effectiveness, much data (some unpublished) has been generated, and an updated inclusive requirement of initial ART efficacy and its connections is acceptable (Stella-Ascariz et al., 2017).

HIV and antiretroviral Resistance

According to WHO (2018), pretreatment HIV drug resistance to the NNRTI drug class is up to 3 times more common in people with previous exposure to antiretroviral drugs. The analysis of data from the Chinese National HIVDR Surveillance and Monitoring Network showed that among 2826 treatment-experienced patients, 33.8% had a viral load ≥1000 copies/mL and 19.2% had resistance mutations identified, virtually all with NNRTI mutations and two-thirds with NRTI mutations (Hui Xing, 2013). Hui Xing, (2013) recommends that countries routinely implement nationally representative HIV drug resistance surveys in different populations, including adults, children and adolescents.

Pharmacological resistance is a critical characteristic that allows pathogens to survive and reproduce even under severe drug pressure (Fletcher, Staskus, Wietgrefe, Rothenberger, Reilly, Chipman and Schacker, 2014). An HIV-1 infected person has a pool of HIV-1 variations derived mostly from one (a "founder") who propagated the virus. In general, a virus generates a pool of "subspecies" by undergoing genetic changes caused by errors produced by HIV-1 when replicating its genetic information, paired with a very high rate of HIV-1 turnover (Fletcher et al., 2014). RT uses RNA- and DNA-dependent DNA polymerization to copy the -10,000 nucleotide RNA HIV-1 genome into dsDNA, requiring-20,000 nucleotide incorporation steps (Fletcher et al., 2014). At the enzyme level, RT is an error-prone enzyme that causes around one disincorporation for every 104 nucleotide incorporations (Fletcher et al., 2014). The inaccuracy rate in viral replication is lower than predicted based on RT disincorporation data. In viral replication, the in vivo mutation rate is 5-10, which is an order of magnitude lower than that seen in RT (Fletcher et al., 2014). Aside from RT, the host RNA polymerase may also exhibit mutations during transcribing viral DNA to mRNA copies that are I translated into viral polyproteins and (ii) processed into viral ssRNA copies. The errors are sporadic, resulting in random sequence variations in the

progeny viruses and viral proteins. Many, if not most, of the mutations may be detrimental to the structural assembly or activities of viral proteins, and hence the virus cannot tolerate them. Fascinatingly, HIV-1 may continue to function while having numerous mutations, and each of these mutant strains can reproduce and generate additional changes. Each replication cycle increases the variability of the viral pool in an HIV-1 infected individual (Fletcher et al., 2014).

The wild-type variations are well adapted to the natural host environment and proliferate as the dominant strains, while different subspecies replicate less effectively. Nonetheless, under pharmacological selection pressure, the replication dynamics change. A typical medication is designed to have a strong impact on the wild-type virus (Smith, Raugi, Pan, Sow, Seydi, Mullins and Gottlieb, 2015). The mutations that are least repressed by the drugs are the most prevalent. Such a drug-resistant version may be less fit for replication or transmission capabilities at times; nevertheless, some of these strains may achieve fitness by accumulating compensating mutations or adding resistance mutations to an existing variant carrying the compensatory mutation background (Smith et al., 2015).

Nucleoside Reverse Transcriptase Inhibitor (NRTI)

There are two groups of inhibitors of reverse transcriptase (RT): The Nucleoside Reverse Transcriptase Inhibitors (NRTIs); and the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). NRTIs insert in evolving viral DNA, which contributes to the DNA chain's termination and blocks additional DNA expansion (D'Cruz, 2016). NNRTIs interrupt the replication of HIV-1 by binding the p66 subunit of the RT enzyme to the hydrophobic pocket to prevent it from reverse transcribing viral RNA into DNA (D'Cruz, 2016). NNRTIs are non-competitive and do not need activation inhibitors of HIV-1 RT. The poor HIV-1 RT fidelity, the high HIV-1, and high recombination rates by RT lead to the development of RT resistance inhibitors (Ho, 2015).

Mechanisms of NRTI Resistance

Discrimination is an NRTI resistor process in which the reverse transcriptase (RT) enzyme can inhibit NRTI binding while preserving the ability to recognize the analogical natural deoxynucleotide triphosphate (dNTP) (Clutter, Jordan, Bertagnolio and Shafer, 2016). Examples are K65R, L74V, Q151M, and M184V point mutation viruses that induce decreased affinity of the RT with or without modifications in the affinity of the dNTP substratum for a particular NRTI, resulting into less drug incorporation incorporated into the DNA chain (Clutter et al., 2016).

Another process involves enhancing the phosphorolysis clearance of the NRTI chain-end of the first 3′ after it was inserted in the viral DNA. NRTI-associated mutations can impact the phosphorolysis behavior of RT in a process called 'key unblocking' in some cases, overwhelming chain termination (Clutter et al., 2016). Mutations improved with zidovudine (ZDV) and stavudine (d4T) action are identified by M41L, D67N, K70R, L2110W, T215Y/F, and K219q/E (Clutter et al., 2016). TAMs include all NRTIs except lamivudine (3TC), but their level of cross-resistance depends on the NRTI and the number of TAMs on the virus considered (Clutter et al., 2016). There are certain interactions between the various resistance systems. Selected by 3TC and emtricitabine (FTC), M184V/I mutations postpone TAM presentation and increase the ZDV and d4T sensitivity in vitro (Clutter et al., 2016).

Protease and the first half of the reverse transcriptase (up to at least nucleotide 215) can be sequenced to decide which new regimes to use in patients in whom ART has been deficient (Koullias, et al., 2017). Integration can be sequenced in situations where an InSTI comprising therapy has collapsed. Baseline InSTI resistance testing is not currently cost-effective, although useful for a deeper understanding of trends during failure (Koullias, et al., 2017). In select patients with TDR proof, such as those with NRTI- or multi-class resistance, however, InSTI resistance testing should be considered. In such patients, the risk of InSTI

resistance transmittal is also higher than in non-TDR patients, and the effect on an InSTI-containing initial regime of virological failure may be more serious (Koullias, et al., 2017).

In the research background, sequencing of other regions (C-terminus of the reverse transcriptase, group antigen) or even a near-complete duration of HIV-1 could be helpful (Manasa, et al., 2017). The third variable loop (V3) sequence of the glycoprotein envelope, gp120, will decide if the R5 tropics virus are R5 and could therefore react to inclusion into ART of a chemokine receptor 5 (CCR5). The output of a genotypic tropic test may be close to phenotypic tropism tests, particularly when NGS is used (Swenson, Mo and Dong, 2011). However, testing for genotypic tropism in the PBMC is less reliable than plasma testing for Peripheral blood mononuclear cells (Swenson and Dong, 2013). The developed sequencing assay yields consistent tropism determinations from HIV RNA and HIV DNA for a wide spectrum of HIV group M subtypes, exhibiting a reliable sequence reproducibility, and, for plasma testing, resulting in a viral load limit comparable to those for sequencing assays for the detection of HIV drug resistance mutations (Paar and Geit, 2011).

Summary of the Identified Knowledge Gap

This chapter reviewed nucleoside reverse transcriptase inhibitor drug-resistance and susceptibility patterns of naive HIV. It has also reviewed the HIV Epidemiology, drug classes, antiretroviral resistance, nucleoside reverse transcriptase inhibitor and mechanisms of NRTI resistance. Studies have been indicating a sharp rise in HIV Drug Resistance (HIVDR) prevalence, from 7.5 percent to 13.2 percent (Sigaloff, et al., 2012), among newly-diagnosed ARV-naive people in four Mombasa Voluntary Counselling and Testing (VCT) clinics, highlighting the necessity for continued demographic sampling and opioid resilience trend monitoring. This study therefore was set out to fill this knowledge gap by profiling drug resistance and sensitivity trends for ingenuous HIV-positive Nucleoside Reverse Transcriptase of patients in hospitals at Machakos levels 5.

CHAPTER THREE

RESEARCH METHODOLOGY

Research Design

This is a descriptive study profiling the NRTI drug resistance and susceptibility patterns of treatment of naïve HIV patients in Machakos level V hospital. Descriptive study was used because the aim of the study was to profile the NRTI drug resistance without manipulating the variables. Naive HIV-1-positive patient samples were obtained randomly, without any knowledge of drug-resistance and susceptibility patterns, from both the sample collection facilities, Machakos Level V hospital, and the diagnostic section at Kenya Medical Research Institute (KEMRI).

Study Population

The research's population was both male and female HIV-positive patients enrolled at Machakos Level 5 hospital. The HIV-positive status of correspondents was based on hospital clinical records. Machakos County has a total population of 1,155,956 people (52% female and 48% male). The general prevalence of HIV or AIDS is 6%, with higher prevalence among women (6.9%) than among men (2.8%). About 74% of the population had never been tested for HIV. Around 26% of the adults are registered for care. Most HIV or AIDS patients use Machakos level 5 comprehensive care hospital for care, testing, and treatment refill. Some supportive care is offered through five support groups and community units. The Aids Health Foundation and other partners provide HIV/AIDS and ARVs support.

Study Area

The study samples were collected from Machakos Level 5 hospital, situated in Machakos town, Machakos County, Kenya. Machakos County covers an area of 6,208 KM^2 It is located on Machakos-Wote Road. Machakos Level 5 Hospital is situated nearby to Tea

Tot, and close to Shalom Hospital. It lies between latitudes 1006’22.8” South and longitudes 37021’23.3” East.

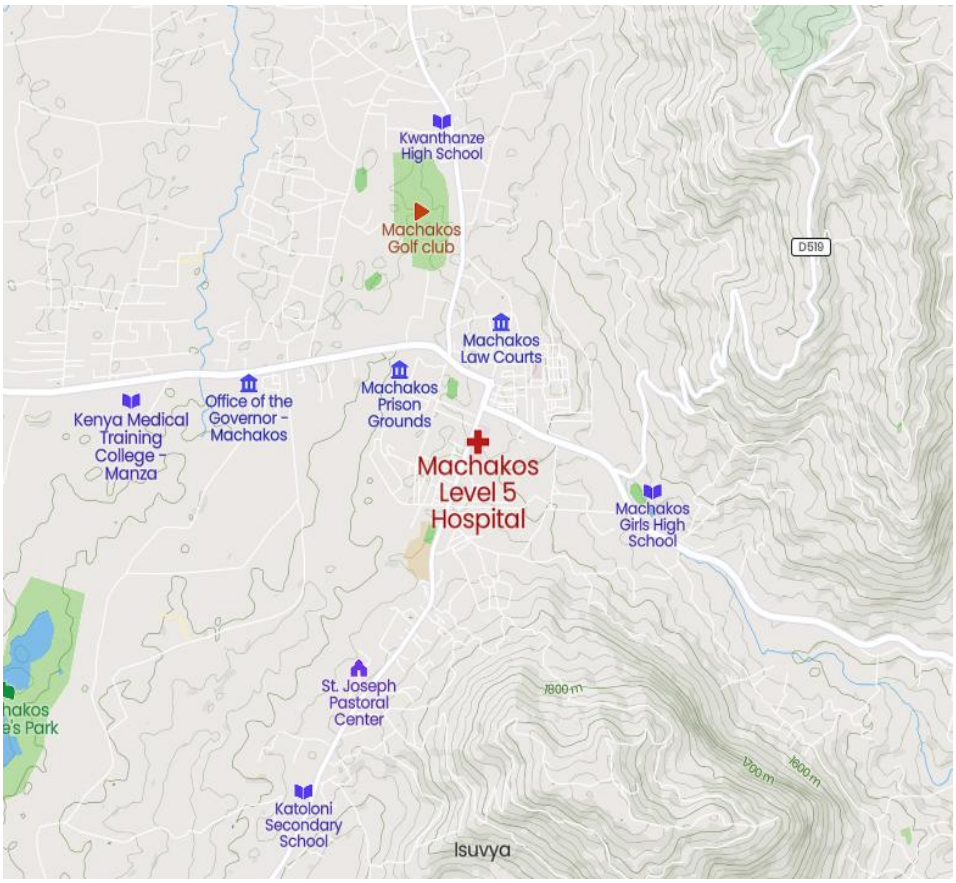


Figure 1: Geographical location of Machakos Level 5 hospital.

Machakos County borders Nairobi and Kiambu counties to the West, Kitui to the East, Embu to the North, Makueni to the South, Murang’a and Kirinyaga to the North West, and Kajiado to the South West. It serves the Eastern region of Kenya as the main town. Machakos Level 5 hospital was established in Kenya by the Ministry of Health and entrusted with the responsibility of managing most of the medical cases in Kenya. Machakos county has a population of 1,155,956 people with the population density at 194 people per square kilometer.

Sample Collection and Sampling technique

Total population sampling technique was applied in this study where all the plasma samples of the naïve HIV positive patients, collected from Machakos Level 5 Hospital were examined. All the available 18 samples were used for the experimental study. The samples show clear characteristics of significance and concern to the study.

18 samples were collected from Machakos Level 5 hospital in 2018. The samples were of naïve HIV positive patients who attend the Machakos Level 5 hospital. These were serum samples which were well stored in a refrigerator at −20°C at KEMRI Nairobi. After the samples had been used by the HIV Lab for CD4 T-cell count and viral load analysis, the samples were then used to perform resistance profiling. The samples basic epidemiological data such as transmission route, CD4 cell count, and HIV-1/AIDS-related symptoms were not provided by KEMRI due to privacy concerns.

Data Gathering Procedures

Ribonucleic Acid Extraction

Ribonucleic acid (RNA) from the plasma samples was extracted using Qiagen RNAmini kit (Qiagen CA, 2016) according to manufacturers' instructions. Briefly 140µl of sample was added to 560µl of viral lysis buffer, incubated at room temperature (15-25^{0}C) for 10 minutes, then 560µl of molecular grade 100% ethanol (Sigma USA, n.d) was added and mixed by vortexing for 15 seconds. This was then centrifuged using a microcentrifuge (Applied Biosystems, USA) briefly to remove drops from inside the Eppendorf tube lid. From the lysed RNA, 630µl of RNA was then placed on to a spin column, spun at 6000 x g, twice binding the RNA to the spin column. The RNA was then washed twice, first with 500µl of wash buffer 1 (AW1) at 6000 x g for 1 minute, then with 500µl of wash buffer 2 (AW2) at 20,000 x g for 3 minutes. The RNA was eluted from the spin column by adding 60µl elution buffer (AVE) and spinning at 6000 x g for 1 minute to a 1.5 ml Eppendorf tube. The eluted

RNA was then stored at -80^0 C until the day when the polymerase chain reaction (PCR) was carried out on the samples (Qiagen CA, 2016).

Polymerase Chain Reaction (PCR)

The reverse transcriptase PCR (RT-PCR) procedure consisted of one-step reverse transcription and PCR amplification, using the one-step RT-PCR kit from QIAGEN (Qiagen CA, 2016). The reaction mixture contained 5µl of 5× RT-PCR buffer, 1µl of 0.4mM dNTPs, 0.75µl of each of the primers (1st round forward and reverse primers final concentration 0.6µM), 9.5µl of nuclease free water and 1µl of enzyme mix. A 2.5µl aliquot of viral RNA was added to give a final volume of 25µl. The cycling conditions for the RT-PCRs were an initial cycle at 50^0C for 30 minutes for the reverse transcriptase. This was followed by incubating at 94^0C for 10 minutes to inactivate the reverse transcriptase and activate the *Taq* polymerase. This was followed by 35 cycles of 95^0C for 30 seconds, 55^0C for 30 seconds, and 72^0C for 1 minute, with a final extension of 72^0C for 10 minutes (Ndembi, et al., 2004). In the 2nd round PCR, the amplification was carried out using the 2mM MgSO4 (Invitrogen), 0.8mM dNTPs (Invitrogen), 0.5 units Taq polymerase (Invitrogen), 10x PCR Buffer (Invitrogen), 2ng of each 2nd round primer (Table 1) and the 2µl of the 1st round DNA template. The PCR cycle conditions consisted of 1 cycle of 95^0C for 10 min and 35 cycles of 95^0C for 30 sec, 55^0C for 30 sec, and 72^0C for 1 min, with a final extension of 72^0C for 10 min (Ndembi, et al., 2004).

The forward primer used for the 1st round PCR was RT18 F1 and its sequence 5'-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3'. For the reverse primer, KS104 R1 was utilized where its sequence was 5'-TGACTTGCCCAATTTAGTTTTCCCACTAA-3' (Ndembi, et al., 2004).

Finally, for the 2nd Round PCR, KS101 F2 was used as a forward primer where its sequence was 5' - GTAGGACCTACACCTGTTCAACATAATTGGAAG-3' and KS102 R2 as a reverse primer where its sequence was 5'-CCCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG-3' (Songok, et al., 2004).

Gel Electrophoresis

After the 2nd round PCR, PCR products were electrophorized in 1.5% agarose gels along with a 100-bp ladder (Gibco N.Y., n.d) and visualized under UV light by ethidium bromide staining. 1.5g of agarose (Sigma USA, n.d) was added to 100ml of tris-borate EDTA (TBE) buffer. This was then heated in a microwave until clear, then later placed on a water bath that was at 48^0C. When cool, 0.5-1µl of ethidium bromide was added to the agarose, then later poured on to a gel tank that had gel combs. This was left to solidify. Once solid the comb was removed, and the gel tank filled TBE. The product 10µl was mixed with gel loading dye (gld) and electrophorised at 100v for 30 minutes. The PCR products were visualized under UV light using an HP AlphaImager® (Alpha Innotech SA, 2010).

QIAquick Gel Extraction Procedure

The QIAquick®gel extraction kit (Qiagen USA, 2016) was used to clean up the PCR products following the procedure described in the manufacturer's manual. The PCR products were excised from the gel, weighed and 3 volumes of buffer QG added for every 1 volume of the gel. These was incubated for 10 minutes at 50^0C to dissolve the gel. Once dissolved, 850µl of the solution was dispensed to a QIAquick spin column (Qiagen USA, 2016) and centrifuged at 17,900 x g for 1 minute to bind the DNA to the matrix of the column. This procedure was repeated once more. After the last spin, 500µl of buffer QG was added to the spin column and centrifuged at 17,900 x g for 1 minute to remove traces of agarose. This was then washed by adding 750µl of buffer PE and centrifuged at 17,900 x g for 1 minute. The

spin column was placed on a 1.5ml Eppendorf tube, 50µl of buffer EB was then added to the column and centrifuged for 1 minute. The eluted DNA was stored at -20^{0}C until nucleotide sequencing was carried out.

Nucleotide Sequencing

The amplified fragments acquired from RT were in several base pairs. These fragments were sequenced by the Sanger sequencing method at KEMRI following the manufacturer's instructions, along with published primers.

Molecular Analysis of Sequences

The pairing of the gained fragments was performed with Vector NTI Advance 11.5 software (Invitrogen Life Technologies, n.d) and compared with the standard database (Tang, et al., 2012).

Gene	Primer name and Sequence 3′ – 5′	Amplicon/Sequence length (bp)	Reference
12S rRNA	12SAL: AAACTGGGATTAGATACCCCACTAT 16SBHnew: CCTGGATTACTCCGGTCTGA	2033/400–700	Palumbi et al. 2002 RTPrimer DB*
D-Loop	t-PHE-L: GAACCAAATCAGTCATCGTAGCTTAAC CR2H: GGGGCCACTAAAAACTGGGGG	~656/~598	Ray and Densmore 2002
LDHA	LDHAI7-F1: TGGCTGAAACTGTTATGAAGAACC LDHAI7-R1: TGGATTCCCCAAAGTGTATCTG	743/697	Gatesy et al. 2004
c-mos	CmosF: ATAGTTGCTGTGAAGCAGGT CmosR: GCTCAGTGATGAACACATTG	388/347	Meganathan et al. 2010
c-myc	Cmyc-Croc-F: GGTGAATGGAGTTGAATCCGG Cmyc-Croc-R: AGCCAAGGTTGTGTAGTTGC	693/642	this study

* Real Time PCR Primer database (www.rtprimerdb.org)

*Figure 2: Comparison of Amplicon/Sequence length(bp) to Standard database for the pairing of gained fragments***.**

To determine whether or not the nucleotide sequences obtained from sequencing were of viral origins, they were initially compared to other sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST). First, the forward and reverse sequences were combined to form contigs, using the contig assembly program (CAP) which is part of the Bioedit® version 7.2 (Hall, 2011). The contigs were then uploaded to BLAST

by accepting the default parameters and searching against the non-redundant data set. Once the sequences were confirmed to be of viral origin, they were then uploaded to the Los Alamos HIV sequence database, the Stanford University HIV drug resistance database (Rhee, et al., 2003; Liu and Shafer, 2006; Shafer, 2006) and the International AIDS Society (IAS) using default parameter algorithms to determine this study's HIV subtypes and resistance patterns of the detected viruses.

To determine the phylogenetic relatedness of this study's sequences to similar sequences in the Los Alamos database, sequence data was saved in a FASTA format and aligned using the Mega Evolutionary Genetic Analysis (MEGA) software version 10 (Kumar, et al., 1994) using the Neighbour Joining (NJ) algorithm and the 1000 Bootstrap resampling algorithm. The NJ algorithm substitution model used was the Kimura 2-parameter method. The generated trees were used to infer and assign HIV-1 subtypes.

Ethical Approval

The researcher sought clearance from the University of Eastern Africa Baraton Review Ethics committee (REC), Office of Director of Graduate Studies and Research and, National Commission for Science Technology and Innovation. The researcher observed privacy and confidentiality during the process of this study. All the blood samples were identified using unique numbers for confidentiality.

CHAPTER FOUR

PRESENTATION OF FINDINGS, ANALYSIS AND INTERPRETATION

Introduction

This study sought to profile Nucleoside Reverse Transcriptase Inhibitor drug-resistant and susceptibility patterns of naive HIV positive patients from Machakos Level 5 hospital.

Profile of Resistance to Nucleoside Reverse Transcriptase Inhibitor Drugs

Table 1 below shows the results from uploading of study's sequence and uploading them to the Stanford University HIV database (https://hivdb.stanford.edu/hivdb/by-sequences/) to govern the nucleoside reverse transcriptase inhibitors (NRTIs) sequences using the default algorithms.

Table 1 NRTI mutations and NRTI drug susceptibility

Sample Id	NRTI Mutation	Drug Susceptibility	Potential Low-level Resistance
MKseq1	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq2	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq3	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq9	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq10	D67E	ABC, FTC, 3TC, TDF	AZT

Key: ABC – abacavir, AZT – zidovudine, FTC – emtricitabine, 3TC – lamivudine, TDF – tenofovir

The main mutation noted in Table 1 above was D67E, with a high-level drug susceptibility to abacavir, emtricitabine, lamivudine, and tenofovir drugs for five samples. However, there were potential low levels of resistance to zidovudine drug.

Markers for Resistance to Non-Nucleoside Reverse Transcriptase Inhibitor Drugs

The sequences obtained from this study were then uploaded to the Stanford University HIV database (https://hivdb.stanford.edu/hivdb/by-sequences/) to determine the non-nucleoside reverse transcriptase inhibitors (NNRTIs) sequences using the default algorithms. The results obtained are shown in table 2 below:

Table 2 Non-Nucleoside Reverse Transcriptase Inhibitor Mutations

Sample Id	NNRTI Mutation	Other Mutations
MKseq1	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q 174K, D177E, I178L, V179I, Q207A, R211S
MKSeq2	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q 174K, D177E, I178L, V179I, Q207A, R211S
MKSeq3	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q 174K, D177E, I178L, V179I, Q207A, R211S
MKSeq9	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q 174K, D177E, I178L, V179I, Q207A, R211S
MKSeq10	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q 174K, D177E, I178L, V179I, Q207A, R211S

Table 2 above shows no major NNRTI mutations noted with no susceptibility to NNRTI drugs. However, other minor mutations were noted, like V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E, I178L, V179I, Q207A, R211S.

Sequence of Genetic Markers for Resistance to Nucleoside Reverse Transcriptase Inhibitor Drugs

Polymerase Chain Reaction

Figure 3 below shows PCR products obtained after second-round amplification with HIV *pol-RT* specific primers. The expected sizes of amplified gene fragments, if positive, were approximately 697bp. The gel picture below shows that; indeed, the amplified fragments were approximately 697bp as expected. All amplified samples from the PCR had the gel removed and cleaned using the QIAquick Gel Extraction equipment protocol as described in the methods section.

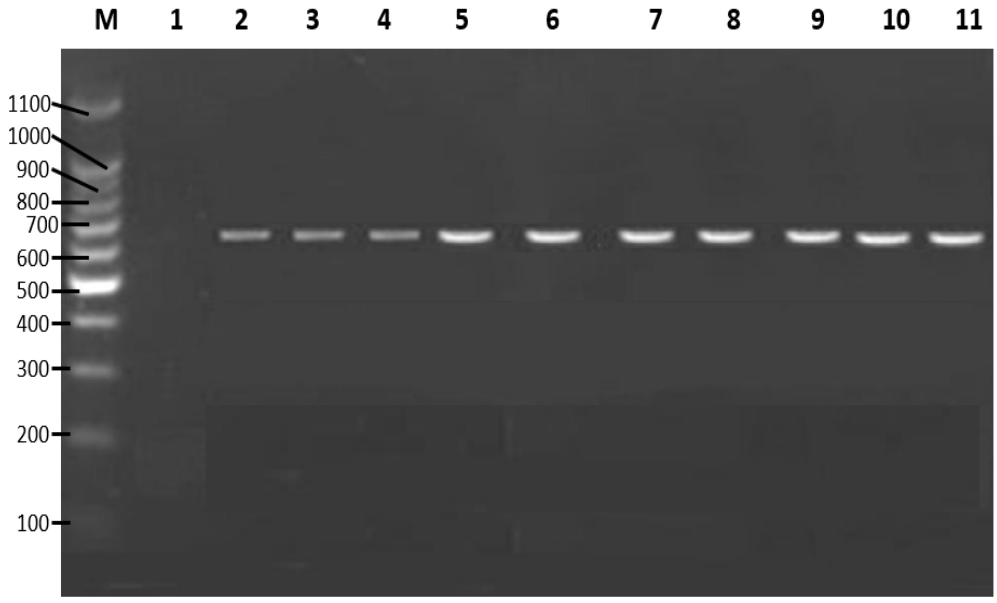


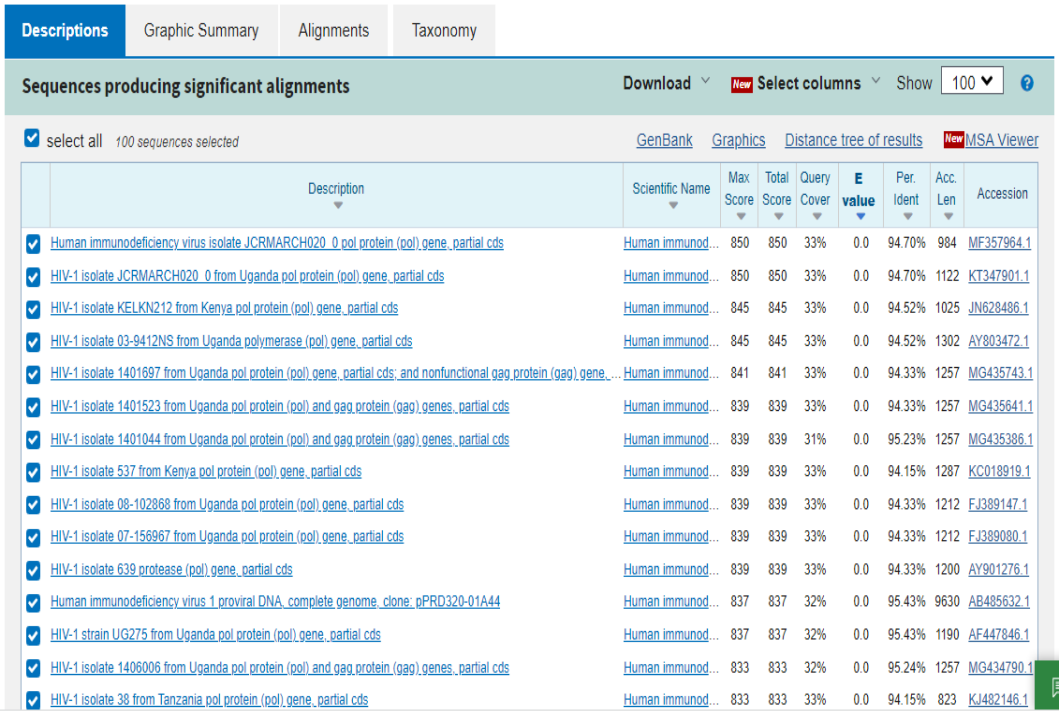
Figure 3: PCR Gel Electrophoresis

The lanes displayed are molecular DNA ladder (100 Mb, Promega, Madison, WI, USA) lane M. The negative control is denoted in lane 1, while the positive control is denoted in lane 2. The field samples are in lanes 3-11.

Analysis of Sequence Obtained

Although specific primers were used to amplify the polymerase reverse transcriptase gene (pol-RT) gene fragments from the patient samples, confirming the nucleotide sequences

obtained from the amplified fragments was always necessary. The sequences obtained from the amplified products from this study were uploaded and compared to related sequences found in the GenBank database using the Basic Local Alignment Search Tool (BLAST) using the default algorithm.



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — New Select columns — Show 100

select all 100 sequences selected — GenBank | Graphics | Distance tree of results | New MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Human immunodeficiency virus isolate JCRMARCH020_0 pol protein (pol) gene, partial cds	Human immunod...	850	850	33%	0.0	94.70%	984	MF357964.1
HIV-1 isolate JCRMARCH020_0 from Uganda pol protein (pol) gene, partial cds	Human immunod...	850	850	33%	0.0	94.70%	1122	KT347901.1
HIV-1 isolate KELKN212 from Kenya pol protein (pol) gene, partial cds	Human immunod...	845	845	33%	0.0	94.52%	1025	JN628486.1
HIV-1 isolate 03-9412NS from Uganda polymerase (pol) gene, partial cds	Human immunod...	845	845	33%	0.0	94.52%	1302	AY803472.1
HIV-1 isolate 1401697 from Uganda pol protein (pol) gene, partial cds; and nonfunctional gag protein (gag) gene, ...	Human immunod...	841	841	33%	0.0	94.33%	1257	MG435743.1
HIV-1 isolate 1401523 from Uganda pol protein (pol) and gag protein (gag) genes, partial cds	Human immunod...	839	839	33%	0.0	94.33%	1257	MG435641.1
HIV-1 isolate 1401044 from Uganda pol protein (pol) and gag protein (gag) genes, partial cds	Human immunod...	839	839	31%	0.0	95.23%	1257	MG435386.1
HIV-1 isolate 537 from Kenya pol protein (pol) gene, partial cds	Human immunod...	839	839	33%	0.0	94.15%	1287	KC018919.1
HIV-1 isolate 08-102868 from Uganda pol protein (pol) gene, partial cds	Human immunod...	839	839	33%	0.0	94.33%	1212	FJ389147.1
HIV-1 isolate 07-156967 from Uganda pol protein (pol) gene, partial cds	Human immunod...	839	839	33%	0.0	94.33%	1212	FJ389080.1
HIV-1 isolate 639 protease (pol) gene, partial cds	Human immunod...	839	839	33%	0.0	94.33%	1200	AY901276.1
Human immunodeficiency virus 1 proviral DNA, complete genome, clone: pPRD320-01A44	Human immunod...	837	837	32%	0.0	95.43%	9630	AB485632.1
HIV-1 strain UG275 from Uganda pol protein (pol) gene, partial cds	Human immunod...	837	837	32%	0.0	95.43%	1190	AF447846.1
HIV-1 isolate 1406006 from Uganda pol protein (pol) and gag protein (gag) genes, partial cds	Human immunod...	833	833	32%	0.0	95.24%	1257	MG434790.1
HIV-1 isolate 38 from Tanzania pol protein (pol) gene, partial cds	Human immunod...	833	833	33%	0.0	94.15%	823	KJ482146.1

Figure 4: A representative nucleotide sequence BLAST analysis result from this study using GENBANK database (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

The sequences were later uploaded and compared to similar sequences found in the Los Alamos HIV database tools, Recombinant Identification Program (RIP) default algorithm. A representation of the analysis results is shown in figure 5 below.

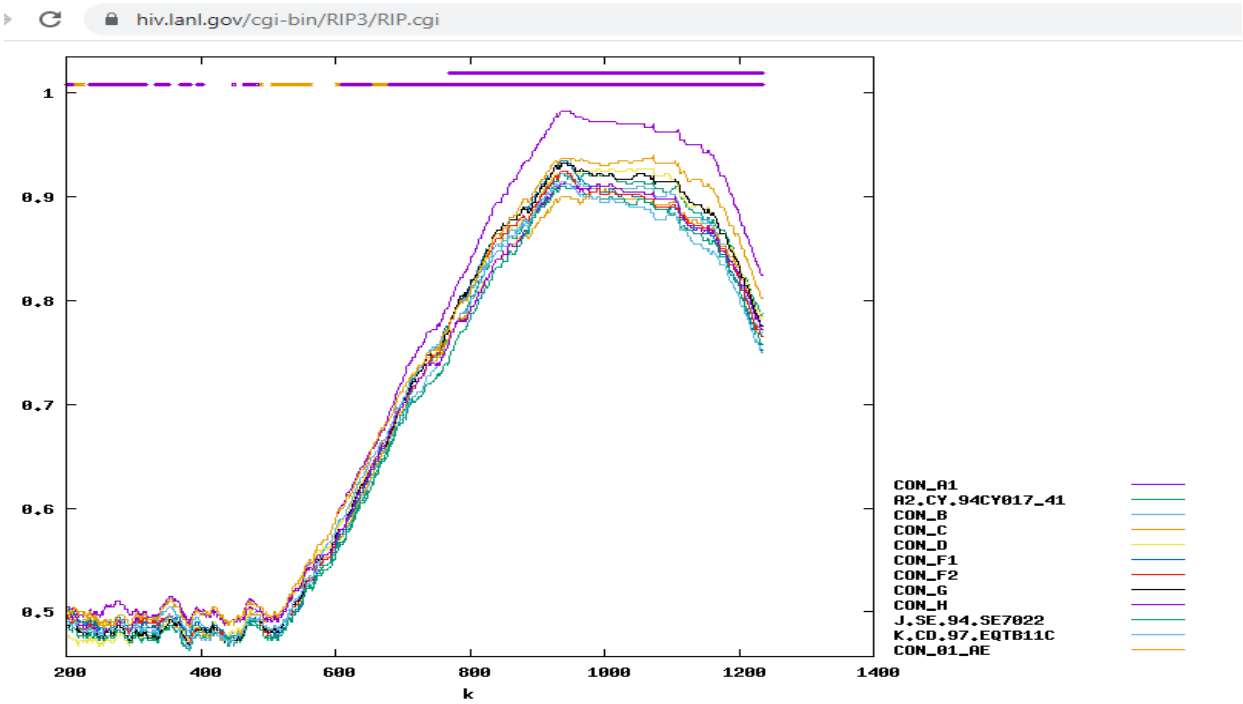


Figure 5: A representative sequence analysis result from this study using the Los Alamos HIV database tool, Recombinant Identification Program (RIP). Ref; https://www.hiv.lanl.gov/cgi-bin/RIP3/RIP.cgi.

From the figure 5 above shows the analysis for determination of recombination in the study's sequence data, the dominant curve was that of A1 sub type.

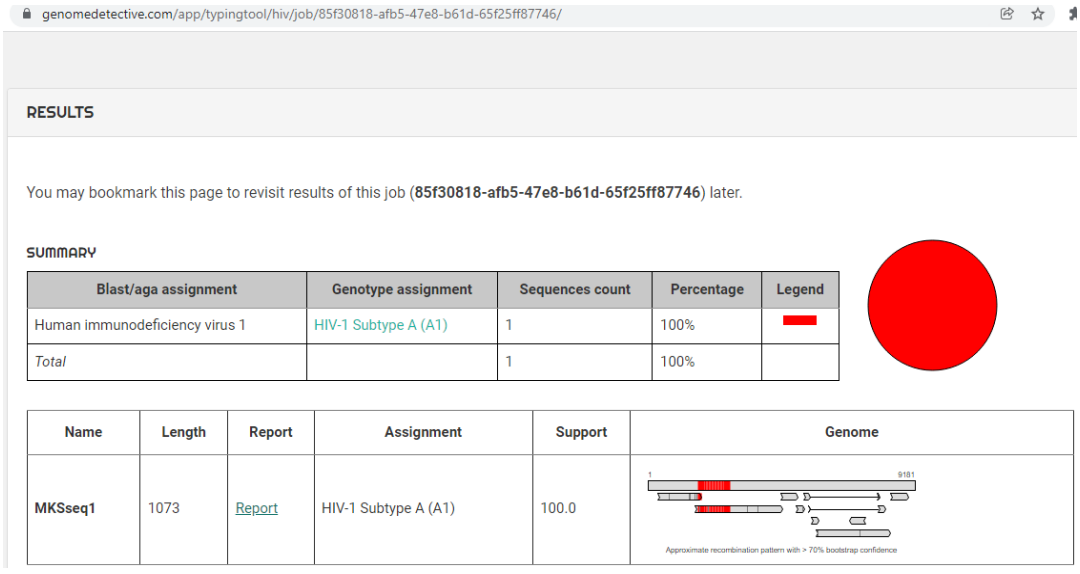


Figure 6: Sequence Analysis from REGA HIV Subtyping https://www.genomedetective.com/app/typingtool/hiv.

This study's sequences were later uploaded to the online REGA HIV, which is an HIV Drug Resistance Database subtyping database using the default algorithm. A representation of the genotyping analysis results is shown in figure 6 above. Figure 6 above confirms that the predominant HIV-1 subtype is, subtype A 1 with 100 percent support.

To determine the phylogenetic relatedness to similar subtype sequences, sequences with high relatedness were fetched in a FASTA format which is a text-based format for representing either nucleotide sequences or amino acid (protein) sequences, and aligned using the Mega Evolutionary Genetic Analysis (MEGA) software version 10 using the Neighbour Joining (NJ) algorithm and the 1000 Bootstrap resampling algorithm. The NJ algorithm substitution model used was the Kimura 2-parameter method. The generated tree to infer and assign HIV-1 subtypes is shown below in figure 7.

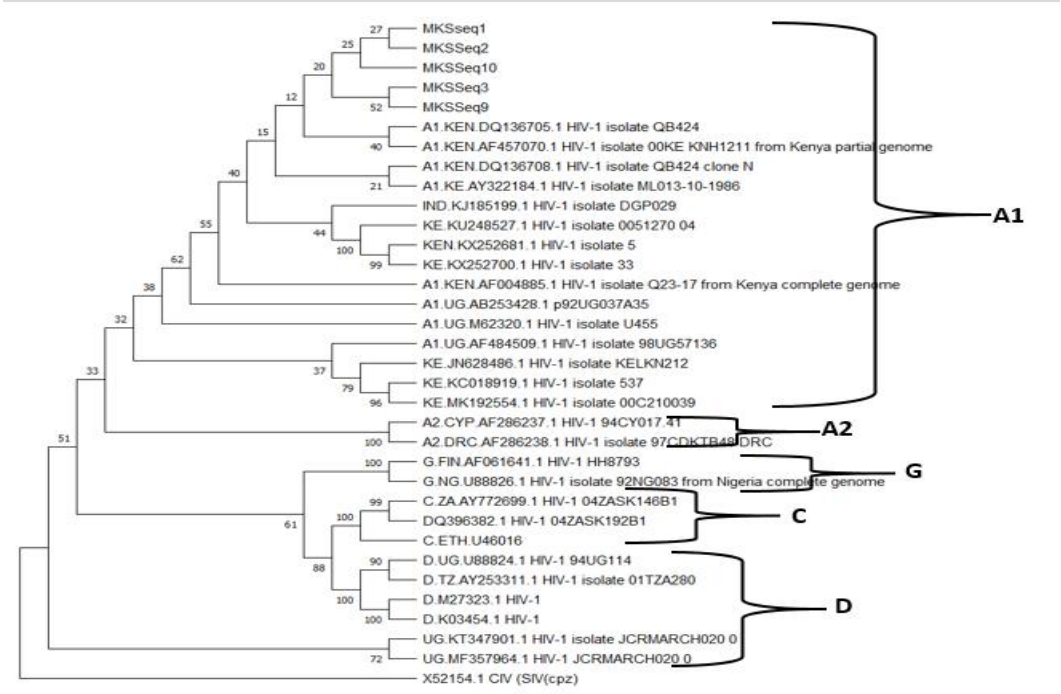


Figure 7: Phylogenetic Relatedness from BLAST and HIV BLAST Databases using MEGA version 10 Neighbor-Joining (NJ) algorithm at 2000 replicates Rooted with SIV (cpz)-X52.

The phylogenetic tree above also confirms the genotyping as of sub-type A1 and it aligned with other Kenyan sub-type A1 sequences. There is a unique observation in clustering of these study's samples on the phylogenetic tree after 2000 replicates.

CHAPTER FIVE

SUMMARY, DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

Summary

This chapter offers the discussion, conclusion, and recommendations. This is in line with the specific research objectives: to profile the Nucleoside Reverse Transcriptase Inhibitor drug-resistant and susceptibility patterns of naive HIV-positive patients from Machakos Level 5 hospital.

Discussion

Research shows that in East Africa, most HIV infections are caused by subtype A 1, plus also subtypes C and D, and some URFs (Bbosa et al., 2019). Subtype A is also distributed in Russia and the former Soviet Union and Central Asia (Bbosa et al., 2019; Akhome, 2021). In Kenya, research investigations conducted across the nation in diverse locations ranging from the coast to western and central Kenya show the detection and, in some cases, preponderance of subtype A, as well as the existence of subtypes C and D. This study's sequences on analysis had shown genetic similarities of between 85 – 97% alignment with HIV subtype A as shown by the representative sequence in the HIV BLAST results. This is similar to what other researchers have shown from previous studies carried out here in Kenya, which show the predominance of subtype A, where they range from 44% to 74% detection (Yang, et al., 2003; Khamadi, et al., 2009; Lihana, et al., 2009; Kageha, et al., 2012; Nyamache, et al., 2013; Adungo, et al., 2014; Koigi, et al., 2014; Kitawi, et al., 2015; Luvai, et al., 2015; Gounder, et al., 2017; Onywera, et al., 2017; Kinyua, et al., 2018; Hassan, et al., 2018; Nduva, et al., 2020; Mabeya, 2021; Adhiambo, et al., 2021). At the time of the study,

the amplified and sequenced samples data indicated the presence of only subtype A and no other subtype was detected from the samples, which is a difference from other studies carried out in Kenya.

When the Recombinant Identification Program (RIP) hosted by the Los Alamos Database was used to analyze for and determine recombination in this study's sequences, from the results, the most dominant curve was that of the A1 sub-subtype. This was also confirmed by the REGA HIV subtyping tool, which also confirmed the genotyping as sub-subtype A1 with 100% support. When the phylogenetic tree was drawn using similar sequences obtained from the Genbank Database, this study's sequences aligned with other Kenyan sub-subtype A1 sequences. A unique observation is the clustering of this study's samples on the inferred phylogenetic tree after 2000 replicates. Previous Kenyan studies carried out on various groups, including expectant mothers, intravenous drug users, and HIV infected individuals, have majorly the sub-subtype A1, which is the most predominant strain detected with detection rates ranging from 44% to 86% (Yang, et al., 2003; Khamadi, et al., 2009; Lihana, et al., 2009; Kageha, et al., 2012; Nyamache, et al., 2013; Adungo, et al., 2014; Koigi, et al., 2014; Kitawi, et al., 2015; Luvai, et al., 2015; Gounder, et al., 2017; Onywera, et al., 2017; Kinyua, et al., 2018; Hassan, et al., 2018; Nduva, et al., 2020; Mabeya, 2021; Adhiambo, et al., 2021). The neighbor-joining method for constructing phylogenetic trees uses pairs of operational taxonomic units (OTU) or neighbors to minimize branch lengths, starting with a starlike tree that is repeatedly run at each replication stage to ensure the integrity of the tree structure (Saitou and Nei, 1987). The Kimura 2 parameter model used in this analysis has the role of estimating genetic distances between different nucleotide sequences during an evolutionary process (Kimura, 1980; Nishimaki and Sato, 2019).

Profile of Resistance to Nucleoside Reverse Transcriptase Inhibitor (NRTI) Drugs

Nucleoside reverse transcriptase inhibitors (NRTIs) are medicines that prevent viral DNA from being reversed within cells. They contain nucleotide base analogs that will cause the chain termination or non-extension of the DNA during reverse transcription of the HIV viral DNA while inside an infected cell using the HIV reverse transcriptase (Immunopedia, 2021). Their method of action is either through description, resulting in a reduction in the binding affinity of NRTI-triphosphate over the natural nucleotide at the reverse transcriptase binding site, or non-discriminatory (Immunopedia, 2021). Again, this may decrease the NRTI-phosphate over the natural nucleotide rate of incorporation of the reverse binding site. The second mechanism is an excision process that relies on adenosine triphosphate (ATP) or pyrophosphate; hence, mutations that improve reverse transcriptase affinity for ATP or increase the rate of analog complex removal are preferred (Immunopedia, 2021). Additionally, changes in the capacity of the residues to translocate from the active site (N-site) to the post-translocation site (P-site), and the rate of separation of the template/primer from the enzyme, may also help to improve the excision route (immunopedia, 2021). Generally, NRTIs are drugs that comprise abacavir (ABC), zidovudine (AZT), emtricitabine (FTC), lamivudine (3TC), tenofovir (TDF), stavudine (d4T), didanosine (ddl), and zalcitabine (ddC) (NASCOP, 2018; Immunopedia, 2021). All the sequenced samples had a D67E mutation in this study, which is a change of amino acid aspartic acid (D) to glutamic acid (E). Aspartic acid (D) and glutamic acid (E) are used to form proteins in the body (Immunopedia, 2021). According to the HIV research, this is a non-polymorphic NRTI selected mutation at position 67 that is a change from aspartic acid (D) to either glutamic acid (E), serine (S), threonine (T), or histidine (H), that is D (67G/E/S/T/H) which has been associated with low-level resistance to AZT and d4T, and reduced susceptibility to ABC, DDL and TDF (Shafer, et al., 2000; Rhee et al., 2003; Liu, and Shafer, 2006; Rhee, et al., 2006; Shafer, 2006;

Immunopedia, 2021). Previous research studies from Kenya have shown resistance to the resistance mutation at position 67, where there was a change from glutamic acid (D) to asparagine (N), thus the acronym D67N. These studies also noted resistance to AZT and other NRTIs (Lihana, et al.,2009; Luvai, et al.,2015; Kinyua, et al.,2018; Mabeya, 2021).

Markers for Resistance to non-Nucleoside Reverse Transcriptase Inhibitor Drugs

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) inhibit reverse transcription by directly attaching to the enzyme reverse transcriptase (RT) and preventing it from working. Typically, they are tiny chemical compounds with a lengthy half-life (Immunopedia, 2021).

Generally, NNRTIs drugs include nevirapine (NVP), efavirenz (EFV), doravirine (DOR), and delavirdine (DLV) (NASCOP, 2018; Immunopedia, 2021). No major NNRTIs resistance mutations were noted in the amplified samples sequenced in this study. Results from table 2 shows that the minor mutations were
V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E, I178L, V179I, Q207A, R211S. Valine is a plant synthesized essential acid used for muscle growth stimulation, regeneration, and energy production, while isoleucine, another essential amino acid, is involved in muscle metabolism and important in immune functions and collagen production (Immunopedia, 2021). This polymorphic mutation is frequently seen in patients receiving etravirine (ETR) and rilpivirine (RPV) but has a little direct effect on NNRTI susceptibility. A study carried out in Kisii County also detected the same V179I mutation in 10 sampled patients (Mabeya, 2021). The most commonly identified resistance mutation noted in other studies was at position 103, where lysine (K) changed to asparagine (N), thus the acronym K103N (Lihana, *et al.,* 2009; Saida, *et al.,* 2013; Koigi, *et al.,* 2014; Luvai, *et al.,* 2015; Hassan, *et al.,* 2018; Kinyua, *et al.,* 2018). However, this mutation was not noted in this study. Other 13 mutations

Conclusions

A high prevalence of drug resistance mutations was found. The most significant predictors of HIVDRM were viral load and treatment duration. The most striking finding was that a subject's sex and treatment-duration independently influenced HIV DR counts, emphasizing the importance of targeted resistance monitoring and switching ART regimens while taking into account the risk of exhausting future treatment options.

Recommendations

- Importantly, patients with triple and dual class drug resistance should alter ART-regimens immediately to avoid the possibility of transmitting multidrug-resistant HIV-1 strains, which would have fewer treatment options.
- More research is needed to determine the variables that contributed to the finding that a subject's sex and treatment time independently influenced HIV-1 drug resistance mutations.

REFERENCES

Adhiambo, M., Makwaga, O., Adungo, F., Kimani, H., Mulama, D.H., Korir, J.C. and Mwau, M., (2021). Human immunodeficiency virus (HIV) type 1 genetic diversity in HIV positive individuals on antiretroviral therapy in a cross-sectional study conducted in Teso, Western Kenya. *The Pan African Medical Journal*, *38*.

Adungo, F.O., Gicheru, M.M., Adungo, N.I., Matilu, M.M., Lihana, R.W. and Khamadi, S.A., (2014). Diversity of human immunodeficiency virus type-1 subtypes in Western Kenya.

Akhome, P. (2021). HIV subtypes. Available online at https://www.aidsmap.com/about-hiv/hiv-1-subtypes accessed 30th October 2021.

Bbosa, N., Kaleebu, P. and Ssemwanga, D., (2019). HIV subtype diversity worldwide. *Current Opinion in HIV and AIDS*, *14*(3), pp.153-160.

Carrico, A. W., Shoptaw, S., Cox, C., Stall, R., Li, X., Ostrow, D. G., ... and Plankey, M. W. (2014). Stimulant use and progression to AIDS or mortality after the initiation of highly active anti-retroviral therapy. *Journal of acquired immune deficiency syndromes (1999)*, *67*(5), 508.

Clutter, D. S., Jordan, M. R., Bertagnolio, S., and Shafer, R. W. (2016). HIV-1 drug resistance and resistance testing. *Infection, Genetics and Evolution*, *46*, 292-307.

Deeks, S. G., Overbaugh, J., Phillips, A., and Buchbinder, S. (2015). HIV infection. *Nature reviews Disease primers*, *1*(1), 1-22.

Fletcher, C. V., Staskus, K., Wietgrefe, S. W., Rothenberger, M., Reilly, C., Chipman, J. G., ... and Schacker, T. W. (2014). Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues. *Proceedings of the National Academy of Sciences*, *111*(6), 2307-2312.

Gounder, K., Oyaro, M., Padayachi, N., Zulu, T.M., de Oliveira, T., Wylie, J. and Ndung'u, T., (2017). Complex Subtype Diversity of HIV-1 Among Drug Users in Major Kenyan Cities. *AIDS research and human retroviruses*, *33*(5), pp.500-510.

Hall, T., Biosciences, I. and Carlsbad, C., (2011). BioEdit: an important software for molecular biology. *GERF Bull Biosci*, *2*(1), pp.60-61.

Hassan, A.S., Esbjörnsson, J., Wahome, E., Thiong'o, A., Makau, G.N., Price, M.A. and 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), p.e0206177.

Immunopedia. (2021). ARV Mode of Action – NRTI. Available online at https://www.immunopaedia.org.za/treatment-diagnostics/hiv-infection-treatment/arv-mode-of-action/ accessed 5th November 2021.

Kageha, S., Lihana, R.W., Okoth, V., Mwau, M., Okoth, F.A., Songok, E.M., Ngaira, J.M. and Khamadi, S.A., (2012). HIV type 1 subtype surveillance in central Kenya. *AIDS research and human retroviruses*, *28*(2), pp.228-231.

Khamadi, S.A., Lihana, R.W., Osman, S., Mwangi, J., Muriuki, J., Lagat, N., Kinyua, J., Mwau, M., Kageha, S., Okoth, V. and Ochieng, W., (2009). Genetic diversity of HIV type 1 along the coastal strip of Kenya. *AIDS research and human retroviruses*, *25*(9), pp.919-923.

Kinyua, J.G., Lihana, R.W., Kiptoo, M., Muasya, T., Odera, I., Muiruri, P. and Songok, E.M., (2018). Antiretroviral resistance among HIV-1 patients on first-line therapy attending a comprehensive care clinic in Kenyatta National Hospital, Kenya: a retrospective analysis. *Pan African Medical Journal*, *29*(1), pp.1-6.

Kitawi, R.C., Nzomo, T., Mwatelah, R.S., Aman, R., Kimulwo, M.J., Masankwa, G., Lwembe, R.M., Okendo, J., Ogutu, B. and Ochieng, W., (2015). HIV-1 subtype

diversity based on envelope C2V3 sequences from Kenyan patients on highly active antiretroviral therapy. *AIDS research and human retroviruses*, *31*(4), pp.452-455.

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. and 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), pp.1211-1217.

Liu, T.F. and Shafer, R.W., (2006). Web resources for HIV type 1 genotypic-resistance test interpretation. *Clinical infectious diseases*, *42*(11), pp.1608-1618.

Luvai, E., Waihenya, R., Munyao, J., Sanguli, L., Mwachari, C. and Khamadi, S., (2015). HIV-1 Drug Resistance Mutations in Patients Failing 1st Line Therapy in a Comprehensive Care Center in Nairobi, Kenya.

Mabeya, S.N., (2021). *HIV-1 Genetic Diversity, Tropism and Drug Resistance Mutations among HIV Infected Patients Attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital, Kenya* (Doctoral dissertation, JKUAT-COHES).

Marrazzo, J. M., Ramjee, G., Richardson, B. A., Gomez, K., Mgodi, N., Nair, G., ... and Chirenje, Z. M. (2015). Tenofovir-based preexposure prophylaxis for HIV infection among African women. *New England Journal of Medicine*, *372*(6), 509-518.

Nael, A., Walavalkar, V., Wu, W., Nael, K., Kim, R., Rezk, S., and Zhao, X. (2016). CD4-positive T-cell primary central nervous system lymphoma in an HIV positive patient. *American journal of clinical pathology*, *145*(2), 258-265.

NASCOP, (2018), Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya, (2018) Edition. Available online at: https://www.nascop.or.ke/new-guidelines/ Accessed 30th November 2021.

Ndembi, N., Takehisa, J., Zekeng, L., Kobayashi, E., Ngansop, C., Songok, E.M., Kageyama, S., Takemura, T., Ido, E., Hayami, M. and Kaptue, L., (2004). Genetic diversity of HIV type 1 in rural eastern Cameroon. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *37*(5), pp.1641-1650.

Nduva, G.M., Hassan, A.S., Nazziwa, J., Graham, S.M., Esbjörnsson, J. and Sanders, E.J., (2020). HIV-1 transmission patterns within and between risk groups in coastal Kenya. *Scientific reports*, *10*(1), pp.1-10.

Nyamache, A.K., Muigai, A.W. and Khamadi, S.A., (2013). Circulating trends of non-B HIV type 1 subtypes among Kenyan individuals. *AIDS research and human retroviruses*, *29*(2), pp.400-403.

Rhee, S.Y., Gonzales, M.J., Kantor, R., Betts, B.J., Ravela, J. and Shafer, R.W., (2003). Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic acids research*, *31*(1), pp.298-303.

Rhee, S.Y., Kantor, R., Katzenstein, D.A., Camacho, R., Morris, L., Sirivichayakul, S., Jorgensen, L., Brigido, L.F., Schapiro, J.M., Shafer, R.W. and International Non-Subtype B HIV-1 Working Group, (2006). HIV-1 pol mutation frequency by subtype and treatment experience: extension of the HIVseq program to seven non-B subtypes. *AIDS (London, England)*, *20*(5), p.643.

Saitou, N. and Nei, M., (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, *4*(4), pp.406-425.

Shafer, R.W., (2006). Rationale and uses of a public HIV drug- resistance database. *The Journal of infectious diseases*, *194* (Supplement_1), pp.S51-S58.

Shafer, R.W., Jung, D.R. and Betts, B.J., (2000). Human immunodeficiency virus type 1 reverse transcriptase and protease mutation search engine for queries. *Nature medicine*, *6*(11), pp.1290-1292.

Smith, R. A., Raugi, D. N., Pan, C., Sow, P. S., Seydi, M., Mullins, J. I., and Gottlieb, G. S. (2015). In vitro activity of dolutegravir against wild-type and integrase inhibitor-resistant HIV-2. *Retrovirology*, *12*(1), 1-9.

Songok, E.M., Lwembe, R.M., Kibaya, R., Kobayashi, K., Ndembi, N., Kita, K., Vulule, J., Oishi, I., Okoth, F., Kageyama, S. and Ichimura, H., (2004). Active generation and selection for HIV intersubtype A/D recombinant forms in a coinfected patient in Kenya. *AIDS research and human retroviruses*, *20*(2), pp.255-258.

Stella-Ascariz, N., Arribas, J. R., Paredes, R., and Li, J. Z. (2017). The role of HIV-1 drug-resistant minority variants in treatment failure. *The Journal of Infectious Diseases*, *216*(suppl_9), S847-S850.

Yang, C., Li, M., Newman, R.D., Shi, Y.P., Ayisi, J., van Eijk, A.M., Otieno, J., Misore, A.O., Steketee, R.W., Nahlen, B.L. and Lal, R.B., (2003). Genetic diversity of HIV-1 in western Kenya: subtype-specific differences in mother-to-child transmission. *Aids*, *17*(11), pp.1667-1674.

Zhao, S., Feng, Y., Hu, J., Li, Y., Zuo, Z., Yan, J., ... and Xing, H. (2018). Prevalence of Transmitted HIV drug resistance in antiretroviral treatment naïve newly diagnosed individuals in China. *Scientific Reports*, *8*(1), 1-8.

APPENDICES

APPENDIX I: NUCLEOTIDE SEQUENCES OF AMPLIFIED STUDY PRODUCTS

>MKSseq1001

GGATGGGGGAAGAGATAGCCTCCCCTTCGAAGCAGGAGCAGAAAGACAGGGAA
CAGGCCCCACCTTTAATTTCCCTCAAATCACTCTTTGGCAACGACCTCTTGTCACA
GTAAGAATAGGGGGACAGCTAAAAGAAGCTCTATTAGATACAGGAGCAGATGAT
ACAGTATTAGAAGACATAGATTTGCCAGGAAAATGGAAACCAAAAATGATAGGG
GGAATTGGAGGTTTCATCAAGGTAAAACAGTATGATCAGATACTTATAGAAATTT
GTGGAAAAAAGGCTATAGGTACAGTATTAGTGGGACCTACACCTGTCAACATAA
TTGGAAGAAACATGTTGACCCAGATTGGTTGTACTTTAAATTTCCCAATTAGTCC
TATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAGGTTAA
ACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGA
TATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC
TCCAATATTTGCTATAAAGAAAAAAGAAAGCACTAAATGGAGGAAGTTAGTAGA
TTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGAAT
ACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGG
GGAAGCATATTTTTCAGTTCCTTTGCATGAAAGCTTTAGAAAATATACTGCATTC
ACCATACCTAGTATAAACAATGAGACACCAGGAATCAGATATCAGTATAATGTG
CTTCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAGTAGCATGACAAAA
ATCTTAGAGCCCTTTAGATCAAAAAATCCAGAATTAATTATCTATCAATACATGG
ATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAG
AAGAGTTAAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAGAAGC
ATCAGAAAGAACCTCCATTCCTTTGGATGGGATATGAGCTCC

>MKSSeq1002

GGATGGGGGAAGAGATAGCCTCCCCTTCGAAGCAGGAGCAGAAAGACAGGGAA
CAGGCCCCACCTTTAATTTCCCTCAAATCACTCTTTGGCAACGACCTCTTGTCACA
GTAAGAATAGGGGGACAGCTAAAAGAAGCTCTATTAGATACAGGAGCAGATGAT
ACAGTATTAGAAGACATAGATTTGCCAGGAAAATGGAAACCAAAAATGATAGGG
GGAATTGGAGGTTTCATCAAGGTAAAACAGTATGATCAGATACTTATAGAAATTT
GTGGAAAAAAGGCTATAGGTACAGTATTAGTGGGACCTACACCTGTCAACATAA
TTGGAAGAAACATGTTGACCCAGATTGGTTGTACTTTAAATTTCCCAATTAGTCC
TATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAGGTTAA
ACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGA
TATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC
TCCAATATTTGCTATAAAGAAAAAAGAAAGCACTAAATGGAGGAAGTTAGTAGA
TTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGAAT
ACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGG
GGAAGCATATTTTTCAGTTCCTTTGCATGAAAGCTTTAGAAAATATACTGCATTC
ACCATACCTAGTATAAACAATGAGACACCAGGAATCAGATATCAGTATAATGTG
CTTCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAGTAGCATGACAAAA
ATCTTAGAGCCCTTTAGATCAAAAAATCCAGAATTAATTATCTATCAATACATGG
ATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAG
AAGAGTTAAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAGAAGC
ATCAGAAAGAACCTCCATTCCTTTGGATGGGATATGAGCTCC

>MKSSeq1003
GGATGGGGGAAGAGATAGCCTCCCCTCAGAAGCAGGAGCAGAAAGACAGGGAA
CAGGCCCCACCTTTAATTTCCCTCAAATCACTCTTTGGCAACGACCTCTTGTCACA
GTAAGAATAGGGGGACAGCTAAAAGAAGCTCTATTAGATACAGGAGCAGATGAT
ACAGTATTAGAAGACATAGATTTGCCAGGAAAATGGAAACCAAAAATGATAGGG
GGAATTGGAGGTTTCATCAAGGTAAAACAGTATGATCAGATACTTATAGAAATTT
GTGGAAAAAAGGCTATAGGTACAGTATTAGTGGGACCTACACCTGTCAACATAA
TTGGAAGAAACATGTTGACCCAGATTGGTTGTACTTTAAATTTCCCAATTAGTCC
TATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAGGTTAA
ACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGA
TATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC
TCCAATATTTGCTATAAAGAAAAAAGAAAGCACTAAATGGAGGAAGTTAGTAGA
TTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGAAT
ACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGG
GGAAGCATATTTTTCAGTTCCTTTGCATGAAAGCTTTAGAAAATATACTGCATTC
ACCATACCTAGTATAAACAATGAGACACCAGGAATCAGATATCAGTATAATGTG
CTTCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAGTAGCATGACAAAA
ATCTTAGAGCCCTTTAGATCAAAAAATCCAGAATTAATTATCTATCAATACATGG
ATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAG
AAGAGTTAAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAGAAGC
ATCAGAAAGAACCTCCATTCCTTTGGATGGGATATGAGCTCC

>MKSSeq1009
GGATGGGGGAAGAGATAGCCTCCCCTCAGAAGCAGGAGCAGAAAGACAGGGAA
CAGGCCCCACCTTTAATTTCCCTCAAATCACTCTTTGGCAACGACCTCTTGTCACA
GTAAGAATAGGGGGACAGCTAAAAGAAGCTCTATTAGATACAGGAGCAGATGAT
ACAGTATTAGAAGACA**T**AGATTTGCCAGGAAAATGGAAACCAAAAATGATAGG
GGGAATTGGAGGTTTCATCAAGGTAAAACAGTATGATCAGATACTTATAGAAAT
TTGTGGAAAAAAGGCTATAGGTACAGTATTAGTGGGACCTACACCTGTCAACAT
AATTGGAAGAAACATGTTGACCCAGATTGGTTGTACTTTAAATTTCCCAATTAGT
CCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAGGTT
AAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACA
GATATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAAT
ACTCCAATATTTGCTATAAAGAAAAAAGAAAGCACTAAATGGAGGAAGTTAGTA
GATTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGA
ATACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTG
GGGGAAGCATATTTTTCAGTTCCTTTGCATGAAAGCTTTAGAAAATATACTGCAT
TCACCATACCTAGTATAAACAATGAGACACCAGGAATCAGATATCAGTATAATG
TGCTTCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAGTAGCATGACAA
AAATCTTAGAGCCCTTTAGATCAAAAAATCCAGAATTAATTATCTATCAATACAT
GGATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAAT
AGAAGAGTTAAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAGAA
GCATCAGAAAGAACCTCCATTCCTTTGGATGGGATATGAGCTCC

>MKSSeq1010
GGATGGGGGAAGAGATAGCCTCCCCTTGGAAGCAGGAGCAGAAAGACAGGGAA
CAGGCCCCACCTTTAATTTCCCTCAAATCACTCTTTGGCAACGACCTCTTGTCACA
GTAAGAATAGGGGGACAGCTAAAAGAAGCTCTATTAGATACAGGAGCAGATGAT
ACAGTATTAGAAGACATAGATTTGCCAGGAAAATGGAAACCAAAAATGATAGGG
GGAATTGGAGGTTTCATCAAGGTAAAACAGTATGATCAGATACTTATAGAAATTT

GTGGAAAAAAGGCTATAGGTACAGTATTAGTGGGACCTACACCTGTCAACATAA
TTGGAAGAAACATGTTGACCCAGATTGGTTGTACTTTAAATTTCCCAATTAGTCC
TATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAGGTTAA
ACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTAACAGAAATTTGTACAGA
TATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC
TCCAATATTTGCTATAAAGAAAAAAGAAAGCACTAAATGGAGGAAGTTAGTAGA
TTTCAGAGAGCTCAATAAAAGAACACAAGACTTTTGGGAAGTTCAATTAGGAAT
ACCGCATCCAGCGGGCCTAAAAAAGAAAAAATCAGTAACAGTACTAGATGTGGG
GGAAGCATATTTTTCAGTTCCTTTGCATGAAAGCTTTAGAAAATATACTGCATTC
ACCATACCTAGTATAAACAATGAGACACCAGGAATCAGATATCAGTATAATGTG
CTTCCACAGGGATGGAAAGGATCACCGGCAATATTCCAGAGTAGCATGACAAAA
ATCTTAGAGCCCTTTAGATCAAAAAATCCAGAATTAATTATCTATCAATACATGG
ATGACTTGTATGTAGGATCTGATTTAGAAATAGGGCAGCATAGAACAAAAATAG
AAGAGTTAAGAGCTCATCTATTGAGCTGGGGATTTACTACACCAGACAAGAAGC
ATCAGAAAGAACCTCCATTCCTTTGGATGGGATATGAGCTCC

APPENDIX II: CURRICULUM VITAE

PERSONAL PROFILE

I am a creative and enthusiastic Biomedical scientist with a background undergraduate study in Biochemistry and Molecular biology and with a recent working experience in a flu laboratory. I have experience in general sample handling, molecular identification and characterization of respiratory viruses, nucleic acid quantification, sanger chain sequence termination technique, serological characterization of influenza virus, isolation of respiratory viruses from specific cell lines, preparation of media reagents for cell culture, virus transportation, recording and reporting all results to data manager I possess strong analytical and communications skills and a dedicated approach to working in a highly controlled working environment.

CAREER OBJECTIVE

To seek a responsible and challenging position in Science Research Institutions, Medical diagnostic laboratories, pharmaceutical companies, and also food, water and drug companies in quality assurance and quality control department, where my knowledge and experience can be shared and enriched.

KEY SKILLS AND PERSONAL ATTRIBUTES

- **Technical skills**: Molecular identification and characterization of respiratory viruses, well use of real time RT- PCR, characterization using Hemagglutination and Immunofluorescence assays, sequencing techniques and recording and reporting all results.
- Fluent in both English and Kiswahili
- **Computer skills proficient** (MS word, MS Excel, MS Access, MS Power-point, Internet, Programming languages <C++> and MS publisher)
- **Statistical tools skills proficient** (Advanced Biostatistics and Instrumental design)
- Excellent in both interpersonal communication skills and organizational skills
- **Personal attributes**: God fearing, self-disciplined, respect for authority, team player, and self-driven.

EDUCATION AND QUALIFICATIONS

2017- to Date: UNIVERSITY OF EASTERN AFRICA, BARATON

MSc. Biology (Biomedical Science option) - ***Thesis project***

Thesis: Profilling nucleoside reverse transcriptase inhibitor drug-resistance and susceptibility patterns of naive HIV positive patients from Machakos level 5 Hospital

2013 -2016: SOUTH EASTERN KENYA UNIVERSITY

BSc. Biochemistry and Molecular Biology - ***Second Class (lower)***

***Senior project*:** Phytochemical screening and antimicrobial properties of *allium sativum* against lactobacillus

.2008-2011: TAMBACH BOYS HIGH SCHOOL

Kenya Certificate of Secondary Education

Mean Grade: **B+** ***(Plus)***

2000-2007: ST MARY'S PRIMARY SCHOOL

Kenya Certificate of Secondary Education

Aggregate Grade B+ (plus)

OTHER TRAINING AND SEMINARS

October 2012: Computer packages (MS Packages) at Mogotio Youth Polytechnic.

January 2013: Digital Opportunity Trust program (DOT) course at Mogotio Youth polytechnic that aims to impart youth with, ICT skills, life skills and Entrepreneurship skills.

May 2013: Baseline Methodology course for the Farmer Managed Natural Resource survey.

August 2012: BCE class driving training at Sydney driving school in Eldama Ravine.

October 2018: Safety training (Blood borne pathogens and infection control, Fire safety and evacuation, Hazardous materials and chemical hygiene and General laboratory safety) course at Kenya Medical Research Institute.

December 2018: Quality control and Quality Assurance (QA/QC)

WORK EXPERIENCE

September 2018 – December 2021

US Army Medical Research Directorate-Kenya (USAMRD-K)

(Kenya Medical Research Institute – Center for Public Health Research)

Medical scientist - Acute Respiratory Infections (ARI) Unit.

Laboratory attached to Influenza laboratory

Key responsibilities

- **Sample reception, handling techniques and inventory of biological specimen including nasopharyngeal swab specimen**
- **Virology:**
  - Cell culture (MDCK, RD, LLCMK2 and Hep-2 mammalian cell lines)
  - Isolation of respiratory viruses including Influenza and Adenovirus among others. Identification of viruses by Immunofluorescence Assays
  - Typing and sub-typing of Influenza viruses by Hemagglutination Inhibition assays
- **Molecular Biology techniques:**
  - Extraction of nucleic acid (RNA/DNA) from various samples
  - Quantification of nucleic acid using Nanodrop and Qubit fluorometer
  - Molecular identification of viruses using Real Time RT-PCR and conventional PCR.
  - Agarose gel electrophoresis
  - Sanger sequencing using the 3500xL Genetic analyzer Basic nucleotide sequence analysis
  - Real - time PCR for screening of influenza A and B and other respiratory viruses.
  - Conventional PCR and Agarose gel electrophoresis
  - Sanger chain termination sequencing methods:
  - Basic bioinformatics manipulation of biological data
- **Trainings:**
  - Biosafety: fire and evacuation, hazard communication and chemical hygiene, Blood borne pathogens and infection control and laboratory safety.
  - Quality Assurance and Quality Control including participation in the WHO sponsored External quality assessment program (EQAP) for the laboratory
- **Others:**
  - General field sample handling
  - Attended internal scientific seminars
  - General cleaning, maintenance of laboratory equipment and working surfaces
  - Recording and reporting all results to data manager

- Training and orientation of new attaches and interns in the laboratory.

October 2017 – October 2017

Independent Electoral and Boundaries Commission (IEBC) as Deputy presiding officer

Key responsibilities

- Conduct training on the polling, counting and tallying process for the clerks.
- Oversee efficient and effective management of the election before, during and after of election.
- Ensure the good conduct of elections in the polling station or tallying center as assigned.
- Counting, tallying and announcement of provisional results in the polling station and sending the same through online transmission to the National and Constituency tallying centers.
- Ensure safe custody all entrusted documents during the 2017 General Elections, document hand over of all election materials and equipment to RO after the General Election.

May 2017 – June 2017

Independent Electoral and Boundaries Commission (IEBC)

Voter Verification and Inspection Assistant – Mogotio Ward

Key responsibilities

- Publicized register of voters' verification activities.
- Distributed verification/inspection materials to the Verification/inspection clerks.
- Supervised Verification/ Inspection Clerks within the Ward.
- Ensured security of the register of voters' inspection materials.
- Confirmed the GPS coordinates and map polling stations.
- Carried out mapping of polling stations, collected and confirmed the GPS coordinates of the polling stations within the ward.

19th January – 26th February 2017

Independent Electoral and Boundaries Commission (IEBC)

Voter Registration Assistant (VRA) – Mogotio Ward

Key responsibilities

- Supervised registration exercise of clerks within Mogotio Ward, met weekly targets of registered voters within ward
- In consultation with the Registration Officer drew a BVR KIT Movement Schedule for the ward of his/her Jurisdiction
- Publicized BVR KIT Movement Schedule and all voter registration activities
- Distributed voter registration materials to the Voter Registration Clerks.
- Supervised Voter Registration Clerks within the Ward of jurisdiction
- Ensured security of the voter registration materials
- Reported the progress and challenges of the voter registration process to the Registration Officer on daily basis.

Other work experiences:

Jan 2015 to April 2015

Prime International Company, Nakuru

Sales representative

Sept 2016 to December

Ngubereti High School, Mogotio

Chemistry and Biology teacher

July 2013 to August 2013

World Vision Kenya – Mogotio IPA

Enumerator

Conducted a Farmer Managed Natural regeneration survey at Mogotio IPA

PUBLICATIONS

Kiprop, **S**., and Muthangya, M. (2021). Phytochemical screening and antimicrobial properties of *allium sativum* against lactobacillus. International Journal of Sciences: Basic and Applied Research (IJSBAR), Vol 60 No 1(2021).

ACHIEVEMENTS

- Acted as a chairman, Agriculture student association from 2010 to 2011 at Tambach High School
- Serving as active member, Chemogoch University Students Association
- During my Internship at USAMRU-KENYA, I did the training and orientation of new attaches and interns in the laboratory.

HOBBIES

- Swimming
- Watching documentaries
- In sports, my particular interest is Badminton

REFEREES

1. Dr. Mutemi Muthangya
   South Eastern Kenya University,
   Cell-phone: 0716 962771,
   P. O. Box 170 - 90200, Kitui
   Email: temi2m@yahoo.com
   Alt. email: mmuthangya@seku.ac.ke

2. Samwel Morris Lifumo Symekher
   Acute Respiratory Infectious Unit, KEMRI
   P.O Box 54628-00200, Nairobi.
   E-mail: slifumo@kemri.org
   Cell: 0712 466 771

3. Michaiah Ojunga
   University of Eastern Africa, Baraton
   Cell-phone: 0713 052 062
   P. O. Box 2500-30100, Eldoret.
   Email: mojungaf@yahoo.co

APPENDIX III: TIMEFRAME 2019/2022

Activity	**April – October 2019**			**December 2019**	**Jan 2020 – Sep 2021**	**Sep 2021 – Jan 2022**	**May – June 2022**	**June 2022**
Proposal Writing								
Proposal defense								
Sample collection and Experimentation								
Data analysis and Thesis Drafting								
Thesis Defense								
Final Corrections								

APPENDIX IV: BUDGET

ITEM	COST(KSH)
Laboratory Items	145,000
DNA extraction kits	143,000
Electrophoresis buffers	98,000
PCR primers	65,000
Gloves	2,500
Others	8,000
Printing & binding	4,500
Transport	6,000
Miscellaneous	8,000
TOTAL	**380,000**

APPENDIX V: UEAB RESEARCH ETHICS COMMITTEE APPROVAL LETTER

OFFICE OF THE DIRECTOR OF GRADUATE STUDIES AND RESEARCH
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. BOX 2500-30100, Eldoret, Kenya, East Africa

B4025032021 March 25, 2021

TO: Kiprop Stanley
School of Science and Technology
Department of Biological Sciences and Agriculture
University of Eastern Africa, Baraton

Dear, Kiprop,

RE: Nucleoside Reverse Transcriptase Inhibitor Drug Resistant Profiles And Their Susceptibility Patterns Of Naïve HIV Patients From Machakos Level 5 Hospital

This is to inform you that the Research Ethics Committee (REC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/REC/40/03/2021. The approval period is 25th March, 2021 – 25th March, 2022.

This approval is subject to compliance with the following requirements;

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

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

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Research Ethics Committee

University of Eastern Africa, Baraton
25 MAR 2021
Research Ethics Committee

APPENDIX VI: OFFICE OF DIRECTOR OF GRADUATE STUDIES AND RESEARCH PERMISSION LETTER

OFFICE OF DIRECTOR OF GRADUATE STUDIES AND RESEARCH
UNIVERSITY OF EASTERN AFRICA, BARATON
P.O. Box 2500, Eldoret, Kenya

April 1, 2021

National Council for Science, Technology, and Innovation
P.O. Box 30623 – 00100
Nairobi, Kenya

Dear Sir/Madam,

Mr. Kiprop Stanley is a graduate student pursuing the degree of Master of Science in Biological Sciences (Bio-Medical Option) at the University of Eastern Africa, Baraton. He is currently writing his thesis entitled: ***Nucleoside Reverse Transcriptase Inhibitor Drug Resistant Profiles and their Susceptibility Patterns of Naïve HIV Patients from Machakos Level 5 Hospital.***

I am asking you to please allow him to conduct his research in selected respondents in Kenya. The research permit you will grant him will surely facilitate his data-gathering.

Any assistance given to Stanley will be greatly appreciated.

Sincerely yours,

UNIVERSITY OF EASTERN AFRICA, BARATON
DIRECTOR
01 APR 2021
GRADUATE STUDIES
BARATON

Dr. Moses Kibarango, PhD
Ag. Director of Graduate Studies

Cc: Chair, Department of Biological Sciences & Agriculture
Office File

A SEVENTH-DAY ADVENTIST INSTITUTION OF HIGHER LEARNING
CHARTERED 1991

APPENDIX VII: NACOSTI RESEARCH PERMIT

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 114300

Date of Issue: 26/April/2021

RESEARCH LICENSE

This is to Certify that Mr.. STANLEY KIPKURUI KIPROP of University of Eastern Africa, Baraton, has been licensed to conduct research in Nairobi on the topic: NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR DRUG RESISTANCE PROFILES AND THEIR SUSCEPTIBILITY PATTERNS OF NAIVE HIV PATIENTS FROM MACHAKOS LEVEL 5 HOSPITAL for the period ending : 26/April/2022.

License No: NACOSTI/P/21/10128

114300

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

APPENDIX III: PLAGIARISM REPORT FROM UEAB LIBRARY

Ouriginal

Document Information

Analyzed document	Stanley Thesis Final.docx (D142071350)
Submitted	2022-07-15 08:29:00
Submitted by	Hellen Magut
Submitter email	maguthe@ueab.ac.ke
Similarity	5%
Analysis address	hellenmagut.unieab@analysis.urkund.com

Sources included in the report

SA **Col_S_Karade_Thesis_NARI.pdf**
Document Col_S_Karade_Thesis_NARI.pdf (D53964738)

W URL: https://www.verywellhealth.com/hiv-2-5112635
Fetched: 2021-10-30 15:19:23

SA **T.R Dinesha.pdf**
Document T.R Dinesha.pdf (D30690201)

SA **Manuscript_HIVDR in Angola_Review.docx**
Document Manuscript_HIVDR in Angola_Review.docx (D69126878)

SA **urkund.docx**
Document urkund.docx (D62212688)

SA **PS Int, RoL, MM & Res - 2.0_2.docx**
Document PS Int, RoL, MM & Res - 2.0_2.docx (D31356139)

SA **HNguyen Thesis 18Jun.docx**
Document HNguyen Thesis 18Jun.docx (D14794910)

SA **Article cientific VIH. Grup 13..pdf**
Document Article cientific VIH. Grup 13..pdf (D129756542)

W URL: https://www.frontiersin.org/articles/10.3389/fmicb.2022.843330/full
Fetched: 2022-04-25 07:59:55

W URL: https://www.frontiersin.org/articles/10.3389/fmicb.2022.846943/full
Fetched: 2022-05-03 15:28:36

W URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0206177
Fetched: 2021-11-10 18:57:38

W URL: https://www.sci-hub.se/10.1371/journal.pone.0206177
Fetched: 2022-07-15 08:29:56

W URL: https://www.ncbi.nlm.nih.gov/sites/ppmc/articles/PMC6061825/
Fetched: 2022-04-25 08:00:03

SA **0209.pdf**
Document 0209.pdf (D55023816)

W URL: https://scholar.google.com/citations?user=OQEC5nEAAAAJ
Fetched: 2022-07-15 08:30:05

Entire Document

PROFILING NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR DRUG-RESISTANCE AND SUSCEPTIBILITY PATTERNS OF NAIVE HIV POSITIVE PATIENTS FROM MACHAKOS LEVEL 5 HOSPITAL
A Thesis Submitted to the Department of Biological Sciences and Agriculture, School of Science and Technology
University of Eastern Africa, Baraton
In Partial Fulfillment of the Requirements For the Degree of Master of Biological Sciences: Biomedical Sciences
Stanley Kipkurui Kiprop
SSTAKI1811
May, 2022
CHAPTER ONE

APPENDIX IX: PUBLICATION

International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Manuscript Information	
Manuscript Number (ID)	14156
Title	To Profile Nucleoside Reverse Transcriptase Inhibitor Drug-Resistance and Susceptibility Patterns of Naive HIV Positive Patients from Machakos Level 5 Hospital

Congratulations! The review process for the International Journal of Sciences: Basic and Applied Research (IJSBAR) has been completed. The journal during its journey which started in 2009 received submissions from 55 different countries and regions, which were reviewed by international experts.

Based on the recommendations of the reviewers and Based on the editorial board decision, we are pleased to inform you that your paper identified above has been accepted for publication in peer reviewed and indexed [Ulrich, Google Scholar , Directory of Open Access Journals (DOAJ),Ulrich's Periodicals Directory, Microsoft academic research, University of Texas (USA),Stanford University (USA), State University Libraries of Florida (included in 11 universities libraries in Florida)(USA), University of Cambridge (United Kingdom), Simon Fraser University (Canada), University of South Australia (Australia),OAIster database, PubZone (ACM SIGMOD), Research gate, OCLC World Cat, IE Library (Spain), Elektronische Zeitschriftenbibliothek (Ezb germany), Simpson University (USA), Columbia University (USA), NEOS library consortium (Canada), University of Melbourne (Australia), Technische Universität Darmstadt (Germany), University of Gronignen (The Netherlands), University of Liverpool (UK), Universität Wurzburg (Germany), Academic research (ourGlocal), Issuu, Researchbib, Journal seek,docstoc, ProLearnAcademy, ectel07, University of Canterbury (New Zealand), University of Hong Kong, Queen's University (Canada), Universität Mainz (Germany), University of Saskatchewan (Canada), The Hong Kong University of Science & Technology, University of Manitoba (Canada), Auckland University of Technology (New Zealand), scribd, prorch, slideshare, mendeley, academia, Genamics JournalSeek, Internet archive, Ebookbrowse, CiteSeer, Physikalisch Technische Bundesanstalt (Germany), University of Twente (The Netherlands), Universität Osnabrück (Germany), Universität Marburg (Germany) , University of IOWA (USA), etc] International Journal of Sciences: Basic and Applied Research (IJSBAR) (ISSN 2307-4531). The acceptance decision was based on the internal and external reviewers' evaluation after internal and external double blind peer review and chief editor's approval.

Finally, we would like to further extend our congratulations to you.
Yours sincerely,
IJSBAR editorial board

International Journal of Sciences: Basic and Applied Research (IJSBAR)
ISSN 2307-4531
(Print & Online)
http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

To Profile Nucleoside Reverse Transcriptase Inhibitor Drug-Resistance and Susceptibility Patterns of Naive HIV Positive Patients from Machakos Level 5 Hospital

Mr. Stanley Kiprop [a], Dr. Gracelyn P. Francis [b], Dr. Mutemi Muthangya [c]

[a]*PG Student, Biomedical Science, University of Eastern Africa Baraton, P.O.BOX. 2500-30100 Eldoret, Kenya*
[b] *Lecturer, Department of Biological Sciences and Agriculture, University of Eastern Africa, Baraton, P.O.BOX 2500 – 30100 Eldoret, Kenya*
[c] *Lecturer, Department of Biological and Physical Sciences, South Eastern Kenya University, P.O.BOX 170-90200 Kitui, Kenya*

[a]*Email: skiprop32@gmail.com*
[b]*Email: gracelynf@ueab.ac.ke*
[c]*Email: mmuthangya@seku.ac.ke*

Abstract

This study focused on Nucleoside Reverse Transcriptase drug-resistance profiling and the susceptibility patterns for the plasma samples obtained from HIV-positive naïve patients enrolled at Machakos Level 5 Hospital. The research's specific objectives were to profile resistance to Nucleoside Reverse Transcriptase Inhibitor drugs and then identify the markers for resistance to Nucleoside Reverse Transcriptase Inhibitor. This study used an experimental research design; DNA was extracted from the plasma samples, and PCR was amplified using polymerase-gene specific primers and later Gel electrophoresis. Then finally, cycle sequencing of the polymerase (pol) gen. The amplified products were sequenced, and drug-resistant mutations were determined using Los Alamos HIV DR database. All amplified samples from the PCR had the gel cut/excised and cleaned using the QIA quick gel extraction kit protocol. Sequences with high relatedness were fetched in a FASTA format and aligned using the Mega Evolutionary Genetic Analysis (MEGA) software version 10 using the Neighbor Joining (NJ) algorithm and the 1000 Bootstrap resampling algorithm. The main HIV strain detected in this study was the HIV A1 subtype, the major sub-subtype in Kenya. No other subtypes were noted in the study. Regarding NRTIs, the major mutation noted was D67E which indicated inadequate level, zidovudine resistance, and drug susceptibility to abacavir, emtricitabine, lamivudine, and tenofovir noted with no resistance to NNRTIs. However, there were minor mutations noted. Drug resistance mutations were found in high numbers associated with viral load and treatment time. Importantly, patients with triple and dual-class drug resistance should immediately alter ART regimens to alter the possibility of transmitting multi-drug-resistant HIV-1 strains.

Keywords: Anti retroviral therapy; Nucleoside Reverse Transcriptase Inhibitor

---

* Corresponding author.

1. Introduction

Globally, antiretroviral therapy coverage has grown to more than 21 million individuals. The antiretroviral therapy coverage in Sub-Saharan Africa has improved greatly [31]. According to World Health Organization, many nations, irrespective of CD4 T-cell count, have taken the advice of the WHO for the initiation of ART in all persons infected with HIV. While HIV mortality and morbidity have been reduced dramatically by ART, a sustained global expansion of HIV-resistant strains can lead to emergence and dissemination [5]. A rise in pretreatment drug resistance (PDR) in low-resource environments has successfully increased ART [7]. The latest World Health Organization study (WHO) found that the prevalence of PDR to NNRTIs in 6 of the 11 countries surveyed was more than 10%. The WHO 10 percent drug resistance level could entail improvements in the country's first line of ART regimes. In 63 countries, a new meta-analytic study showed a global rise in PDR to NNRTI (up to 23% in Southern Africa). HIV-resistant medication strains restrict treatment opportunities and risk the successful escalation of ART to monitor HIV infection by 2030 [32]. Consequently, controlling the population level of HIV drug resistance (HIVDR) is important because it helps to keep the viral load low and the CD4 cell count high. HIV medicine can make the viral load very low by preventing HIV multiplication.

According to National Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya, the recommended first-line ART regimen for treatment-naive adults consists of two nucleoside reverse-transcriptase inhibitors (NRTIs) and an integrase inhibitor [21]. As an alternative, the use of a non-nucleoside reverse-transcriptase inhibitor (NNRTI) or boosted protease inhibitor (PI) is recommended. Despite the effectiveness of ART and considerable efforts to help control the HIV/AIDS epidemic by 2030, ART failure due to drug resistance mutations is proving a challenge for ART provision and HIV care. In 2017, the World Health Organization (WHO) published a report on HIV drug resistance addressing the alarming increase in the prevalence of DRMs in individuals initiating their first-line ART regimen, linking DRMs to treatment failure. According to the National AIDS and STIs Control Program (NASCOP Recommendations of use of ART drugs, HIV resistance testing is recommended for all individuals with HIV infection who are newly diagnosed, before they initiate ART and in People Living With HIV (PLWH) with ART failure. Genotyping DNA-based assays are the most widely used for HIV DRMs detection. In Machakos Level 5 hospital, HIV genotyping is not performed in PLWH failing their first-line regimens; it is not routinely performed for all PLWH who are treatment-naive and starting their first-line. Several studies have reported the prevalence of HIV DRMs in treatment-naive Kenyan PLWH. According to a nationally representative survey, in Kenya the prevalence of any antiretroviral (ARV) resistance drug in treatment-naive PLWH is greater than 10%. Also, this report concluded that PWLH who initiated with NNRTI-based regimens achieved significantly lower levels of viral suppression compared to those who initiated with Protease inhibitor-based regimens. Also, Drug resistance mutations can directly confer resistance to PI, in the absence of detectable DRMs in the PR. Therefore, after reports on the general HIV-1 drug resistance is missing especially in resource limited rural settings with a longer history of ARV drug use, this research focused on drug-resistant profiling and sensitivity trends for ingenuous HIV-positive patients at Machakos levels 5 Hospital of Nucleoside Reverse Transcriptase.

2. Materials and Methods

a) Ribonucleic Acid Extraction:

Ribonucleic acid (RNA) from the plasma samples was extracted using Qiagen RNAmini kit according to manufacturers' instructions. Briefly 140µl of sample was added to 560µl of viral lysis buffer, incubated at room temperature (15-25^{0}C) for 10 minutes, then 560µl of molecular grade 100% ethanol [22] was added and mixed by vortexing for 15 seconds. This was then centrifuged using a micro centrifuge briefly to remove drops from inside the Eppendorf tube lid. From the lysed RNA, 630µl of RNA was then placed on to a spin column, spun at 6000 x g, twice binding the RNA to the spin column. The RNA was then washed twice, first with 500µl of wash buffer 1 (AW1) at 6000 x g for 1 minute, then with 500µl of wash buffer 2 (AW2) at 20,000 x g for 3 minutes. The RNA was eluted from the spin column by adding 60µl elution buffer (AVE) and spinning at 6000 x g for 1 minute to a 1.5 ml Eppendorf tube. The eluted RNA was then stored at −80^0 C until the day when the polymerase chain reaction (PCR) was carried out on the samples.

b) Polymerase Chain Reaction (PCR):

The reverse transcriptase PCR (RT-PCR) procedure consisted of one-step reverse transcription and PCR amplification, using the one-step RT-PCR kit from QIAGEN [22]. The reaction mixture contained 5µl of 5× RT-PCR buffer, 1µl of 0.4mM dNTPs, 0.75µl of each of the primers (1st round forward and reverse primers final concentration 0.6µM), 9.5µl of nuclease free water and 1µl of enzyme mix. A 2.5µl aliquot of viral RNA was added to give a final volume of 25µl. The cycling conditions for the RT-PCRs were an initial cycle at 50^{0}C for 30 minutes for the reverse transcriptase. This was followed by incubating at 94^{0}C for 10 minutes to inactivate the reverse transcriptase and activate the *Taq* polymerase. This was followed by 35 cycles of 95^{0}C for 30 seconds, 55^{0}C for 30 seconds, and 72^{0}C for 1 minute, with a final extension of 72^{0}C for 10 minutes. In the 2nd round PCR, the amplification was carried out using the 2mM MgSO4 (Invitrogen), 0.8mM dNTPs (Invitrogen), 0.5 units Taq polymerase (Invitrogen), 10x PCR Buffer (Invitrogen), 2ng of each 2nd round primer and the 2µl of the 1st round DNA template. The PCR cycle conditions consisted of 1 cycle of 95^{0}C for 10 min and 35 cycles of 95^{0}C for 30 sec, 55^{0}C for 30 sec, and 72^{0}C for 1 min, with a final extension of 72^{0}C for 10 min. The forward primer used for the 1st round PCR was RT18 F1 and its sequence 5'-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3'. For the reverse primer, KS104 R1 was utilized where its sequence was 5'- TGACTTGCCCAATTTAGTTTTCCCACTAA-3' [22]. Finally, for the 2nd Round PCR, KS101 F2 was used as a forward primer where its sequence was 5' -GTAGGACCTACACCTGTTCAACATAATTGGAAG-3' and KS102 R2 as a reverse primer where its sequence was 5'- CCCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG-3' [30].

c) Gel electrophoresis:

After the 2nd round PCR, PCR products were electrophorized in 1.5% agarose gels along with a 100-bp ladder [20] and visualized under UV light by ethidium bromide staining. Briefly, 1.5g of agarose was added to 100ml of tris-borate EDTA buffer (TBE). This was then heated in a microwave until clear, then later placed on a water bath that was at 48^{0}C. When cool, 0.5-1µl of ethidium bromide was added to the agarose, then later poured on to a gel tank that had gel combs. This was left to solidify. Once solid the comb was removed, and the gel tank filled TBE. The product 10µl was mixed with gel loading dye (gld) and electrophorized at 100v for 30 minutes. The PCR products were visualized under UV light using an HP AlphaImager®

d) QIAquick Gel Extraction Procedure:

The QIAquick®gel extraction kit was used to clean up the PCR products following the procedure described in the manufacturer's manual. Briefly, the PCR products were excised from the gel, weighed and 3 volumes of buffer QG added for every 1 volume of the gel. These was incubated for 10 minutes at 50^0C to dissolve the gel. Once dissolved, 850µl of the solution was dispensed to a QIAquick spin column and centrifuged at 17,900 x g for 1 minute to bind the DNA to the matrix of the column. This procedure was repeated once more. After the last spin, 500µl of buffer QG was added to the spin column to remove traces of agarose and centrifuged at 17,900 x g for 1 minute. This was then washed by adding 750µl of buffer PE and centrifuged at 17,900 x g for 1 minute. The spin column was placed on a 1.5ml Eppendorf tube, 50µl of buffer EB was then be added to the column and centrifuged for 1 minute. The eluted DNA was stored at -20^0C until nucleotide sequencing was carried out.

e) Cycle Sequencing:

The amplified fragments acquired for RT were in several base pairs. These fragments were sequenced by the Sanger sequencing method at KEMRI following the manufacturer's instructions, along with published primers.

f) Statistical Data Analysis:

This was an experimental study profiling the NRTI drug resistance and susceptibility patterns of treatment naïve HIV patients where their samples were obtained randomly, without any knowledge of drug-resistance and susceptibility patterns, from both the sample collection facility, Machakos Level IV hospital, and the diagnostic section at Kenya Medical Research Institute (KEMRI).

3. Results

Figure 1.1 below shows PCR products obtained after second-round amplification with HIV *pol-RT* specific primers. The expected sizes of amplified gene fragments, if positive, were approximately 697bp, whereas the gel picture below shows that, indeed, the amplified fragments were approximately 697bp as expected. All amplified samples from the PCR had the gel removed and cleaned using the QIAquick Gel Extraction equipment protocol as described in the methods section.

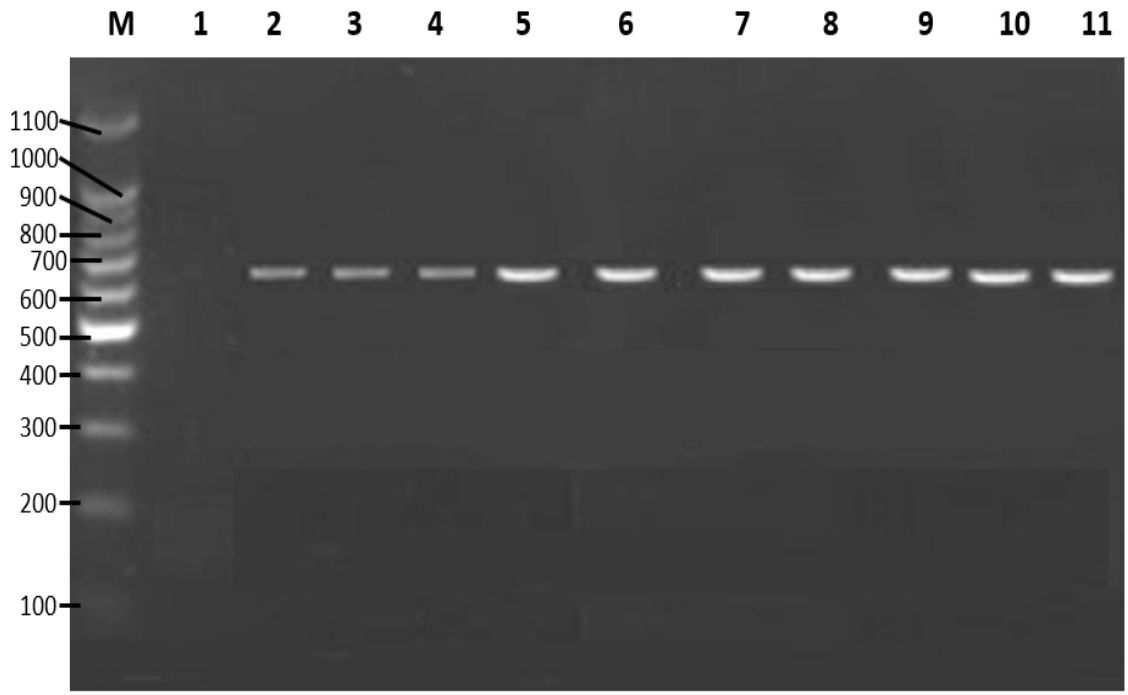


Figure 1.1: Gel electrophoresis

Table 1 below shows the results from uploading of study's sequence and uploading them to the Stanford University HIV database (https://hivdb.stanford.edu/hivdb/by-sequences/) to govern the nucleoside reverse transcriptase inhibitors (NRTIs) sequences using the default algorithms.

Table 1: NRTI mutations and NRTI drug susceptibility

Sample Id	NRTI Mutation	Drug Susceptibility	Potential Low-level Resistance
MKseq1	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq2	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq3	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq9	D67E	ABC, FTC, 3TC, TDF	AZT
MKSeq10	D67E	ABC, FTC, 3TC, TDF	AZT

Key: ABC – abacavir**, AZT –** zidovudine**, FTC –** emtricitabine**, 3TC –** lamivudine**, TDF –** tenofovir

The sequences obtained from this study were then uploaded to the Stanford University HIV database (https://hivdb.stanford.edu/hivdb/by-sequences/) to determine the non-nucleoside reverse transcriptase inhibitors (NNRTIs) sequences using the default algorithms. The results obtained are shown in table 2 below.

Table 2: Non-Nucleoside Reverse Transcriptase Inhibitor Mutations

Sample Id	NNRTI Mutation	Other Mutations
MKseq1	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E , I178L, V179I, Q207A, R211S
MKSeq2	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E , I178L, V179I, Q207A, R211S
MKSeq3	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E , I178L, V179I, Q207A, R211S
MKSeq9	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E , I178L, V179I, Q207A, R211S
MKSeq10	None	V35T, E40D, V60I, D113E, D121H, K122E, D123S, K173S, Q174K, D177E , I178L, V179I, Q207A, R211S

Although specific primers were used to amplify the polymerase reverse transcriptase gene (pol-RT) gene fragments from the patient samples, confirming the nucleotide sequences obtained from the amplified fragments was always necessary. The sequences obtained from the amplified products from this study were uploaded and compared to related sequences found in the GenBank database using the Basic Local Alignment Search Tool (BLAST) using the default algorithm analysis result from this study using the GENBANK database.

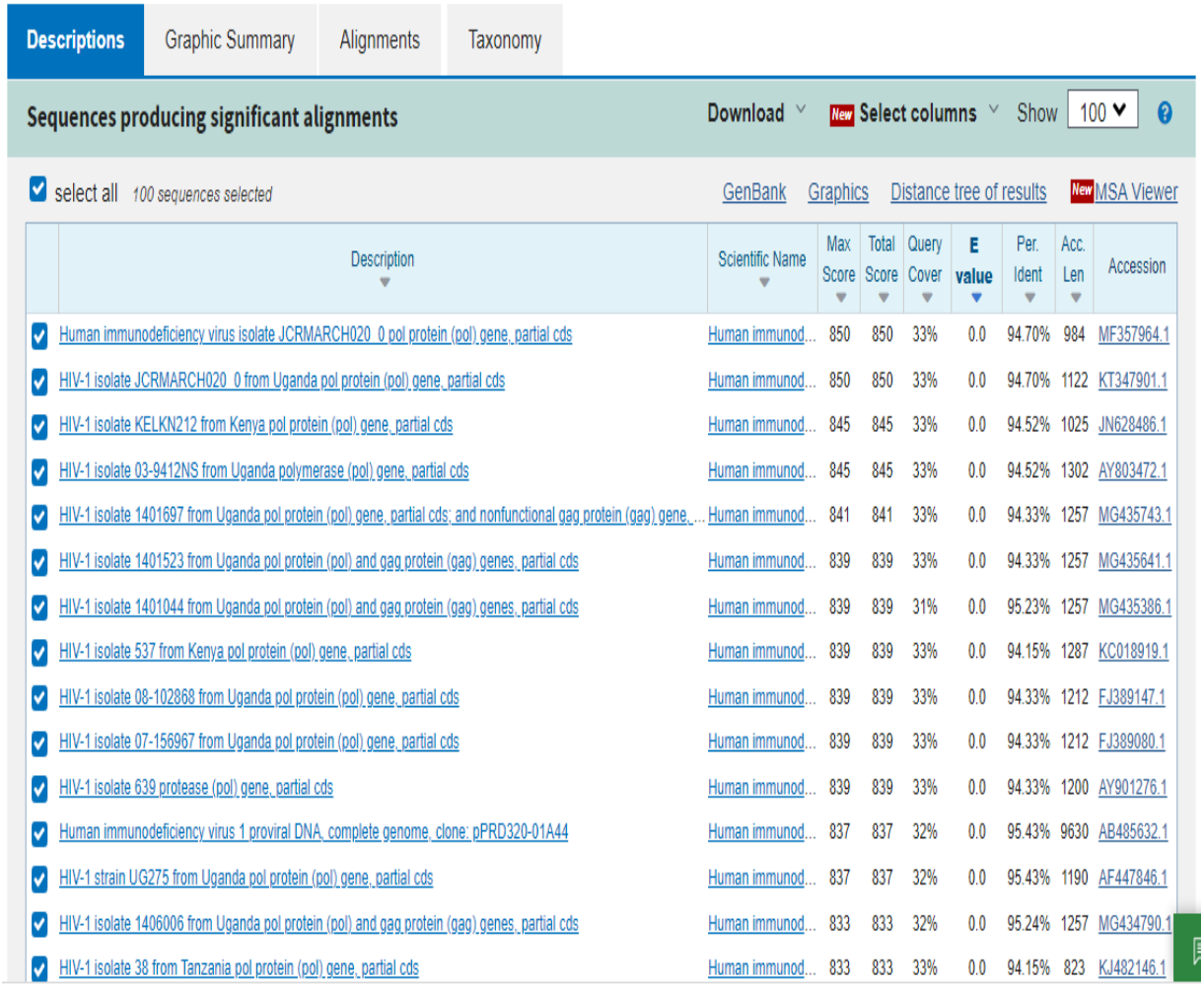


Figure 1.2: A representative nucleotide sequence BLAST analysis result from this study using GENBANK database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

The sequences were later uploaded and compared to similar sequences found in the Los Alamos HIV database tools, Recombinant Identification Program (RIP) default algorithm. A representation of the analysis results is shown in figure 1.3 below.

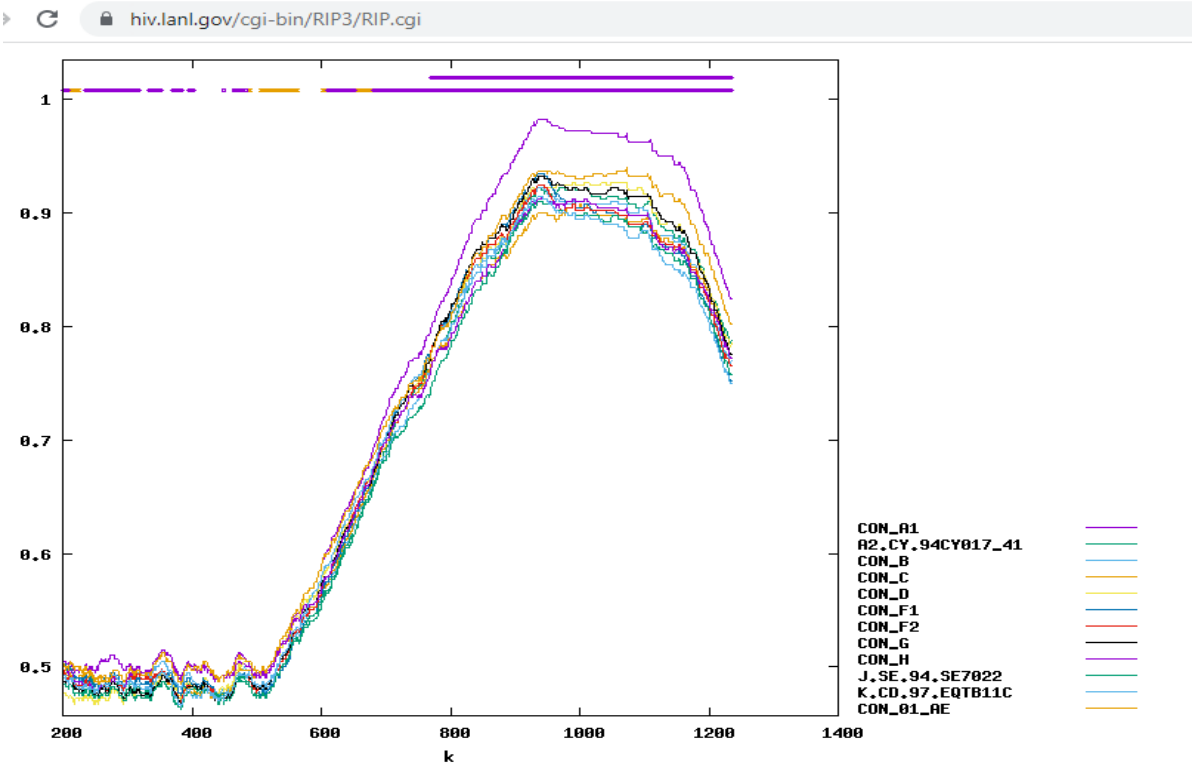


Figure 1.3: A representative sequence analysis result from this study using the Los Alamos HIV database tool, Recombinant Identification Program (RIP). Ref; https://www.hiv.lanl.gov/cgi-bin/RIP3/RIP.cgi.

This study's sequences were later uploaded to the online REGA HIV, which is an HIV Drug Resistance Database subtyping database using the default algorithm. A representation of the genotyping analysis results is shown in figure 1.4 below. Figure 1.4 below confirms that the predominant HIV-1 subtype is, subtype A with 100 percent support.

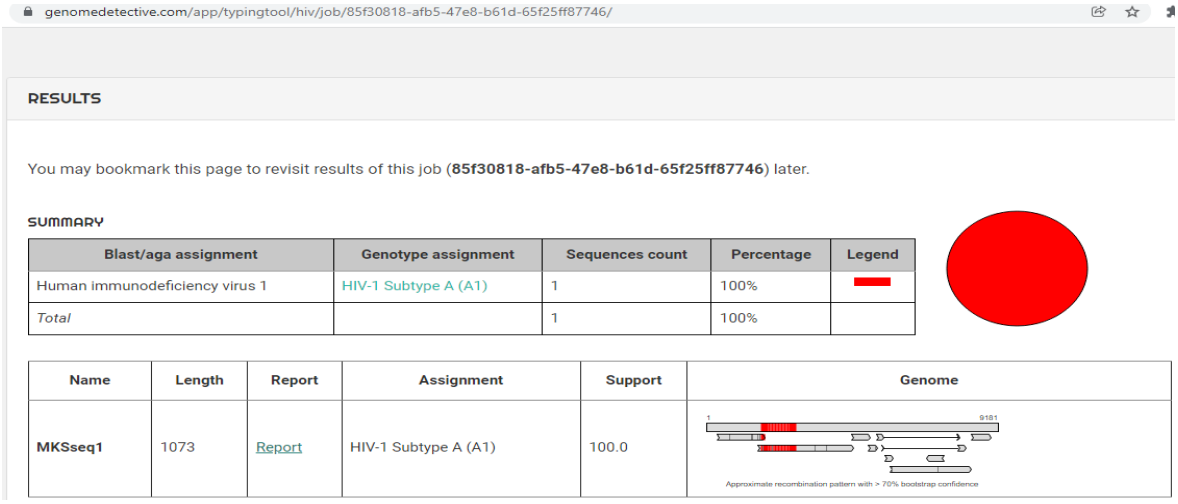


Figure 1.4: Sequence Analysis from REGA HIV Subtyping *https://www.genomedetective.com/app/typingtool/hiv*.

To determine the phylogenetic relatedness to similar subtype sequences, sequences with high relatedness were fetched in a FASTA format which is a text-based format for representing either nucleotide sequences or amino acid (protein) sequences, and aligned using the Mega Evolutionary Genetic Analysis (MEGA) software version 10 using the Neighbour Joining (NJ) algorithm and the 1000 Bootstrap resampling algorithm. The NJ algorithm substitution model used was the Kimura 2-parameter method. The generated tree to infer and assign HIV-1 subtypes is shown below in figure 1.5.

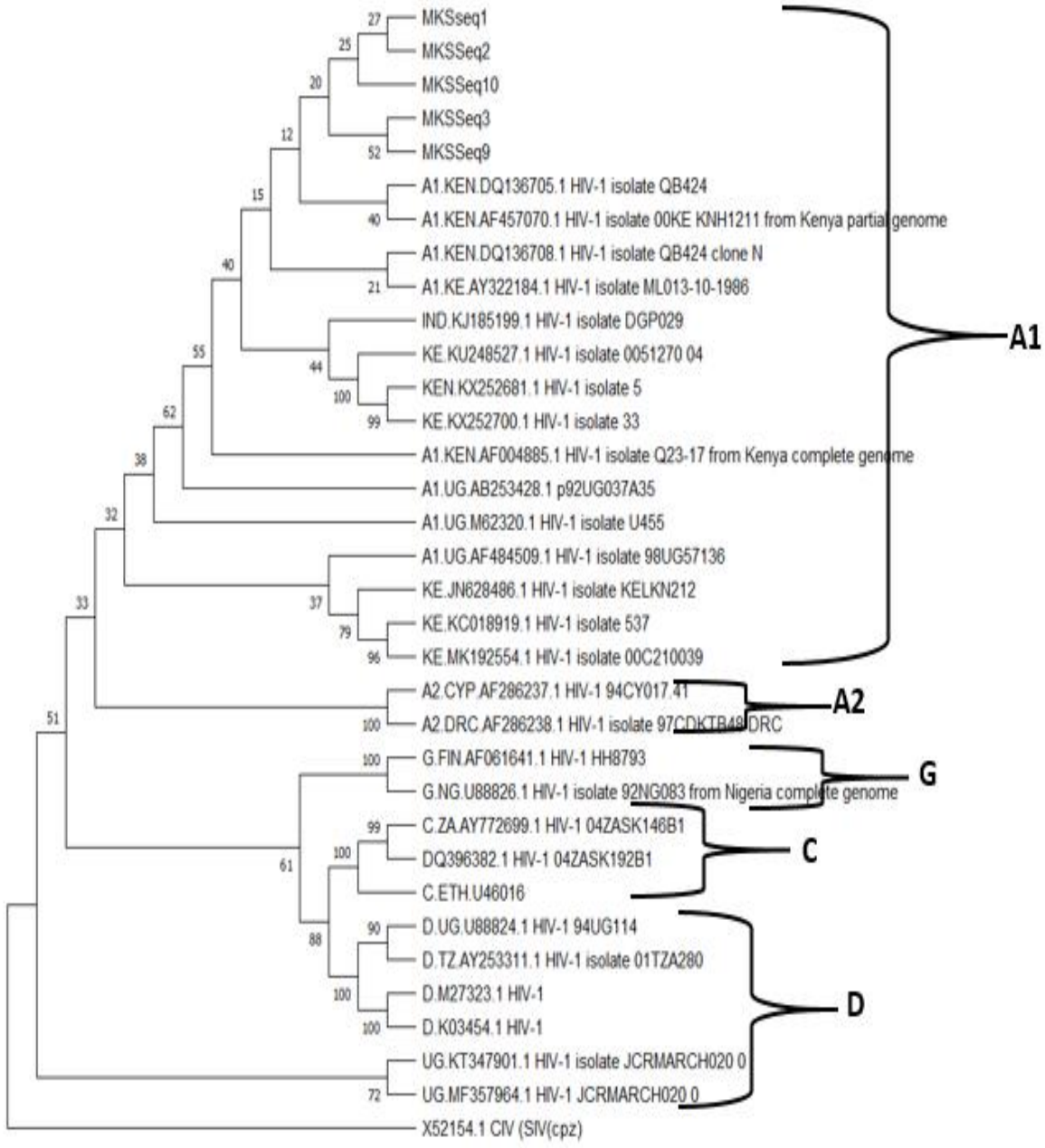


Figure1.5: Phylogenetic Relatedness from BLAST and HIV BLAST Databases using MEGA version 10 Neighbor-Joining (NJ) algorithm at 2000 replicates Rooted with SIV (cpz)-X52.

4. Discussion

Research shows that in East Africa, most infections are caused by subtype A, plus also subtypes C and D, plus some URFs. Subtype A is also distributed in Russia and the former Soviet Union, Central Asia [3,4]. In Kenya, research investigations conducted across the nation in diverse locations ranging from the coast to western and central Kenya show the detection and, in some cases, preponderance of subtype A, as well as the existence of subtypes C and D. This study's sequences on analysis had shown genetic similarities of between 85 – 97% alignment with HIV subtype A as shown by the representative sequence BLAST and HIV BLAST results. This is similar to what other researchers have shown from previous studies carried out here in Kenya, which show the predominance of subtype A, where they range from 44% to 74% detection [1,2,6,8,10,11,13,14,15,18,24,33]. At the time of the study, the amplified and sequenced samples data indicated the presence of only subtype A and no other subtype was detected from the samples, which is a difference from other studies carried out in Kenya.

When the Recombinant Identification Program (RIP) hosted by the Los Alamos Database was used to analyze for and determine recombination in this study's sequences, from the results, the most dominant curve was that of the A1 sub-subtype. This was also confirmed by the REGA HIV subtyping tool, which also confirmed the genotyping as sub-subtype A1 with 100% support. When the phylogenetic tree was drawn using similar sequences obtained from the Genbank Database, this study's sequences aligned with other Kenyan sub-subtype A1 sequences. A unique observation is the clustering of this study's samples on the inferred phylogenetic tree after 2000 replicates. Previous Kenyan studies carried out on various groups, including expectant mothers, intravenous drug users, and HIV infected individuals, have majorly the sub-subtype A1, which is the most predominant strain detected with detection rates ranging from 44% to 86% [1]. The neighbor-joining method for constructing phylogenetic trees uses pairs of operational taxonomic units (OTU) or neighbors to minimize branch lengths, starting with a starlike tree that is repeatedly run at each replication stage to ensure the integrity of the tree structure [28]. The Kimura 2 parameter model used in this analysis has the role of estimating genetic distances between different nucleotide sequences during an evolutionary process [12,23].

a. Profile of Resistance to Nucleoside Reverse Transcriptase Inhibitor (NRTI) Drugs

Nucleoside reverse transcriptase inhibitors (NRTIs) are medicines that prevent viral DNA from being reversed within cells. They contain nucleotide base analogs that will cause the chain termination or non-extension of the DNA during reverse transcription of the HIV viral DNA while inside an infected cell using the HIV reverse transcriptase [9]. Their method of action is either discriminating, resulting in a reduction in the binding affinity of NRTI-triphosphate over the natural nucleotide at the reverse transcriptase binding site, or non-discriminatory. Again, this may decrease the NRTI-phosphate over the natural nucleotide rate of incorporation of the reverse binding site. The second mechanism is an excision process that relies on adenosine triphosphate (ATP) or pyrophosphate; hence, mutations that improve reverse transcriptase affinity for ATP or increase the rate of analog complex removal are preferred. Additionally, changes in the capacity of the residues to translocate from the active site (N-site) to the post-translocation site (P-site), and the rate of separation of the template/primer from the enzyme, may also help to improve the excision route [9]. Generally, NRTIs are drugs that comprise abacavir (ABC), zidovudine (AZT), emtricitabine (FTC), lamivudine (3TC), tenofovir (TDF), stavudine (d4T), didanosine (ddl), and zalcitabine (ddC) [9,21]. All the sequenced samples had a D67E mutation in this study, which is a change of amino acid aspartic acid (D) to glutamic acid (E). Aspartic acid (D) is an important building block used in making proteins in the body, while glutamic acid (E) is used to form proteins in the body [9]. According to the

HIV research, this is a non-polymorphic NRTI selected mutation at position 67 that is a change from aspartic acid (D) to either glutamic acid (E), serine (S), threonine (T), or histidine (H), that is D67G/E/S/T/H) which has been associated with low-level resistance to AZT and d4T, plus also reduced susceptibility to ABC, DDL and TDF [9,17,25,26,29]. Previous research studies from Kenya have shown resistance to the resistance mutation at position 67, where there was a change from glutamic acid (D) to asparagine (N), thus the acronym D67N [13,16]. These previous studies also noted resistance to AZT and other NRTIs.

b. Markers for Resistance to non-Nucleoside Reverse Transcriptase Inhibitor Drugs

Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs) inhibit reverse transcription by directly attaching to the enzyme reverse transcriptase (RT) and preventing it from working. Typically, they are tiny chemical compounds with a lengthy half-life [9].

Generally, NNRTIs drugs include nevirapine (NVP), efavirenz (EFV), doravirine (DOR), and delavirdine (DLV) [33,21]. No major NNRTIs resistance mutations were noted in the amplified samples sequenced in this study. Results from table 2 shows that, even though there was only one mutation at position 179 where valine (V) changed to isoleucine (I), thus the acronym V179I. Valine is a plant synthesized essential acid used for muscle growth stimulation, regeneration, and energy production, while isoleucine, another essential amino acid, is involved in muscle metabolism and important in immune functions and collagen production. This polymorphic mutation is frequently seen in patients receiving etravirine (ETR) and rilpivirine (RPV) but has a little direct effect on NNRTI susceptibility. A study carried out in Kisii County also detected the same V179I mutation in 10 sampled patients in that study [19]. The most commonly identified resistance mutation noted in other studies was at position 103, where lysine (K) changed to asparagine (N), thus the acronym K103N [8,13,15,16,18,27]. However, this mutation was not noted in this study's amplified sample sequences.

5. Conclusion and Recommendation

To summarize, a frighteningly high prevalence of drug resistance mutations was found. Importantly, patients with triple and dual class drug resistance should alter ART-regimens immediately to avoid the possibility of transmitting multidrug-resistant HIV-1 strains, which would have fewer treatment options. The most significant predictors of HIVDRM were viral load and treatment duration. The most striking finding was that a subject's sex and treatment-duration independently influenced HIV DR counts, emphasizing the importance of targeted resistance monitoring and switching ART regimens while taking into account the risk of exhausting future treatment options. More research is needed to determine the variables that contributed to the finding that a subject's sex and treatment time independently influenced HIV-1 drug resistance mutations.

The study recommends the following

1. The provision of fresh samples could help get better amplicons and sequences data.
2. Providing patient details that include age, gender, and treatment regimens would help address research questions regarding which age or gender is mostly affected. Regarding knowing treatment regimens, resistance mutations determined would help improve patient management.

Study Limitations

Fewer samples were sequenced during the time of the study. Though amplifications for most samples occurred, good and reliable sequence results could only be obtained from five (5) samples. More information on circulating

subtypes plus resistance patterns would be obtained if all samples could be amplified. No patient information regarding gender, age, and treatment regimen was provided. This was because of ethical concerns at the health facility.

Acknowledgements

I would like to thank the Almighty God for His agape love during this process. I would also like to take this opportunity to thank my supervisors for their diligent efforts in seeing that this work is properly done by correcting flaws and inaccuracies in the document. I am really thankful to my parents for their financial and emotional support during the entire process. Finally, I want to express my gratitude to my siblings and friends that supported me throughout the tough times.

References

1. Adhiambo, M., Makwaga, O., Adungo, F., Kimani, H., Mulama, D.H., Korir, J.C. and Mwau, M., (2021). Human immunodeficiency virus (HIV) type 1 genetic diversity in HIV positive individuals on antiretroviral therapy in a cross-sectional study conducted in Teso, Western Kenya. *The Pan African Medical Journal*, *38*.
2. Adungo, F.O., Gicheru, M.M., Adungo, N.I., Matilu, M.M., Lihana, R.W. and Khamadi, S.A., (2014). Diversity of human immunodeficiency virus type-1 subtypes in Western Kenya.
3. Akhome, P. (2021). HIV subtypes. Available online at https://www.aidsmap.com/about-hiv/hiv-1-subtypes accessed 30th October 2021.
4. Bbosa, N., Kaleebu, P. and Ssemwanga, D., (2019). HIV subtype diversity worldwide. *Current Opinion in HIV and AIDS*, *14*(3), pp.153-160.
5. Beyrer, C., & Pozniak, A. (2017). HIV drug resistance—an emerging threat to epidemic control. *New England Journal of Medicine*, *377*(17), 1605-1607.
6. Gounder, K., Oyaro, M., Padayachi, N., Zulu, T.M., de Oliveira, T., Wylie, J. and Ndung'u, T., (2017). Complex Subtype Diversity of HIV-1 Among Drug Users in Major Kenyan Cities. *AIDS research and human retroviruses*, *33*(5), pp.500-510.
7. Gupta, R. K., Jordan, M. R., Sultan, B. J., Hill, A., Davis, D. H., Gregson, J., ... & Bertagnolio, S. (2012). Global trends in antiretroviral resistance in treatment-naive individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *The Lancet*, *380*(9849), 1250-1258.
8. Hassan, A.S., Esbjörnsson, J., Wahome, E., Thiong'o, A., Makau, G.N., Price, M.A. and 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), p.e0206177.
9. Immunopedia. (2021). ARV Mode of Action – NRTI. Available online at https://www.immunopaedia.org.za/treatment-diagnostics/hiv-infection-treatment/arv-mode-of-action/ accessed 5th November 2021.
10. Kageha, S., Lihana, R.W., Okoth, V., Mwau, M., Okoth, F.A., Songok, E.M., Ngaira, J.M. and Khamadi, S.A., (2012). HIV type 1 subtype surveillance in central Kenya. *AIDS research and human retroviruses*, *28*(2), pp.228-231.

11. Khamadi, S.A., Lihana, R.W., Osman, S., Mwangi, J., Muriuki, J., Lagat, N., Kinyua, J., Mwau, M., Kageha, S., Okoth, V. and Ochieng, W., (2009). Genetic diversity of HIV type 1 along the coastal strip of Kenya. *AIDS research and human retroviruses*, *25*(9), pp.919-923.
12. Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, *16*(2), 111-120.
13. Kinyua, J.G., Lihana, R.W., Kiptoo, M., Muasya, T., Odera, I., Muiruri, P. and Songok, E.M., (2018). Antiretroviral resistance among HIV-1 patients on first-line therapy attending a comprehensive care clinic in Kenyatta National Hospital, Kenya: a retrospective analysis. *Pan African Medical Journal*, *29*(1), pp.1-6.
14. Kitawi, R.C., Nzomo, T., Mwatelah, R.S., Aman, R., Kimulwo, M.J., Masankwa, G., Lwembe, R.M., Okendo, J., Ogutu, B. and Ochieng, W., (2015). HIV-1 subtype diversity based on envelope C2V3 sequences from Kenyan patients on highly active antiretroviral therapy. *AIDS research and human retroviruses*, *31*(4), pp.452-455.
15. Koigi, P., Ngayo, M. O., Khamadi, S., Ngugi, C., & Nyamache, A. K. (2014). HIV type 1 drug resistance patterns among patients failing first- and second-line antiretroviral therapy in Nairobi, Kenya. *BMC research notes*, *7*(1), 1-6.
16. 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. and 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), pp.1211-1217.
17. Liu, T. F., & Shafer, R. W. (2006). Web resources for HIV type 1 genotypic-resistance test interpretation. *Clinical infectious diseases*, *42*(11), 1608-1618.
18. Luvai, E., Waihenya, R., Munyao, J., Sanguli, L., Mwachari, C. and Khamadi, S., (2015). HIV-1 Drug Resistance Mutations in Patients Failing 1st Line Therapy in a Comprehensive Care Center in Nairobi, Kenya.
19. Mabeya, S.N., (2021). *HIV-1 Genetic Diversity, Tropism and Drug Resistance Mutations among HIV Infected Patients Attending Comprehensive Care Clinic in Kisii Teaching and Referral Hospital, Kenya* (Doctoral dissertation, JKUAT-COHES).
20. Magdeldin, S. (Ed.). (2012). *Gel electrophoresis: Principles and basics*. BoD–Books on Demand.
21. NASCOP, (2018), Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya, (2018) Edition. Available online at: https://www.nascop.or.ke/new-guidelines/ Accessed 30th November 2021.
22. Ndembi, N., Takehisa, J., Zekeng, L., Kobayashi, E., Ngansop, C., Songok, E. M., ... & Ichimura, H. (2004). Genetic diversity of HIV type 1 in rural eastern Cameroon. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *37*(5), 1641-1650.
23. Nishimaki, T., & Sato, K. (2019). An extension of the Kimura two-parameter model to the natural evolutionary process. *Journal of molecular evolution*, *87*(1), 60-67.
24. Onywera, H., Maman, D., Inzaule, S., Auma, E., Were, K., Fredrick, H., ... & Zeh, C. (2017). Surveillance of HIV-1 pol transmitted drug resistance in acutely and recently infected antiretroviral drug-naive persons in rural western Kenya. *PloS one*, *12*(2), e0171124.

25. Rhee, S.Y., Gonzales, M.J., Kantor, R., Betts, B.J., Ravela, J. and Shafer, R.W., (2003). Human immunodeficiency virus reverse transcriptase and protease sequence database. *Nucleic acids research*, *31*(1), pp.298-303.
26. Rhee, S.Y., Kantor, R., Katzenstein, D.A., Camacho, R., Morris, L., Sirivichayakul, S., Jorgensen, L., Brigido, L.F., Schapiro, J.M., Shafer, R.W. and International Non-Subtype B HIV-1 Working Group, (2006). HIV-1 pol mutation frequency by subtype and treatment experience: extension of the HIVseq program to seven non-B subtypes. *AIDS (London, England)*, *20*(5), p.643.
27. Saida, S. J., Manikandan, A., Kaliyaperumal, M., Rumalla, C. S., Khan, A. A., Jayaraman, V. B., ... & Rao, S. V. (2019). Identification, isolation and characterization of dolutegravir forced degradation products and their cytotoxicity potential. *Journal of pharmaceutical and biomedical analysis*, *174*, 588-594.
28. Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, *4*(4), 406-425.
29. Shafer, R.W., Jung, D.R. and Betts, B.J., (2000). Human immunodeficiency virus type 1 reverse transcriptase and protease mutation search engine for queries. *Nature medicine*, *6*(11), pp.1290-1292.
30. Songok, E. M., Lwembe, R. M., Kibaya, R., Kobayashi, K., Ndembi, N., Kita, K., ... & Ichimura, H. (2004). Active generation and selection for HIV intersubtype A/D recombinant forms in a coinfected patient in Kenya. *AIDS research and human retroviruses*, *20*(2), 255-258.
31. UNAIDS. Data Book 2017 - Joint United Nations Programme on HIV/AIDS. Geneva: UNAIDS; 2017.
32. United Nations (UN). Sustainable Development Goals - Goal 3: Ensure healthy lives and promote wellbeing for all at all ages. New York: UN; 2015.
33. Yang, O. O., Lin, H., Dagarag, M., Ng, H. L., Effros, R. B., & Uittenbogaart, C. H. (2005). Decreased perforin and granzyme B expression in senescent HIV-1-specific cytotoxic T lymphocytes. *Virology*, *332*(1), 16-19.