MOLECULAR CHARACTERISATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 AND DETERMINATION OF DRUG RESISTANCE AMONG ANTIRETROVIRAL TREATED PATIENTS ATTENDING KAYANZA DISTRICT HOSPITAL IN BURUNDI

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Molecular Characterisation of Human Immunodeficiency Virus Type 1 and Determination of Drug Resistance among Antiretroviral Treated Patients Attending Kayanza District Hospital in Burundi

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature …….. Date ……....

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This thesis has been submitted for examination with our approval as university supervisors

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Signature…….. Date ……

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DEDICATION

To my wife Lydia BIZIMANA and My children Dav-Lucky MUKUNZI, Belle-Maelle NINZINZA and Angèle Eunice IGIRANEZA with honor and love.

To the countless HIV patients who have suffered at the hands of AIDS epidemic.

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ABBREVIATIONS AND ACRONYMS

3TC	Lamivudine
ABC	Abicavir
ART	Anti-Retro-Viral Therapy
ARV	Antiretroviral
AZT	Zidovudine (also ZDV)
BLAST	Basic local alignment search tool
CRF	Circulating Recombinant Forms
CRI	Co-receptor Inhibitor
DLV	Delavirdine
DNA	Deoxy Ribonucleic Acid
EDTA	Ethylene diamine tetra acetic acid
EFV	Efavirenz
ETR	Etravirine
FTC	Emtricitabine
HAART	Highly Active Antiretroviral Therapy
HIV-1	Human Immunodeficiency Virus type 1
HIVDR	HIV Drug Resistance
KEMRI	Kenya Medical Research Institute
MFbu	Mille franc Burundais
MOH	Ministry of Health

NIAID	National Institute of Allergy and Infectious Diseases
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PEPFAR	The United States President's Emergency Plan for AIDS Relief
PI	Protease Inhibitor
PMTCT	Prevention of Mother-to-Child Transmission
RPM	Revolutions per Minute
RT-PCR	Reverse Transcription PCR
RTI	Reverse Transcriptase Inhibitor
SPSS	Statistical package for the social sciences
TDF	Tenofovir Disoproxil Fumarate
UNAIDS	United Nations' Programme on HIV/AIDS
URF	Unique Recombinant Forms
VF	Virological failure
VL	Viral load
WHO	World Health Organization

ABSTRACT

The use of antiretroviral drugs to prolong lives of people living with HIV and AIDS and in their use in prevention of mother-to-child transmission of HIV is now firmly established in Sub-Saharan Africa .However, virological failure in management of HIV-1 infection has been reported to be between 11 to 24 % after 12 months of treatment; Out of these, acquired or transmitted drug resistance mutations have been reported at 71% to 90%. HIV-1 subtypes and patterns of drug resistance in HIV-infected patients on ART is little known in Burundi. This cross-sectional study was aimed to characterize HIV subtypes and to determine drug resistance mutations in HIV-1 infected patients on ART attending Kayanza district hospital, Burundi. Using purposive sampling technique, 200 participants were recruited. All patients were ARV experienced based on 2008 Burundi National guidelines, the age distribution was unimodal and it peaked at 45 years. The 86% were above 18years and female accounted for 55%. Stratification by religion, 73.5% were Christian-Catholics while 52% were married. On the level of education, 82.5% (n=165) reported completing primary school while farming as occupation was reported by 65% and 94% reporting to earn below BIF 200 .Out of 200 patients, 12.5% were on tuberculosis (TB) medications while 57% had been on antiretroviral therapy for between six to ten years. U4mL of venous blood was collected from each patient that consented to participate in the study. Plasma viral loads were quantified using the Abbott m2000rt system. RT-PCR was done after extraction of nucleic acids from plasma with >1000 copies/mL. Sequencing was done on all amplified samples. Drug resistance was determined using the Stanford University database. Phylogenetic analyses were done using the neighbor joining method. From the two hundred patients; 13% (26/200) had virological failure. This was associated with multiple partners (P =0.016) and inconsistency in taking medications (p=0.014). Fifteen samples were successfully sequenced; of these, (12/15) 80% were HIV-1 subtype C, 13% (2/15) were HIV-1 subtype A1 and 7% (1/15) were subtype A. Of the successfully sequenced, 80% (12/15) recorded at least one mutation (NRTI or NNRTI), while 20% (3/15) did not carry any Drug Resistance Mutations. The most common drug resistance mutations were M184V(7/15), M41L(1/15), E44D(1/15), L74I(1/15), L210W(1/15) and K65R(1/15), K103N(12/15),E138A (/15) and Y188H (1/15). These findings showed potential gaps in the last 90% of the 90-90-90 WHO target by 2020. More strategies are needed so as to improve adherence, while drug resistance mutation testing should be implemented to monitor HIV-1 patients on ART in Burundi.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The HIV/AIDS is one of the world's most serious health and development challenges. (UNAIDS, 2015). In 2018, there were 37.9 million people living with HIV and 24.5 million from these were accessing antiretroviral therapy. Further, around 770000 people died from AIDS-related illnesses worldwide (UNAIDS 2019). The vast majority of people living with HIV are from low- and middle- income countries, with an estimated 68% living in Sub-Sahara Africa (UNAIDS 2019).

The Sub-Saharan Africa carries the largest burden of HIV/AIDS in the world, in part due to a lack of funds for antiretroviral therapy (ART). In 2016, Burundi counted 2200 new HIV infections and 2900AIDS-related deaths. There were 84 000 individuals living with HIV in 2016, among whom 61% were on antiretroviral therapy (UNAIDS 2018). The use of antiretroviral drugs to boost HIV/AIDS patients' lives and their use to prevent HIV transmission from mother-to-child is currently implemented in this part of Africa. The development of antiretroviral (ARV) resistance when viral replication continues in the presence of selective drug pressure may result from impotent regimens, suboptimal adherence, pharmacological hurdles or ineffectively treated compartments is a major factor contributing to treatment failure (Richman, 2001). The HIV-1 chemotherapy with continual treatment failure and most frequent antiretroviral drug changes used to be associated with the increase of mutations that confer resistance and lead to the emergence of multidrug-resistant HIV-1 strains (Richman, 2004)

In 2016, around 84 000 people were infected with HIV in Burundi, among whom 61 % were accessing ARV treatment (UNAIDS, 2018). The use of ARV treatment in 1997 was limited to Bujumbura. In 1999, a program of facilitating access to antiretroviral has been implemented by the Government and several institutions are organized for the support of their staff through solidarity funds. The schemes

proposed for Burundi are inspired by the WHO guidelines of 2015.This represents an important step towards the achievement of universal access to ARVS for therapy and prophylactic purpose This has resulted in upscaling of ARV coverage by treating all HIV-positive person (treat all) (PNLS/IST, 2016)

1.2 Statement of the problem

The use of ARV for HIV/AIDS treatment and mother-to-child transmission prevention is achieved in the Sub-Saharan Africa region. High levels of drug resistance were demonstrated in African countries which pioneered the ARV use (Ndembi *et al*., 2008). However, Virological failure during ART have been reported in 10 % to 30 % of individuals receiving first-line treatment regimen(Barth *et al*., 2010).The majority of these individuals are expected to acquire resistance (Gupta, Hill, Sawyer, & Pillay., 2008). Furthermore, since Burundi initiated ART, little data have been generated on treatment outcomes. This study was conducted to characterize HIV subtypes and to determine drug resistance mutations in HIV-infected patients on ART, attending Kayanza District Hospital, Burundi.

1.3 Justification of the study

The treatment of millions of people with antiretroviral (ARV) drugs is intended to prolong the lives of those who are infected. However, this will inevitably be accompanied by the emergence and transmission of drug-resistant viruses due to both immune and drug pressure. It is now established that monitoring of drug resistance in HIV/AIDS patients under treatment is one of WHO global HIV treatment monitoring strategies.

In Burundi, many interventions for control and prevention, such as Prevention of Mother-to-Child Transmission (PMTCT), care services for treatment with ARV drugs have been implemented. However, Virological, immunological and clinical failure - the most used tools to identify treatment failure have been used intermittently. Although resistance testing is not widely employed in developing countries (due to the costs involved), the need is increasing due to emergence of drug resistance as antiretroviral therapy is scaled up (WHO, 2013). This study aimed to

characterize HIV-1 subtypes and determine drug resistance mutations in patients on ART at Kayanza District Hospital.

1.4 Research questions

i. What is the prevalence of virological failure among HIV-infected patient on ARV attending Kayanza District Hospital?

ii. What are the drug-specific mutations that confer resistance to ART in HIV-1 infected patients on ART attending Kayanza District Hospital?

iii. What are the genotypes circulating in HIV-infected patients on ART attending Kayanza District Hospital?

1.5 Objectives

1.5.1 General Objective

To characterize HIV-1 and determine drug resistance mutations among HIV patients under ART attending Kayanza District Hospital.

1.5.2 Specific Objectives

i. To determine the prevalence of virological failure among HIV-1 positive patients under ART attending Kayanza District Hospital.

ii. To determine drug-specific mutations that confer resistance to ART in HIV-1 positive patients under ART attending Kayanza District Hospital.

iii. To establish HIV-1 virus genotypes in HIV positive patients under ART attending Kayanza district Hospital.

CHAPTER TWO

LITERATURE REVIEW

2.1 Virology of HIV-1

2.1.1 Structural components of HIV Virion

Human Immunodeficiency Virus type belongs to the class of Retroviruses (McGovern *et al* ., 2002). It is around 120 nm in diameter and roughly spherical (Camacho *et al*., 2007). It is composed of two copies of non-covalently linked, unspliced, positive-sense single-stranded RNA enclosed by a conical capsid composed of the viral protein p24 (Kuiken *et al*., 2008) . The single-stranded RNA is tightly bound to nucleocapsid proteins, p7 and the enzymes reverse transcriptase, protease, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid (Kuiken *et al* .,2008) (Figure 2.1). This is in turn, surrounded by the viral envelope composed of two layers of phospholipids. In the viral envelope, there are proteins from the host cell and about 70 copies of a complex HIV protein that protrudes through the surface of the virus particle (Kuiken *et al*., 2008). The proteins gp120 and gp41 help HIV enter a cell to infect it. The viral matrix helps anchor the envelope proteins to the rest of the virus particle. The core contains the HIV genome and some proteins needed for infection. HIV is a retrovirus, meaning it must transform its RNA genome into DNA within a host cell (McGovern *et al*, 2002) .

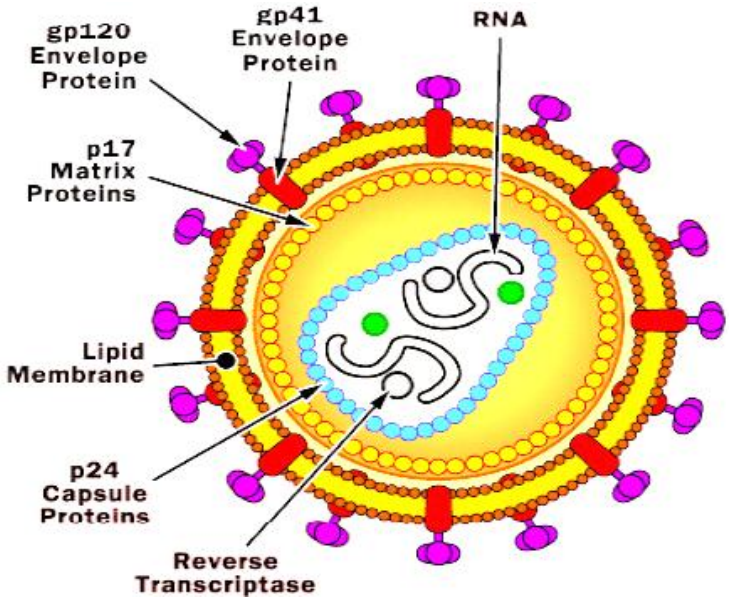

Figure 2.1: Structural components of HIV Virion *(*Woldaregay, *2011)*

2.1.2 HIV-1 Genome

The HIV genome consists of at least nine genes encoding 19 proteins *Gag*, *pol*, and *env* are structural genes while *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* are regulatory genes for proteins that control the ability of HIV to infect cells, replicate and cause disease (Kuiken *et al.,* 2008) .An alternate accessory protein, *rev,* can be produced as a result of mutation of some HIV strains, from the fusion of *tat*, *rev*, and *env* (Peterlin & Trono, 2003). The HIV-1 genome contains nine open reading frames. The *gag* gene encodes a precursor polyprotein Pr55gag which is subsequently cleaved to yield several structural proteins (MA, CA, P2, NC, P1 and P6). The three viral enzymes protease (PR), reverse transcriptase (RT) and integrase (IN) are encoded by the *pol* gene. The *env* gene encodes for the gp 160 glycoprotein, the precursor of the envelope glycoproteins gp 120 and gp41 (Figure 2.2).The other six genes encode for the regulatory proteins, *tat* and *rev*, and the accessory proteins, *vif*, *vpr*, *vpu* and *nef* (Mosha,2008).

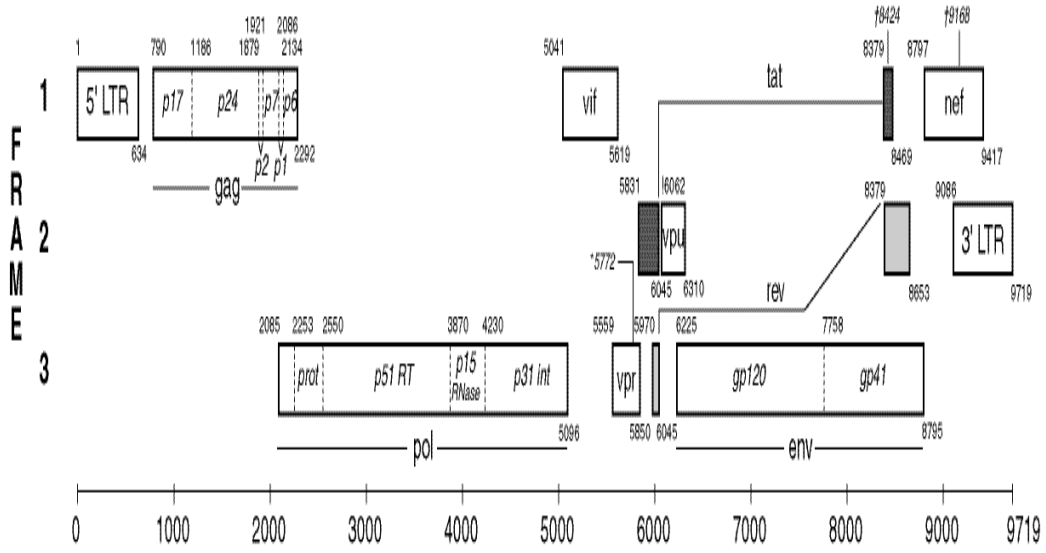

Figure 2.2: HIV-1 Genome structure (Rajarapu, 2014)

2.2 HIV transmission

The human immunodeficiency virus (HIV) cause a disease of acquired immunodeficiency syndrome (AIDS) is a disease in the human immune system. HIV is present in the blood and genital secretions of virtually all infected individuals regardless of whether or not they have symptoms of AIDS (WHO, 2018). The HIV is primarily transmitted through unprotected sex acts, but can also be transmitted from mother to child during pregnancy or breast feeding, through needle sharing for medical purposes or injecting drugs, and blood transfusions with infected blood (Braitstein *et al.,* 2006). Pregnant women infected with HIV can pass the infection to their babies in utero or during birth. Breast milk from infected mothers has also been shown to contain high levels of the virus (WHO, 2018).

2.3 HIV Replication

The HIV-1replication involves six major steps: attachment and fusion, entry and uncoating, integration, translation and synthesis of viral proteins, assembly, maturation and release.

2.3.1 Attachment and fusion

During attachment, the HIV envelope glycoprotein (gp120) attaches to the CD4 T-cell receptor (Briz *et al.,* 2006). The gp120 undergoes the changes, which allows it to bind to the chemokine coreceptor, either CCR5 or CXCR4. CCR5 and CXCR4 bind the V3 region of HIV gp120. Further, this changes in gp120 occur after CCR5 or CXCR4 binding, allowing the HIV envelope gp41 fusion peptide to insert into the cellular membrane, resulting in to the fuse of the virion with the host membrane (Kuiken *et al.,* 2008).

2.3.2 Entry and uncoating

Reverse transcription of viral RNA genome into proviral DNA occurs once the HIV virion enters the cell cytoplasm and is uncoated. Viral uncoating involves cellular factors and the viral proteins matrix protein p17, Nef and Vif (Sierra *et al.*, 2005). The proviral DNA then becomes part of the pre-integration complex, which enters the nucleus through the nuclear pore (Le Rouzic & Benichou, 2005). The HIV integrase enzyme then incorporates the proviral DNA into the host-cell genome. This process involves recognition of specific sequences within the long terminal repeats (LTRs) of viral cDNA by the integrase protein, followed by colinear insertion into chromosomal DNA, resulting in the formation of the provirus (Sierra *et al.*, 2005).

2.3.4 Integration

Following integration into the host chromosome, the integrated provirus serves as the template for the synthesis of the viral RNAs that ultimately encode the full complement of structural, regulatory, and accessory proteins used to direct virus replication (Quashie, 2013). The integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces.

2.3.5 Translation and synthesis of viral proteins

Transcription of viral RNAs is achieved through the RNA polymerase enzyme. The smaller spliced units are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced (Pollard & Malim, 1998).The HIV viral polyproteins are then expressed using host cell machinery. At this stage, the structural proteins Gag and Env are produced from the full-length mRNA.

2.3.6 Viral Assembly

The new HIV-1 virions then begin to assemble at the plasma membrane of the host cell (Figure 2.3). The *env* polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by protease and processed into the two HIV envelope glycoproteins gp41 and gp120 (Jacobs et al, 2014). These are transported to the plasma membrane of the host cell where gp41 anchors the gp120 to the membrane of the infected cell. The *gag* (p55) and *gag-pol* (p160) polyproteins associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell (McNicholl & McNicholl, 2006) Virion maturation either occurs in the forming bud or in the immature virion after it buds from the host cell. During maturation, HIV proteases cleave the polyproteins into individual functional HIV proteins and enzymes. The various structural components then assemble to produce a mature HIV virion (McNicholl & McNicholl, 2006).

2.3.7 Maturation and release

During the maturation process, the protease enzyme cuts the HIV proteins into smaller units that reassemble into a mature virus that can infect other cells (Figure 2.3). Without the enzyme protease, this maturation cannot occur, and HIV cannot go on to infect other cells

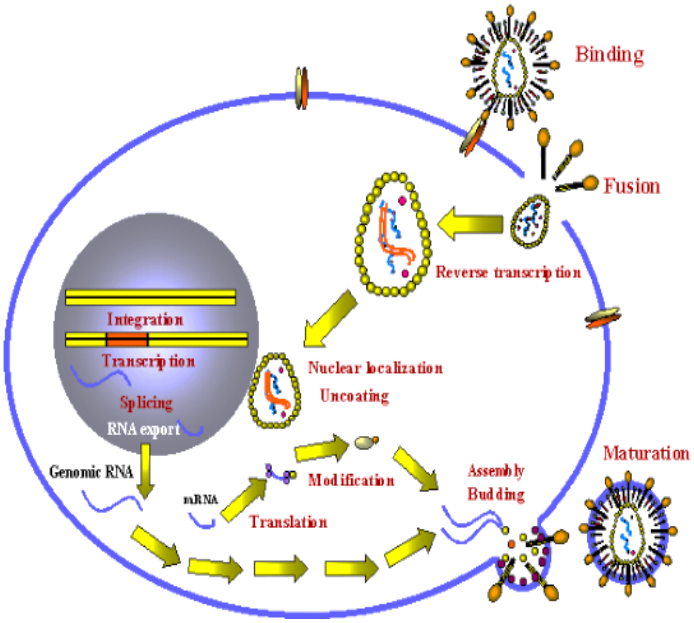

Figure 2.3: The HIV-1 replication cycle

(www.pedaids.org/blog/entry/hiv-101-the-stages-of-hiv-infection)

2.4. HIV Diversity

Human Immunodeficiency Virus is known to be classified into HIV-1 and HIV-2 which have similar genetic structures but differ up to 40% at the DNA sequence level. However Human Immunodeficiency Virus -2 has been found to be less virulent than HIV-1 and its mechanism are not clearly defined (Omobolaji *et al*., 2011). During The distribution of HIV-1 subtypes is significant for epidemiological purposes as well as in clinical settings (Taylor *et al.*, 2008). Globally HIV-1 has been responsible for most of the AIDS pandemic and different subtypes have been shown to have some difference genotypically and phenotypically (Abecasis *et al.,* 2005).The strains of HIV-1 are classified into three major groups due their variability: The "major or main" group M, the "outlier" group O and the "new" group N which are non M and non-O. More than 90 % of the HIV-1 infections worldwide belong to HIV-1 group M while group O and N are mainly restricted to central Africa HIV-1 group M is known to have at least nine genetically distinct subtypes (clades) which include subtype A, B, C, D, G, H, J and K (Santos & Soares, 2010).

In addition to subtypes there are subsubtypes which are made of groups of HIV-1 viral isolates with genetically and phylogenetically related sister clades. Examples of these include clade A classified to subsubtypes (A1, A2, A3, and A4) and clade F classified into subsubtypes F1 to F2 (Taylor *et al.*, 2008). Patients co-infected with strains from two different subtypes can yield inter-subtype recombinants (Abecasis *et al*, 2013). Unique recombinant form (URFs) are inter-subtype recombinant genomes found only in one dually or multiply infected individual patient in which they arose. Circulating recombinant form (CRFs) are inter-subtype recombinant HIV-1 genomes transmitted to three or more people who are not epidemiologically related (Hemelaar *et al.,* 2011). To date 79 CRFs and several URFs have been identified according to the Los Alamos HIV database. The widespread of recombinant forms may affect the global pandemic of HIV-1 (Lau & Wong, 2013).

The outcomes of various epidemiological studies have illustrated the presence of almost all subtypes, CRFs and several URFs in sub-Saharan Africa. For the other parts of the world it has been observed that HIV-1 subtypes have specific geographical distribution (Buonaguro *et al.*, 2007).Human Immunodeficiency Virus (HIV-1) subtypes A, B and C have been observed in studies to be the most prevalent forms globally and 50% of these infections have been found in subtype C. HIV-1 Subtype C is commonly found in Southern Africa and India (Jacobs *et al.*, 2009.).

2.5 Genetic diversity of HIV in Africa

Human Immunodeficiency Virus type 1group M strains are distributed in specific patterns in different regions of Africa. Studies performed on samples collected between 1995 and 2001 have revealed a detailed distribution of HIV subtypes in Africa (Lihana *et al.,* 2012). Most information on genetic subtypes has been based on the envelope, mainly the V3–V5 or C2–V3 regions by the heteroduplex mobility assay (HMA) or sequencing (Peeters & Sharp, 2000). However, some studies have analysed gp41 env, gag (p17 or p24) or pol (protease or reverse transcriptase genes) fragments and near fully genome sequences. (Toure-Kane *et al.,* 2000). The classification of HIV strains is evolving, and depends on the evolution and sensitivity of techniques used in molecular epidemiology. Overall, subtype A (CRF02-AG)

predominates in west and west-central Africa; however, there is a decrease in the proportion of subtype A from west to central Africa. Subtype G (or CRF06-cpx, see below) seems to be the second most prevalent subtype in West Africa. The highest genetic diversity is observed in central Africa, with the Democratic Republic of Congo harboring the highest number of HIV-1 subtypes. All known subtypes have been identified in this country, and a relatively substantial number of strains cannot be classified into the current subtypes (Archer, 2008). In east Africa, subtypes A and D predominate, but subtype C is also present and is the predominant and almost unique subtype in the eastern tip of Africa, Ethiopia. Subtype C also predominates in the epidemic in all countries in southern Africa, where the AIDS epidemic is explosive (Nicole *et al* .,2007).

2.6 Treatment and management of HIV infection

The most significant advance in the medical management of HIV-1 infection has been the treatment of patients with antiviral drugs, which can suppress HIV-1 replication to undetectable levels. Many factors are associated with the emergence of HIVDR. They include viral factors; drug-related factors and programme factors (Santos & Soares, 2010).

The approved drugs available for treatment of HIV-1 infections are distributed into six distinct classes based on their molecular mechanism and resistance profiles: (1) nucleoside-analog reverse transcriptase inhibitors (NNRTIs), (2) non–nucleoside reverse transcriptase inhibitors (NNRTIs), (3) integrase inhibitors, (4) protease inhibitors (PIs), (5) fusion inhibitors, and (6) coreceptor antagonists. These agents target such major steps in the HIV life cycle as attachment, entry, reverse transcription, integration, protein processing, and assembly and release (Figure 2.4).

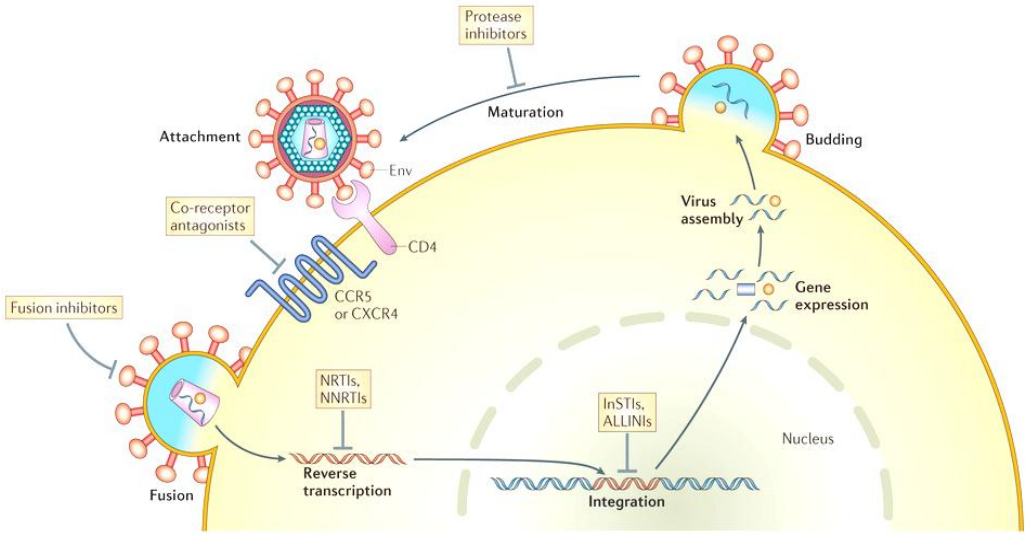

Figure 2.4: Current targets of antiretroviral drugs *(*Sarah & Robert, 2014*)*

2.6.1 Entry Inhibitor

This interferes with binding, fusion and entry of the virus to the host cellular membrane by blocking one of several targets. HIV requires binding to both the CD4 molecule and a co receptor to enable entry into the cell. The chemokine receptors CXCR4 and CCR5 are used as the main co-receptors (Schols, 2004). An example of this drug is maraviroc which works by targeting CCR5. This co-receptor is located on human helper T-cells though there can be a shift in tropism which allows HIV to target an alternative co-receptor such as CXCR4. Some individuals may have a mutation in the CCR5 delta gene which results in a nonfunctional CCR5 co-receptor and resulting in resistance or slow progression of the disease (Sharon & Lieberman-Blum, 2008**).** Another example of this drug is Fuzeon (T20) which is a peptide drug and works by preventing fusion of the virus with the host membrane

2.6.2 Nucleoside Reverse Transcriptase Inhibitors (NRTI)

The first class of drugs to be approved by the FDA were NRTIs and are administered as prodrugs, which require host cell entry and by cellular kinases before enacting an antiviral effect (Maxwell *et al.,* 2012). Nucleoside reverse transcriptase inhibitors (NRTI) and nucleotide reverse transcriptase inhibitors (NtRTI) are nucleoside and nucleotide analogues which inhibit reverse transcription (Maxwell *et al.,* 2012).

The Nucleoside Reverse Transcriptase Inhibitors (NRTI) are chain terminators such that once incorporated, work by preventing other nucleosides from also being incorporated because of the absence of a 3' OH group and both act as competitive substrate inhibitors. Currently, there are eight FDA-approved NRTIs: abacavir (ABC, Ziagen), didanosine (ddI, Videx), emtricitabine (FTC, Emtriva), lamivudine (3TC, Epivir), stavudine (d4T, Zerit), zalcitabine (ddC, Hivid), zidovudine (AZT, Retrovir), and Tenofovir disoprovil fumarate (TDF, Viread), a nucleotide RT inhibitor (Hazuda & Daria, 2012). Non-Nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase by binding to an allosteric site of the enzyme The NNRTIs interact with a specific 'pocket' site of HIV-1 RT that is closely associated with, but distinct from, the NRTI binding site. NNRTIs act as non-competitive inhibitors of reverse transcriptase (Balzarini., 2004).

2.6.3 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

The Nucleoside Reverse Transcriptase Inhibitors inhibit HIV-1 RT by binding and inducing the formation of a hydrophobic pocket proximal to, but not overlapping the active site The binding of NNRTIs changes the spatial conformation of the substrate-binding site and reduces polymerase activity (Tantillo *et al.,* 1994).The NNRTI-binding pocket only exists in the presence of NNRTIs and consists of hydrophobic residues (Y181, Y188, F227, W229, and Y232) and hydrophilic residues such as K101, K103, S105, D192, and E224 of the p66 subunit and E138 of the p51 subunit. Unlike NRTIs, these non/uncompetitive inhibitors do not inhibit the RT of other lentiviruses such as HIV-2 and simian immunodeficiency virus (Hazuda & Daria, 2012). Currently, there are four approved NNRTIs: etravirine, delavirdine, efavirenz, and nevirapine, and several in development, including rilpivirine in phase 3 (Hazuda & Daria, 2012). As with NRTI resistance, complex patterns of NNRTI-resistant mutations can arise and alternative pathways have been observed in nonsubtype B infected individuals. Most NNRTI mutations engender some level of cross resistance among different NNRTIs, especially in the context of additional secondary mutations (Hazuda & Daria, 2012).

2.6.4 Protease Inhibitors

Protease inhibitors interfere with the protease enzyme by blocking it yet it is important in production of mature virions upon budding from the host membrane. Mostly these drugs prevent the cleavage of *gag* and *pol* precursor proteins and thus virus particles produced in their presence are defective and mostly non-infectious (Wensing, 2010). Examples of HIV protease inhibitors are lopinavir (LPV), indinavir (IDV) , nelfinavir (NFV), amprenavir (APV) fosamprenavir (FOS-APV), atazanavir (ATV), darunavir (DRV), ritonavir (RTV), saquinavir (SQV), tipranavir (TPV) and ritonavir. Maturation inhibitors have a similar effect by binding to *gag*. Resistance to some protease inhibitors is high. Second generation drugs have been developed that are effective against otherwise resistant HIV variants (Gulick, 2003).

2.6.5 Integrase Inhibitor

Integrase enzyme is inhibited by integrase inhibitors. Examples of these drugs are raltegravir (RAL), dolutegravir (DTG) and elvitegravir (EVG) (Quashie.2013). These three integrase inhibitors bind to the catalytic core (amino acids 50-212) of the integrase enzyme and target the integration process at the strand transfer step (Maxwell *et al.,* 2012).

2.7 Human immunodeficiency virus Drug resistance

The HIV drug resistance is the ability of HIV to mutate and reproduce itself in the presence of antiretroviral drugs and the consequences of HIVDR include treatment failure and further spread of drug resistant HIV. The HIV drug resistance occurs when the virus starts to make changes (mutations) to its genetic make-up (RNA) that are resistant to certain HIV drugs, or classes of HIV drugs. This can happen either as a result of a prolonged period of time on treatment, or more commonly, as a result of suboptimal treatment adherence. The new mutations make copies of themselves,

gradually increasing the level of the virus (viral load) in the person living with HIV resulting to treatment failure.

Human Immunodeficiency Virus (HIV) drug resistance occurs when there is a change (mutation) in the genetic structure of HIV. This interferes with the blocking of replication of the virus by antiretroviral drugs. The emergence of drug resistant virus posses a risk of ARV drugs becoming partially or fully inactive.HIV drug resistance is divided into three main categories (WHO, 2017). First is the acquired HIV drug resistance (ADR) which emerges due to viral replication in patients receiving ARV drugs.This occurs mainly because of medication nonadherence and suboptimal treatment (De Luca & Zazzi, 2015). Secondly there is transmitted HIV drug resistance (TDR) which happens when uninfected ARV naive individuals are infected with HIV that already has drug resistance mutations (De Luca & Zazzi, 2015). Thirdly is the pretreatment HIV drug resistance which is detected at initiation of first line ART or at re initiation of first line ART. Pretreatment drug resistance can either be transmitted or acquired drug resistance and it can also be both (Kityo C 2017). Antiretroviral drugs act by inhibiting the viral enzymes critical to HIV replication cycle while others act on HIV host cell target (CCR5 or CCR4) which blocks the entry of the HIV virus into the cell. Currently, four classes of over 30 licensed antiretroviral and combination regimens of these ARVs are in use clinically including: reverse transcriptase inhibitors (RTIs) (for example nucleoside reverse transcriptase inhibitors, NRTIs; and non-nucleoside reverse transcriptase inhibitors, NNRTIs), protease inhibitors (PIs), integrase inhibitors and entry inhibitors such as fusion inhibitors and CCR5 antagonists (Lu & Chen, 2010).

2.8. Indicators of drug resistance

The WHO transmitted drug resistance surveys alert program managers to the existence of drug-resistant HIV among recently infected populations in specific geographical areas. The Word Health Organization surveys of acquired HIV drug resistance estimate prevalence and patterns of resistance at treatment initiation, the proportion of people achieving successful virological suppression at 12 months at sentinel sites and describe drug resistance in populations experiencing treatment

failure. The target of ART is to achieve and maintain viral load suppressed. According to WHO 2016, here are three different definitions for ART failure: clinical, immunological and virological. The WHO 2006 global guidelines define: clinical failure when there is a new or recurrent WHO stage 4 condition; immunological failure when CD4 falls to the pre-therapy baseline (or below) or there is a 50% fall from the on-treatment peak value (if known) or CD4 levels are persistently < 100 cells/mm3; and virological failure when plasma VL > 1000 copies/ml. The consensus expert opinion of virological failure of over 1000 copies/ml is not an evidence based recommendation as no data currently exists to make a formal recommendation. (World Health Organization, 2016) .It has been suggested that as long as VL continuously remains < 1000 copies/ml, CD4 cell gains can be expected (WHO, 2016) . It is clear that if the initial goal of treatment is to maximally suppress viral replication, then failure is defined by this goal not being achieved; therefore, failure occurs when virus is detectable at least 6 months after ART has been initiated (upon confirmation that lack of adherence is not a factor).

The two initial indicators of development of resistance to ART are declining CD4+ T cell counts and increasing plasma viral RNA concentrations (VL) (Collazos *et al.,* 2006). Ideally, viral suppression is considered successful if less than 50copies/ml of HIV RNA (considered undetectable) occur in blood after six months of treatment while CD4+ T cell counts above 200 cells/µl or 500 cells/ µl, are defined as an indicator of success. (Maxwell *et al.,* 2012).The CD4+ T cell count remains the strongest predictor of HIV-related complications, even after the initiation of therapy. The baseline pretreatment value is informative: lower CD4+ T cell counts are associated with smaller and slower improvements in counts. However, precise thresholds that define treatment failure in patients starting at various CD4+ T cell levels are not yet established (Garcia *et al.,* 2004). A large proportion with suppressed viral load implies a low rate of onward transmission and is an indicator of treatment success and reduced potential for transmission. Suppressed viral load is defined as <1000 copies/ml after 12 months on ART (WHO, 2016). Some patients fail to respond as expected or may even exhibit clinical deterioration initially. These issues combine to present specific challenges for clinical management and it is important to allow sufficient time on therapy before judging effectiveness of ART.

2.9 Antiretroviral treatment in Burundi

Various intervention activities have been in place to curb the expansion of HIV-1 epidemic in Burundi since its recognition in 1983. Evidence shows that the scale-up of ART in Burundi has greatly improved the longevity of HIV infected persons in the country (PNLS/IST, 2016). The introduction of antiretroviral drugs in 1997 was essentially limited to Bujumbura, the capital. Two years later, in 1999, a program of facilitating access to antiretroviral has been implemented by the Government and several institutions are organized for the support of their staff through solidarity funds. The Burundi initiative for improving access to antiretroviral therapy, the Government has set up a solidarity fund therapeutic nationally in 2000 with as guidelines the combination therapy in standard schema (PNLS/IST, 2016).

Access to antiretroviral therapy has been strengthened as part of the WHO "3 million by 2005" initiative which aimed to put 3 million PLWHA on medication. The schemes proposed for Burundi are inspired by 2015 of the WHO guidelines (Table 2.1 and Table 2.2), and this represents an important step towards the achievement of universal access to ARVS for the treatment and prevention of infection HIV enlargement of ARV coverage by treating all HIV-positive person (treat all) (PNLS/IST, 2016).

Table2.1: Recommended ART Regimens for Adolescents and adults in Burundi

First line treatment	Second line treatment	Third line treatment
	Principle treatment	
2 NRTI+ NNRTI	2 NRTI + PI/r	PI/r + IIs+ 1 NRTI
	Combination of first choice	
TDF/3TC/EFV	ABC/3TC + ATV/r	DRV/r + DTG + 3TC or AZT
	Combination alternative	
TDF/3TC+ NVP	ABC/3TC + ATZ/r	DRV/r + DTG + 3TC or AZT
AZT/3TC/NVP	ABC/3TC + ATZ/r	DRV/r + DTG + 3TC or TDF

NRTI: Nucleoside Reverse Transcriptase Inhibitor; NRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; PI: Protease Inhibitor; Integrase Inhibitor; 3TC: Lamivudine; EFV: Efavirenz; TDF: Tenofovir disoproxil fumarate; NVP: Nevirapine; AZT: Zidovudine; AZT/r: Zidovudine/ritonavir; ATV/r: Atazanavir/ritonavir; ABC: Abacavir; DRV/r: Darunavir/ritonavir; DTG: Dolutegravir. Source: PNLS/IST, 2016.

Table 2.2: Recommended ART Regimens for ARV treatment of 1st, 2nd and 3rd line in infants and children

	Age (years)	Schema used in 1st line	Second line	Third line
1st line with LPV/r	< 3 years	1st choice ABC+3TC+LPV /r	AZT+3TC+RAL	RAL/DTG+ 2NRTIs
		Alternative AZT+3TC+LPV /r	ABC+3TC+RAL	DRV/r+2 INRTs
	>3 years	ABC+3TC+LPV /r	AZT+3TC+EFV	
		AZT+3TC+LPV /r	ABC+3TC+EFV	
1st line with NNRTI	3-10 years	1st choice ABC+3TC+EFV	AZT+3TC+ATV/r	DRV/r+ RAL/DTG+1 NRTIs
		Alternatives ABC+3TC+NVP	AZT+3TC+ATV/r ABC+3TC+ATV/r	
		AZT+3TC+NVP		

NRTI: Nucleoside Reverse Transcriptase Inhibitor; NRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; LPV/r: Lopinavir/ritonavir; RAL: Raltegravir; RAL/DTG: Raltegravir/dolutegravir; 3TC: Lamivudine; EFV: Efavirenz; TDF: Tenofovir disoproxil fumarate; NVP: Nevirapine; AZT: Zidovudine; AZT/r: Zidovudine/ritonavir; ATV/r: Atazanavir/ritonavir; ABC: Abacavir; DRV/r: Darunavir/ritonavir; DTG: Dolutegravir. Source: PNLS/IST, 2016.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was carried at Kayanza District Hospital. This is located at Kayanza Centre, in Kayanza Province, one of the 17 provinces of Burundi in the north.

Kayanza is a city located in northern Burundi. It is the capital city of Kayanza Province. The city is known for its tea and coffee production. (RGPH, 2008). It is one of the areas with higher population density; total population of 585,412 on surface of 1,233.24 km^2 with density of 470/ km^2 (RGPH, 2008). It is subdivided into nine districts from which one is called Kayanza.

Figure 3.1: A map indicating the location of Kayanza province*(GIBU, 2004)*

3.2 Study design

This was a descriptive cross-sectional study of HIV infected patients on ART attending a comprehensive care clinic (CCC) in a district hospital in Burundi. This study was carried out in two months - November 2017 to December 2017.

3.3. Study population

The study population included patients living with HIV and AIDS, males and females, and who were on ART for more than 12 Months. All were attending Kayanza district hospital.

3.3.1 Inclusion criteria

i. HIV infected patients on ART,
ii. Attending Kayanza district Hospital,
iii. Patients on ART over 12 months.
iv. Consented/Assented to take part in the study

3.3.2 Exclusion criteria

i. HIV patients on ART who refused to give consent or assent.
ii. Patient too sick to be enrolled in the study.

3.4. Sample size determination

The sample was determined using 12%, the prevalence of virological failure after the first 12 months on antiretroviral therapy (Ndahimana *et al*., 2016).Using a formula for cross sectional studies (Chadha ., 2006), the sample was calculated as below:

$N = Z^2 \times p \times q / d^2$

Where
N = Minimum sample size required,
Z = Z-score for normal standard deviation for a 95% confidence interval (1.96),
p = the prevalence of drug resistance mutations after the first 12 months on antiretroviral therapy in Rwanda,
q = (1-P)
d = Significance level at 95% confidence interval (0.05).
N = (1.96)2*0.12*0.88/ (0.5)2 = 162.3 = 163

A minimum of 163 patients was required.

3.5 Sampling method

A purposive sampling method was used. All patients who met the inclusion criteria were consented /assented (Appendix I and II) and recruited into the study.

3.6 Data collection and management

3.6.1 Data collection

A questionnaire (Appendix II) was used to collect social and demographic data, clinical, drugs experienced and any other characteristics. More patients' information's were also obtained through desk review of the clinical and laboratory records from the patient's files from the recruitment of the patients.

3.6.2 Sample collection and handling

Up to 4 mL of venous blood was collected from each patient in Ethylene diamine tetra acetic Acid (EDTA) tubes and shipped to Ngonzi regional hospital and Burundi National Reference Laboratory (NRL) for further processing. Plasma samples were stored at -80^{o}C and later shipped to the Kenya Medical Research Institute (KEMRI), in Nairobi – Kenya, where further testing and analysis were performed. This transportation complied with both National and international (IATA) regulations for the transport of the dangerous goods. A triple packaging system with appropriate labeling on the outer packaging was used. Further, a cold chain was maintained by using dry ice.

3.6.3 Specimen Processing and Laboratory Assays

3.6.3.1 Viral Load

Viral load assay for plasma samples was done using Real Time HIV-1 assay Kit Abbott molecular Inc.; Des Plaines, IL 60018, USA), which has an internal Quality Control system, with automated Abbott *m*2000*rt*™ System and was performed according to the manufacturer's instruction. Sample preparation for the automated quantification assay was done manually following the manufacturer's instruction

(Abbott Molecular Inc., Des Plaines, IL 600018, USA) in the same laboratory by extracting and concentrating the target RNA molecules from 200 mL plasma to make the target accessible for amplification and to remove potential inhibitors of amplification from the extract. Although, Virological failure was used as a yardstick in this study instead of clinical failure or immunological failure, because clinical and immunological markers were been shown to be poor predictors of virological suppression (Lihana *et al.,* 2011). Lower limit of detection was 40 copies of HIV RNA /mL. Samples with viral load > 1000 copies/ml were considered for Sequencing and Genotyping.

3.6.3.2 Nucleic acid extraction and Reverse Transcription

Nucleic acid (RNA) was extracted from plasma samples using the Qiagen RNA extraction kit according to the manufacturers' instructions (Khamadi *et al*., 2005). Briefly, 560 µl of prepared Buffer AVL containing carrier RNA was pipetted into a 1.5 ml microcentrifuge tube. A volume up to 140 µl of plasma was added to the Buffer AVL carrier RNA in the microcentrifuge tube. This was mixed by pulse-vortexing for 15 seconds. This was then incubated at room temperature (15-25°C) for 10 min. Then 560 µl of ethanol (96-100 %) was added to the sample, and mixed by pulse-vortexing for l5 seconds. At that stage, 630 µl of the extract was added to a QIAamp Mini spin column (in a 2 ml collection tube) and centrifuged at 6000 x g (8000 rpm) for 1 min and the supernatant discarded. The QIAamp spin column was placed into a clean 2 ml collection tube. The 500 µl of wash buffer (Buffer AW1) was added and centrifuged at 6000 x g (8000 rpm) for 1 min.

The supernatant was discarded and 500 µl of wash buffer (Buffer AW2) added and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. (Maxwell *et al.,* 2012).The QIAamp Mini spin column was placed in a new 2 ml collection tube. 60 µl of elution buffer (Buffer AVE) equilibrated to room temperature was added and incubated at room temperature for 1 min. This was then centrifuged at 6000 x g (8000 rpm) for 1 min to elute the viral RNA. This RNA was kept at -80^{o}C till use (Khamadi *et al*., 2005). The template (10 µl of extracted RNA) was reverse transcribed in a total volume of 20 µl with 1 µl dNTP, 2 µl primer, 4µl 5× RT buffer, 2 µl DTT, 0.25 µl RNase OUT and 1.0 µl Super ScriptTM III RNase H− RT

(Invitrogen, Carlsbad, CA2). The RNA primer and dNTPs were first incubated at 65°C for 5 minutes, then 4° for 5 minutes, and the remaining reagents were added for cDNA synthesis at 25°C for 5 minutes, 50°C for 40 minutes, followed by 70°C 15 minutes (Khamadi *et al*., 2005).

3.6.3.3 R-T Polymerase Chain Reaction (PCR)

The cDNA was used as template to amplify the region of interest for HIV-1 drug resistance. The reagents were thawed on ice to preserve their integrity. The total volume of outer PCR was 25µL reaction volume with a mixture containing 3 µL of the DNA template to be amplified, 2.5 µL of 10X buffer (without magnesium chloride), 0.3 µL of each 0.5µL dNTP, 2.0 µL Mgcl2, 0.2 µL of Taq polymerase and 16.2 µL of distilled water (Table 3.1)(Lihana *et al*., 2009).The cycling conditions were 1 cycle of 95˚C for 10 min and 35 cycles of 95˚C for 30 s, annealing at 55˚C for 30 s, and 72˚C for 1 min, and final extension of 72˚C for 10 min (Nyamache *et al*., 2011). From the first-round PCR products, 3 µL was used as a template to make 25µL for the second reaction volume with the second set of primers ; using the same cycling conditions (Table 3.1)(Nyamache *et al*., 2011).

Table 3.1 Reverse transcriptase (RT) region primer pairs

Primer name	Sequences (5'-3')	Position on HXB2
1st RT PCR		
RT18	GGAAACCAAAAATGATAGGGGGAATTGGAGG	2377-2407
KS104	TGACTTGCCCAATTTAGTTTTCCCACTAA	3355-3327
2nd RT PCR		
KS101	GTAGGACCTACACCTGTTCAACATAATTGGAAG	2481-2512
KS102	CCCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG	3241-3210

3.6.3.4 Analysis of PCR amplicons by gel electrophoresis

After DNA amplification, all products were viewed by conventional agarose gel electrophoresis on a 2.0% agarose (SEakem LE® agarose; FMC Bio Products,

Rockland, Marine, USA) gel in 1X TAE buffer (0.04M Tris acetate, 0.001 M EDTA). The gel was prepared using 2 g of agarose gel into 100 mL of 1X TAE buffer and then heating. This was cooled to about 50°C and 70 µl of ethidium bromide added (Sigma-Aldrich®) to the gel to stain the DNA (Kiptoo *et al.,* 2013). A 100 kb DNA molecular weight marker (exACTGene™ Low Range DNA ladder from Fisher scientific) was used to estimate the DNA band size. The samples were mixed with gel loading dye before loading into the wells on the gel. Electrophoresis was done at a constant voltage of 80 volts/cm using a Mupid®2 plus submarine electrophoresis system power supply source. After electrophoresis, the band size on the gels was visualized under an ultra violet light as previously described (Khamadi *et al*., 2005).

3.6.3.5 Sequencing PCR

The amplicons from the nested PCR were sequenced. Briefly, a reaction mixture containing the following reagents was used for sequencing PCR: Thirteen (13) µl RNase free double distilled H_2O,5x sequencing buffer,1.5 µl primers of 1 µM sense and antisense primer,1.0 µl big dye terminator and 1.0 µl PCR product making up a total reaction volume of 20 µl (Songok *et al*., 2004) . The PCR tubes containing the samples were placed into the thermocycler under the following programmed conditions; Initial PCR activation step 96°C for 2 minutes; 30 cycles of: denaturation for 10 seconds at 96°C, annealing for 10 seconds at 50°C; extension for 4 minute at 60°C and thereafter the samples were held at 4°C (Kiptoo *et al* ., 2013;Lihana *et al*., 2009;).

3.6.3.6 Purification of PCR products

The following reagents were added to a new 1.5ml microtube: 20 µl of the sample,2 µl of 125Mm EDTA, 2 µl 3M sodium acetate and 50 µl 100% ethanol This was then vortexed and kept at room temperature for 15 minutes Centrifugation was done at 14,000 rpm for 20 minutes. The supernatant was discarded, 70 µl of 70% ethanol added and mixed gently. Centrifugation at 14,000 rpm for 10min was done. Supernatant was discarded and sample dried at room temperature for about 10 min.

This was followed by addition of 25 µl of HiDi formaminde to the sample then vortexing and spinning down. Sample was then incubated at 95 ºC for 2 min then placed on ice for 10 min. The sample was span down and transferred to sequencing tube and loaded into the ABI 310 DNA sequence for sequencing (Applied biosystems, Foster City, CA) (Kiptoo *et al*., 2013).

3.6.3.7 HIV-1 Subtyping

The Sequences derived in this study were subjected to BLAST and REGA search tools to determine HIV subtypes. Reference sequences were selected from Los Alamos HIV database. The sequences were aligned and gaps removed using BioEdit. The tree topology was obtained using the neighbour-joining method .A phylogenetic tree was constructed using neighbour joining method in MEGA 6 software (Tamura *et al.,* 2013). Alignment of DNA sequences was performed using the CLUSTALW program (http://www.clustal.org).The pairwise distance matrix was estimated using the Kimura two parameter model with the MEGA 6 software package (http://www.megasoftware.net). Bootstrap re-sampling (1000 data sets) of the multiple alignments was performed to set the statistical robustness of the tree. The tree profile was visualized with tree view version 1.65 (Foley *et al*., 2015).

3.6.3.8 Drug resistance mutations identification

Genotypic drug resistance in the RT region was defined as the presence of one or more resistance-related mutations, as specified by the consensus mutation figures of the International AIDS Society-USA (Johnson *et al.,* 2013) and the online tool on the Stanford HIV algorithm-Software (http://hiv.grade.de). All mutations associated with high level resistance according to Stanford IAS Algorithms were considered.

3.7 Data management and analysis

Analyses were done using SPSS version 23 software. The descriptive statistics was used to analyse socio-demographic data, clinical and immunological outcomes and virological outcomes. The data was reported as percentages. Multinomial logistic regression was used to adjust for odds of viral outcomes associated with various

demographic factors (like gender, age, religion, education status, marital status, income source, presence of other partners and reminders to take ARVs) as well as other medical factors (irregularity in taking drugs, taking TB drugs, line of ARV medication , initial CD4 count). The results were expressed as odds ratios (OR) with accompanying p-values at 95% confidence intervals (CIs). P values $\leq$ 0.05 was considered significant.

3.8 Ethical considerations

Ethical clearance to carry out this study was granted by the Burundian National Ethical Review Committee (BERC: 25/03/2018) (Appendix IV). With further permission granted by the Ministry of Public Health – Burundi and the Kayanza District Hospital. All participants consented to the study by signing a consent form which was duly read and explained to all participants. Participation of the minors to the study was done after consenting and parents assenting on behalf of the minors. All consent forms were translated to the native Kirundi language.

Further confidentiality of the participants was maintained by recording the samples with unique codes that were only known to the principal investigator and the hard copies stored in lockable drawers whereas soft copies were password protected, passwords which were changed at least 3 times in the course of data collection.

3.9 Storage and disposal of biological materials

All the samples used in this study were stored in the freezers (-80ºC) either in Burundi National Reference Laboratory or at KEMRI HIV laboratory. Reagents were also stored in the freezers (-30 ºC) and some in room temperature in the laboratory cabinets according to the manufacturer's instructions. After use all the waste were disposed in a biohazard bag and autoclaved before releasing them to the incinerator.

CHAPITER FOUR

RESULTS

4.1 Baseline characteristics of study participants

A total of 200 patients met the inclusion criteria and were included in the study. All patients were ARV experienced based on 2008 Burundi National guidelines, which proposed combination of Zidovudine (AZT), lamivudine (3TC) and Nevirapine or Efavirenz are the most commonly prescribed first-line. The age distribution was unimodal and it peaked at 45 years (range 3 to 67 years). From respondents, 86% (172/200) (Table 4.1) were above 18years and female accounted for 55% (n=110). Stratification by religion, 73.5% (n=147) were Christian-Catholics while 52% (104/200) were married On the level of education, 82.5% (n=165) reported completing primary school while farming as occupation was reported by 65% (131/200) with 94% (n=188) reporting to earn below BIF 200 (Table 4.1).Out of 200 patients, 12.5% (25/200) were on tuberculosis (TB) medications while 57% (114/200) had been on antiretroviral (ARV) therapy for between six to ten years. In total 51%, (102) did not have detectable viral loads while at the time of starting the ARV medications 68% (137) of the respondents had a CD4 count of >350 cells/$mm^{3.}$ Based on ARV medication, 56.5% (113) of the respondents reported using tenofovir (TDF), lamividune (3TC) and Efavirenz (EFV) drug regimen, but TDF, tenofovor-3TC, lamividune-NVP, nevirapine; zidovudine AZT+lamividune, Duovir; Duovir+ EFV, efavirenz were also used

Table 4.1: Baseline characteristics of study participants in HIV patients on ART attending Kayanza district Hospital

		Frequency	Percent
Age (years)	2-12	16	8.0
	13-17	12	6.0
	18 -67	172	86.0
Gender	Male	90	45.0
	Female	110	55.0
Religion	Catholic	147	73.5
	Protestant	19	9.5
	Muslim	34	17.0
Marital status	Single	37	18.5
	Married	104	52.0
	Divorced	12	6.0
	Widowed	47	23.5
Educational background	Primary	165	82.5
	Secondary	33	16.5
	University	2	1.0
Income Source	Farmer	131	65.5
	Government employer	33	16.5
	private employed	36	18.0
Income level	<200	188	94.0
	200-400	10	5.0
	>400	2	1.0
CD4 results	>350 cells/mm^3	138	68.4
	<350 cells/mm^3	62	31.6
On TB treat	Yes	25	12.5
	No	175	87.5
ARV treatment used	TDF3TCEFV	113	56.5
	Others	87	43.5
Viral load results	< 40 copies/mL	102	51
	40-999 copies/mL	72	36
	>1000 copies/mL	26	13

4.2 Virological outcomes

At the time of the study more than half of the respondents (51%, n=102) did not have detectable viral load (VL < 40 copies/mL), 34%, n=72 were between 400 and 999 copies/ml while 13%, n= 26 had virological failure (VL ≥1000 copies/mL). Virrological faiure was strongly associated with the presence of other partners (adjusted odds ration [aOR] 0.154, p =0.016 (Table 4.2). Viral failure was significantly correlated with irregularity in taking medications (adjusted odds ratio (aOR) 0.4, p=0.014) (Table 4.2).

Table 4.2: Virological outcomes and other medical factors among HIV positive patients attending Kayanza district Hospital

Variable		Viral Failure	aOR	P Value
Taking alcohol	Yes	6	0.8	0.711
	No	20	1	
Missing Dose	Yes	5	0.4	**0.014**
	No	21	1	
Taking TB Drugs	Yes	3	0.3	0.251
	No	23	1	
Line of ARV Medication	First line	19	5.2	0.075
	Second line	7	1	
Presence of other sexual partners	Yes	3 (42.9%)	0.154 (0.02-1.3)	**0.016**
	No	23 (13%)	1	
CD4 initial count	<200 cells/mm^3	9	1.8	0.412
	201-350 cells/mm^3	7	0.881	0.833
	>350 cells/mm^3	10	1	
CD4 Count at study time	<200 cells/mm^3	4	1.7	0.598
	201-350 cells/mm^3	6	0.6	0.409
	>350 cells/mm^3	16	0	1

4.3 Analysis of the generated amplicons

Fifteen samples were successfully amplified at the pol-RT gene region. The PCR products were 697 base pairs in size (figure 4.1).

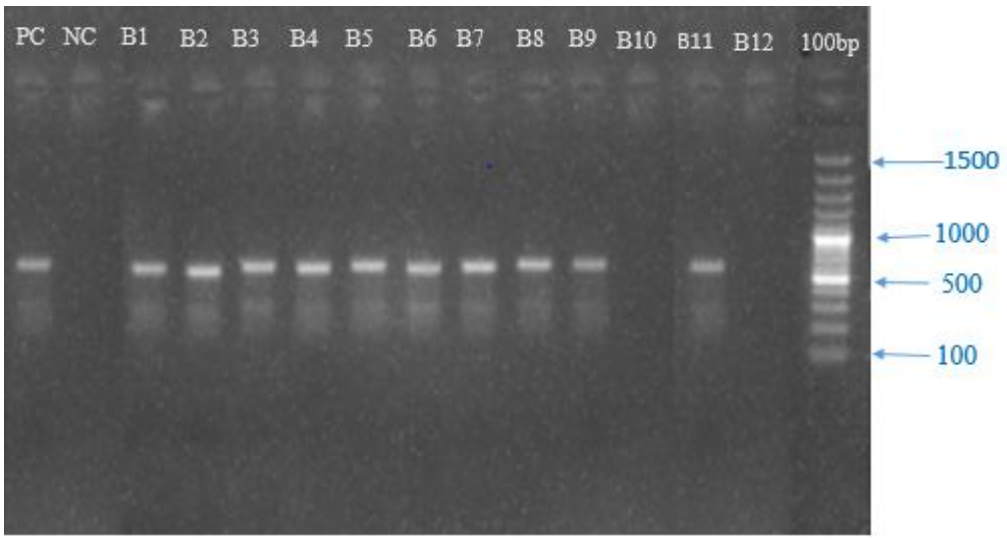

Figure 4.1: Electrophoresis profile of the pol-RT gene

Lane **PC**- positive control, Lane **NC**-Negative control, Lane **B1** to **B9** and lane **B11** -positive samples with the right size of DNA; (697)**, B10 and B12** -Negative samples, Last lane–Molecular marker (100base pairs).

4.4 Phylogenetic characterizations of HIV-1 variant**s**.

Genotype sequencing was performed on 26 samples with virological failure (HIV-1 RNA ≥1,000 cop/mL). Fifteen (15) samples were successfully sequenced. From these, 80% (12/15) were HIV-1 subtype C, 7 % (1/15) were subtype A, and 13 % (2/15) were HIV-1 subtype A1. The Phylogenetic tree is illustrated with reference sequences labelled using their gene bank accession numbers and country of origin show that HIV-1 subtype C clustered with those of Burundi, Rwanda, and Tanzania. The samples with subtype A clustered with those from Burundi and DRC (Figure 4.2).

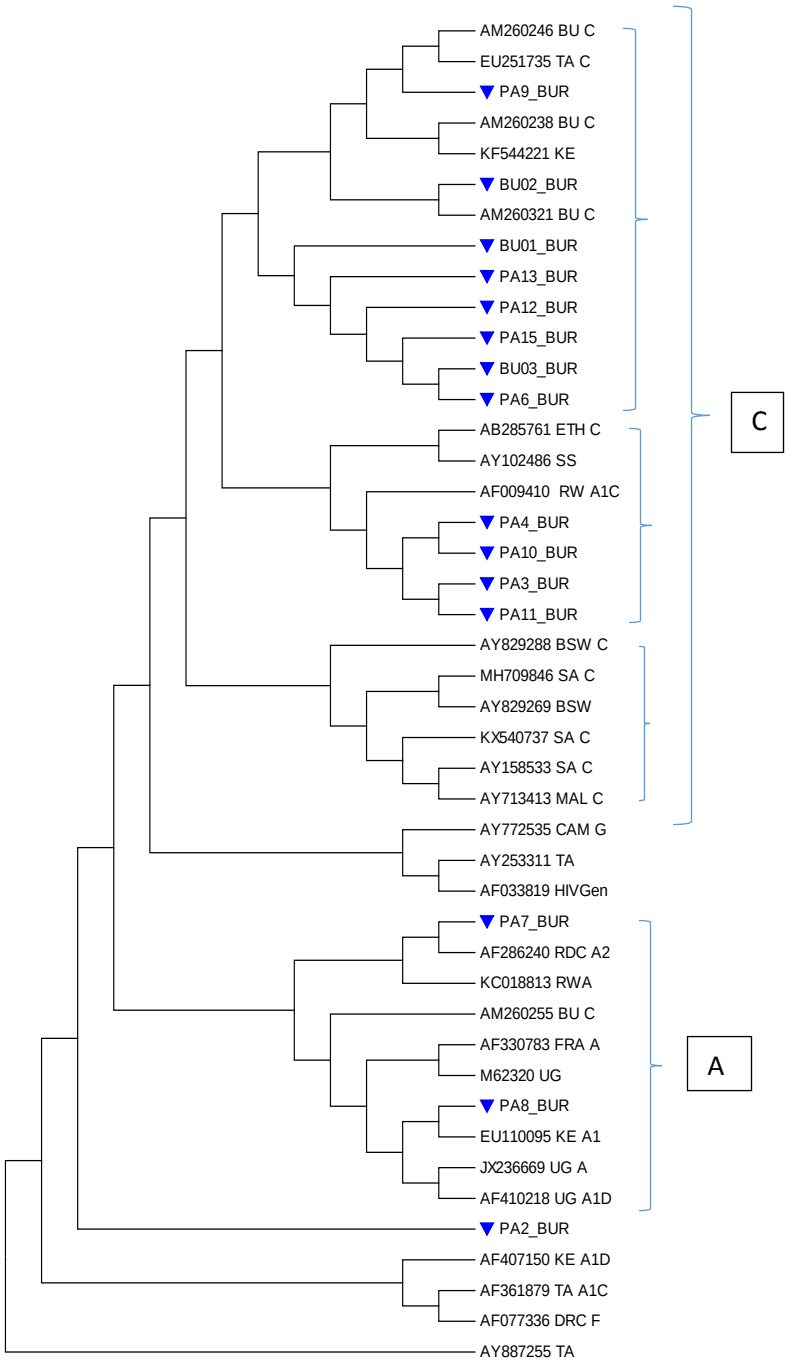

Figure 4.2. Phylogenetic tree illustrated using samples sequences and references from Los Alamos data base.

The samples are labeled with BU followed by the numbers and subtype; and the references from Los Alamos database are labeled by their accession numbers, the subtypes and the country of origin.

4.5 Drug resistance mutations analysis

From the 200 samples, only 26 had suspected virological failure according to WHO virological failure definition. From these 15 were successfully amplified and sequenced. Using the Stanford drug Resistance Data base algorithm, 80% (12/15) had at least one mutation (NRTI or NNRTI), while 20 % (3/15) did not carry any drug resistance mutations. Based on drug class, the most common drug resistance mutations were against NNRTI 80 % (12/15) while DRM against NRTI were 47 % (7/15). The most common drug resistance mutations were M183V, M41L, E44D, L74I, L210W, K65R, K103N, E188A and Y188H (Table 4.3). Finally, minor mutations were found like V179I in two patients, K101Q in 5 patients and S68G in one patient. For NRTIs, the HIV drug resistance mutations found were against FTC, 3TC, D4T and TDF while for the NNRTI the mutations found were against EFV and NVP (Table4.3).

Finally, minor mutations in RT like 131K, V35K, E36D, K13Q, M16V, V21F, K22P, Q23R, 15L, E6D, and V10I were observed. For NRTIs, the HIV drug resistance mutations found were against FTC, 3TC, D4T and TDF while for the NNRTI the mutations found were against EFV and NVP (Table 4. 3).

Table 4.3: Resistance mutations by drug class and subtypes in HIV patients attending Kayanza district hospital

Sequence ID	Subtype	DRMs			
		NRTIs		NNRTIs	
		DRMs	DRUGS	DRMs	DRUGS
BU01	C	M184V	FTC,3TC	K103N	NVP,EFV
BU02	C	None	-	K103N	NVP,EFV
BU03	C	M184V	FTC,3TC	K103N, Y188H	EFV,NVP
BU04	A	K65R,M184V	FTC,ABC, DDI	K103N,	EFV,NVP
BU05	C	None	-	K103N	EFV,NVP
BU06	C	None	-	K103N	EFV,NVP
BU07	C	M184V	3TC,FTC	K103N	EFV,NVP
BU08	A1	None	-	None	None
BU09	A1	None	-	None	-
BU10	C	None	-	None	-
BU11	C	None	-	K103N	EFV,NVP
BU12	C	None	-	K103N	EFV,NVP
BU13	C	M184V M41L, E44D, L74I	FTC,3TC	K103N	EFV,NVP
BU14	C	M184V,L210W	ABC,FTC, DD,3TC	K103N,E138A	EFV,NVP
BU15	C	M184V	FTC,3TC	K103N	EFV,NVP

NRTI: Nucleoside Reverse Transcriptase Inhibitor; NRTI: Non-Nucleoside Reverse Transcriptase Inhibitor; DRM: Drug Resistance Mutation; BU: Burundi; FTC Emtricitabine 3TC: Lamivudine; EFV: Efavirenz; NVP: nevirapine; ABC: abacavir;

CHAPTER FIVE

DISCUSSION

5.1 Discussion

5.1.1 Virological failure and demographic characteristics

This cross-sectional study on HIV-1 subtypes and HIV drug resistance mutation outcome is among the few that have been conducted in Burundi and establishes a prevalence of virological failure of 13%(26/200) whereas prevalence of drug resistance mutation at 80%(12/15). All patients were ARV experienced based on 2008 Burundi National guidelines, which proposed combination of Zidovudine (AZT), lamivudine (3TC) and Nevirapine or Efavirenz as the commonly prescribed first-line treatment combinations. In this unimodal population with 86% above 18 years with 55% mainly females, the treatment failure found raises a concern to the public health system, as low level transmitted resistance could already be taking place in the general population. These finding show that there was no association to virological failure and age. This may be due to the adherence in different age and need to be confirmed by a large sample size. In the study conducted in Rwanda and Kenya, virological failure was associated with age being <25 years and between 15–35 year respectively (Ndahimana *et al*., 2016;Hassan *et al*., 2014).The results from the study report that female accounted for 55% (n=110).This could be that female are likely more to believe adherence and David Riedel et *al* reported the same case in Rwanda in their retrospective cohort study in HIV-infected patients receiving antiretroviral therapy. The results from the study reported that 65% of the respondents were farmer and this was expected as Kayanza district hospital is a rural facility where most of them are illiterate.

However, this success of HIV drug treatment met the WHO recommendation of >85% of patients on ART suppressing HIV-1 VL after 12 months of treatment. At 87 % viral suppression after 12 months on treatment, Burundi exhibits a potential to reach the third 90 % of the 90-90-90 target recently set by UNAIDS. Likewise, studies conducted in other countries in Resources Limited Settings such as Rwanda,

Tanzania show virological suppression of 88.1 % and 86 % respectively (Ndahimana *et al*., 2016; Rusine *et al*., 2013;Hawkins *et al*., 2016) but are high contrary to those found in Latin America and in Ethiopia. (Cesar *et al*., 2015; Endalamaw *et al*., 2018). The reason for geographic area where carried the study should not be excluded in this discordant findings .In this study, Viral failure was significantly correlated with irregularity in taking medications, missing dose. As Kayanza Hospital is a rural health facility, poor adherence to distance-related antiretroviral therapy may be observed and could certainly lead to virological failure. The findings highlight why adherence is good, not only for the prevention of morbidity, mortality and transmission, but also for the prevention of HIV drug resistance. The results were not surprising because it has long been known that low adhesion leads to virological failure and resistance to anti-HIV drugs. (Etta *et al*., 2017, Endalamaw *et al*., 2018).
Although, even the national HIV program in Burundi provides basic membership counseling, outreach activities should be planned to improve adherence interventions for ARV patients and minimize follow-up losses. Moreover, the high rate of VF in our study could be attributed to infrequent VL measurement due shortage stock, thus resulting to delay testing and suboptimal monitoring. At the time of this study, VL was possible only at the National Reference Laboratory and Ngozi regional hospital. More facilities to test VL should be implemented to scale-up access to VL monitoring in Burundi. There was show also association between virological failures with the presence of other partners. This should be the evidence from HIV patients on ARV who have sex with those who have already the acquired or transmitted drug resistance. Also, multiple infections with different strains of the virus and other sexually transmitted infections can lead to ART drug resistance hence virological failure (Dessie., 2011) .This could potentially be due to transmission of resistance virus from other partners ,an assertion that calls for further investigation of the rate of transmitted resistance.

5.1.2 Circulating HIV-1 subtype

Based on the results, high diversity of HIV-1 subtypes circulating in kayanza province, north of Burundi, was observed. It has been shown previously that HIV-1 diversity is high in Burundi, with a dominance of subtype C (Vidal *et al*., 2007) and

have been reported with an important fraction of HIV infection in east Africa(Delatorre & Bello., 2012; Lihana *et al*.,2012). HIV-1 subtype C is the most prevalent worldwide and is the major HIV-1 subtype non subtype B in some countries of southern and eastern Africa, Asia and the Middle East, where most infections occur (Lihana *et al*., 2012). Also sexual transmission of this subtype is also suggested to be more than that of subtype A (Abecasis *et al*., 2013). Although, our finding is particularly not similar to those found in Rwanda , one of borders country (Rusine *et al*., 2013). This should mention the absence of a cross transmission from these two countries. We suggest that a study should be done to reveal the origin of that predominant of subtype C in Burundi, which is not predominant in border countries (Rusine *et al*., 2013).

From this study, subtype C was observed to have a high number of drug associated mutations. The M184V was the highest mutation (33%) in NRTI and is known to confer very high resistance against NRTI. For NRTI, Subtype C viruses develop resistance against non-nucleoside reverse-transcriptase inhibitors of K10N at 77% and this are known to be common in cases of treatment failure (Rusine *et al.,* 2013) . Also, Grossman *et al*., 2004 in their study showed that Subtype C viruses develop resistance against nonnucleoside reverse-transcriptase inhibitors through the K103N mutations. Due to the small number of sample size, it is difficult to determine effective treatment of the different subtypes.

5.1.3 Nucleoside Reverse and Non-Nucleoside Reverse Transcriptase Inhibitors (NRTIs and NNRTIs) and Drug Resistance associated mutations

It has been found that drug resistance mutations were found in had at least one NRTI or NNRTI based on the results. One reason might have been the low sub-optimal adherence. Our results indicate that reliable measurements of drug adherence are needed. This results should not be unexpected as the drug resistance mutations was done from patients with virological failure .Also, the drug resistance mutation between 79 % to 85 % have been reported from many authors in sub-Saharan Africa like in Rwanda, Kenya and Tanzania (Rusine *et al.,* 2013.; Hawkins *et al*., 2016). Although 20 % (3/15) of our findings did not carry any drug resistance mutations. Based on drug class, the most common DRM were against NNRTI (81.25%) while

DRM against NRTI were 62.5 %. Also, the most common mutations in this study were M184V, K65R in NRTI; K103N and Y188H in NNRTI. In this study, the majority of the patients had developed two class drug resistance to both NNRTI and NRTI, which greatly limits optimal treatment choices in this population.

These was common as long as these mutations are known to be common in cases of treatment failure and have been reported elsewhere (Johnson *et al*., 2013). They are associated with the use of 3TC, EFV and NVP, which have low genetic barriers towards resistance. The M184V is known to cause resistance to 3TC and FTC, enhances the susceptibility to AZT, and delays the emergence of mutations associated with AZT and d4T (Johnson *et al*., 2013). The relatively high overall prevalence in this study may have consequences for second line treatment responses in HIV patients attending Kayanza district hospital. The presence of K103N and Y188H which are more likely present in subtypes C (Delatorre & Bello, 2012) was mentioned in this study. The K103N is a non-polymorphic mutation that causes high-level reductions in NVP and EFV susceptibility (Wang *et al*., 2013).

For NRTIs, the HIV drug resistance mutations found were against FTC, 3TC, and TDF while for the NNRTI the mutations found were against EFV and NVP). This should be explained by the use of regimen based in 2NRTI+ 1NNRTI in the first line therapy. The utility of these agents as part of second- or third-line regimens is limited and then this should have an impact on second-line nucleoside analog therapy options with consequences of limit future treatment options. Also, we found HIV drug resistance mutations against D4T which have not even been introduced in Burundi guideline. Further resistance testing on this drug regimen should be conducted before its introduction as a first line regimen in Burundi. Few samples (2/15) were patients under 18 years, from which did not harbour any mutation both in NRTIs and NNRTIs.

Although, due to the relatively small number of children enrolled in our study, these findings should not rule out presence of transmitted or acquired drug resistance mutations in HIV positive children at Kayanza district hospital. A specific study with high number of children should be conducted to confirm our findings. Although our findings may not be generalized to all HIV clinics in Burundi but should indicate that

some strategies to minimize HIVDR are needed .These should include improved availability and utilization of VL-based monitoring of ART response, and evaluation of the potential added value of HIV genotyping at treatment failure .In addition, high quality patient support for treatment adherence will contribute to protecting the efficacy of second line and subsequent therapy and improving overall treatment outcome.

Limitations of the study

The samples analyzed in this study were collected and stored in Burundi before shipped to KEMRI. In the 26 samples with suspected virological failure, 15 samples succeeded to be sequenced. This may be possibly the degradation of RNA virus during transport. Also, we identified drug resistance mutations by comparing the samples sequences and those from Stanford database, this implies that new mutations which are not in database and which are unique in the Burundi HIV sequences would not be identified. Since it is single ART center study, these results may not also be generalized to all Burundian hospitals, or in all HIV patients on ART, further studies are needed to be done in multiple ART centers to conform these results.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The prevalence of virological failure was 13 % and was associated with presence of other partners and irregularity in taking medications.
ii. This study has shown that HIV-1 subtypes in HIV positive patients failing treatment at Kayanza district hospital are diverse with HIV-1 subtype C 80 % (12/15); HIV-1 subtype A 7 % (1/15) and HIV-1 subtype A1 13 % (2/15).
iii. Majority of those on treatment, 80 % of those successfully sequenced (12/15) had at least one mutation (NRTI or NNRTI), while 20 % (3/15) did not carry any drug resistance mutations. The most common drug resistance mutations were against NNRTI 80 % (12/15) while DRM against NRTI were 47 % (7/15). These findings showed possible gaps in the last 90-90-90 WHO target by 2020.

6.2 Recommendations

In the light of the above results from this study, the following recommendations were formulated:

i. Viral load testing is important to monitor the treatment failure hence to evaluate the extensive development of multidrug resistance which can render second- line unsuccessful and ineffective.
ii. Regardless of cost, resistance testing should be used as a guideline of selecting the best regimen in HIV patient to avoid drug resistance mutations in Burundi.
iii. A longitudinal study with high number of sample size should be done to characterize HIV-1 subtypes circulating in HIV-1 positive patient in Burundi and to determine effective treatment of the different subtypes.

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APPENDICES

Appendix I: Informed consent documents in English

TITLE: MOLECULAR CHARACTERISATION OF HIV-1 AND DETERMINATION OF DRUG RESISTANCE MUTATIONS AMONG ARV-TREATED PATIENTS ATTENDING KAYANZA DISTRICT HOSPITAL, BURUNDI.

INVESTIGATORS

1. Principal Investigator: Pascal BUTOYI, MSc (Med Virology) ITROMID, KEMRI
2. Research Supervisors : Dr Lihana Raphael Wekesa , PhD-KEMRI
   Dr Eddy Odari, PhD- JKUAT

My name is …… I wish to introduce you to this study undertaken by Pascal BUTOYI, a Masters student at Jomo Kenyatta University of Agriculture and Technology.

Institute of Tropical Medicine and Infectious Diseases- KEMRI. I would be very grateful if you would volunteer to participate in this study.

Introduction

We are requesting your consent to participate in a research study titled: "**Molecular characterization of HIV-1 and determination of Drug Resistance Mutations among ARV-treated patients attending Kayanza district Hospital, Burundi**."

Some HIVDR will inevitably occur among patients taking ART even when optimal adherence to therapy is supported. The study will be carried out HIV-1 patient, aged from 1 year and over, under ART from twelve months attending Kayanza district Hospital .So we will need consent from you to be included into the study. Being in this study is voluntary and you/ or and your child may decide to withdraw from the study at any time, without suffering any penalty or losing any benefits available for

him/her through this study. This form tells you about the study, read it carefully and feel free to ask any questions you have at any time.

If you agree, we need your informed consent to participate in our research study.

Aims of the study: This study aims to determine the prevalence of virological failure, drug resistance mutations and HIV-1 genotypes in HIV patients attending Kayanza district Hospital. Then we will be able to come up with solutions that can help save you or your child's live and improve health.

Benefits of the study: You will not have any further direct benefits. However, the results of this study will assist the Doctors and nurses to improve your treatment and management of HIV/AIDS.

Risks and discomforts being in the study: Only 4 ml of whole blood will be drown with small harm from this. The relevant medical and social data will be collected from your medical records and more information should be collected during enrolment.

Permission to access your Medical records: We are requesting your consent to access and use your medical records to obtain the following information: age, gender, when they joined the health facility, when you start ART drugs, weight, ART regimen and CD4+ T-cell count.

Privacy of your information: We will protect your privacy by keeping this signed consent form and your medical information under lock and key. Moreover, after you have signed the consent form, we shall assign you a serial number to identify you. We will NOT use your name during this study or in the publication of our results. Also, any information entered into our computer databases will be password-protected, and only the principal investigator of the study will have that password.

Your right to refuse participating or withdraw in the study: You do NOT have to take part in this research study if you do not wish to do so. Also, this will NOT affect your participation in this ART service care.

Procedures: Written consent will be sought after giving a verbal explanation about the study and will be filling a simple questionnaire on demographic characteristics.

Thus Approximately 4 milliliters of whole blood specimen will be obtained and put in an EDTA bottle to be used for analysis. Furthermore, once the analysis is done a copy of patients' results will be given to Doctor or your service care for improving management of treatment.

What are the costs: You do not pay anything to participate in this study.

If you agree to give consent to participate in this research study, and for the results to be published, please read and sign the following consent form.

In case of any questions regarding this research study, please contact the principal investigator Pascal BUTOYI, by calling (+254) 0703-409935/ (+257) 7992614.

You may also contact the chairman of the BURUNDI Ethical Review Commission using the following address and telephone number:

Chairman: Doctor John Baptiste SINDAYIRWANYA

BURUNDI/ Ethical Review Committee (BERC)/Telephone: +257 921173/+257 22226961.

Signed, informed Consent Form for adults

I……., do hereby agree to participate in the proposed research study led by Pascal Butoyi. I have been given all the necessary information to understand the purpose and nature of this research study. I have also been assured that I can withdraw from the study at any time, without penalty or loss of benefits that I enjoy as a participant at Kayanza district Hospital. The research proposal has been explained to me in the language I understand.

Name of patient …… patient's signature…….

Date……. Place……..……

Name of principal investigator……..

Principal investigator's signature……..

Date……. Place……..……

Signed, informed Assent Form for minors

I……., do hereby agree to participate in the proposed research study led by Pascal BUTOYI ……... I have been given all the necessary information to understand the purpose and nature of this research study. I have also been assured that I can withdraw from the study at any time, without penalty or loss of benefits that I enjoy as a participant at Kayanza district Hospital. The research proposal has been explained to me in the language I understand.

Name of minor ……Minor's signature…….

Date…….

Place……..……

Name of principal investigator……..

Principal investigator's signature……..

Date…….

Place……..……

Signed parental /guardian informed permission for minors

I……, do hereby give permission to Pascal BUTOYI for my child to participate in the proposed research study. I have been given all the necessary information to understand the purpose and nature of this research study. I have also been assured that I can withdraw my child from the study at any time, without penalty or loss of benefits that my child enjoys as a participant at Kayanza district Hospital. The research proposal has been explained to me in the language I understand.

Name of parent / guardian……..

Parent's / guardian's signature……..

Date…….

Place……..……

Name of principal investigator……..

Principal investigator's signature……..

Date…….

Place……..……

Signed parental/Guardian informed consent Form for children

I…… do hereby give consent to …… for my child to participate in the proposed research study. I have been given all the necessary information to understand the purpose and nature of this research study. I have also been assured that I can withdraw my child from the study at any time, without penalty or loss of benefits that my child enjoys as a participant at Kayanza district Hospital. The research proposal has been explained to me in the language I understand.

Name of parent / guardian……

Parent's / guardian's signature…….

Date……. Place…….

Name of principal investigator……

Principal investigator's signature……

Date……. Place……..…….

Appendix 2: Informed consent documents in English

UGUSABA ATAGAHATO GUKORANA MU BUSHAKASHATSI

UMUTWE W'ICIRWA: "Amoko y'umugera wa SIDA n'Imyitwarire y'imiti iwuvura mu badwayi bagendana uwo mugera kandi bari k'Umuti bitura ibitaro vy'akarere ka KAYANZA mu Burundi muri."

Umushakashatsi mukuru: Pascal BUTOYI umunyeshuri muri kaminuza y'uburimyi n'ubuhinga yitiriwe Jomo Kenyatta mu gihugu ca KEKYA, Telefone: +254 703409935/+257 79926114

Abagenduzi babahinga: Muganga Lihana Raphael/KEMRI

Muganga Eddy Odari/JKUAT.

Intangamarara: Nshaka kugusaba gukorana atagahato m'ubushakashatsi ku ndwara ya SIDA mubagendana iyi ndwara bitura ibitaro vy'akarere ka KAYANZA. Ubu bushakashatsi buzofasha kumenya amoko y'umugera wa SIDA n'Imyitwarire y'imiti iwuvura mu badwayi bagendana uwo mugera kandi bari k'umuti, kugirango mushobore kuronka imiti nyayo umubiri wakira neza.Ici cirwa kizokorerwa kuba dwayi bafise kuva k'umwaka umwe niyirenga, bari kumuti mukiringo kirenga amezi cumi n'abiri; bitura ibitaro vy'akarere ka Kayanza.

Rero dukeneye uruhusha rwawe kugirango dushobore gukorana iki cigwa.Kwitabira muri iki cigwa ni kubushake kandi ushobora kukivamwo umwanya wose ushakiye, nta gahato kandi ntanakimwe uzotakaza muvyo bahora bagufasha ngaha.Uru rupapuro rurabasobanurira ibijanye n'ubu bushakashatsi, rusome neza witonze, kandi ntutinye kubaza aho udasobanukiwe igihe cose ushakiye.

Intumbero y'ubushakashatsi: Kumenya Amoko y'umugera wa SIDA n'Imyitwarire y'imiti iwuvura mu badwayi bagendana uwo mugera kandi bari k'Umuti bitura ibitaro vy'akarere ka KAYANZA**.** Ibizovamwo bizofasha ababakurikirana kumenya neza umuti bobaha.

Inyungu y'ubu bushakashatsi: umurwayi ubwiwe nta nyungu iboneka ubwo nyene aca aharonkera, ariko inyishu zizotuma abaganga n'abaforma babakurikirana neza. Kwitaba iki cirwa bizofasha kandi kuvura no gukurikirana neza abadwayi ba SIDA ngaha kuri ibi bitaro no mukarere kavyo.Ibitangenda neza vyose bijanye nukudakora neza kw'imiti bizoboneka.

Ingaruka mbi z'ubu bushakashatsi: Ubu bushakashatsi ntangaruka mbi bufise, ariko urashobora kubabara buhorobuhoro bivanye n'urushinge dukoresha mu gufata amaraso kandi ubwo bubabare bumara akanya gatoyi cane. Vya nkenerwa bimwe bijanye niki cirwa tuzobiraba kuma fishi yanyu canke tubibabaze turiko turakorana.

Uruhusha rwo gukoresha ibiri kw'Ifishi yawe: Nagira ngusabe atagahato gushobora gukoresha ibiri kw'ifishi yawe ndaba ibi bikurikira: Imyaka, igitsina, ibiro, aho watanguriye kwitura ibitaro, umuti uriko, aho watanguriye gufata imiti, urugero rw'abasoda b'umubiri nibindi.

Gukingira ibijanye nibibaraba: Tuzobika neza uruhusha wadusinyiye hamwe nivyo twakuye ku fishi yawe.Kandi uhejeje kudusinyira tuzoguha inimero isubirira izina ryawe. Ibibdi bozobikwa mu nyabwonko, iyo nayo tuzoyugarana, ntawundi muntu numwe azoshobora kuyugurura Atari jewe nyene icirwa hamwe**.**

Uburenganzira bwo kwemera no guhaka kwitabira icirwa: Witabira iki cirwa aruko ubishatse kandi ntangaruka mbi zihari kubijanye nivyo bahora bagukorera

ngaha.Wemeye kwitabira icirwa, ushobora kukivamwo igihe cose ushakiye atankurikizi.

Ukwo bigenda: Uruhusha atagahato rushinye turusaba duheje gusigura ibijanye niki cirwa tugaca twuzuza urupapuro r'wibibazo bijanye n'imibereho.Dukoresheje agacupa kabigenewe, turafata amaraso makeya (4ml) kukuboko, tuzoyakoreshe mugukora ivyo twabasiguriye hejuru.Hanyuma inyishu zibonetse, inyishu yumw'umwe tuzoyiha muganga kugirango ayikorereko mukubavura no kubakurikirana.

N'ikihe kiguzi co kwitabira iki cirwa?

Ntakintu nakimwe uriha mukwitabira iki cirwa.

Wemeye atagahato kwitabira iki cirwa, ukanatwemerera ko inyishu zizovamwo tuzozishira mubinyamakuru kugirango zikorerweko mukwitaho abadwayi, soma uce udusinyira urwo rupapuro rukurikira rwo gusaba kwitabira atagahato.

Mugihe ugize ikibazo kirabana n'iki cirwa, gerageza urondere umushakashatsi mukuru, Pascal BUTOYI, mukumuterefona kuri terefone: +254 0703-409935/+257 7992614.

Urashobora no kurondera umukuru w'igisata c'Uburundi kijwejwe Gusubiramwo kuraba kw'icirwa gikurikije amategeko kubijanye nabo kiraba bose: **Umukuru w'igisata:** Muganga Yohani Baptista SINDAYIRWANYA,

Telephone: +257 921173/+257 22226961.

UBWEMEZI ATAGAHATO BUSINYWE N'UMUVYEYI/UWUHAGARARIYE UMWANA, BWO KWITABIRA ICIRWA KUVA K'UMWAKA GUSHIKA KUMYAKA CUMI N'IBIRI (1-12)

Jewe…..**.....** (Amazina y'umuvyeyi/uwuhagarariye umwana), nk'umuvyeyi/uwuhagarariye umwana …… (Amazina) nemeye atagahato ko yokwinjira muri iki cirwa ca Pascal BUTOYI

Umukono (igikumu) w'umuvyeyi/uwuhagarariye ……..

Igenekerezo n'ikibanza ……

Umukono w'umushakashatsi …….

Igenekerezo n'ikibanza ……**.....**

UBWEMEZI ATAGAHATO BUSINYWE N'UMUDWAYI, BWO KWITABIRA

Jewe……, nemeye kwitabira iki cirwa gikorwa na Pascal BUTOYI

Nasiguriwe bikwiye kubijanye nuko kizokorwa.Natahujwe kandi ko nshobora kukivamwo igihe cose nshaka kandi ibijanye niki cirwa nabisiguriwe mururimi numva.

Umukono (igikumwe) c'uwemeye kwitabira icigwa …...……..

Igenekerezo n'ikibanza ……

Umukono w' umushakashatsi ……

Igenekerezo n'ikibanza …….....

UBWEMEZI ATAGAHATO BUSINYWE N'UMWANA W'IMYAKA 13-17, BWO KWITABIRA ICIRWA ATAGAHATO

Jewe……, nemeye kwitabira iki cirwa gikorwa na Pascal BUTOYI

Nasiguriwe bikwiye kubijanye nuko kizokorwa.Natahujwe kandi ko nshobora kukivamwo igihe cose nshaka kandi ibijanye niki cirwa nabisiguriwe mururimi numva

Umukono (igikumwe) c'umwana yemeye kwitabira icigwa …...……..

Igenekerezo n'ikibanza ……

Umukono w' umushakashatsi ……

Igenekerezo n'ikibanza …….....

UBWEMEZI BW'URUHUSHA ATAGAHATO BUSINYWE N'UMUVYEYI/UWUHAGARARIYE UMWANA, BWO KWITABIRA ICIRWA KUVA KUMYAKA 13-17

Jewe…..... (Amazina y'umuvyeyi/uwuhagarariye umwana), nk'umuvyeyi/uwuhagarariye umwana …… (Amazina) ndamuhaye uruhusha atagahato ko yokwinjira muri iki cirwa ca Pascal BUTOYI

Umukono (igikumu) w'umuvyeyi/uwuhagarariye ……..

Igenekerezo n'ikibanza ……

Umukono w'umushakashatsi …….

Igenekerezo n'ikibanza …….....

Appendix III: Research questionnaire

TITLE: MOLECULAR CHARACTERISATION OF HIV-1 AND DETERMINATION OF DRUG RESISTANCE MUTATIONS AMONG ARV-TREATED PATIENTS ATTENDING KAYANZA DISTRICT HOSPITAL, BURUNDI.

INVESTIGATORS

1. Principal Investigator: Pascal BUTOYI, MSc (Med Virology) ITROMID, KEMRI
2. Research Supervisors : Dr Lihana Raphael Wekesa , PhD-KEMRI
   Dr Eddy Odari, PhD- JKUAT

KAYANZA District Hospital

Questionnaire number: ……

Date of specimen collection ……..…

Age: ……

Gender: Male ☐ Female ☐

Marital status: Single ☐ Married ☐ Divorced ☐ Widowed ☐

Educational attainment:

Primary school ☐ Secondary school ☐ University ☐

Working status:

Farmer ☐ Government employer ☐ Private employer ☐

On TB treatment Yes ☐ No ☐

Taking Alcohol: Yes ☐ No ☐

Any irregularity of medication at least one month: Yes ☐ No ☐

Multiple sexual partners: Yes ☐ No ☐

Date of Starting treatment: ……...

CD4 T cell count (Initial) ……

CD4 T cell count (on study) ……

Viral load: Not detected ☐ < 1000 copies/ml ☐ >1000copies/ml ☐ Failure ☐

Appendix IV: Sequence Names and GenBank ID

Sequence name	GenBank sequence ID
BU01_BUR	MK580981
BU02_BUR	MK580982
BU03_BUR	MK580983
PA2_BUR	MK580984
PA3_BUR	MK580986
PA4_BUR	MK580985
PA6_BUR	MK580987
PA7_BUR	MK580988
PA8_BUR	MK580989
PA9_BUR	MK580990
PA10_BUR	MK580991
PA11_BUR	MK580992
PA12_BUR	MK580993
PA13_BUR	MK580994
PA15_BUR	MK580995

Appendix V: Burundi National Ethical Review Committee Letter

BURUNDI NATIONAL ETHICAL COMMITTEE FOR THE PROTECTION OF HUMAN BEINGS PARTICIPATING IN BIOMEDICAL AND BEHAVIORAL RESEARCH

Bujumbura, 14th October 2017

FROM: BURUNDI NATIONAL ETHICAL COMMITTEE

TO: **Pascal BUTOYI** (Principal Investigator)

RE: Decision of the Burundi National Ethical Committee

Dear Sir,

The Burundi National Ethical Committee analysed your research project

«Molecular characterisation of HIV-1 and determination of drug resistance mutations among antiretroviral treated patients attending Kayanza District Hospital in Burundi».

After analysis of the Ethical aspects of the project in accordance with the International Regulations in the field, the Burundi National Ethical Committee has approved the project. The approval is valid for a period of one year from 14th October 2017 to 13th October 2018.

However, note that any changes to the protocol must be reported to the Burundi National Ethical Committee prior to implementation. This includes changes to research design or procedures that could introduce new or more than minimum risk to human subjects.

You may embark on your study.

Sincerely

Comité National d'Ethique

Prof. Jean Baptiste SINDAYIRWANYA

THE BURUNDI NATIONAL ETHICAL COMMITTEE PRESIDENT

Appendix VI: Abstract of the published Letter

Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) DOI: 10.7176/JBAH
Vol.9, No.10, 2019

Molecular Characterization of HIV-1 And Drug Resistance Among HIV-1 Infected Patients Attending Kayanza District Hospital, Burundi

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This study was funded by the East Africa Public health Laboratory Network Project (EAPHLNP)/Burundi.

Abstract
Virological failure in management of HIV-1 infection has been reported to be between 11 to 24% after 12 months of treatment. Out of these, acquired or transmitted drug resistance mutations have been reported at 71% to 90% in Sub-Sahara Africa. In this cross-sectional study we aimed to determine virological failure and drug resistance mutations in HIV-1 infected patients on ART attending Kayanza district hospital, Burundi. Patients were recruited using a purposive sampling technique. After informed consent, 4mL of venous blood was collected from each patient. The blood was separated into plasma and cells for various laboratory assays. Plasma viral loads were quantified using the Abbott m2000rt system. Polymerase chain reaction using gene specific primers was done after extraction of nucleic acids from plasma with >1000 copies/ mL, followed by sequencing of all amplified samples. Drug resistance was determined using the IAS and Stanford University database, with phylogenetic analyses done using the neighbor joining method.Two hundred patients were recruited; 13% of the respondents had virological failure associated with multiple sex partners (adjusted odds ratio (aOR, 0.154 , p =0.016) and irregularity in taking medications (aOR: 0.4 , p=0.014). Fifteen samples were successfully sequenced; 80% (12/15) were HIV-1 subtype C, 7% (1/15) subtype A, and 13% (2/15) were HIV-1 subtype A1. Of these, 87.5% had at least one mutation (NRTI or NNRTI), while 12.5 % did not carry any Drug Resistance Mutations. The most common drug resistance mutations were M183V, T215V M41L, E44D, L74I, L210W and K65R, K103N, Y188H. The prevalence of virological failure was established at 13%.Our findings showed possible gaps in the last 90% of the 90-90-90 WHO target by 2020. The results highlight the need for intense viral load and resistance testing for patients to improve overall treatment outcome. Some strategies are needed to improve adherence counselling and drug resistance mutation testing should be implemented to monitor HIV-1 patients on ART in Burundi.

Keywords: HIV-1, antiretroviral therapy, Virological failure, DRMs, Burundi.
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