GENETIC CHARACTERIZATION OF ANTIRETROVIRAL RESISTANCE AND THEIR EFFECT ON HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS INITIATING THERAPY IN KISUMU -KENYA

BY

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university or institution of learning. I have done all the work carried herein and all sources of information have been duly acknowledged by means of references.

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DEDICATION

To my family and the ones I love.

ABSTRACT

Experiences of early antiretroviral treatment failures are increasingly linked to escalating pretherapy HIV resistance mutations. Regular update of epidemic status and impact of pretherapy resistance are needed in resource limited countries, including Kenya. Since pretherapy resistance survey has not been conducted and factors associated remain unknown in the high-HIV prevalence western Kenya region, this study aimed to establish the prevalence of pretherapy antiretroviral resistance, the associated factors, the mutations' impact on treatment, the HIV subtypes and phylogenetic relationships in the major urban establishment of Kisumu. Two hundred and forty HIV-1 infected persons were consecutively recruited and followed-up for 12 months at 2 facilities between 2013 through to 2015. Blood samples along with demographic information were obtained at both study baseline and follow-up end-point. Genetic sequence analysis of partial *pol* gene was performed on all baseline, and the end-point samples that had viral RNA≥1000 copies/mL. Calibrated Population Resistance tool and Drug Resistance Database algorithms for HIV were used in the interpretation of baseline and end-point resistance mutations respectively. Subtyping was performed using REGA v3.0, RIP v3.0 and in addition phylogenetic relationship analysis was performed using MEGA 6.0. Prevalence were calculated in percentages, categorical variables compared by x^2 and fisher's exact test while continuous variables were compared by Mann-Whitney U test. Factors that had p<0.2 by univariate analysis were fitted into regression models to determine associations and impacts of resistance. A moderate prevalence of 8.8% pretherapy ARV resistance was established, that seemed more likely among younger patients although age was not significant. HIV-1 subtype A1 was found to be dominant (64%), and 71% of the Kisumu's subtype C tended to cluster more closely with the southern Africa viral infection. Although pretherapy resistance increased the likelihood of treatment failure (p=0.029), the NNRTI mutations only did not imply virologic failure at least in the short-term for 47% of the patients. The presence of multiclass HIV drug mutations was associated with heightened virologic failure (OR=4.3; p=0.025) amongst the patients who had pretherapy resistance. Receipt of ARV regiment with GSS<2.00 was likely to result in treatment failure (hazard ratio=3.5). A modereate prethrapy resistance found in Kisumu is adversely impacting on treatments. The moderate pretherapy resistance necessitates regular antiretroviral resistance surveillance. More intense monitoring of HIV-infected patients initiating first-line treatment in Kisumu is necessary to identify the increased patients at risk of early virologic failure. Conclusive analyses will require large survey studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
CD4	Cluster of differentiation 4
CDC	Centres for Disease Control and Prevention
CI	Confidence Interval (normally =95%)
DR	Drug resistance
FACES	Family AIDS Care and Education Services, Kenya
GSS	Genetic Sensitivity Score
HIV-1	Human Immunodeficiency Virus type-1
HIV DR	HIV-1 Drug Resistance
KAIS	Kenya AIDS Indicator Survey
KEMRI	Kenya Medical Research Institute
NASCOP	National AIDS/STIs Control Program, Kenya
NACC	National AIDS Control Council
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
OR	Odds Ratio
PIs	Protease Inhibitors
RNA	Ribonucleic acid
TDR	Transmitted Drug Resistance
UNAIDS	Joint United Nations Program on HIV/AIDS
WHO	World Health Organisation

DEFINITIONS OF OPERATIONAL TERMS

Pretherapy drug resistance(pretherapy DR) was the detection of any one of the mutations listed in the WHO 2015 surveillance mutations list; this list contained only resistance mutations associated with protease inhibitors (PI) and (non-) nucleoside reverse transcriptase inhibitors [(N)NRTI] adjusted for non-B subtype polymorphisms according to Stanford University's HIV Calibrated Population Resistance (CPR) online tool (http://cpr.stanford.edu/cpr.cgi).

Acquired PI/(N)NRTI resistance mutations were defined as the detection of any one of the mutations listed in the Stanford University HIV Drug Resistance Database updated in 2015.(Available online at http://sierra2.stanford.edu/sierra/servlet/JSierra?action=sequenceInput)

Virologic suppression/success was defined as HIV-1 viral load <1000 RNA copies/mL.

Virologic failure was defined as HIV-1 viral load ≥1000 RNA copies/mL.

Baseline was defined as the enrolment point into the study at which patient information and demographic information were obtained.

End-point was defined as the 12-month follow-up outcome or otherwise; treatment switch to second-line/death/loss-to-follow-up or transfer-out to another healthcare facility

Injecting drug users introduce drug into the bloodstream via a hollow hypodermic needle and a syringe, which is pierced through the skin into the body, a practice more often abused and is thus associated with numerous health and social harms

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CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Human Immunodeficiency Virus (HIV) type-1 disease prognosis has greatly improved since approval of the first antiretroviral drug in 1987 (Kiertiburanakul *et al*., 2013). Notably, morbidities and mortalities related to the HIV type-1 (HIV-1) disease have dramatically reduced (Novak *et al.*, 2005). These great developments are a result of evolving knowledge and expanding options of antiretroviral therapy (ART) that have yielded potent, tolerable, and simpler treatment regimens (Thompson *et al*., 2012). ART has transformed HIV-1 infection, once considered invariably fatal within a median survival period of 12 months after diagnosis, into a manageable chronic disease whose youthful patients on antiretroviral (ARV) treatment may survive much beyond 30 years (Enriquez *et al*., 2011). Evidence from studies have also shown that using ART in suppressing viral replication reduces the chances of HIV-1 transmission, thereby protecting uninfected persons from contracting the disease (Cohen *et al*., 2013). These life-saving attributes of antiretroviral drugs only became substantial beyond 2% of the infected population who could afford it then, after a 2003 World Health Organization (WHO) - championed commitment (UNAIDS 2014; Harries *et al*, 2010). These efforts to improve ART access have been sustainably premised on WHO public health strategy that recommended affordable standardised first-line antiretroviral drug combinations (Bennett *et al*., 2008). By 2013, UNAIDS report indicated that Acquired Immune Deficiency Syndrome (AIDS) related deaths had decreased by 35% (UNAIDS 2014). The major urban setting in the high-HIV prevalence region of Nyanza –Kenya, where the uptake of this WHO ART strategy is well received and established, is Kisumu (NACC 2015) whose epidemic needs to be understood.

This strategy that made treatment available for most HIV-1 infected patients in developing countries is however increasingly under threat due to transmission of acquired antiretroviral resistance to persons who are newly infected, and yet to initiate therapy (Kiertiburanakul *et al*., 2013). Although a relevant current data on resistance to ARV prior to treatment initiation for Kisumu is not available, the phenomenon of pretherapy HIV-1 drug resistance (HIV DR) has been escalating in countries that have scaled-up ART, resulting in increased rates of early ARV treatment failure (Gupta *et al*., 2012; Kiertiburanakul *et al*., 2013). Several resource-

limited countries have reported increased pretherapy HIV-1 drug resistance associated mutations (Hamers *et al*., 2012; Hamers *et al*., 2011; WHO 2012). The pretherapy antiretroviral resistance data on Kenya is limited and cannot project latest trends. Surveys conducted before 2006 in Nairobi, Mombasa, North Rift, Western province and Nyanza province (Hassan *et al*., 2013; Kiptoo *et al*., 2013; Kontor *et al*., 2014; Zeh *et al*., 2016), most were done then, do not reflect the situation after the decade-old scaled-up national treatment program (Hamers *et al*., 2011). The recent findings reported for rural Siaya near Kisumu (Zeh *et al*., 2016), within the high-HIV burden western Kenya, for a cross-sectional study done in 2005 and earlier, would not be informative of the magnitude and pattern of ARV resistance in the urban establishment of Kisumu.

HIV exhibits extensive and dynamic genetic diversity that characterises it into distinct molecular subtypes and recombinant forms. This diversity affects the viral transmissibility, pathogenesis, and diagnosis and has profound implication for vaccine development (Buonaguro *et al*., 2007). Previous studies in Kenya have reported complex variability in subtype and recombinants distribution, with subtype A1 being dominant, along with subtype C, D and their recombinant forms with A1 constituting majority of the infecting virus (Adungo *et al*., 2014; Hassan *et al*., 2013, Kageha *et al*., 2012, Khoja *et al*., 2008, Khamadi *et al*., 2005). The distribution frequency of HIV-1 subtype D in Kenya has been characterized to be more than that of subtype C except in the northern part of the country where it is responsible for 39% of the viral infection (Kamadi *et al*., 2005). The recombinant HIV forms have been found to consist of more substantial proportion of the transmitted virus (56.9%)in Kericho–Kenya, than those reported for patient samples in other parts of the country (Hassan *et al*., 2013). These evidences showing molecular complexity in the HIV-1 epidemic in Kenya compel a need to understand the viral characteristics responsible for AIDS, especially in the high-HIV prevalence regions like Kisumu that has not been reported for the general population previously. The variance in subtype distributions also point to peculiar phylogenetic transmission relationships that has not been well characterized for HIV infections in Kisumu.

The factors associated with likelihood of harbouring HIV resistance mutations at pretherapy vary across regions, and depend on contextual social dynamics. Even with differences in methodologies of previous studies, some of the factors identified to be associated with increased pretherapy HIV drug resistance include; transmitted viral subtypes, previous ART

exposure, HIV transmission route, duration of HIV infection and local ART regimens (Novak *et al*., 2005; zuKnyphausen*et al*., 2014). Although a previous study has hinted transmission risk in injecting drug users, the study methodology did not systematically show the factor (Osman *et al*., 2013). The role of sex and residence has been highlighted in developed country cities (Frentz *et al*., 2014). With the patterns of changing HIV transmission risk, most studies on pretherapy resistance conducted in Kenya are outdated and in addition, the few resistance cases identified were from small sample sizes that could not meaningfully support analyses of associations in pretherapy resistance. Limited effort has been made to investigate the dynamics associated with increasing pretherapy HIV DR in Kenya, thus these factors continue to compromise HIV treatment unabated in Kisumu.

Data on impact of pretherapy resistance mutations on treatment outcomes are scarce; some studies have yielded inconsistent results, partly because of the different thresholds for effect of pretherapy resistance mutations, level of resistance or absolute numbers of drug-resistant mutations (zuKnyphausen*et al*., 2014; Lai *et al*., 2012). Nonetheless, studies have reported significantly higher rates of virologic failure in subjects with pretherapy HIV DR if treatment regimen comprises at least one drug showing reduced efficacy (Wittkop *et al*., 2011; Bansi *et al*., 2010). The studies reported that the presence of NNRTI resistance mutations was associated with a 1.5-fold increased risk for treatment failure in the first 48 weeks after ART initiation (Li *et al*., 2011). By contrast, some studies have also demonstrated desirable treatment response at least in the short-term (zuKnyphausen *et al*., 2014; Lai *et al*., 2012; Lee *et al.*, 2014). These conflicting reports are largely from patient tailored treatments of developed countries, the outcomes from developing countries are not substantive yet. The impact of pretherapy HIV DR for the pattern of HIV resistance mutations observed in Kenya has not been reported and therefore remains to be established.

With an estimated over 1.6 million country HIV-1 infection burden (Billings *et al*., 2015), the present study is aimed to update the prevalence and pattern of pretherapy HIV-1 drug resistance in Kisumu for the period between 2012 and 2013. In addition to investigating the factors associated, this study also investigated the impact of resistance mutations characterized and the HIV subtype phylogenetic relationships.

1.2 Statement of the Research Problem

Kisumu is a major town in Kenya's HIV-1 high-prevalence region where wide ARV use has been accepted since 2003, and pretherapy HIV resistance has never been surveyed despite the escalating rates of treatment failures linked to resistance risks prior to treatment initiation. Because of absence of this critical background, the factors associated with pretherapy HIV resistance have not been established. Although an analysis of the local viral subtype peculiarities and their phylogenetic relationships has not been performed, such knowledge would lead to understanding of the epidemic in Kisumu and the transmission risks implicated. Conflicting reports and scarcity of substantive data on implications of pretherapy HIV resistance has made prediction of treatment outcomes for the patients in Kisumu difficult, thus the need for a systematic investigation. A genetic analysis of HIV's partial *pol* gene sequences provide molecular basis for ascertaining possible viral resistance to ARVs. The current study therefore aimed to establish pretherapy resistance to antiretroviral, the factors associated with them, implications of the resistance mutations, the viral subtypes in Kisumu and their phylogenetic relationships.

1.3 General Objective

To assess mutations associated with first-line ARV drug resistance at therapy initiation and their implication on virologic outcomes within one year of treatment, in addition to characterising the HIV infection in Kisumu –Kenya.

1.3.1 Specific Objectives

1. To determine the overall prevalence of HIV-1 mutations conferring resistance to protease- and reverse transcriptase-inhibitor drugs among patients initiating first-line therapy.
2. To investigate the key factors associated with the likelihood of pretherapy HIV-1 drug resistance mutations in a patient.
3. To determine the subtypes responsible for HIV infections, and the phylogenetic analysis of geographic relationships in the Kisumu viral epidemic.
4. To investigate the effect of pretherapy antiretroviral resistance mutations on treatment outcomes, within 12 months of initiating first-line therapy.

1.4 Research Questions

1. What is the overall prevalence of HIV-1 mutations conferring resistance to therapy among patients initiating first-line antiretroviral treatment?
2. What are the key factors associated with the likelihood of pretherapy HIV-1 drug resistance mutations in a patient?
3. Which subtypes are responsible for HIV infections, and what can be inferred from the phylogeographic relationships of the viral epidemic in Kisumu?
4. What are the effects of pretherapy antiretroviral resistance mutations on treatment outcomes, within 12 months of initiating first-line therapy?

1.5 Justification of the Study

Majority of virologic failures in Kenya, as in other sub-Saharan countries, are associated with HIV resistance mutations. An increasing proportion of these resistance mutations resulting in virologic failures are being linked to presence of the viral resistance prior to therapy initiation. Following intensification of efforts to improve antiretroviral access for deterrence of new transmissions and for overall long-term survival of patients, the need to provide efficacious treatments is being adversely affected by the currently increasing early treatment failures resulting from pretherapy antiretroviral resistance levels. This cause for concern, is reverting the gains made and also making treatments costly, thus this escalation of resistance needs to be arrested. Although Kisumu is experiencing increased cases of early antiretroviral treatment failures, an updated evaluation of the pretherapy HIV resistance has not been performed for the region. The knowledge of factors associated with the risk of pretherapy resistance has enabled some developing countries to mitigate the rates of escalating pretherapy resistance, however in Kisumu, the informed mitigation plan is not available since the risk factors are not known. Information on the characteristics of HIV subtypes is also not up to date since recombination events and introduction of new viral clades are expected to have shifted the regional disease dynamics following previous decade's last survey conducted on women. Further, the phylogenetic analysis of geographic relationships of the virus transmitted has not been conducted in Kisumu previously, this would help to understand the local epidemic, characterized by sustained high HIV incidence rates over the years. Although pretherapy HIV resistance is known to compromise treatment outcomes, standard ARV treatment is being administered regardless of underlying viral resistance. The impact of

administered therapy on patterns and extend of resistance mutations observed in Kisumu has not been keenly considered. The present study therefore aims to establish the prevalence of pretherapy HIV resistance, the factors associated, characterize the viral subtypes, phylogenetic relationships and investigate implications of the resistance mutation pattern in Kisumu.

1.6 Significance of the study

Assessing the prevalence levels, trends and clinical implications of pretherapy HIV DR is critical in providing the evidence-based support required for optimal performance of ART roll-out programs. Ultimately, this analysis would help clinicians and policy-makers to maximize potency, minimize side effects and cross resistance, preserve future treatment options, and increase overall duration of viral suppression. Phylogenetic analysis of subtypes responsible for the HIV epidemic in the region would help epidemiologists in understanding of the disease spread and characteristics. The assessment of factors associated with resistance before treatment in this study highlights aspects within implementation programs that require continued monitoring and adjustments by program implementers, especially those that lead to preventable emergence and spread of ARV resistance. The subsequent intervention actions on the factors that might be aggravating drug resistance situation averts the high cost that would be encumbered by the government in sustaining the survival of HIV-1-infected persons who accumulate adverse resistance to the available sustainably-accessed low-cost ARV. Monitoring pretherapy HIV DR and their impact would subsequently ameliorate the apparent possibility of accumulating overwhelming transmissions of HIV-1 resistance that could eventually necessitate radical overhaul of the first-line regimen drugs, for replacement treatments that are currently very costly and complicated. Unchecked HIV resistance prevalence trends could compel the treatment programs into a costly patient management practice of mandatory resistance screening on the overwhelming numbers of HIV-1 infected patients being initiated on ARV treatment. Further, this analysis is important because the limited therapeutic choices available to majority of HIV infected patients in Kenya necessitate careful consideration of potential resistance impacts for future optimized treatments.

CHAPTER TWO

LITERATURE REVIEW

2.1 The HIV epidemic

Currently, HIV-1 is the world's leading infectious cause of adult deaths in low- and middle-income countries, accounting for 90% of deaths attributed to infectious diseases (UNAIDS 2013). The HIV disease epidemiology can be linked to combination of factors including poor socioeconomic conditions, lack of access to healthcare, risky cultural practices and gender inequalities (Shao *et al*., 2012).

In sub-Saharan Africa, the region that host's two-thirds of the world's HIV infected persons, the epidemic has been devastating (UNAIDS 2010). Assessment of severity of the epidemic in the region shows most of southern African countries (Zambia, Zimbabwe, Malawi, Mozambique, Namibia, Botswana, Lesotho, South Africa, and Swaziland) having an adult (15–49 years) prevalence of >10%, and countries in the East and Central Africa (Uganda, Kenya, Tanzania, Cameroon, and Rwanda) having less severe epidemics, with adult HIV prevalence ranging between 5% and 10% (Shao *et al*., 2012). According to the latest Kenya AIDS Indicator Survey (KAIS) 2012 statistics, the national HIV prevalence is at 5.6%, after having dropped from 10.5% observed during the peak of disease's onslaught in 1996 (Kimanga *et al*., 2014). Despite this encouraging trend, Kenya's HIV epidemic situation is the fourth-largest (1.6 million infected people) in the world (UNAIDS 2013). Kisumu is the major urban establishment within Nyanza, where highest HIV-1 prevalence rates (15.1%) have been reported in Kenya (Goldblatt *et al*., 2015). The infected persons in this region benefit from a free government-supported ART access based on a simplified standard WHO recommended protocol (Kimanga *et al.*, 2014).

2.2 Treatment of HIV in Kenya

In 2003, Kenya's Ministry of Health collaboration with other agencies culminated in availing free access to ART based on cost-effective WHO- recommended guidelines suggested to be sustainable for the developing countries (Odhiambo *et al.*, 2014). Based on immunologic threshold of CD4 <350 cells/μL, an estimated 28.6% of persons eligible for ART received treatment in 2007 (NACC 2015). The number of adults receiving ART has since increased by

more than 3-fold, about 525,000 persons (representing 61% of estimated persons eligible for ART) out of the 1.6 million people estimated to have been infected in 2013 (Billings *et al*., 2015). Within the context of statistics above, extent and duration of population level massive ART uptake have been suggested to impact on prevalence and extent of viral resistance to ARV treatment (Hamers *et al.*, 2011) In the effort towards adopting the 2013 WHO guideline for initiating ART in Kenya, the HIV-1 infected persons in need of ART rose by an additional 19%, corresponding to a projected estimate of an additional 214,000 HIV-infected persons, based on December 2012 statistics (Odhiambo *et al.*, 2014).

2.3 Treatment of HIV and resistance to antiretrovirals

Since characterization of this virus that causes AIDS, knowledge about its characteristics and appropriate efficacious treatment strategies have evolved (Thompson *et al*., 2012). The goal of ARV treatment has expanded to include prevention of HIV transmission in addition to averting AIDS-related morbidities and mortalities (UNAIDS, 2013). The use of antiretroviral drugs for treatment in developed countries is widespread and has resulted in substantial improvement in survival of HIV-1 patients. A previous analysis projected that access to ARV treatment could extend life expectancy of young HIV-1-infected adult in a developed country by 39 years (Enriquez *et al*., 2011).

In the resource-poor settings of Africa, Asia, and Latin America, where 90% of the people with HIV/AIDS live however, access to ART remained constrained by resources, owing to the high cost of drugs and the lack of infrastructure that could enable universal delivery of ART (Montaner *et al*., 2006). Following the operationalisation of the WHO '3 by 5' initiative in 2003 (that meant 3 million patients treated by 2005), antiretroviral therapy delivery and monitoring tools were simplified and standardized in an effort to increase access to treatment in the developing countries (Jordan *et al.*, 2008). The commitment prompted increased HIV/AIDS support funds, and sharp drop in the cost of proprietary drugs conveniently accessed as potent low-cost generic preparations (Egger *et al*., 2005). These milestones have been achieved through strategies that have adopted standard treatment protocols, simplified monitoring of patients, and decentralization of service delivery as prescribed in the WHO recommendation (Gupta *et al.*, 2012). The standard treatment consist of triple combination therapy in which the first-line regimens are based on a non-nucleoside reverse transcriptase inhibitor (NNRTI) and nucleotide reverse transcriptase inhibitors (NRTIs) while the second-line treatment consist a protease inhibitor (PI) along with NRTIs (Lima *et al*., 2007). The

successes realised through this ART access strategy in developing countries have been met with challenging responsibility of closely monitoring and weighing risk of inevitable increase in HIV DR being transmitted among treatment naïve infected persons against benefits of simplified standard regimen administered (WHO, 2012).

Escalating levels of early ART failure following the roll-out of standard HIV treatment is being reported in sub-Saharan Africa (Hamers *et al*., 2013). More than half of the virologic failure outcomes currently reported is being attributed to clinically important viral resistance to NNRTIs and NRTIs, which essentially reflect the occurrence of mutation within the viral genome region that affects these ARV drugs (Gupta *et al.*, 2012). The onward transmission of these resistance mutations has been noted to jeopardize subsequent and long-term effectiveness of the standard ARV drugs prior to therapy initiation (pretherapy). The standardization of ARV is a key element of the HIV-1 treatment programs that has enabled developing countries to sustainably implement ART scale-up (Mbisa *et al*., 2011).

2.4 Prevalence of pretherapy HIV-1 resistant to ARV

The prevalence of HIV resistant to ARV among treatment-naïve patients (variedly referred as pretherapy, transmitted, pre-treatment or primary drug resistance, depending on context) varies widely. Previous surveys in the developed countries, including United States and Europe have reported pretherapy resistance prevalence rates averaging between 10% and 15% (Rhee *et al*., 2015; Frentz *et al*., 2014). The estimated prevalence rates of pretherapy drug resistance (transmitted drug resistance) for earlier surveys in Europe ranged between 3.3% and 14.2% (zuKnyphausen *et al*., 2014). These rates of HIV DR among treatment naïve populations have since stabilized at around 10%, and others having improved even further in these replete patient-tailored treatment programs of European countries (Rhee *et al*., 2015; zuKnyphausen *et al*., 2014; Jakobsen *et al*., 2010). The superior drug choices and better health services offered to patients have suppressed the resistance levels in developed countries, a contrast from the situation in developing countries.

The developing countries are resource-constrained, thus simplified standard treatment protocols are preferred (as in Kenya). The increased use of standard antiretroviral drugs is linked with increased risk of selection and transmission of resistant HIV variants to newly infected patients (Hamers *et al*., 2011). The problem of increasing HIV-1 resistant to ARVs before therapy initiation (pretherapy) has been highlighted by several reports. WHO HIV

drug resistance report, documented an increasing percentage of surveys reporting moderate pretherapy resistance prevalence of 5%–15% over time (WHO 2012), levels<5% being low and that beyond 15% being categorized as high as outlined in the same document. This moderate resistance prevalence was 18% in the period betwween 2004 and 2006 compared to 32% between 2007 and 2010 (WHO 2012). The report particularly indicated increased percentages of surveys reporting moderate pretherapy resistance in Africa: 17.6 (3 out of 17) in 2004–2006 up to 40.7 (11 out of 27) in 2007–2010 (WHO 2012). Other reports generated around this time also indicated steady increase in pretherapy resistance in east and southern Africa, in parallel with the ART coverage (Gupta *et al*., 2012). A report particularly highlighted an increasing trend in non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance in Africa from 43 surveys conducted in 18 countries (Cambiano *et al*., 2013). These reports relied on limited studies conducted a few years after ART roll-out hence the need for an updated analysis when ART-use and coverage has become more established.

Although data on Kisumu is not available, survey analysis performed in 2008–five years after ART roll-out in Nairobi and Mombasa–Kenya reported the increasing development of pretherapy HIV resistance prevalence of 4.7% (Hamers *et al*., 2011). Another study conducted around the same time in Kilifi, 60 Km from Mombasa, reported a prevalence of 1.1% (Hassan *et al*., 2013), revealing regional variations in country's pretherapy HIV resistance rates. A study done two years later in Mombasa reported a pretherapy HIV DR prevalence of 7.4% (Sigaloff *et al*., 2012), a remarkable increase in comparison to the previous survey in the same region. Another study among injecting drug users within the same town and year reported 13.8% pretherapy resistance, thus highlighting the differences inherent in apparent transmission risks (Osman *et al*., 2013). Previous surveillance nearest to the study region conducted (before May 2005) in Gem-Asembo (Siaya County) reported 1% pretherapy HIV-1 resistance (Zeh *et al*., 2016). However, the setting of the study was in remote rural villages just around the time when ART was being rolled-out. These findings therefore cannot reflect the resistance situation of the urban Kisumu city, a decade later after ART scale-up.

2.5 Distribution of HIV subtype

Based on animal source and genetic profiles of HIV distribution pattern, HIV type-1 (HIV-1) and type-2 have been characterized. HIV type-2 is less virulent and largely restricted to the west of Africa, in contrast to HIV-1 that is almost exclusively responsible for global AIDS

pandemic (Cohen *et al*., 2008). HIV-1 variants are classified into three phylogenetically distinct groups: M (main), O (outlier), and N (non-M/non-O). Group M is the major viral clade responsible for the AIDS pandemic; it is subdivided into at least 12 distinct lineages, designated as subtypes and sub-subtypes (A1, A2, B, C, D, F1, F2, G, H, J, K and L) and at least 48 circulating recombinant forms (CRF) (Nyamache *et al*., 2012) These forms display an uneven global distribution with the most prevalent being subtypes A, B, and C (Buonaguro et al., 2007). Subtype C accounts for almost 50% of all HIV-1 infections worldwide. Subtype A predominates eastern and central Africa; subtype B being the main genetic form found in western and central Europe, the Americas, and Australia; and subtype C being found in southern Africa and Asia (Buonaguro *et al.,* 2007). Previous studies on HIV isolates in Kenya have reported complex mixture of several subtypes including: A1, A2, C, D, G and several circulating recombinant forms, that are dynamic and varying in regional distribution frequency. Subtype A1 is dominant, while subtype C, D, and their recombinant forms with A1 constitute substantial proportions of the transmitted virus (Zeh *et al*., 2016; Adungo *et al*., 2014; Hassan *et al*., 2013; Kageha *et al*., 2012; Khamadi *et al*., 2009; Khoja *et al*., 2008; Khamadi *et al*., 2005; Yang *et al*., 2004). The HIV-1 subtype D is more prevalent in most parts of Kenya than subtype C except in northern Kenya where it is the vice versa (Khamadi *et al*., 2005). Highest frequency of HIV-1 recombinant forms (56.9%) have been reported for patients sampled from the Kericho –Kenya (Billings *et al*., 2015). These regional variance in subtype and HIV recombination dynamic complexities illustrate the realistic challenges in trying to predict the Kisumu HIV disease. The previous known survey in Kisumu was conducted before 2003 amongst pregnant women (Yang *et al*., 2004), hence not likely to reflect the characteristics of HIV infection in the general population more than a decade later.

2.6 Phylogenetic analysis of HIV isolates for regional geographic relationships

Divergence of HIV epidemic in a community or village can be considered as a series of sub-epidemics caused by phylogenetically distinct HIV lineages that are likely to represent viral transmission chains (Novitsky *et al*., 2015). Mapping of HIV phylogenetic lineages/clusters has been conveniently utilized to trace and associate viral transmissions with spread of particular HIV variants (Ragonnet-Cronin *et al*., 2016). Despite HIV phylogenetic relationships being time and epidemiological context-sensitive, scarcity of data has limited the meaningful contextual applicability. The limited data have shown HIV-1 subtype C in Kenya clustering with those of Ethiopian and southern African countries depending on sampling region (Lihana *et al*., 2012; Nyamache *et al*., 2012). Previous phylogenetic analysis

conducted in 2007 on samples collected in Nairobi, revealed the clustering of Kenyan HIV-1 subtype A with sequences from Uganda, Kenya, Sweden, Rwanda, South Africa, Australia, India, China and Democratic Republic of the Congo (Khoja *et al*., 2008). These geographic relationships in diversity of small sample (n=69) from high-end Hospital (Aga Khan) in Nairobi is unlikely to show the transmission relationships associated with the Kisumu HIV epidemic. An analysis of the HIV relationships of a region neighbouring Kisumu, remote villages of Gem and Asembo –in Siaya county, western Kenya– has recently revealed a phylogenetically distinct localized HIV-1 disease epidemiology (Zeh *et al*., 2016). This survey however, was conducted in a village before mid-2005 and the scope of the report did not reveal geographic relationships. An indigenous study to establish the geographic relationships that fuel the HIV transmissions in Kisumu therefore needs to be conducted.

2.7 The effects of pretherapy HIV drug resistance on treatment outcome

Data on the effect of pre-therapy HIV drug resistance is still scarce; some studies have yielded inconsistent results, partly because of the different thresholds for the effect of pretherapy resistance mutations; either the level of resistance or the absolute numbers of drug-resistant mutations (Bertagnolio *et al*., 2013; Lai *et al*., 2012). Although resistant virus strains transmitted to new hosts can subsequently lead to antiretroviral treatment failure (zuKnyphausen *et al*., 2014), inconsistent data exist regarding the impact of transmitted drug resistance to first-line treatment response. At least from studies with short duration of observation; a comparable efficacy of first-line ART has been reported in patients with and without pretherapy HIV DR if regimens comprised only active drugs (Oette *et al*., 2006; Shet *et al*., 2006). Other studies found higher proportion of virologic failure in the participants with pretherapy resistance mutations even when they were receiving fully active regimen (Wittkop *et al*., 2011; Little *et al*., 2002). These previous studies have availed conflicting data from developed countries where treatments at therapy initiation are tailored based on medical review of patient resistance reports at baseline.

For developing countries however, patients on NNRTI-based regimens have been reported to be particularly more vulnerable to early treatment failure especially if they reported baseline pretherapy DR, compared to treatments based on boosted protease inhibitors (PIs), possibly due to their low genetic barrier and wide usage (Lai *et al*., 2012). Observations from other studies showed virologic response regardless of presence of pretherapy resistance associated mutations among some patients, however their sample sizes were limited (Wadonda-

Kabondo *et al*., 2012; Ugbena *et al*., 2012). Studies have supported this further by reporting that pretherapy drug resistance does not result in excess mortality or AIDS events at least in a short term use of partially potent first-line ART (Boender *et al*., 2015; Lee *et al*., 2014). These data on implication of pretherapy mutations are conflicting and limited in showing the extent treatment outcomes are currently affected within the context of low-cost therapy strategy practised in the developing countries, an analysis of the treatment outcome from Kisumu will therefore provide vital data.

2.8 Factors associated with pretherapy resistance

Pretherapy resistance are higher in countries where the ARV roll-out was initiated earlier, a situation aggravated by weaknesses within treatment programs (Hamers *et al*., 2011). The associated factors alluded to in studies include; concurrent diagnoses of sexually transmitted infections (Weng *et al*., 2016), and HIV transmission route (Pham *et al*., 2015) –higher risk among injecting drug users (IDU) and men who have sex with men (MSM). Previous ART exposure such as in prevention of mother to child HIV transmission program (Hamers *et al*., 2013), and the duration of HIV infection i.e. higher pretherapy mutations among recently infected individuals (Taiwo, 2009) have also been implicated. Local ART regimens such as widespread NNRTI use increase the resistance associated with that drug class in sub-Saharan Africa (Gupta *et al*., 2012). HIV-1 subtype i.e. higher mortalities in subtype D influence the HIV-1 isolate proportions (Kiertiburanakul *et al*., 2013). Notably, these systematic studies that have reported these data are mainly not of sub-Saharan Africa. A study on IDU in Kenya might have hinted at showing higher pretherapy prevalence among IDU however, the methodology used could not systematically lead to this conclusion (Osman *et al*., 2013).

Higher prevalence of pretherapy HIV-1 mutations is reported in men in some European regions (Frentz *et al*., 2014), this sex bias is partly explained by increased risk contributed by MSM, a sexual orientation that is not common in Kisumu. A slight bias in pretherapy resistance has been reported for HIV-1 infections relative to subtype D in Uganda (Lee *et al*., 2014). Subtype D is suspected be to substantial in Kisumu (Zeh *et al*., 2016). Studies in developed countries have reported varying pretherapy HIV resistance across various cities and subsequently also shown differences in HIV-1 resistance rates for the health facilities serving these residents (Frentz *et al*., 2014). The few studies conducted in Kenya have identified ≤3 pretherapy resistance cases using small sample sizes (Ssemwanga *et al*., 2015) which cannot meaningfully support analysis of associations in pretherapy resistance. With the absence of data on Kisumu, and only few studies having highlighted the pretherapy ARV

resistance situation in Kenya, the data that hint on factors associated the observed phenomenon are even more scarce. The scarcity of knowledge on factors associated with escalating pretherapy resistance in Kisumu therefore needs to be addressed.

2.9 Pathogenesis of HIV-1 infection

The HIV-1 virus gains entry into host system by its surface-associated gp120 that attaches to the host's cell membrane by first binding to the CD4+ receptor, followed by fusion with chemokine receptors before release of the viral core into host cell cytoplasm (Fauci, 2007). The core is disassembled and subsequently, reverse-transcription of the viral genome into cDNA by the virus' own reverse transcriptase enzyme immediately follows. The viral integrase enzyme in conjunction with host DNA repair enzymes then inserts the viral genome into a gene-rich transcriptionally active domain of the host's chromosomal DNA. The host cell is eventually turned into a potential virus producer with the help of both host- and virus-driven transcription. Finally, the viral protease generates mature infectious virions through specific cleavage (Simon *et al*., 2006).

Infection with HIV eventually leads to AIDS within a median approximate time of 12 years (Weber, 2001). Early phase of the infection is characterised by temporal intense replication of the virus in the host system and dissemination to lymphoid tissue (Murooka *et al*., 2012). This period may be accompanied by either symptoms of a 'sero conversion illness', or more often this period is clinically asymptomatic. The primary host immune response soon spontaneously resolves the viraemic peak that occurs in 2–4 weeks after infection, followed by a chronic asymptomatic HIV infection phase (Maartens *et al*., 2014). The chronic asymptomatic phase is characterized by sustained immune activation that advances to a slow CD4 depletion. At the late stage of HIV-1 infection when the CD4 count drop has reached < 200 x 10^9 cells/L, the depletion rate of CD4 cells become more rapid leading to AIDS (Weber, 2001).

2.10 Molecular basis underlying prevalence of HIV-1 resistance to ARVs

During ARV treatment, the level of viral suppression predicts ART response durability, immunological restoration and most importantly, it reduces the chances of allowing the evolution of drug-resistant virus (Enriquez *et al.*, 2011). Nonetheless, development of resistance to ARV drugs is largely inevitable due to the error-prone nature of HIV reverse transcriptase (RT) coupled with the enzyme's lack of proofreading function (Duffy *et al*., 2008). Also, the high drug resistance rates result from sheer number of replication cycles

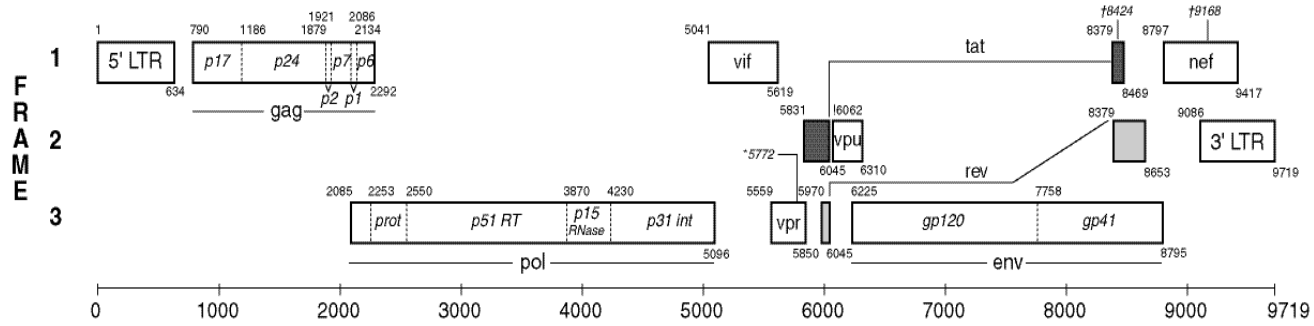
occurring in an infected individual coupled with high rate of RT-mediated recombination events between the diploid viral genomes (Gianella *et al*., 2010). Certain tissue compartments also seem to permit proliferation of resistance mutations due to presence of low drug concentrations (Wainberg, 2012).

The occurrence of mutations on the genes that encode antiretroviral drug targets, sometimes result in serendipitous successful production of gene products that are altered in both structure and function. Although, some of these proteins may be altered, they may still retain their functional role in HIV replication, and as such may proffer survival fitness in normally inhibitive drug pressure of antiretroviral compounds (Wanger *et al*., 2012).

2.11 Significance of genotyping a partial *pol* gene

Selection of survival-fit mutant viral strains sometimes confer reproductive advantage especially under certain replication-inhibiting pressure. Replication of drug-resistant HIV-1 variants during combination therapy is considered a major cause of treatment failure (Kiertiburanakul *et al*., 2009). Genotypic, rather than phenotypic, tests are preferred for establishment of treatment failures in clinical settings because of their wider availability, lower cost, and more rapid turnaround time (Gianella *et al*., 2010). The genetic basis of most resistance mechanisms are already known, therefore making it possible to determine the resistance profile of a strain by means of gene sequence analysis (Kiertiburanakul *et al*., 2009). Expert panels recommend drug resistance testing to enable selection of the optimal drug therapy, in effect genotypic drug resistance testing is routinely performed in the developed countries before initiation of treatment and thereafter for monitoring any viral changes for timely detection and mitigation of virological failure situations (Hamers *et al*., 2012). However this ideal genotyping practice is not feasible in resource-constrained settings such as Kenya, hence the implementation of simplified standard WHO recommended protocol in periodic surveys is favoured (Hong *et al.*, 2011; Jordan *et al*., 2008). The gene regions of interest are represented in the Figure 1 below. They entail the enzymes targeted by drug classes used widely in the HIV-1 treatment –protease inhibitors (PI), and reverse-transcriptase inhibitors (NRTI and NNRTI) –and are transcribed by adjoined sections within the HIV-1 *pol* gene (Aitken *et al.*, 2013; Zhou *et al*., 2011). Almost the entire protease transcribing gene (*prot*) between nucleotides 2280 to 2550 and partial reverse transcriptase gene (*p51 RT*) between nucleotides 2551 to 3210 are target regions for genetic resistance analysis (Bertagnolio *et al*., 2008).

Figure 1.The HIV-1 gene map



Landmarks of the HIV-1 genome HXB2 (K03455) adopted from Los Alamos HIV database (Los Alamos HIV database). The numbers on the upper left corner represent the start codon for the gene while that on the lower right records the last position of the stop codon.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

This research analysis was made up of two parts involving; a retrospective cross-sectional baseline survey of HIV-1 resistance associated mutation prevalence and a longitudinal follow-up analysis for the effects of the resistance associated mutations on the first-line ARV regimens. This multi-site sentinel survey study was conducted at two health facilities in Kisumu [Kisumu County Hospital and Lumumba Family AIDS Care and Education Services (FACES)], Kenya.

3.2 Study Participants

The participants considered for this analysis were obtained from a prospective multi-site cohort study designed to understand the clinical characteristic of HIV-infected patients initiating therapy, treatment outcomes and programmatic factors potentially associated with less-than-optimal ARV performance at the ART site and programme levels.

The study participants were serologically confirmed HIV-1 positive patients,that had presented with clinical conditions that necessitated initiation of first-line antiretroviral treatment. Patients' samples, together with demographic information obtained using a questionnaire (including age, sex, residence, and clinical information) were taken at both baseline and study end-point. The baseline participant samples coincided with the study enrolment time-point, it represented the study participants' pre-therapy time point. At the baseline, the participant blood specimen collected in ethylene diamine tetra-acetic acid (EDTA) tubes, and the study participant's data that included: socio-demographic, clinical, prescribed regimen, and also, any additional information on previous ARV experience were obtained. The end-point was either; 12 months appointment follow-up on first-line regimen, treatment switch, death, having transferred-out, got lost-to-follow-up or stopped taking ARV, as the censure point, in the course of study follow-up period.

3.3 Survey sites

The survey was conducted at two study sites in Kisumu, that lies at 0^{o} 6′0″S 34^{o} 45′0″E on the globe. The two health facilities selected for this study; Kisumu County Referral Hospital and

Lumumba FACES Health Centre represented two major patient support centres where ART and HIV patient management support were provided. Although the two sites were not representative of ART sites in Kenya, they represented functional ART sites in western region of Kenya, and would nonetheless reveal valuable information about the region's management of HIV epidemic. Selection of these sites was based on meeting certain criteria i.e. typical, mature HIV treatment programs, capacity to handle the study without being disrupted, offering health services to patients from almost the entire county, among others.

3.4 Research Study Population

This study population belonged to the Nyanza region previously reported to have the highest HIV-1 prevalence of 15.1 (95% confidence interval: 11.4 to 18.8) and the prevalence trend still remained highest compared to other parts of the country since previous 2007 survey (Kimanga *et al*., 2014). Kisumu county's adult HIV prevalence is 19.3%, the third countrywide. The HIV transmissions are largely fuelled by heterosexual relationships (NACC 2015). At the point of treatment initiation, they received HIV care according to local treatment guidelines in Kenya at the designated hospitals. Medical costs related to HIV care, including ART and management of opportunistic illnesses were supported free-of-charge by government of Kenya in collaboration with partner agencies.

3.5 Enrolment criteria

The population of participants considered for this survey were HIV-infected patients enrolled at the 2 outpatient clinics in Kisumu.

3.5.1 Inclusion Criteria

HIV-1 infected patients who visited the selected health facilities for HIV treatment, and had met the criteria that included: being at least 18 years of age and having agreed to participate in the study by written informed consent. Inclusion also considered eligibility to initiate and had not initiated adult first-line ART regimen at a participating site. Those previously on ART elsewhere but were eligible to initiate first-line ART at the site were also enrolled.

3.5.2 Exclusion Criteria

A HIV-infected patient was excluded from this study if the individual had; previously started and stopped first-line ART at the designated (monitoring) sites. Patients restarting first-line ART, transferred-in from another ART site from where at the time of transfer, the individual had been taking a three- or four-drugs of the first-line regimen were excluded. Also excluded were patients involved in clinical trials or cohort evaluations that required increased follow-

up compared to other patients taking ART at the site, and patients that were known to be infected with HIV type-2.

3.6 Sampling Technique

Consecutive consenting patients were enrolled into the study until the sample size of 120 individuals was reached for each participating facility. The study participant samples and information collected then formed the baseline parameters. The participants' treatment progresses were tracked for 12 months following the initiation of first-line treatment for collection of the study end-point parameters.

3.6.1 Sample Size Calculation

Based on a WHO generic HIV drug resistance protocol, a sample size of N=96 was established to be "effective" to provide a 95% confidence interval of +/- 10% regardless of HIV-1 DRM prevalence (Jordan *et al.*, 2008). With this sample size, HIV-1 DRM prevalence above or below 50% was estimated with better precision than +/-10%. However, since this survey required a 12-month follow-up for the 96 participants, an adjustment to make for loss-to-follow-up was made. After factoring a conservative retention rate of at least 80% based on other similar studies in the same setting that had realized even much higher retention rates by 12 months, a reasonably adjusted sample size of N=120 participants per selected health facility was determined to support precision of the estimated prevalence with sufficient confidence. To arrive at this number of participants, the survey sample size required in order to obtain an estimate of unknown proportion having a probability of (1-α) of being no farther than *e* from the true population proportion, and the following formula (based on normal approximation) was applied:

$$n = \frac{N \times (p \times (1-p))}{(N-1) \times \left(\frac{e^2}{z^2}\right) + (p \times (1-p))}$$

Where z was the α/2 point of the normal distribution and ignoring the finite population correction (WHO 2010), this formula simplified to:

$$n = \frac{z^2 \times (p \times (1-p)}{e^2}$$

The estimated unknown proportion within 0.1 (i.e. 10%) of the true population ($\boldsymbol{p}$) proportion within a probability of 95% (i.e. α =0.05), assuming true prevalence of 50% resulted in this conservative estimate of the sample size (WHO 2010):

$n = \frac{1.96^2 \times (0.5 \times (1-0.5)}{0.1^2}$=3.8416 × 0.25/0.01

=96

To retain this effective sample size at the end of the follow-up period, the total number of the enrolled participants was adjusted to 120 patients to account for patients (at most 20%) who would be lost or transferred. Large studies within settings in Kenya and sub-Saharan Africa having reported overall retention rates of at least 89% and 80% respectively at one year (May *et al*., 2010; Fox *et al.*, 2010).

3.7 Ethical Considerations

Ethical and scientific clearance for this study were separately sought through Kenya Medical Research Institute (KEMRI) –SSC protocol No. 1371, and the Centres for Disease Control and Prevention (CDC) Human Subjects Research Protection office (Appendices 4 & 5).

Risk to participant: This study posed minimal risks to study participants. The primary risk associated with venepuncture was necessary in order to obtain blood sample for patients' routine clinical tests, and therein sufficient patient sample was obtained for this study test.

Direct Benefits: Since individual genotyping of HIV was not commonly performed at treatment initiation, the participants benefited from genotyping result guiding treatment decisions taken.

Indirect Benefits: The information on levels and determinants of primary HIVDR from this analysis study were intended to inform policy on public health HIV-1 treatment guidelines. This knowledge will help strengthen ART policy guidelines regarding prevention of drug resistance.

Informed consent: Following explanation of study to eligible participants, informed consent was obtained at enrolment. The informed consent process described the purpose of the study, procedures to be followed and the risks and benefits of participation. Eligible participants who were unable to read or write had the informed consent form transcribed and read to them in the local language they preferred. Upon agreeing to participate in the study, the participant consented by a written signature or thumb print. The consent process for such participants was witnessed by a third person who also signed the consent form as a witness.

3.8 Methods of Blood collection and processing

The HIV-1 infected patients who presented at the health facilities and required ART treatment initiation qualified to be enrolled in this study following the fore mentioned criteria.

Their whole blood samples were collected in tubes containing ethylene diaminetetra-acetic acid as anticoagulant, and transported to CDC/KEMRI's clinical research facility at the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOORTH) in Kisumu County, where plasma separation was performed. The plasma samples were stored at -80^{0}C and later transported to the genotyping laboratory in frozen state prior to analysis. The HIV isolates of all the 240 baseline participant samples were genotyped. In addition, the end point samples of the particular study participants who experienced virological failure (viral load >1000cp/mL) at 12 month follow-up end point or before then, were also obtained. Viral load testing was performed using the Roche COBAS AmpliPrep/ COBAS TaqMan HIV-1 test, version 2.0 by Roche (CAP/CTM v2.0).

3.9 HIV RNA extraction, amplification and sequencing analysis

An in-house technique of genotyping was used for analysis of the samples. The analysis technique is published (Zhou *et al.*, 2011). The viral RNA was extracted using the QIAGEN QIAamp Viral RNA Mini Kit (QIAGEN, Valencia CA) then reverse transcribed before polymerase chain reaction (PCR) amplified and sequenced (Zhou *et al*., 2011). Briefly, 140 µL plasma was added to 560 µL of buffer AVL containing carrier RNA in a microcentrifuge tube. Following incubation at room temperature for 10 minutes (min), 560 µL of ethanol was added and mixed. The mixture was transferred to QIAamp spin column in two successive aliquots of 630 µL and centrifuged at 8,000 revolutions/min (rpm) for 1 min. The QIAamp spin column was be transferred into a clean 2 mL collection tube followed by the addition of 500 µL of Buffer AW1 and subsequent centrifugation at previous speed. Again, 500 µL of Buffer AW2 was added before another centrifugation at 14,000 rpm for 3 min. The QIAamp spin column was finally placed in a clean 1.5 mL microcentrifuge and incubated with 60 µL of buffer AVE at room temperature for 1 min before the viral RNA were harvested by a final centrifugation step at 8,000 rpm for 1 min. The samples were then amplified by PCR.

Amplification involved using primers PRTM-F1 and RT-R1 in a one-step reverse transcription before PCR using Superscript III RT-enzyme. Subsequently, two microliter of the amplified sample was used as template for a nested PCR that utilized primers; PRT-F2 and RT-R2 aided with AmpliTaq Gold polymerase (Roche -Switzerland) as described previously (Zhou *et al*., 2011). The primers targeted gene region spanning nucleotides 2268 through to 3303 occurring within the *pol* gene. The successful amplification of the approximately 1.0 kb product was confirmed by gel electrophoresis. DNA sequencing of

HIV-1 *pol* gene was performed using the BigDye Terminator sequencing chemistry method (Applied Biosystems, California USA) employing in ABI 3130*xl* Genetic Analyzer (Applied Biosystems). Six sequencing primers overlapping the entire amplicon length were used to generate a *pol* gene fragment encoding protease (amino acid 6-99) and the reverse transcriptase (amino acids 1-251), essentially the entire adjoined region affected by protease- and reverse transcriptase-based antiretroviral treatment pressure. The obtained sequences were assembled and edited using stand-alone ReCall software (University of British Columbia, USA).

Table 1.A table showing various primers utilized in the in-house HIV-1 genotyping assay

Primer name	Sequence (5'- 3')	Location (based on HXB2)	Purpose
PRTM-F1*	F1a –TGAARGATGYACTGARAGRCAGGCTAAT F1b –ACTGARAGRCAGGCTAATTTTTTAG	2057 – 2085 2068 – 2092	RT-PCR RT-PCR
RT-R1	ATCCCTGCATAAATCTGACTTGC	3370 – 3348	RT-PCR
PRT-F2	CTTTARCTTCCCTCARATCACTCT	2243 – 2266	Nested PCR & Sequencing
RT-R2	CTTCTGTATGTCATTGACAGTCC	3326 – 3304	Nested PCR & Sequencing
SeqF3	AGTCCTATTGARACTGTRCCAG	2556 – 2577	Sequencing
SeqR3	TTTYTCTTCTGTCAATGGCCA	2639 – 2619	Sequencing
SeqF4	CAGTACTGGATGTGGGRGAYG	2869 – 2889	Sequencing
SeqR4	TACTAGGTATGGTAAATGCAGT	2952 – 2931	Sequencing

*PRTM-F1 is a mixture of primers F1a and F1b at a ratio of 1:1 (w/w); RT: reverse transcriptase; PCR: polymerase chain reaction. Published in-house HIV-1 genotyping assay primers (Zhou et al., 2011).

3.10 HIV Drug resistance interpretation

To rule out PCR contamination, phylogenetic analysis was performed on all newly obtained sequences by MEGA 6 (Tamura *et al*., 2013). Sequence quality was checked by Stanford HIVdb program. For HIV DR monitoring, the drug resistance-associated mutations in PR and RT gene regions were classified based on the 2015 WHO DRM surveillance list (Wensing *et al*., 2015). Any mutation or combination of mutations that produced low, intermediate or high level resistance to an ARV or drug class was used to define HIVDR. In this study, the Stanford HIVdb algorithm (http://hivdb.stanford.edu/) which assigned genetic sensitivity score (GSS) of 1.00, 0.75, 0.50, 0.25 and 0.00 to the five levels of resistance (susceptible, potential low-level, low-level, intermediate-level and high-level resistance, respectively), was further used to evaluate the genotypic resistance data. The GSS of each regimen was

calculated as the sum of the individual scores for the specific agents prescribed, as described previously (Lai *et al.*, 2012).

3.11 Subtype and phylogenetic relationship analysis

HIV-1 subtypes were determined based REGA HIV-1 Subtyping Tool Version v3.0 (Pineda-Pena *et al*., 2013) and also were 97% identical to the Los Alamos RIP 3.0 recombinant identification program with following settings: window size 400, confidence threshold 95% (www.hiv.lanl.gov/content/sequence/RIP/RIP.html). BLAST analysis was performed to identify nearest sequences using the HIV BLAST tool found in the Los Alamos HIV Sequence Database. Reference strains chosen were those that showed nucleotide identity >95% to query strain. Phylogenetic relationships analysis was performed using molecular evolutionary genetics analysis (MEGA) version 6.0 for Macintosh (Tamura *et al*., 2013). The query nucleotide sequences together with the sequences of the protease and RT genes from reference strains representing the different genetic subtypes were aligned with the CLUSTAL W program. The analyses were performed bearing in mind the protein sequences and minimal Hamming distance compared to subtype reference sequences of HIV-1 *pol* from the Los Alamos HIV Sequence database (www.hiv.lanl.gov). Phylogenetic trees were constructed by the neighbour-joining method and the reliability of the branching orders obtained by the bootstrap approach (1000 replicates) implemented with the CLUSTAL W program. Genetic distances were calculated by the two-parameter method of Kimura (Tamura *et al*., 2013).

3.12 Statistical analysis

Characteristics of the patients were described using percentages for categorical data and median and inter-quartile ranges (IQR) for continuous data (such as age). Prevalence values were calculated with a 95% confidence interval (CI). Student *t* test was used compare differences in median age between the groups. To analyse differences in proportions between patients with HIV-1 resistance versus those without, Fisher's exact test and chi-squared test (x^2) were utilized appropriately. The comparison of these proportions were expressed as odds ratios (ORs) with 95% confidence interval (CI) and two-sided p values, with $p<0{\cdot}05$ being considered statistically significant. The univariate outcomes with $p<0.2$ were considered for subsequent multivariate logistic regression analysis linking associated factors. Fisher's exact test and Cox proportional hazard model were used to describe resistance mutation implications on treatment outcomes. All the analyses were performed using Stata version 12.0 (StataCorp LP; College Station, Texas).

CHAPTER FOUR

RESULTS

4.1 Baseline characteristics of the study population

A total of 240 treatment-naïve HIV-infected patients were recruited into the study. The HIV status of these subjects had previously been confirmed to be positive based on HIV-1/2 rapid antibody test. One hundred and twenty eight (53.4%) study subjects were females. The median age of the patients enrolled at baseline was 32years (IQR 27-40). The initial regimens for all the study patients were primarily based on NNRTI (Nevirapine and Efervirenz) in combination with lamivudine (3TC) and zidovudine (AZT). Their baseline characteristics has been broken down and summarized in Table 2.

Table 2.Baseline characteristics of the HIV-infected persons enrolled into the ARV resistance survey by resistance status

Variables	Patients without HIVDR (n=219)	Patients with HIVDR (n=21)	Total (n=240)	*p*-value
Median age (IQR), years	33 (27-40)	28 (26-33)	32 (27-40)	**0.036**[a]
Gender				0.93[b]
Male, No. (%)	102 (46.6)	10 (47.6)	112 (46.7)	
Female, No. (%)	117 (53.4)	11 (52.4)	128 (53.3)	
Health facility				0.83[b]
Kisumu County Hosp., No. (%)	109 (49.8)	11 (52.4)	120 (50.0)	
Lumumba FACE, No. (%)	110 (50.2)	10 (47.6)	120 (50.0)	
HIV-1 subtype				0.76[c]
Subtype A1, No. (%)	141 (64.4)	13(61.9)	154 (64.2)	
Subtype D, No. (%)	30 (13.7)	2 (9.5)	32 (13.3)	
Recombinant A1, D; No. (%)	19 (8.7)	3 (14.3)	22 (8.8)	
Others, No. (%)	29 (13.2)	3 (14.3)	32 (13.3)	
Residence *				0.35[c]
Urban setting	19 (14.7)	3 (27.3)	22 (15.7)	
Peri-urban setting	38 (29.5)	4 (36.4)	42 (30.0)	
Rural setting	72 (55.8)	4 (36.4)	76 (54.3)	

Abbreviations: IQR, interquartile range; No., number; HIVDR, Human Immunodeficiency Virus Drug Resistance;*, some data missing: n=140. Superscripts indicate statistical methods used for comparing proportions: a-Mann Whitney U test, b-Chi squire, c-Fisher exact test.

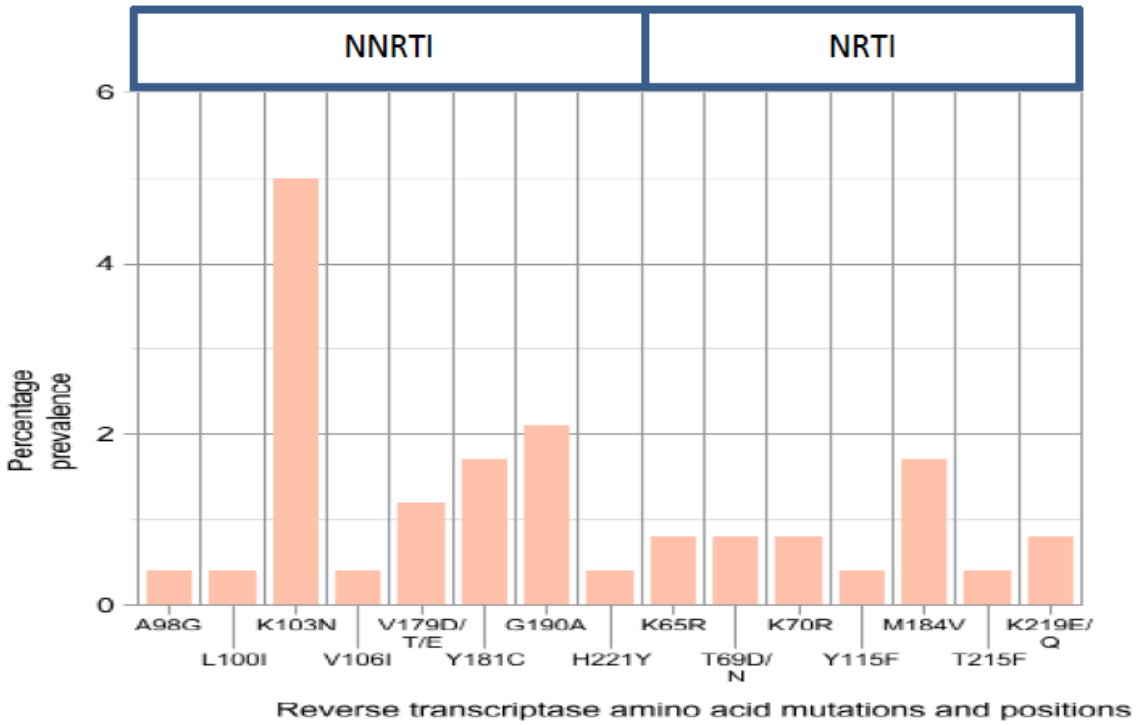
4.2 The prevalence of HIV drug resistance at treatment initiation

All the 240 study samples collected at baseline were successfully genotyped giving a sequencing success of 100%. Drug resistance associated mutations were detected in 21 of the 240 patients–a prevalence of 8.8% (confidence interval [CI]: 4.6-14.7). At least one NNRTI

class mutation was detected in the study participants who had any resistance to ARV (shown in detail –Table 5); the NNRTI drug class resistance had occurred alone in 16(6.7%), while in the rest 5(2.1%), it had occurred along with NRTI mutations as multiclass drug resistance to ARV. None of the study participants had resistance associated mutations to NRTI alone, neither was a major PI mutation detected in any of the study participants at baseline.

Amongst the study participants who were found to harbour resistance mutations, the notable prevalent resistance mutations included; K103N 12 (5%), G190A 5 (2.1%), Y181C 4 (1.7%), M184V 4(1.7%), and V179D/T/E 3 (1.2%). Some study participants hand multiple pretherapy resistance mutations at baseline. The prevalence of several other mutations were <1%; this information has been summarized in Figure 2.

Figure 2. The list of mutations detected among the study participants at baseline and their prevalence percentage



Abbreviations: NNRTI –non-nucleoside reverse transcriptase inhibitor, NRTI - nucleoside reverse transcriptase inhibitor. Letter before numerical digits denote wildtype amino acid abbreviation, numerical digits identify the mutation position, the letter after numerical digits denote the amino acid mutation detected.

4.3 Factors associated with resistance mutations

The prevalence of pretherapy resistance by sex, health service facility, residential set-up and HIV-1 infection subtype did not show any significance. Notably however, the median age of patients who had resistance mutations was younger when compared to the contrasting group, the difference was not significant when systematic analysis was performed by categorisation of age sets.

Table 3. Analysis of the risk factors associated with genotypic drug resistance

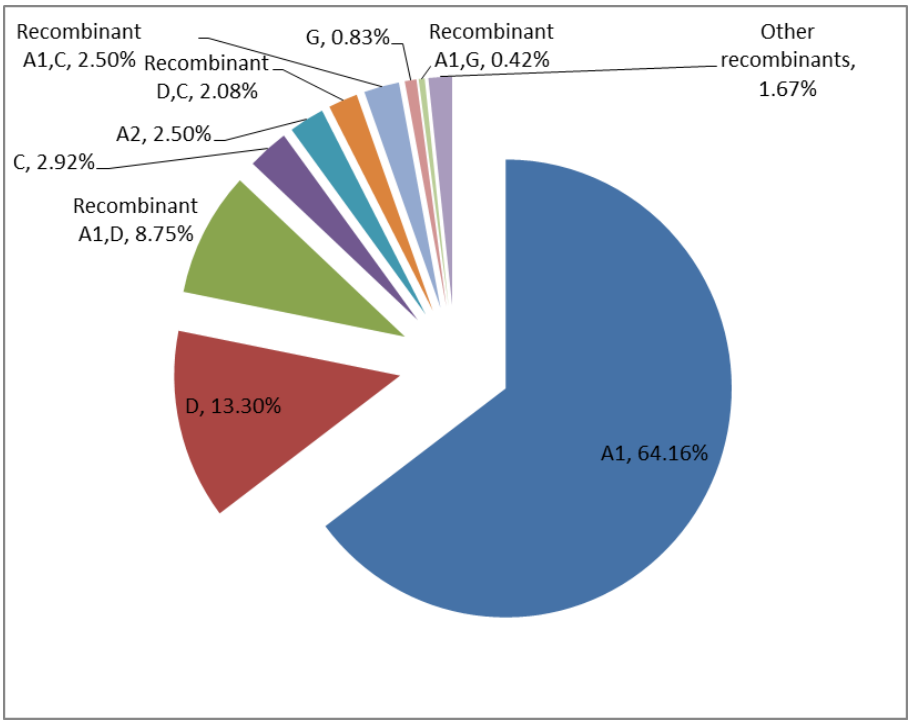
Variable	Adjusted OR (95% CI)	*p*-value
Gender		0.93
Female	ref	
Male	1.04 (0.43 – 2.56)	
Health Facility		0.82
Kisumu County Hospital	ref	
Lumumba FACES	1.11 (0.45 – 2.72)	
Age in Years		0.24
20 - 29	ref	
30 - 39	0.48 (0.17 – 1.35)	
40 - 49	0.30 (0.06 – 1.41)	
≥50	0.33 (0.04 – 2.72)	

Legend Table 3. OR=odds ratio; CI=confidence interval; ref=reference

4.4 HIV-1 subtype analysis

All the 240 sequences were identified as belonging to HIV-1 group M. Amongst the subtypes and circulating recombinant forms identified; the dominant HIV-1 subtype was A1, 154 (64%) followed by subtype D and the recombinant subtype of A1 and D, 32 (13%) and 21 (9%) respectively. These 3 HIV-1 clades cumulatively made up 86.3% of the viral infection in the patients. Subtypes C, A2 and G HIV-1 variants in their pure forms were also detected (2.9%, 2.5% and 0.8% respectively). A part from recombinants A1,C and D,C that occurred in 2.5% and 2.1% respectively, the prevalence of a few other recombinant forms were <1.0% as summarized in Figure 3 below.

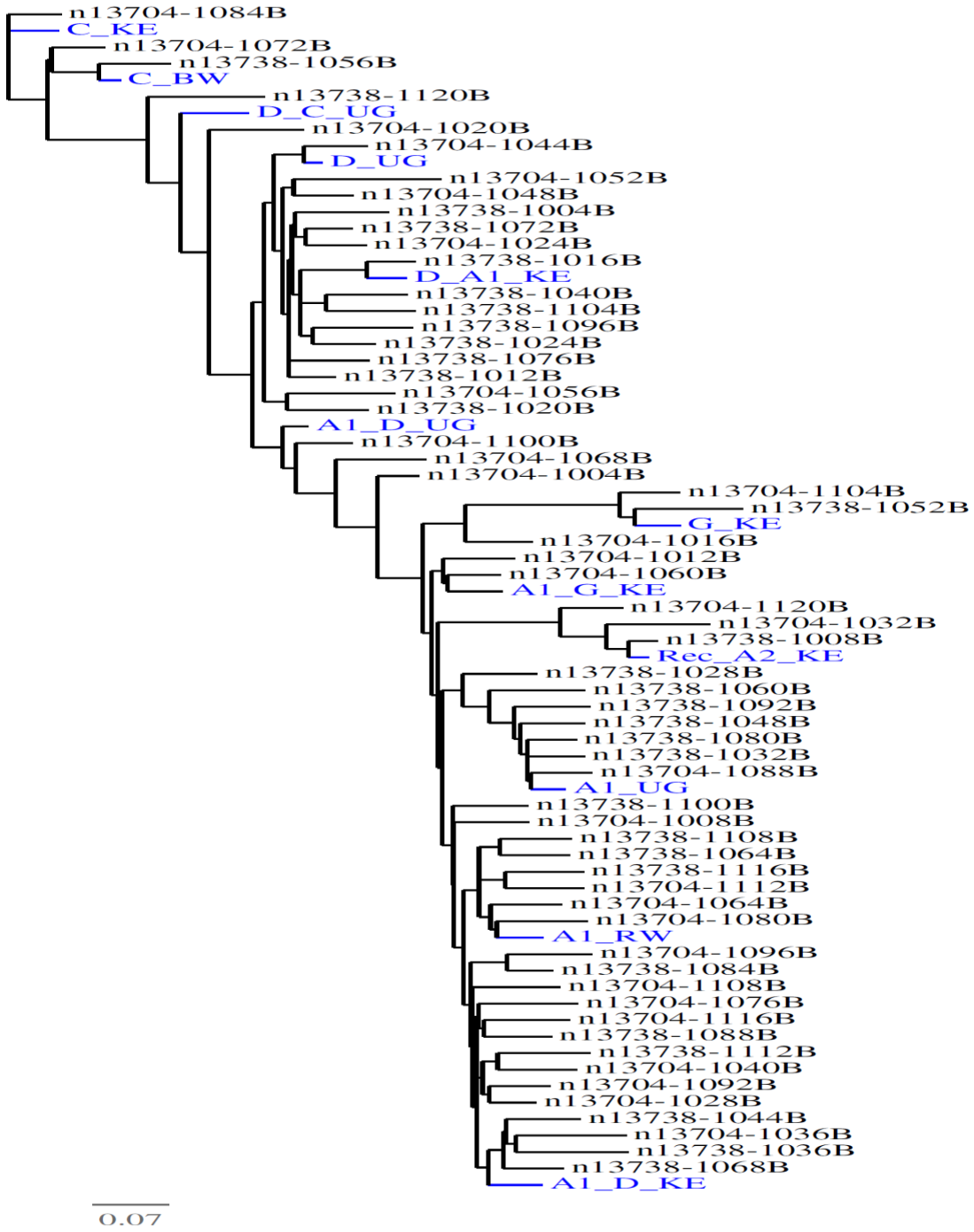
Figure 3. A pie chart representation of percentage prevalence of the HIV-1 subtypes and circulating recombinant forms among the Kisumu patients



4.4.1 Phylogenetic clustering for HIV isolates relationships

Analysis of the HIV-1subtypes A1 and D, which are the viral clades responsible for majority of infections in Kisumu showed phylogenetic clustering with the East African variants. Although the subtype A1 sequences from Kisumu majorly showed multiple sub-clustering with those of other Kenyan viral subtype, some clustered with sequences sampled from Uganda and Rwanda. Subtype D sampled along with some of its recombinants from Kisumu clustered more closely with the Ugandan HIV-1 sequence. More interestingly however, 5 out of all the 7 subtype C found in Kisumu clustered with the sequences from southern Africa (Zambia, Botswana and South Africa) rather than with the Indian and Ethiopian sequences. Analysis of the clustering outcomes realized pairwise sequence similarities of >95% nucleotide match. The recombinants of HIV-1 subtype A/D exhibited phylogenetic cluster that were distinctly different; while some clustered with the Kenyan HIV-1 epidemic, others clustered with those HIV isolates from Uganda.

Figure 4. Phylogenetic tree representing subtype characterization and relationships in the HIV among the patients sampled from Kisumu



Legend figure 4. Neighbour-joining analysis using MEGA 6.0 for the *pol* gene of representative sequences. The blue tree branches denote reference sequences, the HIV-1 subtype and the country of origin; from Kenya (KE), Botswana (BW), Rwanda (RW) and Uganda (UG) The branch lengths correspond to relative percentage of nucleotide substitutions per site.

Figure 5. A radial unrooted phylogenetic tree showing the relationships of the Kisumu HIV-1 subtype C

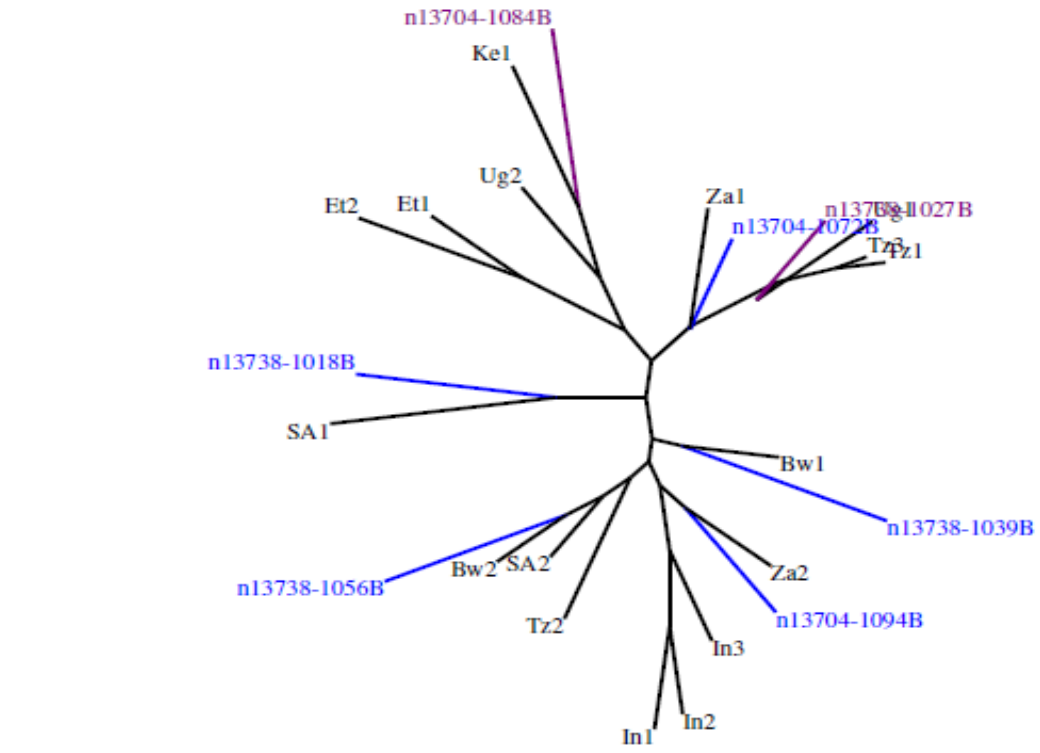
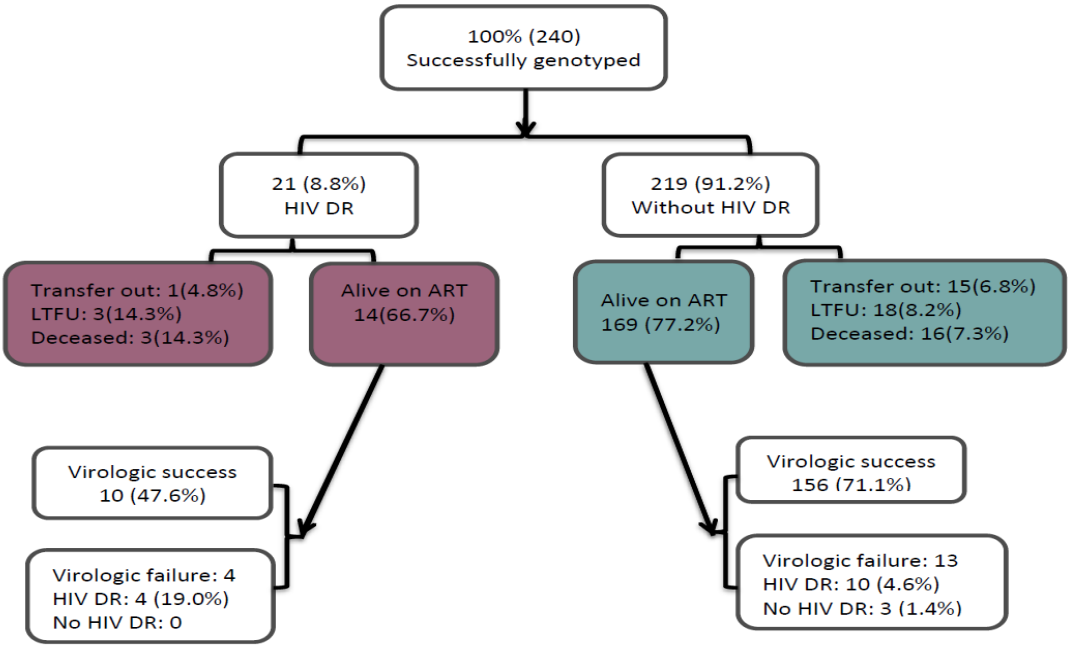


Figure 5 Legend: A radial phylogenetic tree of 24 HIV-1 subtype C sequences constructed by maximum parsimony using MEGA 6.0. The pol gene sequences of the 7 subtype C sequences from Kenya (Ke), Botswana (Bw), Zambia (Za), Ethiopia (Et), South Africa (SA), Tanzania (Tz) and India (In). The numerical suffixes on country code have been used for convenient sequence distinction. The branch lengths correspond to relative percentage of nucleotide substitutions per site. The blue tree branches represent the Kisumu samples that clustered with southern Africa viral clade, while the purple coloured branched represent the samples that clustered with the east African clade.

4.5 Twelve month treatment outcomes of study participants

One hundred and eighty three study participants followed-up to the 12-month end-point were alive, fourteen of whom, were those found to harbour ARV resistance at study baseline. The schematic Figure 6 below summarizes the data.

Figure 6. A flow chart representing the study patient groups



Abbreviations: HIV =Human Immunodeficiency Virus; HIV DR= HIV drug resistance; LTFU=Lost-to-follow-up; ART= antiretroviral therapy.

One third (7 out of 21) of the study participants in whom pretherapy resistance mutations were detected were not available at the study end-point; three (14.3%) had died, and an equal proportion as well, had been lost-to-follow-up (LTFU). In relative comparison to the participants who did not harbour any detectable resistance associated mutations at baseline; only about half that percentage (7.3%) had died, and those lost-to-follow-up were 8.2%. The odds of being deceased (odds ratio[OR]=2.1[95%CI=0.56-7.91], p=0.39) or LTFU (OR=1.9[95%CI=0.50-6.9], p=0.41) within the one year follow-up after initiating ARV treatment was higher among study participants with pretherapy resistance associated mutations.

Also shown in Figure 6, of the 14 participants with baseline drug resistance followed-up to study end-point, ten (47.6%) out of these 21 subjects achieved virologic suppression (RNA <1000 copies/mL) and 4 (19.0%) experienced virologic failure (RNA>1000copies/mL). These four study participants who experienced virologic failure had acquired additional resistance associated mutations within the 12 month treatment follow-up duration (Table 5). In relative comparison, only 5.9% (OR=4.8[95%CI=1.32 -17.44], *p*=0.029) amongst the participants who did not harbour resistance at treatment initiation did not achieved virologic suppression.

Table 4.Survey end-point and follow-up outcomes at 12 months after antiretroviral therapy initiation by baseline HIV drug resistance status

	Resistant	Susceptible	Total	*P*-value
Viral Characteristics at Baseline, No. (%)	21 (8.75)	219 (91.25)	240(100)	
Outcome at 12 months				0.37
Alive on ART, No. (%)	14 (66.7)	169 (77.2)	183 (76.3)	
Lost-to-follow-up, No. (%)	3 (14.3)	18 (8.2)	21 (8.8)	
Deceased, No. (%)	3 (14.3)	16 (7.3)	19 (7.9)	
Transfer out, No. (%)	1 (4.8)	15 (6.8)	16 (6.7)	
Laboratory results				**0.031**
Specimen collected, No. (%)	14 (66.7	169 (77.2)	183(76.3)	
HIV RNA >1000 copies/mL, No. (%)	4 (19.0)	13 (5.9)	17 (7.1)	
VL response and HIV DR analysis				**0.031**
Undetectable VL <40 copies/mL, No. (%)	7 (33.3)	129 (58.9)	136 (56.7)	
Detectable VL >40 and <1000 copies/mL	3 (14.3)	27 (12.3)	30 (12.5)	
Detectable resistance VL >1000 copies/mL, No. (%)	4 (19.0)	10 (4.6)	14 (5.8)	
Without resistance VL >1000 copies/mL, No. (%)	0 (0.0)	3 (1.4)	3 (1.3)	

Table 4 legend. ART: antiretroviral therapy; HIV: human immunodeficiency virus; HIVDR: HIV drug resistance; VL: viral load; No.: number; RNA: ribonucleic acid. Baseline n=240, endpoint n=183.

Curiously, there seemed no peculiar baseline viral characteristic, amongst the 10 patients who had exhibited virologic success regardless of NNRTI mutations, that predicted virologic success for the ARV treatment provided. The 3 out of 4 participants who experienced virologic failure had the very mutations (K103N and G190A) that their corresponding counterpart match had managed to successfully suppress with the common standard ARV treatment provided.

In the comparative group, only 10 (4.6%) study participants who did not have ARV resistance at study baseline freshly acquired resistance associated mutations within the 12 month treatment follow-up course. The patterns of resistance associated mutations characterized amongst them are represented on the Table 6 below.

Table 5.The end-point outcomes of participants with HIV resistance associated mutations at the study baseline

Sample ID	Sex	Age(yrs)	Baseline HIV DR Mutations		GSS	End-point	End-point VL	End Point HIV DR Results Mutations	
			NRTI	NNRTI				NRTI	NNRTI
KEN 13704-1006E	M	20		K103KN,V179EV	2.00	LTFU			
KEN 13704-1010E	F	22		K103N	2.00	12mo	< LDL cp/ml		
KEN 13704-1015E	F	20	K65KR,Y115FY, M184MV	V106IV,V179D, Y181CY,G190AG	1.00	Deceased			
KEN 13704-1030E	F	28		K103N	2.00	12mo	< LDL cp/ml		
KEN 13704-1067E	M	32		Y181C,H221HY	2.00	Transferred			
KEN 13704-1070E	M	45		Y181C, G190A	2.00	12mo	< LDL cp/ml		
KEN 13704-1077E	F	27	T69N,K70R,M184V,T215F,K219Q	L100I,K103N,V179T	0.00	Deceased			
KEN 13704-1078E	F	20		K103N	2.00	12mo	1654 cp/ml	K65R, M184V	K103N, V108I, Y181C
KEN 13704-1085E	M	36		G190AG	2.00	12mo	59798cp/ml	K65KR,M184V	K101E,Y181C,Y188L,G190A
KEN 13704-1091E	M	26		K103N	2.00	12mo	60 cp/ml		
KEN 13704-1120E	F	34	T69D	A98AG	2.25	Deceased			
KEN 13738-1001E	M	26		K103N	2.00	12mo	92 cp/ml		
KEN 13738-1020E	F	30		K103N	2.00	12mo	74 cp/ml		
KEN 13738-1021E	M	29		G190A	2.00	12mo	< LDL cp/ml		
KEN 13738-1042E	F	28		K103N	2.00	12mo	< LDL cp/ml		
KEN 13738-1053E	F	28		G190A	2.00	12mo	1280 cp/ml	K70DEKN, M184V	A98G, G190A
KEN 13738-1063E	M	26		K103N	2.00	LTFU			
KEN 13738-1075E	F	29		K101E	2.00	12mo	< LDL cp/ml		
KEN 13738-1079E	M	58	K70R, M184V, K219E	A98G, Y181C	0.25	Treatment Switch	191,566 cp/ml	K70R,M184V,T215I,K219E	A98G, K101E, V108I, Y181C,G190A
KEN 13738-1097E	M	33		K103N	2.00	12mo	< LDL cp/ml		
KEN 13738-1103E	M	40	K65R,M184V	K101P,K103N	1.00	LTFU			

Table 5 legend. HIV DR: Human Immunodeficiency Virus drug resistance; VL: viral load; LDL: lowest detection limit; cp/ml: copies/millilitres; mo: month; LTFU: lost-to-follow-up; NNRTI: non-nucleoside reverse transcriptase; NRTI: nucleoside reverse transcriptase; GSS: genetic sensitivity score; F: female gender; M: male gender.

Table 6. **Resistance mutations detected amongst study participants who had acquired ARV resistance over the 12-month treatment follow-up course.**

Sample ID	Sex	Age (yrs)	Baseline HIV DR Mutations		End Point	End-point VL	End Point HIV DR Mutations	
			NRTI	NNRTI			NRTI	NNRTI
KEN 13704-1009E	F	30			12mo	7,768 cp/ml	**M41L,M184V,T215Y**	**K103N,E138EQ,Y181CY**
KEN 13704-1083E	M	45			12mo	15,390cp/ml	**K70N,M184V**	**K103N,V108I,F227L**
KEN 13704-1110E	M	26			12mo	73,850 cp/ml	**K65R**	**K101EQ,Y181C,G190S**
KEN 13704-1115E	F	25			12mo	32,094 cp/ml		**K103KN**
KEN 13704-1118E	M	30			12mo	254,364 cp/ml	**K70EK,L74LV,Y115F,M184V**	**Y181C,H221HY**
KEN 13738-1002E	M	38			Switch	39,472 cp/ml	**K165R, M184V**	**L100I, K103N**
KEN 13738-1029E	F	27			12mo	73,604 cp/ml	**K65R, D67DG, K70EK, M184MV, K219E**	**E138A, G190Q**
KEN 13738-1031E	F	28			12mo	7,790 cp/ml		**K103N**
KEN 13738-1034E	F	27			12mo	18,860 cp/ml	**L74IL, M184V**	**V90IV, A98G, K103N, V108IV, P225H**
KEN 13738-1040E	F	30			12mo	109,728 cp/ml	**K65KR, D67DG, K70EK, Y115F, M184V**	**Y181C**

Legend table 6. HIV DR: Human Immunodeficiency Virus drug resistance; VL: viral load; LDL: lowest detection limit; cp/ml: copies/millilitres; mo: month; LTFU: lost-to-follow-up; NNRTI: non-nucleoside reverse transcriptase; NRTI: nucleoside reverse transcriptase; F: female gender.

Most of them (8 out of 10) acquired multiclass resistance mutations and all had resistance to NNRTI drug class. As noticed with the baseline samples, K103N was the most prominent resistance associated mutation, occurring in more than half of the patients who acquired resistance.

3.6 Comparison of treatment outcomes within participant group with pretherapy resistance at baseline

The increased risk of poor virologic outcome (odds ratio=4.27) observed among study subjects that had multiclass drug resistance mutations at baseline was significant (p=0.025). Amongst the 5 out of 21 patients that had resistance to multiclass drugs, none of the 4 that were followed to the study endpoint was successfully sustained on first-line treatment regimen until 12 months. Three had died and one was lost-to-follow-up, while the only individual who remained alive presented deteriorating clinical conditions that warranted antiretroviral treatment switch to second-line regimen. On the other hand, none of the 13 participants who had resistance mutations to only one drug class, NNRTI mutations, and was not lost-to-followed-up to the end of study had died; ten(10/13) achieved virologic success (viral copies<1000).

The likelihood of virologic failure or fatal outcome (death, clinical deterioration) among the patients who had baseline ARV resistance yielding genetic sensitivity score (GSS) of <2.00, the 3/3(100%) was higher, although not significant (p=0.051), compared to 4/14(28.6%) in the group that had GSS ≥2.00 (Table 5). The risk of virologic failure linked to detection of M184V mutation at pretherapy was concurrent with that of GSS<2.00, showed equivalent prognostic value as GSS which had a hazard ratio of 3.5.

CHAPTER FIVE

DISCUSSION

5.1 The pretherapy HIV-1 resistance prevalence in Kisumu

This survey, conducted on 240 HIV-infected patients initiating ARV treatment at the two key health facilities in Kisumu, revealed an estimated pretherapy HIV drug resistance prevalence of 8.8% (CI: 4.6% - 14.7%), categorized as being of moderate level according to WHO (WHO 2012). Previous surveys for pretherapy resistance conducted before 2008 had reported low level (<5%) resistance prevalence (Hamers *et al*., 2011; Hassan *et al*., 2013). The only documented survey conducted after then, in Mombasa, had reported moderate prevalence levels (Sigaloff *et al*., 2012). Despite the methodological differences that render these studies not entirely comparable in the projecting the country's precise pretherapy resistance situations then, this study reports a high pretherapy resistance prevalence ever documented in a Kenyan regional population. The highest pretherapy HIV-1 resistance ever reported in the country previously (13.8%) was amongst a high-risk small cohort of injecting drug users, a group of peculiar transmission risk isolated from general population (Osman *et al*., 2013), an observation reported by others too (Pham *et al*., 2015).

The findings of this study highlight an increase in a potential cause for early virologic failure on patients initiating first-line ARV treatment. These findings corroborate the trends already observed in other developing countries, that adopted the standard simplified treatment strategy proposed by WHO, demonstrating the rising pretherapy HIV-1 resistance to ARV (Hamers *et al*., 2012). The notable upsurge in resistance contributed by increased prevalence of NNRTI drug class associated mutations in this survey is in concordance with the trends recorded for latest studies in the region (Cambiano *et al*., 2013; Hamers *et al*., 2012; Gupta *et al*., 2012). This escalation of pretherapy resistance has been linked to rapid scale-up of poorly functioning programs (Hamers *et al*., 2011) the observation also demonstrated the cost incurred in deriving the benefits of Nevirapine orEfervirenz, the two NNRTI drugs used widely as backbone for the low-cost standard first-line HIV treatment sustainably afforded in resource-limited settings. Despite their low genetic barrier to resistance, NNRTI-based regimens are recommended by WHO as the preferred first-line combination ART. Many

regimens for prevention of mother-to-child transmission also contain an NNRTI (Cambiano *et al*., 2013). The dominance of NNRTI associated mutations, in all the 21 participants who had resistance at baseline, was not surprising since these mutations tend not to impair viral replication capacity–in part explaining their prolonged persistence up to therapy initiation time, in contrast to NRTI associated mutations (Novak *et al*., 2005; Lee *et al*., 2014). The occurrence of NRTI associated mutation M184V on the other hand is known to be fitness-reducing, readily diminishing when drug pressure is lifted, thus its presence partly indicating a possible recent sustained drug pressure (Novak *et al*., 2005). In this study, the presence of this mutation was detected amongst the patients who also exhibited multiclass resistance (4 out of 21) suggesting previous exposure to treatment, unfortunately some belatedly admitted being previously on ART drugs, when their disease condition had already advanced to a fatal clinical health deterioration.

5.2 The Factors associated with pretherapy HIV-1 resistance mutations in Kisumu

Although the median age associated with pretherapy resistance seemed younger by univariate analysis, the further systematic analysis by age categorization did not indicate any significance in the age factor. The slight bias in detection of HIV drug resistance among younger population has previously been highlighted by a study done at the Kenyan coast (Hassan *et al*., 2014). The higher likelihood of acquisition of HIV resistance in age set <35 years could be translated to the biased subsequent transmission of HIV of the same characteristics within the same age sets. The observation supports the WHO guideline requiring transmitted drug resistance surveys to be conducted among persons of younger age (Jordan *et al.,* 2008). Noting the frequency of HIV resistance among study participants of subtype D, a bias might have been introduced because subtype D has been shown, with limited evidence though, to have a more rapid disease progression before treatment initiation in Africa (Lee *et al*. 2014). Although it was not significant in that study, this analysis as well did not find statistically significant difference between D and non-D subtypes.

5.3 The characterization of HIV-1 subtypes in Kisumu

The dominance of HIV-1 subtype A1, followed by D and circulating recombinants of A1 and D was in concordance with the previous studies across various localities in Kenya (Kiptoo *et al*., 2013; Hassan *et al*., 2013; Khoja *et al*., 2008, Khamadi *et al*., 2005). This study however, has supported indications of a remarkable variability in the distribution prevalence of subtypes C, D and the circulating recombinant forms (CRF). The current study reports a prevalence of 63.7% for HIV-1 subtype A1 and a lower prevalence of 2.9% for the viral

subtype C in infected patient in Kisumu. Conversely, Hamadi et al. (2005) reported a relatively lower prevalence of 50% for HIV-1 subtype A1 and the highest subtype C prevalence of 39% among infected persons in northern Kenya (Khamadi *et al*., 2005). The prevalence of CRFs shown in this study is relatively lower compared to that reported by a study (56.9%) done in Kericho –Kenya (Billings *et al*., 2015). The higher prevalence of HIV-1 subtype D in western Kenya is in concordance with a recent publication (Zeh *et al*., 2016), and supporting the possibly high social interaction with neighbour country –Uganda, where the subtype's frequency is much higher (Lee *et al*. 2014; Stoneburner *et al*., 2004). Subtype distribution characterised among the subjects in this study represent the viral clades actively responsible for AIDS in Kisumu (Yang *et al*., 2004), there was no peculiar clade that showed different characteristics from those previously identified in other parts of Kenya. The varying prevalence in subtype distribution frequency is linked to regional neighbour influences and cultural differences (Nyamache *et al*., 2012).

5.4 The phylogenetic analysis for geographic relationships

The clustering of HIV-1subtypes A1, D and recombinant A1/D responsible for majority of infections in Kisumu support the previously documented findings (Khoja *et al*., 2008) that indicated these subtypes are dominant in eastern Africa (Adungo *et al*., 2014). Subtype D appeared to cluster closely with the Ugandan HIV isolate, possibly supporting the introduction the founder strain through the neighbouring country route (Lihana *et al*., 2012). These subtypes have nucleotide signature patterns characteristic for the epidemic in east Africa. Interestingly though, more than half of the subtype C detected among Kisumu patients neither clustered with the Indian subtype C despite substantial population of Indians residing in Kisumu, nor the Tanzanian or Ethiopian, where it is substantial –and the other nearest possible sources of infection for Kisumu –but clustered with the southern Africa subtype C HIV epidemic (Zambia, Botswana and South Africa). This finding supports an analysis by previous investigators (Lihana *et al*., 2012; Nyamache *et al*., 2012) and suggests possible level of influence of a founder HIV-1 subtype C from the region. Implementation of a robust southern-Africa-specific epidemic intervention, such as promising vaccine candidate, may produce at least a partially favourable outcome in Kisumu. The distinctly different clusters of subtype A1/D recombinants observed can be explained by differences in founder viral clade introduced, and actively being transmitted in the region.

5.5 The impact of pretherapy resistance mutations in Kisumu

Varied treatment outcomes resulted amongst individuals that had drug resistant mutants depending on potency of ARV and how extensive the detected mutants potentially affected the treatment administered. The elevated risk of virologic failure among HIV infected persons with resistance associated mutations despite good adherence, shown in this study was in concordance with the findings of other investigators (Lai *et al*., 2012; Wadonda-Kabondo *et al*., 2012; Wittkop *et al*., 2011). This study however, was even more precise in demonstrating the much heightened risk of virologic failure in persons who bore multiclass resistance to antiretroviral drugs of first-line regimens. None of the individuals who had multiclass resistance that affected both drug classes of the first-line regimen (NNRTI and 2NRTI) at baseline realized favourable treatment outcome. In support of a surprise outcome previously noted with Uganda and Rwandese patients, almost half of persons who had pretherapy resistance (10/21), especially those with single NNRTI resistance mutations like K103N, G190A and Y181C, achieved and sustained virologic success in the 12 month follow-up (Lee *et al*., 2014;Wadondo-Kabondo *et al*., 2012). This study participants proportion in whom the occurrence of mutations had not compromised their short-term virologic responsiveness (10 patients) was equivalent to the overall number that acquired resistance associated mutations over the 12 month course of treatment. This observation support the finding by other investigators (Lee *et al*. 2014; Wadondo-Kabondo *et al*., 2012) that demonstrated the presence of pretherapy ARV resistance not always implying imminent treatment failure, at least in the short term. Although these observations are not well understood, they highlight the weight that should be dedicated to the following-up of patients within the first year of initiating treatment, to identify and intervene on adverse resistance to ARV situations that affect the treatments in resource limited settings. Possible predictability of ARV treatment outcomes based on baseline GSS in the persons with pretherapy resistance mutations was alluded to by this study; a poor disease prognosis was associated with GSS<2.00 cut-off index, although the observation was not statistically powered. This prediction of virological outcomes based on baseline GSS as established in this study has been supported previously by other researchers. Lai *et al*. (2012) demonstrated that a baseline GSS index of ≤2.5 was a poor predictor of treatment outcome (a 5.31 times higher risk of treatment failure), notably their GSS threshold being higher compared to that of this study due to the differences in study designs (Lai *et al*., 2012), Lai *et al*. (2012) had incorporated 4 ARV drugs in their regimens, while our study treatments consisted 3 ARV drugs.

Possible underestimation of resistance mutations prevalence at baseline cannot be ruled out due to relatively lower sensitivity of standard genotyping assays only capable of population-based sequencing and therefore detects minority species >20% in population dominance (Johnson *et al*., 2008), as is the case in study. The findings of this study may not be generalized to represent the national HIV drug resistance prevalence level because the participants involved in this study were recruited from 2 hospital facilities in the residential establishment of Kisumu, Kenya. Some of the interesting findings especially on the implications of pretherapy mutations, which are important in informing patient treatment could not be subjected to multivariate logistic regression because of small number for outcomes of interest, and will still require further studies.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The pretherapy resistance prevalence of 8.8% detected is considered moderate level. The dominance NNRTI resistance is largely attributed to wide usage of this drug in general treatment and prophylactic practices. Although this analysis observed likelihood pretherapy resistance among younger patients yet to initiate treatment, it was not significantly associated with young age. The HIV subtype A1 is dominant in Kisumu, just as previously concluded by other studies, followed by subtype D. The HIV subtype D along with its recombinants with subtype A1 found in Kisumu showed closer association to the Ugandan epidemic, suggesting a likely source origin. The subtype C showed phylogeographic relationship to the southern Africa epidemic. Although the presence of pretherapy resistance increased the likelihood of treatment failure, almost half achieved virologic success regardless of the resistance mutations for especially those without multiclass drug resistance.

6.2 Conclusions

i. A moderate level of pretherapy HIVDR found after a decade of rapidly scaling-up ART in Kenya necessitate more watchfulness by clinical care experts. The dominance of NNRTI resistance necessitate the search for alternative replacement drug choices

ii. Although the risk of pretherapy ARV resistance was tending toward younger age in Kisumu, it was not significant.

iii. HIV-1 group M, subtype A1 was found to be responsible for almost two thirds of the disease in Kisumu. The public health experts should however watch the impacts of molecular complexity of emerging recombinant forms, for instance on diagnostic assays.

iv. The detection of pretherapy resistance mutations did not translate to treatment failure even if the patient was put on a regimen that was partially contraindicated based on pretherapy resistance mutation(s) detected. However, the 12 month prognosis for study participants who had multi-drug class resistance to ARV before initiating treatment was poor.

6.3 Recommendations

i. There is need for continued surveillance of HIV drug resistance at treatment initiation, and need to increase access to second-line ART alternatives in the light of escalating pretherapy resistance
ii. The efforts to find factors escalating pretherapy HIV resistance should be identified
iii. Impact of the regionally varying HIV subtype and molecular recombinants should be monitored with respect to diagnosis, treatment and vaccine development
iv. Multiclass drug-resistant HIV-1 adversely impact patient survival, therefore the monitoring of response to ARV treatment following the initiation of therapy should be performed more aggressively

6.4 Future perspectives

i. Future studies should focus on these counties with the highest HIV prevalence like Homa-Bay and Siaya counties
ii. Future studies of larger sample sizes for younger age sets are required to derive better understanding of pretherapy resistance tending toward younger median age and the additional factors that may be escalating the HIV resistance situation
iii. Future studies should focus on understanding the consequences of shifting dynamics in HIV-1 subtype characteristics and the phylogeographic relationships that are varying regionally across Kenya with respect to diagnosis, treatment and vaccine development
iv. Future studies should also examine the long-term detriment of the lack of post-therapy resistance testing on clinical outcome and survival in resource-limited settings especially in the light of escalating pretherapy HIV drug resistance prevalence

The larger studies will meaningfully assist in identifying the factors that drive pretherapy resistance situation in Kenya and as well help to substantively support the observations made with regard to impact of resistance mutation patterns conclusively.

REFERENCES

Adungo F, Gicheru M, Adungo N, MatiluM, Lihana R,Khamadi S. Diversity of Human Immunodeficiency Virus Type-1 Subtypes in Western Kenya. *World J AIDS* 2014; 4: 365-372.

Aitken S, Bronze M, Wallis C, Stuyver L, Steegen K, Balinda S, Kityo C, Stevens W, Rinke de Wit T, Schuurman R. A pragmatic approach to HIV-1 drug resistance determination in resource-limited settings by use of a novel genotyping assay targeting the reverse transcriptase-encoding region only. *J Clin Microbiol* 2013; 51(6):1757-1761.

Bansi L, Geretti A, Dunn D, Hill T, Green H, Fearhill E, Gazzard B, Nelson M, Porter K, Philips A, Sabin C, UK Collaborative Group on HIV Drug Resistance and UK Collaborative Cohort (CHIC) Study. Impact of transmitted drug –resistance on treatment selection and outcomeof first-line Highly Active Antiretroviral Therapy (HAART). *J Acquir Immune Defic Syndr* 2010; 53(5): 633-9.

Bennett D, Bertagnolio S, Sutherland D, Gilks C. The World Health Organization's global strategy for prevention and assessment of HIV drug resistance. *Antivir Ther* 2008; 13 (Suppl l): 21–13.

Bertagnolio S, Federico Perno C, Vella S, PillayD.The Impact of HIV Drug Resistance on the Selection of First- and Second-Line ART in Resource-Limited Settings. *J Infect Dis* 2013; 207 (suppl 2): S45-S48.

Bertagnolio S, Derdelinckx I, Parker M, Fitzgibbon J, Fleury H, Peeters M, Schuurman R, Pillay D, Lynn Morris, Tanuri A, Gershy-Damet G, Nkengasong J, Gilks C, Sutherland D, Sandstrom P. World Health Organization/HIVResNet Drug resistance laboratory strategy. *Antivir Ther* 2008; 13(Suppl 2): 49-57.

Billings E, Sanders-Buell E, Bose M, Bradfield A, Lei E, Kijak G, Arroyo M, Kibaya R, Scott P, Wasunna M, Sawe F, Shaffer D, Birx D, McCutchan F, Michael N, Robb M, Kim J, Tovanabutra S. The number and complexity of pure and recombinant HIV-1 strains observed within incident infections during the HIV and Malaria Cohort Study Conducted in Kericho, Kenya, from 2003 to 2006. *PLoS One* 2015; 10(8): e0135124.

Boender T, Hoenderboom B, Sigaloff K, Wellington M, Siwale M, Kityo C, Akanmu A, Botes M, PASER study group, Rinke de Wit T. Pretreatment HIV drug resistance increases regimen switch in sub-saharan Africa. Abstract: XXIV International HIV Drug Resistance workshop; CROI 2015. Available at:

https://www.informedhorizons.com/resistance2015/pdf/Presentations/Boender.pdf. Last accessed: May 2015.

Buonaguro L, Tornesello M, Buonaguro F. Human Immunodeficiency Virus Type 1 Subtype Distribution in the Worldwide Epidemic: Pathogenetic and Therapeutic Implications. *J Virol* 2007; 81(19): 10209–10219.

Cambiano V, Bertagnolio S, Jordan M, Lundgren J, Phillips A. Transmission of Drug Resistant HIV and Its Potential Impact on Mortality and Treatment Outcomes in Resource-Limited Settings. *J Infect Dis* 2013; 207 (suppl 2): S57-S62.

Cohen M, Smith M, Muessig K, Hallett T, Powers K, Kashuba A. Antiretroviral treatment of HIV-1 prevents transmission of HIV-1: where do we go from here? *Lancet* 2013; 9903: 1515-1524.

Cohen M, Hellmann N, Levy J, DeCock K, Lange J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J Clin Invest* 2008; 118(4): 1244–1254.

Duffy S, Shackelton L, Holmes E. Rates of evolutionary change in viruses: patterns and determinants. *Nat Rev Genet* 2008; 9: 267-276.

Egger M, Boulle A, Schechter M, Miotti P. Antiretroviral therapy in resource-poor settings: scaling inequalities? *Int J Epidemiol* 2005; 34:509–512.

Enriquez M, McKinsey . Strategies to improve HIV treatment adherence in developed countries: clinical management at the individual level. *HIV/AIDS - Res Pal Care* 2011; 3:45–51.

Fauci A. Pathogenesis of HIV disease: opportunities for new prevention interventions. *Clin Infect Dis* 2007; 45(Suppl 4): S206-12.

Frentz D, van de Vijver D, Abecasis A, Albert J, Hamouda O, Jørgensen L, Kücherer C, Struck D, Schmit J, Vercauteren J, Asjö B, Balotta C, Bergin C, Beshkov D, Camacho R, Clotet B, Griskevicius A, Grossman Z, Horban A, Kolupajeva T, Korn K, Kostrikis L, Linka K, Nielsen C, Otelea D, Paraskevis D, Paredes R, Poljak M, Puchhammer-Stöckl E, Sönnerborg A, Stanekova D, Stanojevic M, Vandamme A, Boucher C, Wensing A; SPREAD Programme. Patterns of transmitted HIV drug resistance in Europe vary by risk group. *PLoS One* 2014; 9(4): e94495.

Fox M, Rosen S. Patient retention in antiretroviral therapy programs up to three years on treatment in sub-Saharan Africa, 2007-2009: systematic review. *Trop Med Int Health* 2010; 15(Suppl 1): 1-15.

Gianella S, Richman D. Minority variant of drug-resistant HIV. *J Infect Dis* 2010; 202 (5): 657-666.

Goldblatt A, Kwena Z, Lahiff M, Agot K, Minnis A, Prata N, Lin J, Bukusi E, Auerswald C. Prevalence and Correlates of HIV Infection among Street Boys in Kisumu, Kenya. *PLoS One* 2015; 10(10): e0140005.

Gupta R, Jordan M, Sultan B, Hill A, Davis D, Gregson J, Sawyer A, Hamers R, Ndembi N, Pillay D, Bertagnolio S. Global trends in antiretroviral resistance in treatment-naïve individuals with HIV after rollout of antiretroviral treatment in resource-limited settings: a global collaborative study and meta-regression analysis. *Lancet Infect Dis* 2012; 380(9849): 1250-1258.

Hamers R, Wallis C, Siwale M, Mandaliya, Conradie F, Botes , Wellington M, Osibogun A, Sigaloff K, Nankya I, Shuurman R, Wit F, Stevens W, Vugt M, Rinke de Wit TF, PASER. HIV-1 drug resistance in antiretroviral-naive individuals in sub-Saharan Africa after rollout of antiretroviral therapy: a multicentre observational study. *Lancet Infect Dis* 2011; 11(10):750-759.

Hamers R, Schuurman R, Sigaloff K, Wallis C, Kityo C, Siwale M, Mandaliya K, Ive P, Botes M, Wellington M, Osibogun A, Wit F, Vugt M, Stevens W, Rinke de Wit TF, PASER. Effect of pretreatment HIV-1 drug resistance on immunological, virological, and drug-resistance outcomes of first-line antiretroviral treatment in sub-Saharan Africa: a multicentre cohort study. *Lancet* 2012; 12(4): 307-317.

Hamers R, Sigaloff K, Kityo C, Mugyenyi P, Rinke de Wit T. Emerging HIV-1 drug resistance after roll-out of antiretroviral therapy in sub-Saharan Africa. *CurrOpin HIV AIDS* 2013; 8(1):19–26.

Harries A, Zachariah R, Lawn S, Rosen S. Strategies to patient retention on antiretroviral therapy in sub-Saharan Africa. *Prop Med Int Health* 2010; 15(Suppl): 70-5

Hassan A, Nabwera H, Mwaringa S, Obonyo C, Sanders E, Rinke de Wit T, Cane P, Berkley J. HIV-1 virologic failure and acquired drug resistance among first-line antiretroviral experienced adults at a rural HIV clinic in coastal Kenya: a cross-sectional study. *AIDS Res and Ther* 2014; 11: 9.

Hassan A, Mwaringa S, Obonyo C, Nabwera H, Sanders E, Rinke de Wit T, Cane P, Berkley J. Low Prevalence of Transmitted HIV Type 1 Drug Resistance Among Antiretroviral-Naive Adults in a Rural HIV Clinic in Kenya. *AIDS Res Hum Retroviruses* 2013; 29(1): 129–135.

Hong S, Nachega J, Kelley K, Bertagnolio S, Marconi V, Jordan M. The global status of HIV drug resistance: clinical and public-health approaches for detection, treatment and prevention. *Infect Disord Drug Targets* 2011; 11(2):124–133.

Jakobsen MR, Tolstrup M, Sogaard OS, Jorgensen LB,Gorry PR, Laursen A, Ostergaad L. Transmission of HIV-1 drug-resistant variants: Prevalence and effect on treatment outcome.*Clin Infect Dis* 2010; 50:566–573

Johnson J, Li J, Wei X, Lipscomb J, Irlbeck D, Craig C, Smith A, Bennett D, Monsour M, Sandstrom P, Lanier E, Heneine W. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naïve populations and associate with reduced treatment efficacy. *PLoS Med* 2008; 5(7): e158.

Jordan M, Bennett D, Bertagnolio S, Gilks C, Sutherland D. World Health Organization surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. *Antivir Ther* 2008; 13 (suppl 1): 15–23.

Kageha S, Lihana R, Okoth V, Mwau M, Okoth F, Songok E, Ngaira J, Khamadi S. HIV type 1 subtype surveillance in central Kenya. *AIDS Res Hum Retroviruses* 2012; 28(2): 228-31.

Khamadi S, Lihana R, Osman S, Mwangi J, Muriuki J, Lagat N, Kinyua J, Mwau M, Kageha S, Okoth V, Ochieng W, Okoth F. Genetic diversity of HIV type 1 along the coastal strip of Kenya. *AIDS Res Hum Retroviruses* 2009; 25(9):919-23.

Khamadi S, Ochieng W, Lihana R, Kinyua J, Muriuki J, Mwangi J, Lwembe R, Kiptoo M, Osman S, Lagat N, Pelle R, Muigai A, Carter J, Oishi I, Ichimura H, Mwaniki DL, Okoth F, Mpoke S, Songok E. HIV type 1 subtypes in circulation in northern Kenya. *AIDS Res Hum Retroviruses* 2005; 21(9): 810-4.

Khoja S, Ojwang P, Khan S, Okinda N, Harania R, Ali S. Genetic Analysis of HIV-1 Subtypes in Nairobi, Kenya *PLoS One* 2008; 3(9): e3191.

Kiertiburanakul S, Chaiwarith R, Sirivichayakul S, Ditangco R, Jiamsakul A, Li PC, Kantipong P, Christopher Lee, Ratanasuwan W, Kamarulzaman, Sohn A, Sungkanuparph S, for the TREAT Asia Studies to Evaluate Resistance Surveillance and Monitoring Studies. Comparisons of Primary HIV-1 Drug Resistance between Recent and Chronic HIV-1 Infection within a Sub-Regional Cohort of Asian Patients. *PLoS One* 2013; 8(6): e62057.

Kiertiburanakul S, Sungkanurph S. Emerging of HIV drug resistance: Epidemiology, diagnosis, treatment, and prevention. *Curr HIV Res* 2009; 7(3): 273-278.

Kimanga D, Ogola S, Umuro M, Ng'ang'a A, KimondoL,Murithi P, Muttunga J, Waruiru W, Mohammed I, Sharrif S, De Cock K, Kim A, KAIS Study Group. Prevalence and incidence of HIV infection, trends, and risk factors among persons aged 15-64years in Kenya: Results from a nationally representative study. *JAIDS 2014*; 66(suppl 1): S13-26.

Kiptoo M, Brooks J, Lihana R, Sandstrom P, Ng'ang'a Z, Kinyua J, Lagat N, Okoth F, Songok E. HIV-1 drug resistance-associated mutations among HIV-1 infected drug-naïve antenatal clinic attendees in rural Kenya. *BMC Infect Dis* 2013; 13: 517.

Kantor R, DeLong A, Balamane M, Schreier L, Lloyd R, Injera W, Kamle L, Mambo F, Muyonga S, Katzenstein D, Hogan J, Buziba N, Diero L. HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in western Kenya. *J Int AIDS Soc* 2014; 17: 19262.

Lai C, Hung C, Chen M, Sun H, Lu C, Tseng Y, Chang S, Su Y, Liu W, Hsieh C, Wu P, Chang S, Chang S. Trends of transmitted drug resistance of HIV-1 and its impact on treatment response to first-line antiretroviral therapy in Taiwan. *J Antimicrob Chemother* 2012; 67(5): 1254-60.

Lee G, Bangsberg D, Muzoora C, Boum Y, Oyugi J, Emenyonu N, Bennett J, Hunt P, Knapp D, Brumme C, Harrigan P, Martin J. Prevalence and virologic consequences of transmitted HIV-1 drug resistance in Uganda. *AIDS Res Hum Retroviruses* 2014;30(9):896-906.

Li J, Paredes R, Ribaudo H, Svarovskaia E, Metzner K, Kozal M, Hullsiek K, Balduin M, Jakobsen M, Geretti A, Thiebaut R, Ostergaard L, Masquelier B, Johnson J, Miller M, Kuritzkes D. Low-frequency HIV-1 drug resistance mutations and risk of NNRTI-based antiretroviral treatment failure: a systematic review and pooled analysis. *JAMA* 2011; 305(13): 1327-1335.

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

Lima V, Hogg R, Harrigan P, Moore D, Yip B, Wood E, Montaner J. Continued improvement in survival among HIV-infected individuals with newer forms of highly active antiretroviral therapy. *AIDS* 2007; 21: 685-692.

Little S, Holte S, Routy J, Daar E, Markowitz M, Collier A, Koup R, Mellors J, Connick E, Conway B, Kilby M, Wang L, Whitcomb J, Hellmann N, Richman D. Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med* 2002; 347(6): 385-394.

Los Alamos HIV database, http:/www.hiv.lanl.gov/. Last accessed: Jan, 2016.

May M, Boulle A, Phiri S, Messou E, Myer L, Wood R, Keiser O, Sterne J, Dabis F, Egger M; IeDEA Southern Africa and West Africa. Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. *Lancet* 2010; 376(9739):449-457.

Maartens G, Celum C, Lewin S. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* 2014; 384(9939): 258-71.

Mbisa J, Gupta R, Kabamba D, MulengaV, Kalumbi M, Chintu C, Parry C, Gibb D, Walker S, Cane P, Pillay D. The evolution of HIV-1 reverse transcriptase in route to acquisition of Q151M multi-drug resistance is complex and involves mutations in multiple domains. *Retrovirology* 2011, 8: 31.

Montaner J, Hogg R, Wood E, Kerr T, Tyndall M, Levy A, Harrigan R. The case for expanding access to highly active antiretroviral therapy to curb the growth of the HIV epidemic. *Lancet* 2006; 368: 531–36.

Murooka T, Deruaz M, Marangoni F, Vrbanac V, Seung E, von Andrian U, Tager A, Luster A, Mempel T. HIV-infected T-cells are migratory vehicles for viral dissemination. *Nature* 2012; 490(7419): 283-287.

National AIDS Control Council (NACC). Kenya AIDS Indicator Survey 2012 Final Report. Available at http:// http://nacc.or.ke/wp-content/uploads/2015/10/KAIS-2012.pdf. Accessed: June, 2016.

Novak R, Chen L, McArtur R, Baxter J, HupplerHullsiek K, Peng G, Xiang Y, Henely C, Schmetter B, Uy J, van den Berg-Wolf M, Kozal M; Terry Beirn Community Programs for Clinical Research on AIDS 058 Study Team. Mutations in chronically HIV-infected, treatment-naïve patients: implications for routine resistance screening before initiation of antiretroviral therapy. *Clin Infect Dis* 2005; 40:468–74.

Novitsky V, Kühnertb D, Moyoc S, Widenfeltc E, Okuic L, Essex M. Phylodynamic analysis of HIV sub-epidemics in Mochudi, Botswana. *Epidemics* 2015; 13: 44-55.

Nyamache A, Muigai A, Nganga Z, Khamadi S. HIV Type 1 genetic diversity and naturally occurring polymorphisms in HIV type 1 Kenyan isolates: implications for integrase inhibitors. *AIDS Res Hum Retroviruses* 2012; 28(8): 933-6.

Odhiambo J, Kellogg T, Kim A, Ng'ang'a L, Mukui I,Umuro M, Mohammed I, De Cock K, Kimanga D, Schwarcz S; KAIS Study Group. Antiretroviral treatment scale-up among persons living with HIV in Kenya: Results from a nationally representative survey. *J Acquire immune Defic Syndr* 2014; 66(suppl 1): S116-S122

Oette M, Kaiser R, Daumer M, Petch R, Fatkenheuer G. Primary HIV drug resistance and efficacy of first-line antiretroviral therapy guided by resistance testing. *J Acquir Immune Defic Syndr* 2006; 41: 573–581.

Osman S, Lihana R, Kibaya R, Ishizaki A, Bi X, Okoth F, Ichimura H, Lwembe R. Diversity of HIV type 1 and drug resistance mutations among injecting drug users in Kenya. *AIDS Res Hum Retroviruses* 2013; 29:187–190

Pham Q, Do N, Le Y, Nguyen T, Nguyen D, Huynh T, Bui D, Van Khuu N, Nguyen P, Luong A, Bui H, Nguyen H, McConnell M, Nguyen L, Zhang L, Truong L. PretreatmentHIV-1 drug resistance to first-line drugs: results from a baseline assessment of a large cohort initiating ART in Vietnam, 2009-10. *J Antimicrob Chemother* 2015; 70(3): 941-947.

Pineda-Pena A, Faria N, Imbrechts S, Libin P, Abecasis A, Deforche K, Gómez-López A, Camacho R, de Oliveira T, Vandamme A. Automated subtyping of HIV-1 genetic sequences for clinical and surveillance purposes: performance evaluation of the new REGA version 3 and seven other tools. *Infect Genet Evol* 2013; 19: 337-348.

Ragonnet-Cronin M, Lycett S, Hodcroft E, Hué S, Fearnhill E, Brown A, Delpech V, Dunn D, Leigh Brown A; United Kingdom HIV Drug Resistance Database. Transmission of Non-B HIV Subtypes in the United Kingdom Is Increasingly Driven by Large Non-Heterosexual Transmission Clusters. *J Infect Dis* 2016;213(9): 1410-1418.

Rhee S, Blanco J, Jordan M, Taylor J, Lemey P, Varghese V, Hamers R, Bertagnolio S, Rinke de Wit T, Aghokeng A, Albert J, Avi R, Avila-Rios S, Bessong P, Brooks J, Boucher C, Brumme Z, Busch M, Bussmann H, Chaix M, Chin B, D'Aquin T, De Gascun C, Derache A, Descamps D, Deshpande A, Djoko C, Eshleman S, Fleury H, Frange P, Fujisaki S, Harrigan P, Hattori J, Holguin A, Hunt GM, Ichimura H, Kaleebu P, Katzenstein D, Kiertiburanakul S, Kim J, Kim S, Li Y, Lutsar I, Morris L, Ndembi N, Ng K, Paranjape R, Peeters M, Poljak M, Price M, Ragonnet-Cronin M, Reyes-Terán G, Rolland M, Sirivichayakul S, Smith D, Soares M, Soriano V, Ssemwanga D, Stanojevic M, Stefani M, Sugiura W, Sungkanuparph S, Tanuri A, Tee K, Truong H, van de Vijver D, Vidal N, Yang C, Yang R, Yebra G, Ioannidis J, Vandamme A, Shafer R. Geographic and temporal trends in the molecular epidemiology and genetic mechanisms of transmitted HIV-1 drug resistance: an individual-patient- and sequence-level meta-analysis. *PLoS Med* 2015; 12(4): e1001810.

Shao Y, Williamson C. The HIV-1 Epidemic: Low- to middle-income countries. *Cold Spring Harb Perspect Med* 2012; 2 (3): a007187.

Shet A, Berry L, Mohri H, Mehandru S, Chung C, Kim A, Jean-Pierre P, Hogan C, Simon V, Boden D, Markowitz M. Tracking the prevalence of transmitted antiretroviral drug-resistant HIV-1: a decade of experience. *J Acquir Immune Defic Syndr* 2006;41(4):439-46.

Sigaloff K, Mandaliya K, Hamers R, Otieno F, Jao IM, Lyagoba F, Magambo B, Kapaata A, Ndembi N, Rinke de Wit TF. High prevalence of transmitted antiretroviral drug resistance among newly HIV-1 diagnosed adults in Mombasa, Kenya. *AIDS Res Hum Retroviruses* 2012; 28(9): 1033-1037

Simon V, Ho D, Karim Q. HIV/AIDS epidemiology, pathogenesis, prevention, and treatment. *Lancet* 2006; 368(9534): 489 – 504.

Ssemwanga D, Lihana R, Ugoji C, Abimiku A, Nkengasong J, Dakum P, Ndembi N. Update on HIV-1 acquired and transmitted drug resistance in Africa. *AIDS Rev* 2015; 17(1): 3-20.

Stanford University Drug Resistance Database. http://hivdb.stanford.edu/.
Accessed; Dec, 2014.

Sungkanurph S, Sukasem C, Kiertiburanakul S, Pasomsub E. Emergence of HIV-1 drug resistance mutations among antiretroviral-naïve HIV-1-infected patients after rapid scaling up of antiretroviral therapy in Thailand. *J Int AIDS Society* 2012; 15:12.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013; 30(12): 2725-2729.

Taiwo B. Understanding transmitted HIV resistance through the experience in the USA. *Int J Inf Dis* 2009; 13 (5): 552–559.

Thompson M, Aberg J, Hoy J, Telenti A, Benson C, Cahn P, Eron J, Gunthard H, Hammer S, Reiss P, Richman D, Rizzardini G, Thomas D, Jocobsen D, Volberding P. Antiretroviral Treatment of Adult HIV Infection: 2012 Recommendations of the International Antiviral Society–USA Panel. *JAMA* 2012; 308(4):387-402.

Ugbena R, Aberle-Grasse J, Diallo K, Bassey O, Jelpe T, Rottinghaus E, Azeez A, Akpan R, Muhammad M, Shanmugam V, Singh S, Yang C. Virological response and HIV drug resistance 12 months after antiretroviral therapy initiation at 2 clinics in Nigeria. *Clin Infect Dis* 2012; 54(suppl4):S375-S380.

UNAIDS. Financing the Response to HIV in Low- and Middle-Income Countries: International Assistance from Donor Governments in 2013; July 2014.
UNAIDS. 2014 Epidemiological slides – GAP report; 2014.

UNAIDS. UNAIDS Report on the Global AIDS Epidemic 2013. Geneva, Switzerland: UNAIDS, 2013. Available at: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf. Accessed: Oct, 2014.

United Nations Programme on HIV/AIDS & World Health Organization: Global report (2010) UNAIDS report on the global AIDS epidemic 2010. WHO Library Cataloguing-in-Publication. Available: http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf . Accessed: Sept, 2014.

Wadonda-Kabondo N, Bennett D, van Oosterhout J, Moyo K, Hosseinipour M, Devos J, Zhou Z, Aberle-Grasse J, Warne T, Mtika C, Chilima B, Banda R, Pasulani O, Porter C, Phiri S, Jahn A, Kamwendo D, Jordan M, Kabuluzi S, Chimbwandira F, Kagoli M, Matatiyo B, Demby A, Yang C. Prevalence of HIV drug resistance before and 1 year after treatment initiation in 4 sites in the Malawi antiretroviral treatment program. *Clin Infect Dis* 2012; 54(Suppl 4): S362-8.

Wainberg M. The need for development of new HIV-1 reverse transcriptase and integrase inhibitors in the aftermath of antiviral drug reistance. *Scientifica* 2012; 238278: 28.

Wanger B, Garcia-Lerma J, Blower S. Factors limiting the transmission of HIV mutations conferring drug resistance: fitness costs and genetic bottlenecks. *Nat Scientific Rept* 2012; 2: 320.

Weber J. The pathogenesis of HIV-1 infection. Br Med Bull 2001; 58 (1): 61-72.

Weng Y, Tsai H, Lee S, Wu K, Sy C, Chen J, Chen Y. Prevalence and associated factors for HIV-1 transmitted drug resistance in voluntary clients for counseling and testing in Southern Taiwan. *J Microbiol Immunol Infect* 2016; 49(4):487-493.

Wensing A, Calvez V, Günthard H, Johnson V, Paredes R, Pillay D, Shafer R, Richman D. 2015 Update of the Drug Resistance Mutations in HIV-1.*Top Antivir Med*. 2015;23(4):132-141.

Wittkop L, Gunthard HF, de Wolf, Dunn D, Cozzi-Lepri A, de Luca A, Kucherer C, Obel N, von Wyl V, Masquelier B, Stephan C, Torti C, Antinori A, Garcia F, Judd A, Chene G, EuroCoord-CHAIN study group. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV EuroCoord-CHAIN joint project): a European multicohort study. *Lancet Infect Dis* 2011; 11(5): 363-71.

World Health Organization. World health organization indicators to monitor HIV drug resistance prevention at antiretroviral treatment sites. WHO: April 2010 update. Available at: http://www.unaids.org.br/biblioteca/links/OPAS%2014.pdf. Accessed: Oct, 2014.

World Health Organization. WHO HIV drug resistance report 2012. Available at: http://apps.who.int/iris/bitstream/10665/75183/1/9789241503938_eng.pdf. Last accessed: Oct, 2014.

Yang C, Li M, Shi Y, Winter j, van Eijk A, Ayisi J, Hu D, Steketee R, Nahlen B, Lal R. Genetic diversity and high proportion of intersubtype recombinants among hiv type 1-infected pregnant women in kisumu, western Kenya. *AIDS Res Hum Retroviruses* 2004; 20(5): 565–574.

Zeh C, Inzaule S, Ondoa P, Nafisa L, Kasembeli A, Otieno F, Vandenhoudt H, Amornkul P, Mills L, Nkengasong J. Molecular Epidemiology and Transmission Dynamics of Recent and Long-Term HIV-1 Infections in Rural Western Kenya. *PLoS One* 2016;11(2):e0147436.

Zhou Z, Wagar N, DeVos J, Rottinghaus E, Diallo K, Nguyen D, Bassey O, Ugbena R, Wadonda-Kabondo N, McConnell M, Zulu I, Chilima B, Nkengasong J, Yang C. Optimization of a low cost and broadly sensitive genotyping assay for HIV-1 drug resistance surveillance and monitoring in resource-limited settings. *PLoS One* 2011; 6: e28184.

zuKnyphausen F, Scheufele R, Kücherer C, Jansen K, Somogyi S, Dupke S, Jessen H, Schürmann D, Hamouda O, Meixenberger K, Bartmeyer B. First Line Treatment Response in Patients with Transmitted HIV Drug Resistance and Well Defined Time Point of HIV Infection: Updated Results from the German HIV-1 Seroconverter Study. *PLoS One* 2014; 9(5): e95956.

APPENDICES

Appendix 1: E4. Screening form

HIV DR in Kenya SSC 1371

E4. Screening form

National AIDS and STI Control Programme	ADULT HIVDR MONITORING STUDY	SCREENING FORM.
Site Code:	Patient Unique Number _____ Patient MID _____	

Date of visit [1]: |__|__|-|__|__|-|__|__||__||__|
dd mm yyyy

Screening for Patient eligibility

1. Inclusion criteria *(Please tick Yes or No, and specify if applicable)*

	• Individuals who are at least 18 years of age	☐ Yes	☐ No
	• The written informed consent obtained	☐ Yes	☐ No
	• Patient is eligible to initiate, and do initiate an adult first-line ART regimen at a participating site. This includes individuals who have had previous ART elsewhere (including in the private sector) if they are eligible to initiate first-line ART at the survey site.	☐ Yes	☐ No

If one or more of the criteria above were answered 'No', patient cannot be included in Adult HIVDR Monitoring

2. Exclusion criteria *(Please tick Yes or No, and specify if applicable)*

a.	Individuals who have previously started and stopped 1st line ART at your site where they are now restarting 1st line ART	☐ Yes	☐ No
b	Individuals transferring in from another ART site who are currently taking a three or four – drug 1st line ART regimen	☐ Yes	☐ No
c	Patient involved in clinical trials	☐ Yes	☐ No

If one or more of the criteria above were answered 'Yes', the patient cannot be included in Adult HIVDR Monitoring

If informed consent has not been obtained, the patient cannot be included in Adult HIVDR Monitoring

Appendix 2: E5. Enrolment Form

HIV DR in Kenya SSC 1371

E5. Enrolment Form

National AIDS and STI Control Programme	ADULT HIVDR MONITORING STUDY	ENROLLMENT FORM
Site Code:	Patient Unique Number |__|__|__|__| / |__|__|__|__|__| Patient MID |__|__|__|__| / |__|__|__|__|__|	

Date of visit [1]: |__|__|-|__|__|-|__|__|__|__|
dd mm yyyy

Enrollment and Blood draw for Eligible Patients

1. Demographic data

Age and date of birth : |__|__|-|__|__|-|__|__|__|__|

Sex	☐ Male ☐ Female
Main occupation	
Residence and telephone contact	
Marital status	
HIV exposure category *(Tick all that apply)*	☐ Heterosexual contact ☐ Sex worker ☐ MSM ☐ IDU ☐ Perinatal transmission ☐ Military ☐ Unknown ☐ Not willing to answer

	☐ Other, specify:
Education level *(Please tick one)*	☐ No education ☐ Primary incomplete ☐ Primary school complete ☐ Secondary incomplete ☐ Secondary school complete ☐ Tertiary (college, university)

2. HAART eligibility categories *(Please tick one and take appropriate actions)*

☐ **Initiation of first-line HAART Please specify criteria** *(Tick all that apply)*

☐ **Current WHO Clinical Stage** *(Tick one)*	☐ 1	☐ 2	☐ 3	☐ 4
☐ **Immunological status** *(Please tick one)*	CD4 cut-off	☐ <350/mm^3	☐ <250/mm^3	

3. Clinical data

a. General condition

Weight	|__|__|__| Kg	**Height**	|__|__|__| cm

WHO Performance Scale *(Please tick one)*

☐	0: Able to carry out all normal activity without restriction.
☐	1: Restricted in physically strenuous activity, but ambulatory and able to carry out light work.
☐	2: Ambulatory and capable of all self-care, but unable to carry out work; up and about > 50% of waking hours.
☐	3: Capable only of limited self-care; confined to bed > 50% of waking hours.
☐	4: Completely disabled; cannot carry out any self-care; totally confined to bed or chair.

5. Laboratory data *(Please record lab results of the current patient visit)*

Sample date : \|__\|__\|-\|__\|__\|-\|__\|__\|__\|__\|
dd mm yyyy

	Test	Value	Unit		ND*
a. Hematology	Haemoglobin		☐ g/dL	☐ mmol/L	☐
|__|-|__|-|__|__|__| *date (if different from above mentioned sample date)*	Leucocytes		☐ /mm^3 or 10^6/L	☐ 1000/µL or 10^9/L	☐
	Lymphocytes		☐ /mm^3 or 10^6/L	☐ 1000/µL or 10^9/L	☐
b. Chemistry	Creatinine		☐ mg/dL	☐ µmol/L	☐
|__|-|__|-|__|__|__| *date*	ALAT/SGPT		☐ IU/L		☐
c. Immunology	CD4+ cell count		☐ /mm^3 or 10^6/L	☐ 1000/µL or 10^9/L	☐
|__|-|__|-|__|__|__| *date*	CD4%				☐

BLOOD SAMPLE	**For HIV drug resistance** ☐ *(Please tick if taken)*	
|__|-|__|-|__|__|__| *date*	**Sample ID** *(Please fill out)*	
Time sample taken:		Name of responsible person:

Appendix 3: E7. Endpoint data form

HIV DR in Kenya SSC 1371

E7. Endpoint data form

National AIDS and STI Control Programme	ADULT HIVDR MONITORING STUDY	End point
Site Code:	Patient Unique Number |__|__|__|__| / |__|__|__|__|__| Patient MID ||__|__|__|__| / |__|__|__|__|__|	

Date of End Point:

Total duration of HIVDR Follow – Up _____months

ACTION:

- **Please fill out a Follow-up visit form (as complete as possible)**
- **Take HIVDR blood sample (if feasible)**

Most important reason for end of program (Please tick only one)

↑ Normal completion of protocol	
↑ Stop HAART	
↑ Switch to third-line HAART regimen	
↑ Transfer out	
↑ Loss to follow - up	

↑ Death, specify HIV related: ↑Yes ↑No ↑Unknown	
↑ Patient decision	
↑ Physician decision	

Details

Declaration by the investigator

I have reviewed all pages of the CRF for this patient and believe the data is complete and accurate.

Name of investigator:

Date of signature:

Signature of investigator:

Appendix 4: KEMRI Ethics Review Committee approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **March 24, 2014**

TO: **PROF. MATILU MWAU (PRINCIPAL INVESTIGATOR)**

THROUGH: **FREDA ANDAYI,**
ACTING DIRECTOR, CIPDCR,
BUSIA

Dear Sir,

RE: **SSC PROTOCOL No. 1371 - (*3rd AMENDMENT*) POPULATION-BASED MONITORING OF HIV DRUG RESISTANCE EMERGING IN ADULTS DURING TREATMENT AND RELATED PROGRAM FACTORS IN SENTINEL ART SITES IN KENYA.**

This is to inform you that at the 225th meeting of the KEMRI Ethics Review Committee held on 18th March 2014, the request for amendment to the above referenced research proposal was discussed.

The Committee noted the following amendments:

(a) **Survey Site:** Change from Coptic Hospital to EDARP Komarock Clinic to avoid the possible competition for study participants with a similar study funded by the Coptic Hospital.

(b) **Processing of Blood Samples:** The blood samples will be collected in EDTA-tubes to separate the plasma and then the samples sent directly to the CDC Reference Laboratory (WHO Accredited Lab) instead of to the National HIV Reference Laboratory.

It is noted that the above amendments are justified and do not alter the risk/benefit assessment of the study participants and are therefore **granted approval** for implementation.

Please note that you are required to submit any further amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

Please submit a revised proposal and ensure that the document is version controlled e.g. Version 1.0 dated 16 March 2014 to facilitate tracking of the document of record.

Yours faithfully,

DR. ELIZABETH BUKUSI
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

Appendix 5: CDC Approval document

CGH HSR Tracking #:

Request for Project Determination & Approval – Center for Global Health (CGH)

This form should be used to submit proposals to the CGH Office of the Associate Director for Science/Laboratory Science (ADS/ADLS) for research/nonresearch determination and requirements for IRB review/approval.
Approval Chain: Investigator → Branch Chief/Country Director →Division ADS → CGH Human Subjects Mailbox

☐ **New Request** ☒ **Amendment** ☐ **Laboratory Submission**

Project Title: POPULATION-BASED MONITORING OF HIV DRUG RESISTANCE EMERGING IN ADULTS DURING TREATMENT AND RELATED PROGRAMME FACTORS IN SENTINEL ART SITES IN KENYA [#AM-05-2014]		Project Location/Country(ies): Kenya Nairobi
CDC Principal Investigator(s): Evelyn Ngugi		
CDC Project Officer(s): Lucy Nganga	Division: DGHA	Telephone:
Proposed Project Dates: Start: June 2014	End: June 2016	

Please check appropriate category and subcategory:

☐ **I. Activity is NOT human subjects research. Primary intent is public health practice or a disease control activity (Check one)**
- ☐ A. Epidemic or endemic disease control activity; if applicable, Epi-AID #
- ☐ B. Routine surveillance activity (e.g., disease, adverse events, injuries)
- ☑ C. Program evaluation activity
- ☐ D. Public health program activity*
- ☐ E. Laboratory proficiency testing

*e.g., service delivery; health education programs; social marketing campaigns; program monitoring; electronic database construction and/or support; development of patient registries; needs assessments; and demonstration projects intended to assess organizational needs, management, and human resource requirements for implementation.

☐ **II. Activity is research but does NOT involve human subjects (Check one)**
- ☐ A. Activity is research involving collection or analysis of data about health facilities or other organizations or units (NOT persons).
- ☐ B. Activity is research involving data or specimens from deceased persons.
- ☐ C. Activity is research involving unlinked or anonymous data or specimens collected for another purpose.
- ☐ D. Activity is research involving data or specimens from animal subjects.*

*Note: Approval by CDC Institutional Animal Care and Use Committee (IACUC) may be required.

☐ **III. Activity is research involving human subjects but CDC involvement does not constitute "engagement in human subject research." (Check one)**
- ☑ A. This project is funded under a grant/cooperative agreement/contract award mechanism. Award #
  ALL of the following 3 elements are required:
  - ☑ 1. CDC employees or agents will not intervene or interact with living individuals for research purposes.
  - ☑ 2. CDC employees or agents will not obtain individually identifiable private information.
  - ☑ 3. Supported institution must have a Federalwide Assurance (FWA) and project must be reviewed by a registered IRB linked to the supported institution's FWA.

Supported Institution/Entity Name:	NASCOP MOH		
Supported Institution/Entity FWA #	00006828	FWA Expiration Date (mm/dd/yyyy):	
Expiration Date of IRB approval:		(Attach copy of the IRB approval letter)	

- ☐ B. CDC staff provide technical support that does not involve possession or analysis of identifiable data or interaction with participants from whom data are being collected (No current CDC funding).
- ☐ C. CDC staff are involved only in manuscript writing for a project that has closed. For the project, CDC staff did not interact with participants and were not involved with data collection (No current CDC funding).
- ☐ D. Activity is research involving linked data, but CDC non-disclosure form 0.1375B is signed.*

*Access to linked data is permitted under any of the above sub-categories if CDC investigators and the holder of the key linking the data to identifiable human subjects enter into an agreement using CDC form 0.1375B, prohibiting the release of the key to CDC investigators under any circumstances. The purposes of the planned research do not contradict the terms of consent under which the information or specimens were collected, whether that consent was documented or not documented.

☐ **IV. Activity is research involving human subjects that requires submission to CDC Human Research Protection Office (Check one)***
- ☐ A. Full Board Review (Use forms 0.1250, 0.1370-research partners)
- ☐ B. Expedited Review (Use same forms as A above)
- ☐ C. Exemption Request** (Use forms 0.1250X, 0.1370-research partners)
- ☐ D. Reliance**
  - ☐ 1. Request to allow CDC to rely on a non-CDC IRB (Use same forms as A above, plus 0.1371)
  - ☐ 2. Request to allow outside institution to rely on CDC IRB (Use same forms as A above, plus 0.1372)

*There are other types of requests not listed under category IV, e.g., continuation of existing protocol, amendment, incident reports.
**Exemption and reliance request is approved by CDC Human Research Protection Office (HRPO).

CGH HSR Tracking #:

Amendment: If this request is an amendment to an existing project determination. Please include a brief description of the substantive change or modification below and attach both clean and marked copies of the amended protocol or project outline.

Start date changed to accommodate the different timings in the 2 geographical areas i.e Kisumu and Nairobi

EDARP Komarock replaces Coptic Hospital which was dropped due to a similar HIV drug resistance study that may affect client enrollment.

Revised: The selected sites are: Lumumba Health Centre and Kisumu (East) District Hospital in western Kenya, and

Submission: Attach a protocol or project description (See standard format below) in enough detail to justify the proposed category. Submit your request to your branch chief (or country director for DGHA country staff).

Approval Chain
Investigator → Branch Chief/Country Director →Division ADS → CGH Human Subjects Mailbox

CGH ADS/ADLS Review **Date received in CGH ADS /ADLS office:**

☐ **Project does not require human subject research review beyond CGH at this time.**

☐ **Project constitutes human subject research that must be routed to CDC HRPO.**

Comments/Rationale for Determination:

Approvals/Signatures:	**Date:**	**Remarks:**
Investigator		Expedited review since this is an Amendment with minor changes
Cathy Toroitich-Ruto Digitally signed by Cathy Toroitich-Ruto DN: cn=Cathy Toroitich-Ruto, o=CDC-Kenya, ou=Science & Ethics Team, email=crvto@ke.cdc.gov, c=KE Date: 2014.05.30 15:59:56 +03'00' Branch Chief/Country Director		
ADI, DGHA Division Human Research Protection Coordinator Division ADS/ADLS or Director	1/23/15	Cont. upon local IRB approval of this amendment.
CGH Human Research Protection Coordinator CGH ADS/ADLS or Deputy ADS/ADLS		

Note: Although CDC IRB review is not required for certain projects (categories I,II & III) approved under this determination, CDC investigators and project officers are expected to adhere to the highest ethical standards of conduct and to respect and protect to the extent possible the privacy, confidentiality, and autonomy of participants. All applicable country, state, and federal laws must be followed. Informed consent may be appropriate and should address all applicable elements of informed consent. CDC investigators should incorporate diverse perspectives that respect the values, beliefs, and cultures of the people in the country, state, and community in which they work.

Appendix 6: Amino acid letter codes

Name of Amino Acid	Three letter code	One letter code
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V