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Trend of Pre-antiretroviral Therapy HIV-1 Drug Resistance in Kilombero and Ulanga Antiretroviral Cohort, South-Western Tanzania, for over 15 years (2005-2020)

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UNIVERSITY OF THE WITWATERSRAND

JOHANNESBURG



FACULTY OF HEALTH SCIENCES

Trend of Pre-antiretroviral Therapy HIV-1 Drug Resistance in Kilombero and Ulanga Antiretroviral Cohort, South-Western Tanzania, for over 15 years (2005-2020).

Alex NTAMATUNGIRO

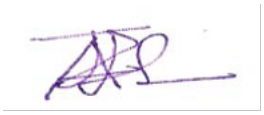
A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy

JANUARY 2024

DECLARATION

I, **Alex Ntamatungiro** declare that this thesis is my original work and has not been submitted before for any degree or award at this or any other university. Where information has been derived from other sources, I confirm that this has been duly acknowledged. Parts of this work have been published as open access articles, I (and my co-authors) retained the copyright for that work.

Alex Ntamatungiro



17th January 2024

I dedicate this work to **my wife** Maria, and our **children** Ethan, and Alvin

PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT

1. **Ntamatungiro AJ**, Kagura J, Weisser M, Francis JM. Pre-treatment HIV-1 drug resistance in antiretroviral therapy-naive adults in Eastern Africa: a systematic review and meta-analysis. *J Antimicrobial Chemotherapy. 2022 Nov 28;77(12): 3231-3241.doi: 10.1093/jac/dkac338. PMID: 36225089*. https://pubmed.ncbi.nlm.nih.gov/36225089/

   My contribution to the paper

   Conceptualization of the study, generation of the hypothesis, conducting literature searches and articles reviews for inclusion eligibility, statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, addressing co-authors' and peer reviewers' comments and checking proofs for the final accepted and published manuscript.

2. **Ntamatungiro AJ**, Anna Eichenberger, James Okuma, Fiona Vanobberghen, Robert Ndege, Namvua Kimera, Joel M. Francis, Juliana Kagura, Maja Weisser, on behalf of the KIULARCO Study Group: Transitioning to dolutegravir in a programmatic setting: Virological outcomes and associated factors in the Kilombero and Ulanga antiretroviral cohort in rural Tanzania: *Open Forum Infectious Diseases, Volume 10, Issue 7, July 2023, ofad321,https://doi.org/10.1093/ofid/ofad321. https://pubmed.ncbi.nlm.nih.gov/37520425*

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   Conceptualization of the study, generation of the hypothesis, planning, and running all lab experiments, and statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, addressing co-authors' and peer reviewers' comments and checking proofs for the final accepted and published manuscript.

3. **Ntamatungiro AJ**, Kagura J, Mnzava D, Ndege R, Okuma J, Vanobberghen F, Francis JM, Weisser M, on behalf of the KIULARCO Study Group: Recent HIV-1 infection and pre-treatment drug-resistance in recently infected, treatment-naïve persons in rural Tanzania: Submitted to the *Journal of Public Health* - http://jpubhealth.oupjournals.org (under review)**.**

   My contribution to the paper

   Conceptualization of the study, generation of the hypothesis, planning, and running all lab experiments, and statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, and addressing co-authors' comments and checking proofs for the final submitted manuscript.

4. **Ntamatungiro AJ**, Francis Joel, Ndege Robert, Okuma James, Tschumi Nadine, Vanobberghen Fiona, Metzner Karin, Paris Daniel, Weisser Maja and Kagura Juliana on behalf of the KIULARCO Study Group: Trends in prevalence of the nucleoside/nucleotide reverse-transcriptase inhibitor (NRTI) associated mutations in newly diagnosed ART naive patients in the Kilombero and Ulanga Antiretroviral Cohort during the 15 years after ART rollout in Tanzania. To be submitted to the *Journal of AIDS.*

   My contribution to the paper

   Conceptualization of the study, generation of the hypothesis, planning, and running all lab experiments, and statistical analyses, synthesizing and presenting findings, writing all drafts of the paper, addressing co-authors' comments and preparing the final manuscript for submission.

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

1. **Ntamatungiro A**, Eichenberger A, Okuma J, Vanobberghen F, Ndege R, Kimera K, Francis J, Kagura J, Weisser. *Transitioning to dolutegravir: virological outcomes in an HIV cohort in rural Tanzania* (AS-EACS-20221-00296): 18th European AIDS Conference (EACS) October 27-30, 2021, London, United Kingdom (ePoster presentation).

2. **Ntamatungiro A**, Eichenberger, Okuma J, Vanobberghen F, Mapesi H, Ndege R, Mnzava D, Kimera N, Weisser M. *Transitioning to Dolutegravir in a programmatic setting: Virological outcomes in the Kilombero and Ulanga Antiretroviral Cohort in rural Tanzania*: 9th Annual Joint Advanced Seminars Conference 29th July 2022, Kampala, Uganda (Oral presentation).

3. **Ntamatungiro A**, Neumann k, METZNER K, PARIS D, and WEISSER M. *HIV-1 Drug Resistance Surveillance in Tanzania*: Swiss TPH Virtual Symposium 25th October 2022 (Oral presentation).

4. **Ntamatungiro A**, Francis JM, Mnzava D, Ndege R, Njau PS, Okuma J, Vanobberghen F, Kagura J, Weisser M, on behalf of the KIULARCO Study Group. *Recent HIV-1 Infection and Pre-Treatment HIV Drug-Resistance in Recently Infected Adults Initiating Antiretroviral Therapy in Rural Tanzania*: Wits School of Public Health Research Day and CARTA Conference 14-15 September 2023, Johannesburg, South Africa (Poster presentation).

ABSTRACT

Introduction

Pre-treatment HIV drug-resistance (PDR) may result in increased risk of virological failure and subsequently acquisition of new HIV drug resistant mutations. With recent increase in antiretroviral therapy (ART) coverage and periodic modifications of the guidelines for HIV treatment, monitoring changes in levels of PDR is critical, particularly in under-sampled areas, such as rural Tanzania. This PhD project aimed to determine the trend and patterns of PDR in the Kilombero and Ulanga antiretroviral cohort (KIULARCO), analyse the impact of recent HIV-1 infection, and dolutegravir rollout in rural Tanzania.

Methods

The study comprised a systematic review and meta-analysis of primary studies about prevalence of PDR among ART-naive people living with HIV (PLHIV) (≥15 years old), published between 2017 and 2022. The data had to be in one or several of the countries of Eastern Africa, namely, Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda. Thereafter, cross-sectional analyses of data on newly HIV-1-diagnosed ART-naïve adults (aged ≥ 15 years), enrolled in the on-going prospective clinic-based observational rural antiretroviral cohort-KIULARCO focusing on various aspects of PDR. Multivariate logistic regressions were used to determine the factors associated with recent HIV-1 infection, and viral suppression at 12-months in patients initiating dolutegravir-based ART in the KIULARCO.

Results

Overall, the pooled prevalence estimate of any PDR was 10.0% (95% CI: 7.9%–12.0%, I^2=88.9%) among 22 studies in the general adults' population, which was higher than the previously reported prevalence of 8.7% using data available until 2016 in the Eastern Africa region. PDR was mainly driven by non-nucleoside reverse transcriptase inhibitors (NNRTI); whereas the pooled prevalence of PDR to nucleoside reverse transcriptase inhibitors (NRTI) was 2.6% (95% CI: 1.8%–3.4%, I^2=69.2%). Remarkably, PDR to NRTIs in a sub-population of recently HIV-1 infected PLHIV in the KIULARCO was high at 12.5%. Also, there was a notable tendency to an increasing prevalence of PDR to NRTI, with the overall prevalence of 2.1% in the first five-year period (2005-2009) of the ART program in Tanzania, and 3.4 % in the most recent period (2019-2022). Moreover, there was no PDR to the dolutegravir co-administered NRTI in those with viremia ≥50 copies/mL, at one year, in patients initiating dolutegravir-based ART in the KIULARCO 2 years after dolutegravir roll. Notably, dolutegravir-based ART was associated with >2 times the odds of viral suppression compared to NNRTI-based ART with an adjusted odds ratio (aOR) of 2.10 (95% CI 1.12-3.94).

Conclusions

There is notable level of PDR to NRTI among general adults' population in Eastern Africa region, that was high among recently HIV-1 infected PLHIV in a representative rural Sub-Saharan Africa setting. Hence, routine surveillance of pre-existing resistance to the DTG co-administered NRTI remains particularly important, in resource-limited settings, to prevent risk of failure of newer antiretroviral agents such as dolutegravir, which would be detrimental to Tanzania and other low- and middle-income countries for the aim to "end AIDS by 2030". Our results underline the benefit of programmatic uptake of dolutegravir -based ART in low- and middle-income countries.

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LIST OF ABBREVIATIONS

ABC	Abacavir
ART	Antiretroviral Therapy
CDCI	Chronic Diseases Clinic
CTC	Care and Treatment Centre
d4T	Stavudine
ddI	didanosine
DTG	Dolutegravir
EFV	Efavirenz
FTC	Emtricitabine
HIV-1	Human Immunodeficiency Virus type 1
HIVdb	HIV Drug Resistance Database
HIVDR	HIV-1 Drug Resistance
HVL	HIV viral load
INSTI	Integrase Inhibitors
KIULARCO	Kilombero & Ulanga Antiretroviral Cohort
3TC	Lamivudine
LMIC	Low- and Middle-Income Countries
NACP	National AIDS Control Care Programme
NGS	Next-Generation Sequencing
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
PCR	Polymerase Chain Reaction
PDR	Pre-treatment HIV-1 drug resistance
PI	Protease Inhibitors
PLHIV	People living with HIV.
PMTCT	Prevent mother-to-child transmission
PR	Protease
RHI	Recent HIV infection
RLS	Resources Limited Settings
RNA	Ribonucleic Acid
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase polymerase chain reaction
SSA	Sub-Saharan Africa

TDF	Tenofovir Disoproxil Fumarate
TDR	Transmitted HIV-1 drug resistance
TLD	Tenofovir/Lamivudine/Dolutegravir
WHO	World Health Organization

PART I

GENERAL INTRODUCTION

CHAPTER 1

1.1. BACKGROUND OF STUDY

1.1.1 HIV burden and the evolution of antiretroviral therapy initiation guidelines

Currently about 38 million people are living with HIV globally and two thirds of these reside in Sub-Saharan Africa (SSA)(1). The HIV prevalence in Tanzania has stabilized at 4.7 % (about 1.7 million people living with HIV (PLHIV) in 2021) and new infections have been reduced to a still high number of 54 000 new HIV infections in 2021. The global epidemiology of HIV infection has changed markedly because of the rapid expanding access to antiretroviral therapy (ART). For example, by the end of 2021, 28.7 million people were accessing ART(2), up from 7.8 million [6.9–7.9 million] in 2010. Also, the ART coverage increased from 24% in 2010 to 75% in 2021(2). By the end of 2021 about 86% of PLHIV in Tanzania were receiving antiretroviral treatment(1).

There has been major improvement in antiretroviral medication with replacement of nucleoside analogues (NRTIs) with long-term toxicity like stavudine (d4T) and didanosine (ddI) to more favorable NRTIs like tenofovir disoproxil fumarate (TDF)(3). Until recently most low- and middle-income countries (LMIC) were using 2 NRTIs and 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) as first line treatment recommended by World Health Organization (WHO)(4,5). The preferred regimen was tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV) as a fixed dose combination.

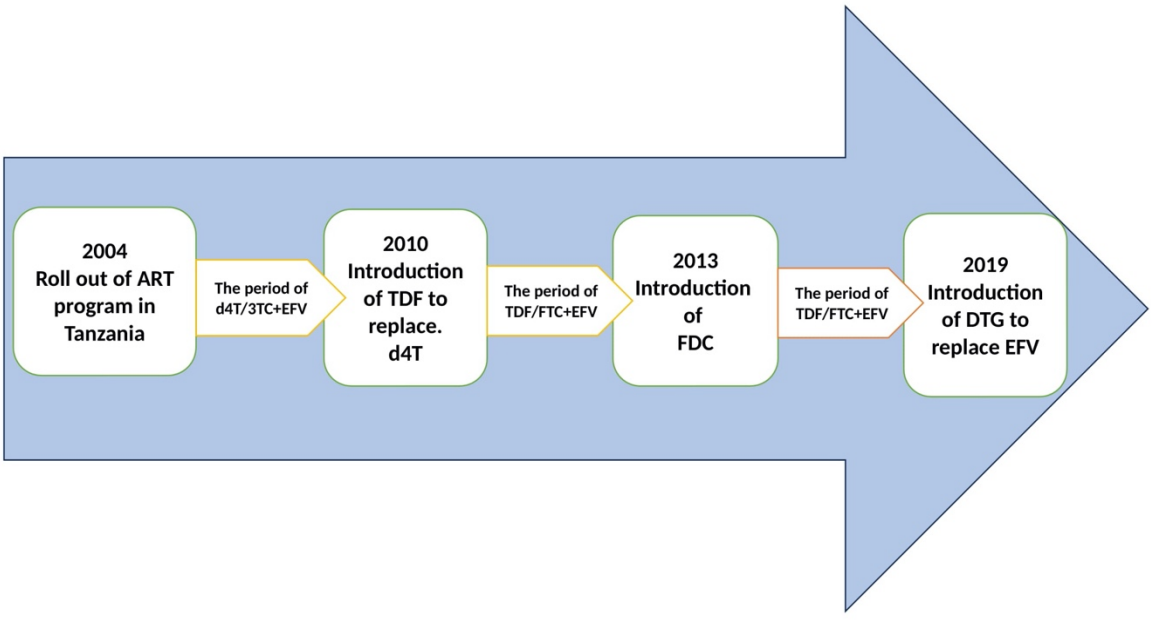


Figure 1.1 Evolution of first line ART in Tanzania

In many high-income countries, integrase-inhibitors as a new drug class have already replaced NNRTIs in first line treatment(6). Available since 2014, integrase inhibitors (INSTI) in several randomized clinical trials have shown faster viral suppression, higher genetic barrier to developing resistance and fewer side effects compared to NNRTIs in treatment-naïve and experienced patients(7,8). Dolutegravir (DTG) an integrase inhibitor for treatment of HIV infection became available to LMIC by the end of 2017, as the generic fixed dose combination tenofovir/lamivudine/dolutegravir (TLD), and since then has been rolled-out in many countries in SSA(9,10). Tanzania has been transitioning to dolutegravir-based first-line regimens since January 2019.

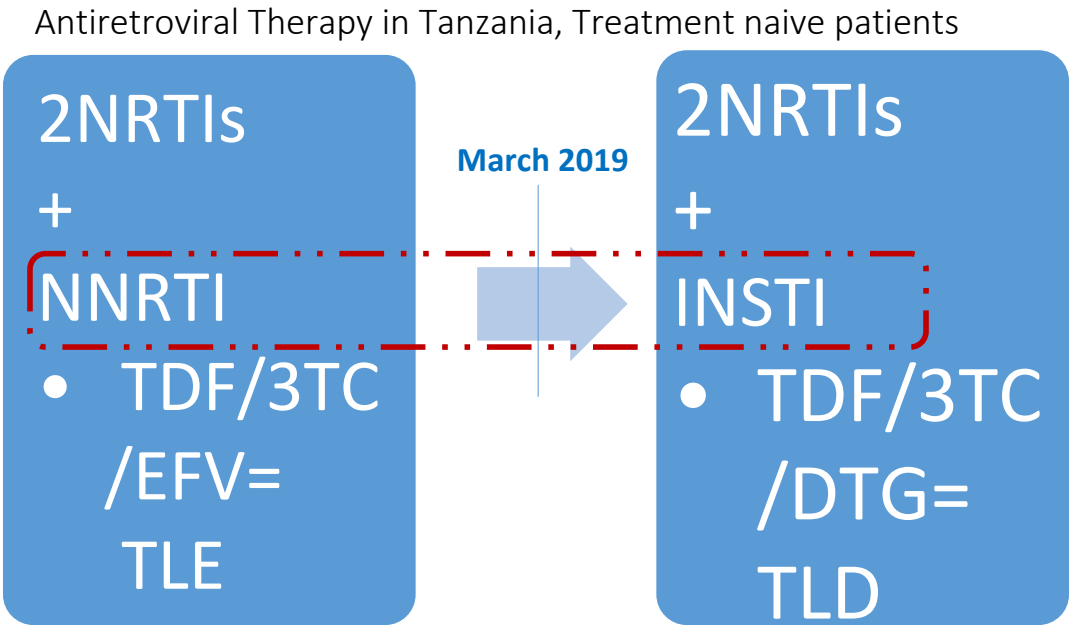


Figure 1.2 WHO recommended programmatic shift to integrase-based therapy.

1.1.2 HIV drug resistance

HIV drug resistance refers to the ability of viruses to continue multiplying even in presence of antiretroviral drugs that usually inhibits its replication. The phenomenon occurs mainly when the virus develops changes in its genetic information, referred to as “mutations”, which alter HIV proteins, that the virus uses to replicate, including the reverse transcriptase, protease, and integrase enzymes.

HIV drug resistance mutations may naturally emerge due to random errors occuring because of the high viral replication rates, or during episodes of incomplete viral suppression, occurring mainly due to suboptimal compliance and irregular drug taking behaviour, which allows the virus to replicate and develop new HIV drug resistant mutations over time. This is known as acquired drug resistance. In some cases, people acquire a strain of HIV that already has the mutations conferring resistance to antiretroviral (ARV), that is known as transmitted drug resistance (TDR). While TDR are resistances detected in patients with no prior history of HIV drug exposure, a synonym term "pre-treatment HIV drug resistance" (PDR) OR "pre-antiretroviral therapy" refers to resistance detected in ARV drug-naive people initiating ART or people with prior ARV drug exposure initiating or reinitiating first-line ART(11).

1.1.2.1 Factors associated with development of HIV drug resistance

The main factor that can lead to uncontrolled viral replication, is poor adherence to ART(12). If ART are taken at sub-optimal level—and it is not diagnosed quickly, the virus can replicate and develop HIV drug resistant mutations. Other factors may include poor absorption: HIV drugs need to be taken consistently on schedule. Additionally, drugs must be absorbed properly into the bloodstream to an optimal drug level, to adequately inhibit the virus replication. Another factor is the pharmacokinetics profile of the antiretrovirals: which refers "to how the drugs are absorbed, distributed, broken down and eliminated from the body. Interactions between drugs can interfere with absorption. Some HIV medications require a booster to maintain high enough levels in the body (for example, protease inhibitors boosted with ritonavir)"(13,14).

1.1.2.2 HIV drug resistance testing

HIV drug resistance can be tested by examining the virus genetic sequences to see whether it contains mutations on the drug targets regions of the viral genome, mainly in the reverse transcriptase, protease, and integrase enzymes. For example, the K65R mutation that reduces TDF and ABC susceptibility, is resulted from the lysine (K)-to-arginine (R) substitution at residue 65 (K65R) in human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT), results from a single nucleotide G-to-A transition (AAA to AGA)(15). Genotypic HIV drug resistance testing is achieved by using genetic analyzers equipment. The main sequencing methods are viral population-Sanger bases Sequencing (SBS), and high throughput deep sequencing method or Next Generations sequencing (NGS). Additionally, to archive genotypic resistance testing there need to be established laboratory protocols and bioinformatics tools for mutations annotations, such as the Stanford University's HIV Drug Resistance Database (HIVdb)(16,17). NGS compared to SBS allows for the detection of low-frequency mutation not identified by SBS. Also, NGS

generates massive genomic data with large genome coverage compared to SBS, which allows details analysis of variants(18).

Phenotypic test is another method to test for the HIV drug resistance, that measures the behaviour of HIV in the presence of specific medications. A sample of HIV from a blood test is exposed to various concentrations of different drugs in a laboratory to see whether the virus can still replicate. Its ability to do so is compared against wild-type virus, which is known to be susceptible to all antiretrovirals(19).

1.1.3 The burden of pre-treatment HIV drug-resistance

Recent data from ART-naïve cohorts indicate varying trends in prevalence of PDR across regions globally. For instance, the analysis report of over 33,000 pooled sequences worldwide between 1996 and 2016, indicated an increasing trend of HIV drug resistance within non-B subtype virus populations(20). Importantly, evidence from European HIV-cohorts where HIV is predominantly subtype B, PDR reports indicate a stable at <7%, to a declining trend overtime(21–23). The declining prevalence of PDR in recent studies among western population, is likely to be explained by the routine virologic monitoring including HIV drug resistance testing, allowing early detection of HIV drug resistant mutations and switch to an active drug regimen, as well as an increasing usage of ART regimens with a higher genetic barrier to developing resistance, for example, the use of INSTI(24).

Conversely, available data from studies in SSA as in many other LMICs show a rise of PDR. A systematic review and meta-analysis involving ART-naïve adult populations in Eastern and Southern Africa reported a significantly increased PDR over time(11,25,26). However, the study declares several important limitations: For example, in Eastern Africa less than 30% of included studies come from rural settings, so the analysis reflects mostly the prevalence of PDR primarily in urban and peri-urban areas. Elsewhere, differences in HIV drug resistance burden between urban and rural settings have been documented(27,28). Also, the large amount of the included studies is not nationally representative, which makes extrapolation challenging. Similarly, sampling in the East African studies encompassed only studies published in 2005-2009, during which period ART represented in the current WHO guidelines, e.g., tenofovir-based ART were still relatively new in many LMICs.

In Tanzania like many other settings in SSA, data on PDR is scarce and geographically scattered(29–36). This situation may threaten the HIV control program, including the implementation of the test and treat policy, where newly diagnosed HIV patients are put on a lifelong ART. Elsewhere, evidence show that expansion of ART program appear to coincide with increase in PDR rates(37). Hence, a comprehensive analysis of the burden and trends of PDR for the period of ART rollout is of paramount importance.

As for the current ART regimen recommendation in most LMICs, there is high risk of some patients remaining on dolutegravir monotherapy. Despite of high genetic barrier to developing resistance of dolutegravir, resistance to dolutegravir monotherapy has been reported in some studies in western countries(38–40), where this drug has been in use for some-time. Similarly, it is essential to monitor the rate of emergence and spread of PDR to protease inhibitors (PI). This is

because, under the current recommendation, HIV infected infants are started by default on PI based ART regimen. Thus, it's equally essential to monitor the trend of PI associated HIV drug resistant mutations, for effective implementation of the Prevention of Mother to Child Transmissions (PMTCT) programme program(41–43).

1.2 LITERATURE REVIEW

1.2.1 Introductory Information

The purpose of this literature review was to find out all published information related to the PDR and gaining insight on the rates of emergence and spread of HIV-1 drug resistant strains- among ART-naïve population, with the focus on SSA. The review was to establish the gap in knowledge on this topic, and assist focusing the research direction, to be able to generate useful scientific information that may substantially contribute to the existing body of knowledge on the topic among the scientific community. Furthermore, the literature review was aimed at familiarizing with various methodological approaches that has been employed to study the emergence of PDR among ART-naïve HIV infected populations, with the aim of selecting and employing the most appropriate method(s) for the current research study.

To archive the literature review, a systematic organized search notes was developed in a literature review matrix, as an invaluable writing resource for literature review chapter. The literature review matrix includes information about the author, year of publication, sampling year, country, features of study population, sample size, proportions (time-point prevalence) of HIV-1 drug resistance mutations by ART class and HIV-1 subtypes. Eligible studies had to report data about prevalence of pre-antiretroviral(s) therapy in adults (> 15years) with no prior history of ART exposure; quantitative studies involving genotyping of the reverse transcriptase with or without protease region of the pol gene and the intergrase gene.

Furthermore, in this chapter, the prevalence of PDR among newly diagnosed HIV patients in low- and middle-income countries (LMIC) is described, with a particular emphasis on Tanzania. We do so by describing the research gap, which is pertaining to the sparsity of PDR data in Tanzania as in other SSA. Such information is essential not only for any individual appropriate HIV management, but also for the national control program to be able to evaluate the control intervention(s), as HIV-1 drug-resistance may affect the control efforts, for both reducing HIV transmission, morbidity and mortality attributed to HIV(44,45)

The following section, include description of the research gaps, also the keys concepts and issues are explained, these includes TDR and PDR; Minority HIV-drug resistance mutations; Recent HIV infections with pre-ART drug resistant mutant virus, and dynamics of HIV-1 subtypes and circulating strains in Tanzania. The concept map is described in the next section, it depicts the conceptual relationship between different exposures and PDR status among HIV infected individuals. Thereafter, in the following sections, the various methods used to study the PDR are discussed. The overall focus of the study is on ART naïve PLHIV.

1.2.2 Research Gap

The ART programme in Tanzania has completed 17 years(35), a period in which the national guidelines for ART initiation have undergone periodic modifications based on the WHO recommendations. Likewise, there has been nearly 200 folds increase in the number of PLHIV on ART to date, from about 5000 PLHIV that were on ART at the beginning on the free ART program in 2005 in Tanzania(1). The rapid expansion of ART coverage may coincide with changes in the prevalence of HIV drug resistance among HIV infected ART-naïve population(37). Also, ART regimens may impact differently on the prevalence rates of HIV drug resistance among PLHIV(46). This is mainly because different ART regimens differ in their genetic barrier to developing resistance, pharmacokinetic-profile, and tolerability(47).

Even so, PDR data in Tanzania is scarce, mainly based in urban areas and come from studies with only small numbers of participants being evaluated in each study(29,30,34–36,48–52) Therefore, it is difficult to generalize the trends in prevalence of PDR in individual demographic and population groups. This situation could limit the effectiveness of HIV prevention approaches, such as those limiting onwards HIV transmission. This thesis aimed to improve availability of local evidence and support to adapt preventive measures and effective response to the threats of HIV drug resistance.

Additionally, as dolutegravir rollout continues in LMIC, information from this thesis contributes to evidence demonstrating the efficacy of dolutegravir alongside a compromised NRTI backbone under programmatic conditions in resources limited setting. Information generated in this study provides additional knowledge on the change in prevalence of PDR for over a full decade of ART rollout, in a representative African rural and semi urban setting, from the era of roll-out to the scaling up of ART in many places of Africa. Data generated are of great importance for the Tanzanian National AIDS Control Program, as well as for the region. The study contributes to the essential data needs for mitigating transmitted HIV-1 drug resistance in resources limited setting.

1.2.3 Key concepts

1.2.3.1 Transmitted and pre-treatment HIV-1 drug resistance

HIV transmitted drug-resistance (TDR), or primary HIV drug-resistance, refers to drug resistance in HIV infected persons with no prior history of ART exposure, mainly acquired during initial infection with HIV drug-resistant variant(s), or "during subsequent re-infection with a resistant virus, referred to as superinfection"(11). Whereas pretreatment HIV drug-resistance (PDR) or pre-antiretroviral therapy is drug-resistance in HIV infected person prior ART initiation, which can either be a result of transmitted drug-resistant variant(s) or acquired during previous ART exposure, such as in women given ART to prevent mother-to-child transmission of HIV. PDR can arise either through transmission of drug-resistant HIV-1 variants from a person with acquired drug resistance, or by transmission of primary drug-resistant HIV from another ART naïve person(11).

PDR may compromise the effectiveness of the initial ART, as evidenced in previous systematic reviews, that the odds of virological failure is higher in patients with PDR mutant variants compared to their counterpart infected with wild-type virus only [45]. Likewise, maternal PDR may diminish efficacy of the regimen to prevent mother-to-child transmission (PMTCT) and future treatment options. Thus, information of the levels and trends of PDR is essential for monitoring of HIV control programs, such as PMCTC.

Pre-ART HIV drug-resistant variant(s) may revert to wild-type virus, or be overgrown by wild type variants, as such may exist at low levels(53–55). Hence PDR studies not considering patient populations with early HIV infection may underestimate the levels of mutations. Similarly, some HIV drug resistance associated mutations are known to confer viral fitness equally or more than the wild HIV variant(s). For example, the K103N mutations have been shown to persist for years even without drug pressure, which is not surprising that K103N is commonly detected in recently infected individuals(23). This may partly explain the majority of NNRTI transmitted mutations, observed among ART-naïve patients infected with HIV drug resistant variants.

Until recent, PDR was mainly driven by NNRTI associated HIV drug resistant mutations. In many setting in LMICs prevalence of PDR to NNRTIs exceeds 10%(56), which underscore the importance of WHO recommendation to replace NNRTI with a much more potent-the intergrase inhibitors, i.e., Dolutegravir-based ART regimen. Notably, NRTI associated PDR is reportedly rising, but in many setting to a level of about 5%. Similarly, PI associated PDR are reassuring low in LMICs. PI are characterized by relatively high genetic barrier to developing resistance, also until recent the usage of PI was relatively still very low, thus with recent increasing patients switched or started on PI-based regimen, there is need to systematically evaluate emergence and spread of mutations conferring resistance to PI.

1.2.3.2 HIV-1 drug-resistant minority variants

HIV-1 Minority variants are virus that exist in very low proportional within the viral population, that may go undetected when using standard viral population sequencing methods(57). For example, in a detection methods comparison study, HIV-1 drug-resistance prevalence rates of 8% and 31.3% were detected among recently infected HIV-1 patients using standard population-based Sanger sequencing and Next-Generation sequencing (NGS) respectively(18). Similarly, a pooled analysis of 10 studies involving ART-naïve patients with no detectable HIV-1 drug-resistance mutations using population based-Sanger Sequencing, showed that 14% harbored NRTI/NNRTI mutations by ultra-sensitive assay(18).

Clinical significance of minority HIV-1 variants is still an active area for investigation. However, some studies have shown that minority HIV drug resistant mutations prior to ART initiation are associated with more than two-folds increase in the risk of virologic failure [(55,58–62). For example, a retrospective samples analysis of the anonymized population in Uganda, reported 64% of failing patients without detectable HIV-1 drug resistance associated mutations as determined by population-based Sanger sequencing, harbored minority HIV drug resistant variants. Which may explain the continued virology failure observed in that population(63).

Likewise, Rupérez et al., while studying a population of pregnant women in Mozambique, observed that all emerged drug resistance associated mutations during pregnancy were minority mutants, underscoring the importance of minority HIV-1 variants(64). Nevertheless, such virologic/clinical effects of minority HIV-1 variants have not been apparent in some studies(65).

Minority HIV-1 drug resistant variants may go undetected with standard population-based genetic analysis methods. However, may emerge especially under drug pressure. Pimenta *et al*, while studying a population of HIV-1 infected ART-naive pregnant women in Sao Paulo, Brazil, did not find any HIV drug resistant mutations (66). A similar, recent study in Uganda did not detect HIV drug resistant mutations among ART-naive Ugandan women initiating Option B+ (67). The levels

of pretreatment HIV drug resistant mutations in above studies, may have been underestimated. Firstly, the analytical technique employed detect the majority viral population, at about 20% of the virus population(18). Whereas HIV drug resistant mutations may exist at low levels, particularly in the absence of drug pressure. A similar study in Mozambique, found that 10% of pretreatment HIV drug resistant mutations among pregnant women using NGS, which is the genetic analysis method able to detects minority HIV drug resistant mutations to as lower threshold as 1% of the viral population. In this study, all the emerged HIV drug resistant mutations during pregnancy were minority variants, which underscore the importance of minority HIV drug resistant mutations (64).

1.2.3.3 Recent HIV infections testing assays and their limitations.

Several assays and testing strategies based on HIV antigens, or HIV antibodies and HIV viral RNA have been developed over the years to distinguish recent from chronic HIV infections. The advantage of these assays is that they can be carried out retrospectively on stored blood samples from cross-sectional studies, and they are cheaper and simpler to perform than following cohort studies. However, there are several limitations of the assays that currently limit their widespread applicability, the most important of which relate to the misclassification of the tests- resulting in false recent HIV infection, estimation of the window period, which varies substantially by HIV subtype and host population and low reproducibility. To overcome these misclassification of tests, new HIV-1 recent infection testing algorithm for HIV-1 Limiting Antigen (LAg) Avidity test have included HIV viral load test, and antiretroviral test results(68).

1.2.3.4 Recent HIV infection and drug resistance

Recent HIV infection (RHI) is considered the phase of ≤6 months after infection, during which anti-HIV antibodies become detectable(69). People with RHI may be more likely to transmit HIV to others due to relatively high HIV viral load (HVL) in blood and genital secretions, absence, or few HIV specific blocking antibodies, fewer viral quasispecies diversity and possibly high transmissible, and which may possibly contain HIV-1 drug resistant variants(70).

Data on PDR among recently HIV infected ART-naive patients from routine surveillance is crucial to inform interventions to control the HIV epidemic. However, prevalence estimates of HIV-1 drug resistance among recently HIV infected ART-naive patients in Tanzania has not yet been described. Previous studies show that HIV resistant variants are significantly higher in recently HIV infected population than in those with chronic HIV infections(70). For example, PDR prevalence of 20.9% vs 8.6 % was documented in recently HIV infected population and chronic HIV infection respectively(71). Also, a study in North-West India reported a moderate to high prevalence of PDR at 16% in patients with RHI, in setting where earlier studies have shown lower level of PDR prevalence(72). Likewise, a study in Rio de Janeiro, Brazil documented TDR

prevalence rate of 16.3% among acutely/recently HIV-infected individuals, which was higher than those found in chronically HIV-infected individuals in Brazil(73).

Similarly, relatively higher prevalence of TDR was reported in a population-based household survey, that involve adult patients with RHI in 2012 in Kenya(74). Hypothetically, higher levels of PDR may be observed when targeting the recent HIV infected population, which is key consideration while estimating the burden of PDR.

1.2.3.5 HIV-1 subtypes and circulating strains in Tanzania

The distribution and dynamics of HIV-1 viral subtype, matched to HIV drug resistant mutations or changing ART regimens over time has not been described in Tanzania, and in many other SSA countries. Previous studies conducted in Tanzania at the beginning of rollout of free ART in Tanzania have reported subtype A1 being the most prevalent, followed by subtype C(32,34,35) and D, CRF-CD and URF have been reported in the most recent studies(32). Nonetheless, these studies were only conducted in urban settings and had only small numbers of participants been evaluated. Therefore it is difficult to generalise the trends of HIV variants-linked to HIV drug resistance mutations or changing ART regimens over time, in individual demographic and population groups.

Likewise, previous studies have evidently demonstrated that certain HIV subtype are more prone to developing mutations. For instance, HIV subtype C is known to be more prone to develop K65R mutation, which confer intermediate to high level resistance to Tenofovir. Thus, it is essential to monitor the emergence and spread of the mutations such as K65R. Thus, molecular epidemiological studies of HIV subtypes linked to long-term clinical and epidemiological data from under-sampled areas such as rural Tanzania, are key component in assessing and addressing the threat of HIV drug resistance.

1.2.3.6 Pre-treatment HIV drug resistance to the dolutegravir co-administered Nucleoside reverse transcriptase inhibitors

The current new first line treatment recommended by the WHO, the integrase inhibitor dolutegravir in a fixed dose combination with tenofovir/lamivudine (TLD). Dolutegravir is known for its high and rapid viral suppression and a high genetic barrier to developing resistance. As such, TLD is predicted to curb the number of new HIV infections and the spread of resistance – leading the way to control of the HIV pandemic.

Most evidence on efficacy of dolutergravir-based regimens comes from randomized controlled trials conducted in high-income countries, with a low background level of HIV-drug resistance. In

resource-limited settings, with limited or absent viral load and HIV drug resistance testing, long delays in the management of uncontrolled viraemia, could facilitate emergence of resistance to the co-administered NRTIs and possibly dolutegravir.

Also, pre-existing HIV resistances to drugs used in combination might lead to dolutergravir functional monotherapy and potential for emergence of dolutergravir resistance(75). European cohorts have shown that, virological efficacy of dolutergravir monotherapy associates with virologic failures and risk of dolutegravir resistance(76). Analysis from the NADIA and DAWNING trial shows that no evident positive effect of inactive NRTI backbone used in combination with dolutegravir; Although observed emerging intermediate -or -high level dolutegravir resistance within the 48 months follow-up raises concern(77,78). Certainly, surveillance of pre-existing resistance to the co-administered NRTIs remains particularly important.

1.2.3.7 Trends of HIV-1 pretreatment HIV drug-resistant mutations

The wider coverage of ART may increase the rates of disorderly use of ART, resulting into increased rates of acquired HIV-DRM, which may in turn be transmitted to individuals who have never been exposed to ART(37). Thus, analysis of time-trend in PDR is essential to monitor the ART regimen and evaluate the effectiveness of the program.

Available data in SSA shows increasing trend of PDR among general adult population, which is mainly driven by NNRTI associated HIV drug resistant mutations (25,26,79). Also, there has been noticeable increase in NRTIs, particularly the Tenofovir associated HIV drug-resistance mutation(26). e.g., a study conducted in two clinics in Nairobi, Kenya in the year 2006 and 2014 respectively, reported a nearly tripled number of cases of K65R mutations(80). The reverse transcriptase (RT) mutation K65R results in a four-fold decrease in TDF susceptibility and is selected by TDF, didanosine (ddI), d4T and Abacavir (ABC)(81).

A pooled analysis of studies on PDR in South Africa shows a relatively stable prevalence of PDR at <5% until 2009, and afterward increased to 11.9% in 2015(26). Similarly, a study involving seven West African and 2 Asian countries, where the prevalence of pre-ART drug-resistance ranged from 9.6% -24.6%(82). On the contrary, data from European HIV cohorts shows a decline or rather stable prevalence of PDR, at <7% (21–23)Examples of such cohorts are United Kingdom (UK) national representative PDR database which show stable or declining prevalence rates of PDR(83,84). Similar trend has been documented by Sallam, et al while studying a population in Iceland between 1996-2012(22), and Machnowska et al, in German with a PDR prevalence declining between 1996-2017(85). The declining prevalence of PDR in recent studies among western population, may be explained by the routinely virologic monitoring, including HIV drug

resistance testing and increasingly usage of ART regimen which have higher genetic barriers, e.g., intergrases. Most European HIV cohorts are characterized with increased genetic diversity of HIV variants, where HIV subtype other the traditionally mostly prevalent, subtype B are becoming increasingly(84,86).

1.2.4 Conceptual framework and concept maps

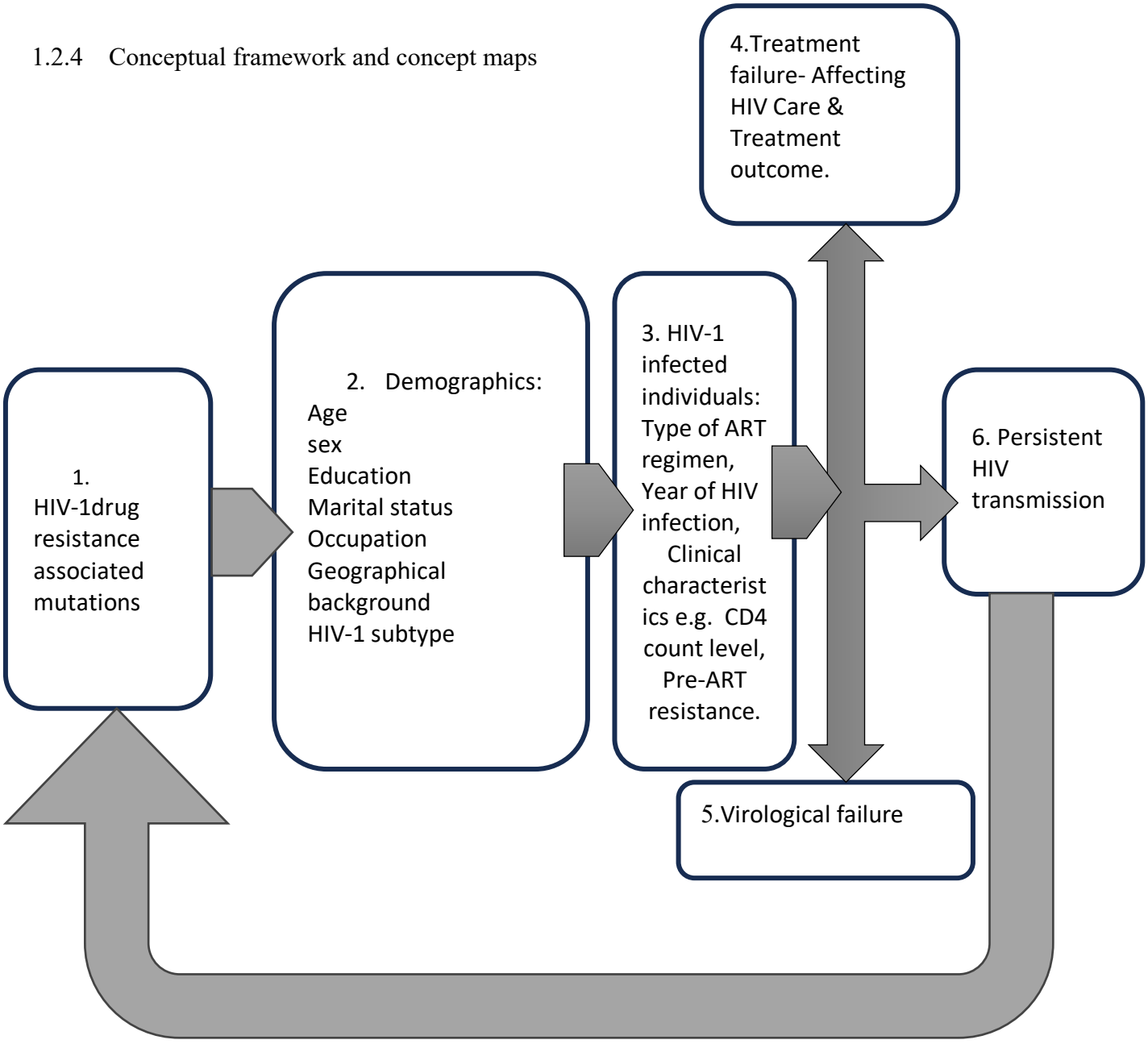


Figure 1.3 Concept map of pre-Antiretroviral Therapy HIV-1 drug resistance and associated factors.

The concept map depicts the conceptual relationship between different exposures and PDR status among HIV infected patients. The concept map show that different demographic factors may be associated with risk of being infected with a drug-resistance HIV virus, e.g. young age. Likewise, year of HIV infection may be associated with the coverage of ART, whereby increased ART access

may increase use at uncontrolled level, thus increase circulation of resistant virus, which may infect an individual who have never been exposed to ART. The concepts map shows the risk of transmission of resistant virus.

1.2.5 Methods for studies of HIV drug resistance prevalence

Temporal analysis of HIV drug resistance associated mutation (HIV-DRM) among newly diagnosed HIV patients, enables the evaluation of HIV diversity overtime, including emergence of new HIV variants, e.g., the circulating recombinant forms (CRFs)(87), understanding the rate of change of burden of HIV drug resistant mutations and emergence of new HIV drug resistant mutations (88). Previous observational studies on molecular dynamics of pre-ART HIV-DRM have employed a variety of methods, including prospective observational methodological approach of newly diagnosed HIV infected patients. On the other hand, retrospective study methods, have been used to generate information by analyzing bio-banked specimen(89), and or analysis of data generated during routine treatment and care(90–92).

Methods for assessing prevalence and incidence of HIV-DRM are mainly quantitative. Quantitative prospective and retrospective methodological approaches have been used to determine the prevalence and incidence of HIV-DRM over-time. Machnowska et al, and Siemieniu et al, employed prospective and retrospective quantitative methods, respectively(23,93). In a prospective cohort study, Machnowska et al, studied the prevalence of transmitted HIV-DRM considering the changing landscape of HIV-1 subtypes in the German HIV-1 Seroconverter Study Cohort between 1996 and 2017, and assessed the proportion of study patients potentially at risk of ART-failure due to these transmitted HIV drug resistant mutations. Elsewhere, in a retrospective analysis Siemieniu et al., documented an increase in non-subtype B, with notable increase in subtype C and CRFS, in southern Alberta, Canada. However, the major limitation of such retrospective studies, is that there is frequently an absence of data on potential confounding factors as the data was recorded in the past. Similarly, it may be difficult to identify an appropriate exposed cohort and an appropriate comparison group.

Data from prospective studies can inform control programs on the rate of emergence of HIV-DRM in programmatic interventions, such as Option B+ which represent a challenge to PMTCT, and emergence of new circulating HIV strains. Prospective methodological approaches allow setting inclusion criteria and may enable to determine if HIV drug resistant mutations are lost during follow-up. Prospective longitudinal studies are expensive and may take long time to accomplish. Conversely, retrospective longitudinal analysis of bio-banked specimen or previously collected data, may provide invaluable information, within short period and at a low cost.

1.3 AIM OF THE PhD WORK

The overall aim of this thesis was to determine the trend and patterns of pre-Antiretroviral therapy HIV-1 drug resistance in Kilombero and Ulanga Antiretroviral Cohort South-western-Tanzania.

1.4 OUTLINES OF THIS PhD THESIS

As an introduction to the thesis, the first two chapters; Chapter 1 include a review of data on PDR, with focus on SSA, that were available before the start of the PhD research (before 2020); and Chapter 2 focus of the study methodology.

In chapter 3, A systematic review and meta-analysis was undertaken to assess the trends in PDR associated mutations in Eastern African countries, during a five-year period (2017-2022).

In chapter 4, A cross-sectional study was undertaken to determining the proportion of recent HIV-1 infection, its predictors, and PDR in recently HIV-1 infected persons in the Kilombero and Ulanga antiretroviral cohort, in rural Tanzania.

In chapter 5, A cross-sectional study was undertaken to measure the change in prevalence of PDR to NRTIs in the KIULARCO during the 15 years after ART rollout in Tanzania.

In chapter 6, A cross-sectional study was undertaken to assess the virological-outcomes at 12 months, and its predictors after initiating DTG-based ART, and prevalence of PDR among patients failing on DTG-based ART.

The final chapter 7, is a summary and general discussion of the main research findings of this thesis, followed by some concluding remarks.

Appendices are included after the references.

Appendix 1 contains a copy of the ethical approval form for conducting my PhD study, **Appendix 2** contains the publication (paper I) in the *Journal of Antimicrobial Chemotherapy*, **Appendix 3** contains the publication (paper IV) in the Journal of *Open Forum Infectious Diseases.* **Appendix 4** contains the publication (paper II) under review in the *Journal of Public Health*.

CHAPTER 2

2.0 Methodology

2.1 Research settings

The study consists of two parts;

Firstly, a systematic review and meta-analysis of primary studies reporting prevalence of pretreatment HIV-1 drug resistance (PDR) in antiretroviral therapy-naïve adults in seven Eastern Africa: Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda (*paper I*).

Secondly, the studies in paper (II-IV) were embedded in the on-going prospective clinic-based observational Kilombero and Ulanga antiretrovirals cohort (KIULARCO) study of Peole living with HIV (PLHIV) in rural Tanzania. KIULARCO includes participants from two districts, namely, Kilombero and Ulanga in Morogoro region, South-Western Tanzania (Figure 4). The longitudinal follow-up of the cohort is conducted within the Tanzanian National AIDS Control Care Programme (NACP) framework. KIULARCO has been described in detail elsewhere(94–99). Briefly, this study was carried out within the Chronic Disease Clinic of Ifakara (CDCI), the HIV Care and Treatment Centre (CTC) of the St. Francis Referral Hospital in Ifakara, Southwestern Tanzania. CDCI is a joint project of the hospital, with the Ifakara Health Institute (IHI), the St. Francis Referral Hospital, the Swiss Tropical and Public Health Institute and the University Hospital Basel, Switzerland. CDCI was among the first rural CTCs in Tanzania started in 2005 to provide HIV care and free ART within the NACP framework. CDCI runs a patient cohort, the KIULARCO described above.

For more detailed information about patients, ethical permits, and statistical analysis, see respective papers.

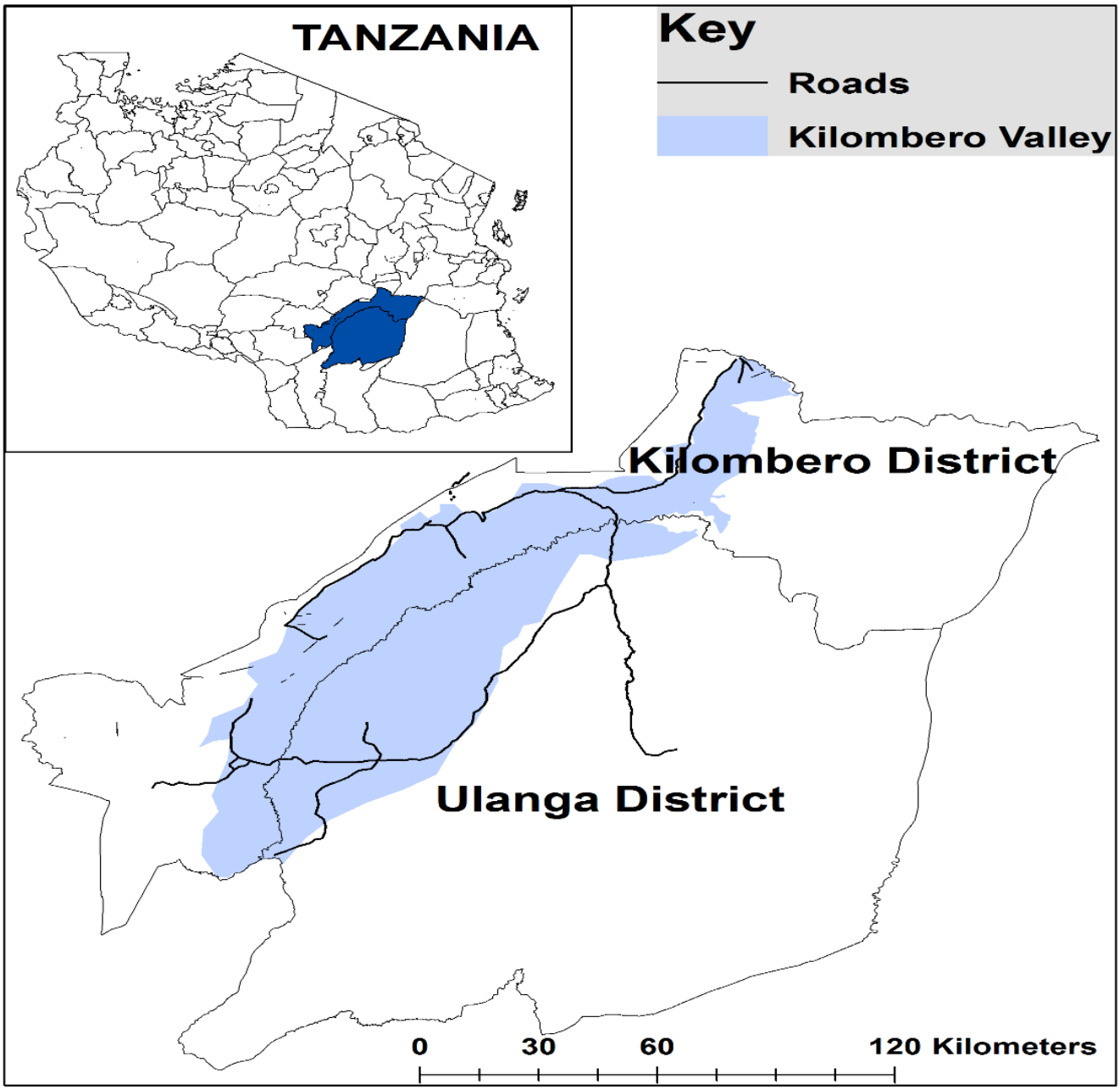


Figure 2.1. Study area for paper II-IV; Kilombero and Ulanga districts in Morogoro region, South-Western Tanzania.

2.2 Study population

In paper I, A Systematic Review and Meta-Analysis of eligible observational studies published between 1 January 2017 and 30 April 2022 about prevalence of PDR among ART-naive PLHIV (>15 years old)(56).

In paper II- IV, studies were be embedded in the KIULARCO of the IHI in Tanzania. KIULARCO is one of the largest long-term patients' cohorts in the East African region, started in 2005(96)(Figure 1). KIULARCO offers a clinical database and a biobank of yearly collected plasma samples collected during routine visits of patients in the past 20 years (have been stored in

-80°C freezers at the IHI laboratory in Ifakara, Tanzania), from more than 12,000 PLHIV within a population of about 600,00 inhabitants and an estimated 30,000 patients living with HIV/AIDS in the area (Figure 2). The longitudinal follow-up of the cohort is conducted within the Tanzanian National AIDS Control Care Programme (NACP) framework(96). Newly HIV-1-diagnosed adult individuals (aged ≥ 15 years) prior to ART initiation, who are enrolled in the KIULARCO were included. Study population detailed descriptions is provided in respective papers.

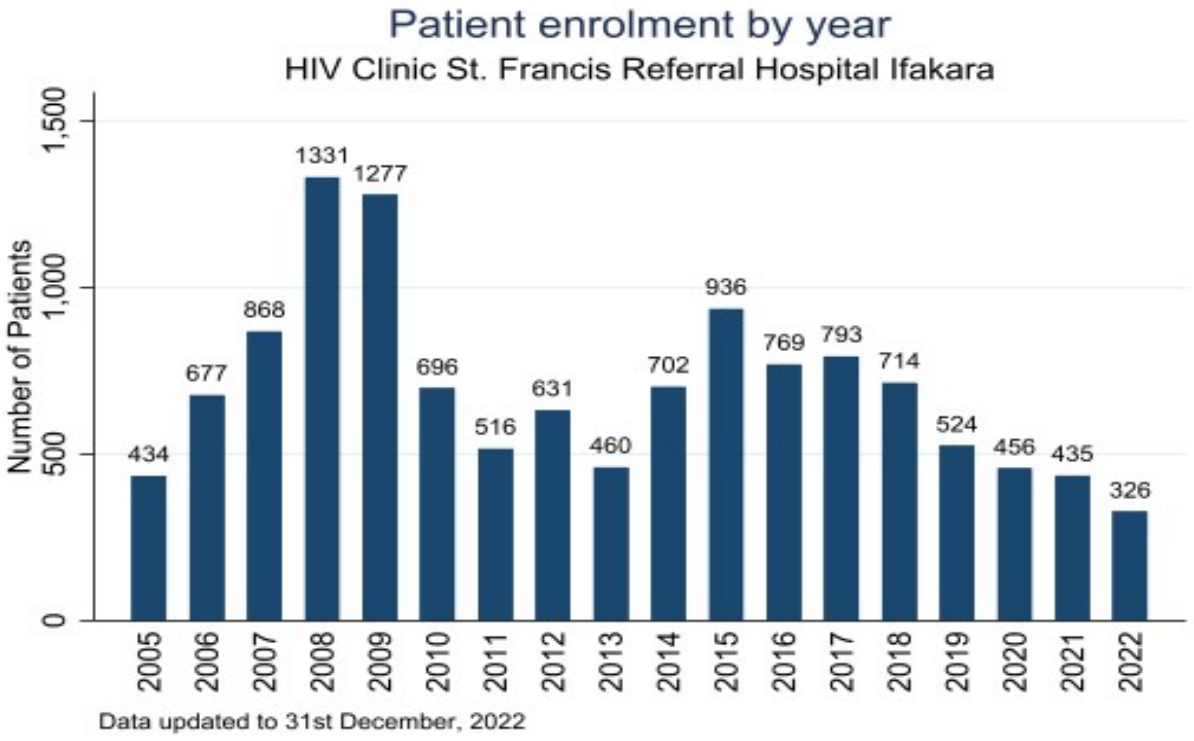


Figure 2.2. Patients' enrolment into the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) by year.

2.3 Sample size estimation

The estimated sample size (In paper III) was calculated based on the expected prevalence of pre-treatment HIV-1 drug resistance of 10%, with prevalence between 3.3% and 5% based on existing published data. Sampling period was, the first five-year period (2005-2009) of the ART program in Tanzania, in the most recent period (2019-2022), in each batch 130 randomly selected samples was included in the study. With this sample, the assumption was to be able to detect the prevalence ≥ 7 %, one sided 5% level of significance, with the power of 80% (Table 1). An addition of 20% for sequencing failure rate to ensure minimal sample size for power is met.

Table 1. Sample size calculations

Established Prevalence	Expected Prevalence	Statistical Power	Calc. Sample Size*
3.3%**	7%	80%	310
		90%	389
	7.5%	80%	252
		90%	315
	10%	80%	122
		90%	149
5%***	7%	80%	1168
		90%	1519
	7.5%	80%	784
		90%	1013
	10%	80%	239
		90%	301

*the caculated sample sizes are calculated with a one-sided test.
**according to the last prevalence study in the KIULARCO cohort in 2009 [14]
***reported transmitted HIVDR prevalence across different sites in Africa [7]

In paper IV, sample size estimation was based on the assuming the rate of VF at 10%, a 95% confidence and 80% statistical power, sample size is 230 patients. Adding 20% sequencing failure rate minimal sample size is 276, rounded-up to 280 total sample size is required.

2.4 Study procedures

In paper I, a systematic review and meta-analysis of pre-treatment HIV-drug resistance (PDR) in antiretroviral therapy-naïve I reviewed all relevant articles, the supervisors independently reviewed 20% of randomly selected articles. Standardized data abstraction form was used to systematically collect data from scientific published articles. Core set of information retrieved from the articles, included bibliographic details, study design, inclusion and exclusion criteria, country and their main demographic, ART exposure history, the year(s) of sample collection, study type, study population, proportion of participants gender, and method for determining pre-treatment HIV-drug resistance (PDR), and reported prevalence of PDR. The systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 18/02/2022 (registration number CRD42022304207).

According to the KIULARCO protocol, patients who signed informed consent have blood samples drawn at routine clinic visits, prior to ART initiation and every 12 months thereafter. Plasma is cryopreserved at -80^{0}C for research purposes. In brief, data on demographics, clinical presentation, therapy outcome and adherence of ART and treatment outcome are being prospectively captured within an electronic database over the years. In paper II-IV, blood plasma samples of eligible patients were identified from the KIULARCO biobank, unfrozen and tested as detailed in respective papers.

2.5 Laboratory analysis

2.5.1 RNA Extraction

Briefly, RNA was extracted from 150 µl of plasma using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol(100).

2.5.2 Reverse Transcription and PCR

RNA extracts were retrotranscribed and amplified using the HIV-1 Genotyping Kit Amplification Module (Applied Biosystems™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol(101). The PCR products were visualized on a gel visualization system (To confirm that the 2892 bp length of the pol gene) after being run in a 1% agarose gel at 130 V.

2.5.3 HIV Genotypic resistance testing

In Paper II- IV, Sequencing was conducted to determine the presence of HIV drug resistance-associated mutations in the *pol* gene.

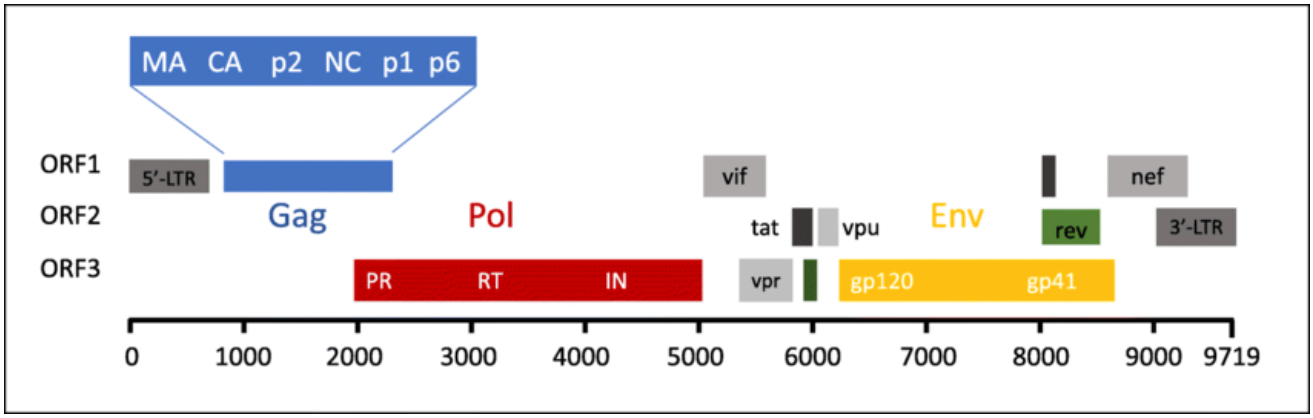


Figure 2.3 Schematic presentation of HIV-1 viral genome

Sequencing was performed using a validated in-house PCR protocol to determine the HIV-1 drug resistance-associated mutations for Reverse Transcriptase (RT), Protease (PR) and *Integrase* Inhibitor (INSTI) – mutations(42,95,99,102) A direct sequencing reaction was done using 6 overlapping primers (Table 1).

Table 2. Sequencing primers information

#	Primer Name	Sequence
§1	F-2150	AGCCAACAGCCCCACCAG
2	F-2232	GGAACTGTATCCTTTAGCTTCCC
3	F-2610	GTTAAACAATGGCCATTGACAGAAGAAA
4	F-3007	GGAAAGGATCACCAGCAATATTCCA
5	R-2605	GGGCCATCCATTCCTGG
6	R-3010	CCATCCCTGTGGAAGCACATTG
7	R-3541	TTCTGTATTTCTGCTATTAAGTCTTTTGATGGGTCA

2.6 Bioinformatics analysis

2.6.1 Sequence reads assembly, analysis, and annotation

In Paper II-IV sequence reads assembly and analysis was performed using BioEdit 7.2-program(103), each sequence was manually inspected to ensure sequence quality, and finally annotated according to the Stanford HIV Drug Resistance Database, current version (https://hivdb.stanford.edu/)(104)

2.6.2 Drug Resistance analysis

The assessment of HIV drug resistance associated mutations were interpreted according to the Stanford University's HIV Drug Resistance Database (HIVdb) Program version 9.2 (http://hivdb.stanford.edu). HIV drug resistant mutations conferring low, intermediate, or high-level resistance were considered. The reported protease and reverse transcriptase sequences are deposited in GenBank accession numbers are provided in respective papers.

2.7 Ethical approval

This study received ethics approval from the University of the Witwatersrand- Human Research (Medical) Ethics Committee. Ethical approval for the KIULARCO cohort has been obtained from

Ifakara Health Institute Review Board IHI/IRB/No: 16 – 2006 and the National Health Research Committee of the National Institute of Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378). Written informed consent for KIULARCO is sought from all participants ≥18years, and for children and adolescents aged <18 years from caregivers.

PART 2

EMPIRICAL CHAPTERS

CHAPTER 3

A systematic review and meta-analysis to assess the levels of pretreatment HIV-1 drug resistance in antiretroviral therapy- naïve in Eastern Africa (2017-2022).

Introduction

This chapter describes the prevalence of pre-treatment HIV-1 drug resistance among ART-naïve adult general population in Eastern African countries, within a systematic review and meta-analysis, during a five-year period (2017-2022). Results are presented in research ***paper I***, entitled "Pre-Treatment HIV-1 Drug Resistance in Antiretroviral Therapy-Naïve Adults in Eastern Africa: A Systematic Review and Meta-Analysis", this has been published as an original article in the *Journal of Antimicrobial Chemotherapy.*

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Journal of Antimicrobial Chemotherapy

Pre-treatment HIV-1 drug resistance in antiretroviral therapy-naive adults in Eastern Africa: a systematic review and meta-analysis

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Background: Pre-treatment HIV drug resistance (PDR) may result in increased risk of virological failure and acquisition of new resistance mutations. With recently increasing ART coverage and periodic modifications of the guidelines for HIV treatment, there is a need for an updated systematic review to assess the levels of the PDR among adults newly initiating ART in Eastern Africa.

Methods: We conducted a systematic search for studies published between 1 January 2017 and 30 April 2022 in the MEDLINE Complete and CINAHL Complete, searched simultaneously using EBSCOhost, and Web of Science. To determine the overall PDR prevalence estimates, we extracted data from eligible articles and analysed prevalence estimates using Stata 14.2.

Results: A total of 22 eligible observation studies were selected. The studies included a total of 5852 ART-naive people living with HIV. The overall pooled prevalence of PDR was 10.0% (95% CI: 7.9%–12.0%, $I^2 = 88.9\%$) and 9.4% (95% CI: 7.0%–11.9%, $I^2 = 90.4\%$) for NNRTIs, 2.6% (95% CI: 1.8%–3.4%, $I^2 = 69.2\%$) for NRTIs and 0.7% (95% CI: 0.3%–1.2%, $I^2 = 29.0\%$) for PIs. No major integrase strand transfer inhibitors (INSTI)-related mutations were identified.

Conclusions: We observed a moderate overall PDR prevalence among new ART initiators in this study. PDR to NNRTIs is more prevalent, underscoring the importance of the current WHO recommendation for replacement of NNRTIs by INSTIs. PDR to NRTIs was low but notable, which warrants continuous surveillance of pre-existing resistance to the dolutegravir co-administered NRTI in Eastern Africa.

Background

The Antiretroviral Therapy programme in countries in Eastern Africa: has been running for more than 15 years. Over these years, the guidelines for HIV care and treatment including ART initiation have undergone periodic modifications based on the WHO recommendations(105). Also, there has been remarkable increase in the number of people living with HIV (PLHIV) on ART to date. For example, globally the ART coverage increased from 24% in 2010 to 65% in 2018. Also 73% of PLHIV East and Southern Africa were on ART by the end of 2019(106). The scale-up of ART rollout may coincide with changes in the prevalence of pre-treatment HIV drug-resistance (PDR)(46). This is mainly due to the existing gaps in HIV preventions interventions, care, and *treatment* cascade, also potency of antiretroviral regimen and patient factors associated with emergence and spread of HIV drug-resistance mutations, such as inadequate adherence to ART(107,108)

Major improvement in antiretroviral medication in terms of safety and pill count have been achieved over the past 15 years of ART rollout worldwide and in low- and middle-income countries (LMIC) – e.g., replacement of NRTI with long-term toxicity like stavudine (d4T) and didanosine (ddI) to more favourable NRTIs like tenofovir disoproxil fumarate (TDF). Until recently, most LMIC were using 2 NRTI and 1 NNRTI as first line treatment according to the recommendations by the WHO. The preferred regimen was tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) or emtricitabine (FTC) + efavirenz (EFV) as a fixed dose combination. Dolutegravir (DTG), one of the first developed integrase inhibitors for treatment of HIV infection became available to LMIC by the end of 2017, as the generic fixed dose combination tenofovir/lamivudine/dolutegravir (TLD). Since then, TLD has been rolled out in many countries in sub-Ssaharan Africa (SSA). It is worth noting that, ART regimens may impact differently on the prevalence rates of PDR. This is mainly because ART regimens differ in their genetic barrier to developing resistance, pharmacokinetic-profile, and tolerability

PDR may compromise ART efficacy resulting in increased risk of virological failure, acquisition of new HIV drug resistant mutations and need of ART switch(109,110). Previous review study using available data in eastern Africa, include studies published until 2016(11). However, this was a period during which ART was not widely available, also ART represented in the current WHO guidelines, such as Tenofovir-based ART was still relatively new in many LMIC. Thus, with increasing ART coverage and periodic modifications of the guidelines for HIV care and treatment including ART initiation, there is the need for an updated systematic review to assess the levels of the PDR associated mutations among adults newly initiating ART in eastern Africa countries from 2017 until 2022.

Methods

Study design

We conducted a systematic search for studies published during January 1,2017 to April 30, 2022, about prevalence of PDR among ART-naïve PLHIV (>15 years). The data had to be collected in one or several of the countries of eastern Africa (Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda). The systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 18/02/2022 (registration number CRD42022304207).

Search Strategy and Selection Criteria

An electronic search was conducted from three databases: MEDLINE Complete and CINAHL Complete searched simultaneously using EBSCOhost and Web of Science. The search advanced search approach was carried out according to PRISMA guidelines (*Figure 1*)(111), using the following search terms: (HIV OR 'Human Immunodeficiency Viruses) AND ('Pre- Antiretroviral Therapy' OR 'Pre-Antiretroviral OR 'Pre-treatment' OR 'Pretreatment' OR antiretroviral OR ART OR drug OR antiviral OR ARV OR ART OR 'Nucleoside RT Inhibitor' OR NRTI OR NNRTI OR INSTI OR 'integrase inhibitors' OR 'protease inhibitors' OR 'non-nucleoside reverse transcriptase inhibitor' OR 'nucleoside reverse transcriptase inhibitor') AND ('HIV-1 Drug Resistance' OR 'transmitted HIV drug resistance' OR 'drug resistance' OR 'Resistance Mutations') AND (Burundi OR Ethiopia OR Kenya OR Malawi OR Rwanda OR Mozambique OR Tanzania OR Uganda OR eastern Africa). We screened studies for eligibility based on title and abstract. Eligible studies had to report original data about the proportion of PDR in a sample of PLHIV (>15 years), with no prior history of ART exposure. Also, studies had be published in English. Publications without primary data such as review articles and letters to editors were excluded. We included quantitative studies involving HIV-1 drug resistance mutations for Reverse Transcriptase (RT), Protease (PR) and, integrase strand transfer inhibitors *(INSTI)*- mutations.

Study Selection process and Extraction

Standardized data abstraction form was used to systematically collect data from journal articles. AJN and JMF independently screened articles for selection. This involved an assessment of articles based on titles and abstract. In the case of insufficient information in the title and abstract, the full text of the specific article was retrieved and assessed. JK was involved in case the two reviewers failed to reach a consensus to include the article. We recorded the source, year of sampling, country origin, gender proportion, study period, number of study participants, prevalence of PDR to NRTI, NNRTI, PI, INSTI and HIV subtypes.

Data Analysis

The statistical analysis was done using STATA 14.2. We assessed heterogeneity using I^2 statistics, where I^2> 50% heterogeneity was high. Studies quality assessment was done using JBI's critical appraisal tools. (https://jbi.global/critical-appraisal-tools). A funnel plot and Egger's test were applied to detect any publication bias. A *p*-value of <0.05 was considered statistically significant.

Results

Characteristics of Included studies

We identified 889 potential research articles from three databases according to our search strategy. A total of 22 observational studies were selected, representing a total of 5852 PLHIV with no prior history of ART exposure (Figure 1), with available resistance data derived from RT and/or PR and/or Integrase sequences in seven eastern African countries; Ethiopia, Kenya, Malawi, Rwanda, Mozambique, Tanzania, and Uganda (Table 1). All the research articles included in this study involved heterosexual patients' population, expect one research article that included men who have sex with men (MSM)(112).

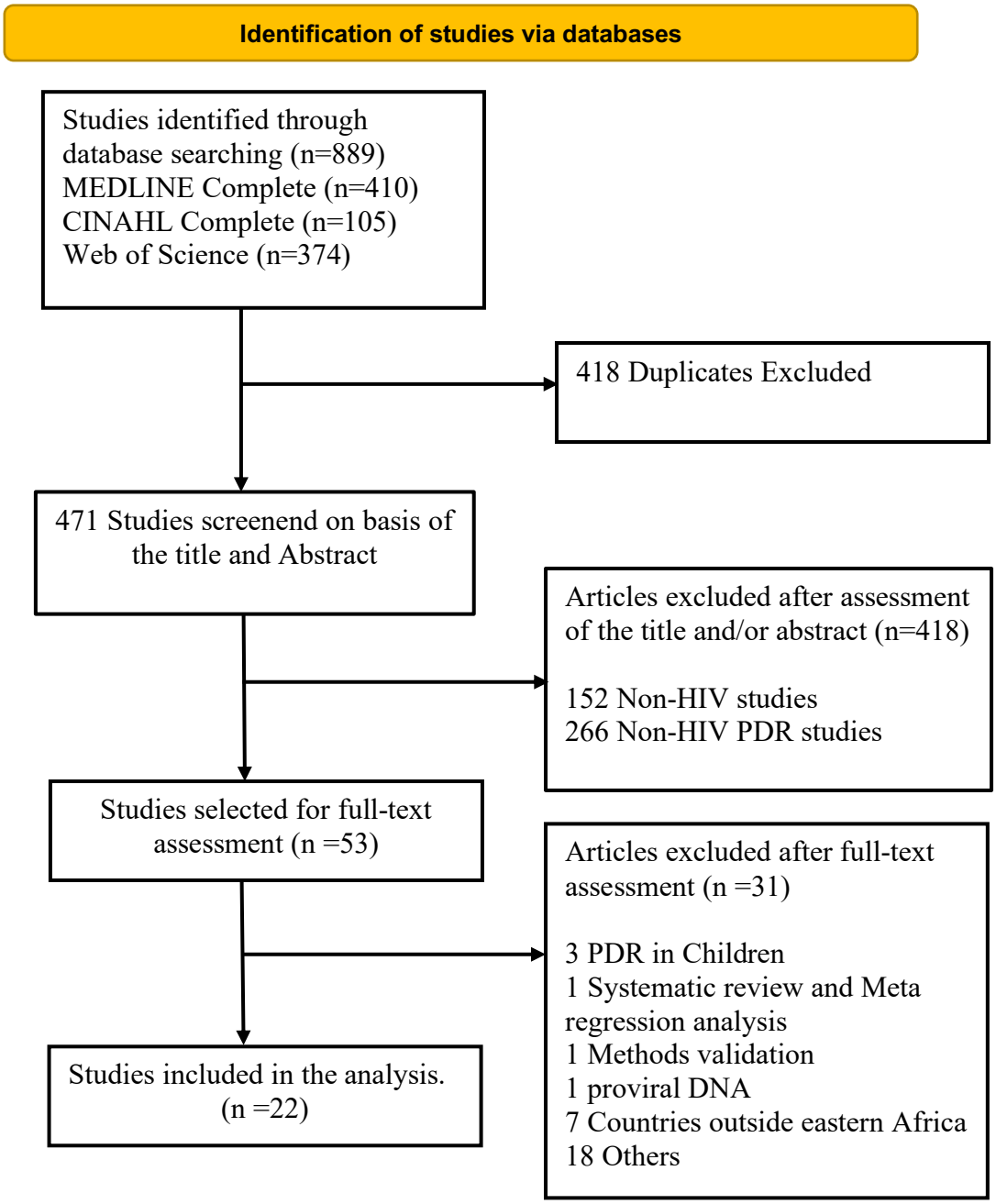


FIGURE 1 | Flow diagram of search results and screening

Table 1: The characteristics of included datasets with reverse transcriptase and/or protease sequences from ART-naïve adults.

AUTHOR, PUBLICATION YEAR, REFERENCE	Year of Sampling	Country Origin	Study Population	Females (%)	Study Design	A Point Prevalence Estimate (%)				GRT-Method	HIVDRM Identification-Method
						Any	NNRTI	NRTI	PI		
HASSAN *ET AL*., (2019)(113)	2008	Kenya	50	72%	Retrospective hospital-based cohort study	24	6.0	8.0	12	NGS	*HIVdb*
TELELE *ET AL*., (2018)(114)	2009-2011	Ethiopia	461	58%	Prospective hospital-based cohort study	3.9	1.5	2.0	0.4	PBSS	HIVdb
HASSAN *ET AL*., (2018)(112)	2005-2017	Kenya	97	0 %	Prospective population-based cohort study	8.2	7.2	2.1	0.0	PBSS	HIVdb
CROWELL *ET AL*., (2020)(115)	2013- 2019	Tanzania	154	59.6%	Prospective hospital-based cohort study	8.0	6 .0	3.0	1.0	PBSS	HIVdb
	2013-2019	Kenya	254	59.6%	Prospective hospital-based cohort study	7.0	7.0	2.0	0.0		
	2013-2019	Uganda	291	59.6%	Prospective hospital-based cohort study	14	10.0	5.0	1.0		
BARABONA *ET AL*., (2019)(29)	2017	Tanzania	47	75.7%	Cross-sectional hospital-based study	29.8	25.5	8.5	0.0	PBSS	HIVdb
RUDOVICK *ET AL*., (2018)(30)	2013-2015	Tanzania	235	75.7%	Cross-sectional hospital-	4.7	3.0	1.7	0.0	PBSS	HIVdb

					based study						
VON BRAUN *ET AL*., (2018)(116)	2015	Uganda	152	62.8%	Cross-sectional hospital-based study	5.9	5.3	3.3	0.0	PBSS	HIVdb
RUTSTEIN *ET AL*., (2019)(117)	2012–2014	Malawi	45	39%	Prospective hospital-based cohort study	24.4	20	6.7	0.0	PBSS	HIVdb
SILVERMAN *ET AL*., (2017)(118)	2013–2014	Kenya	815	65.1%	Cross-sectional hospital-based study	9.4	9	0.7	0.0	PBSS	OLA
MILNE *ET AL*., (2020)(119)	2006-2010	Kenya	97	100%	Prospective hospital-based cohort study	6.2	.	.	.	PBSS	HIVdb
ONYWERA *ET AL*., (2017)(74)	2012	Kenya	87	72%	Cross-sectional population-based study	9.2	6.9	4.6	1.2	PBSS	HIVdb and WHO 2009 Mutation list
CARNIMEO *ET AL*., (2021)(120)	2017–2018	Mozambique	196	58.7%	Cross-sectional hospital-based survey	16.8	14.8	4.6	0.5	PBSS	HIVdb
			258	66.7%	Cross-sectional hospital-based survey	26.4	25.6	4.3	0.4	PBSS	
KIROS *ET AL*., (2020)(121)	2018	Ethiopia	51	54.9%	Cross-sectional hospital-based study	9.8	7.8	2.0	0.0	PBSS	*HIVdb and IAS-USA 2019 Mutation list*
WATERA *ET AL*., (2021)(122)	2016	Uganda	335	61.5%*	Cross-sectional survey	18.0	13.7	6.0	1.5	PBSS	HIVbd

BECK *ET AL*., (2020)(123)	2006	Kenya	303	66.7%	Cross-sectional hospital-based cohort analysis	2.6	.	.	.	PBSS	OLA
	2010	Kenya	97	100%	Cross-sectional hospital-based cohort analysis	6.2	.	.	.		
	2013–2014	Kenya	679	65.7%	Cross-sectional hospital-based cohort analysis	4.4	.	.	.		
MCCLUSKEY *ET AL*., (2019)(124)	2005-2013.	Uganda	738	69%	Cross-sectional hospital-based cohort analysis	3.5	2.8	1.0	0.0	PBSS	HIVbd
NEUHANN *ET AL*, (2020)(125)	2014-2015	Malawi	197	61.9%	Cross-sectional hospital-based cohort analysis	14.2	13.7	0.5	0.0	NGS	HIVdb
ZHOU *ET AL*., (2018)(126)	2008	Malawi	20	.	Cross-sectional hospital-based cohort analysis	.	9	2	0	NGS	.
ARIMIDE *ET AL*., (2018)(127)	2011–2013	Ethiopia	67	87%	Cross-sectional hospital-based survey	6.0	6.0	0.0	0.0	PBSS	HIVdb
NDASHIMYE *ET AL*., (2018)(128)	2007-2011	Uganda	87	100%	Retrospective hospital-	8.7	8.7	.	.	PBSS	HIVdb

					based cohort study						
UMVILIGIHOZO *ET AL*., (2021)(129)	2005-2011	Rwanda	21	.	Prospective hospital-based cohort study	19	19	.	.	PacBio RSII	HIVdb
SANGEDA *ET AL*., (2019)(52)	2010	Tanzania	18	.	Prospective hospital-based cohort study	0.0	0.0	0.0	0.0	PBSS	HIVdb

HIVdb, HIV Drug Resistance Database; **IAS-USA**, International *AIDS Society-USA; NGS, Next Generation Sequencing; PBSS-Population based Sanger Sequencing*; data not analysed or not provided; **OLA**, Oligonucleotide Ligation Assay; **HIVDRM**, HIV drug resistance mutation; **GRT**, Genotypic Resistance Testing.

The Overall Prevalence of Pretreatment Drug Resistance-Associated Mutations

The overall prevalence of PDR was 10.0% (95% CI: 7.9%–12.0%, I^2 =88.9%) among 22 studies (*Figure 2*). Sub-analysis by country shows that the prevalence of PDR in Ethiopia 4.6% (95% CI: 2.4%–6.9%, I^2 =12.0%, in 3 studies), in Kenya 7.0% (95% CI: 4.6%–9.3%, I^2=79.6%, in 7 studies), in Malawi 17.6% (95% CI: 8.2%–27.1%, I^2 =54.9%, in two studies), in Mozambique 21.6% (95% CI: 12.2%–30.9%, I^2 =83.8%, in two studies), in Rwanda 19.0% (95% CI: 2.3%–35.8%, in one study), in Tanzania 10.8% (95% CI: 3.0%–18.6%, I^2 =86.0%, in three studies) and in Uganda 10.0% (95% CI: 4.1%–16.0%, I^2 =93.6%, in five studies). HIV-1 subtypes data is reported in 14(63.6%) of the selected studies; the presence of HIV-1 subtype in Ethiopia, Malawi and Mozambique are predominantly subtype C; in Kenya subtype A and D; in Tanzania subtype A, C and D, whereas in Uganda subtype A and D are prevailing.

Publication bias, as examined by the funnel plot and Egger's test, showed no publication bias in this analysis.

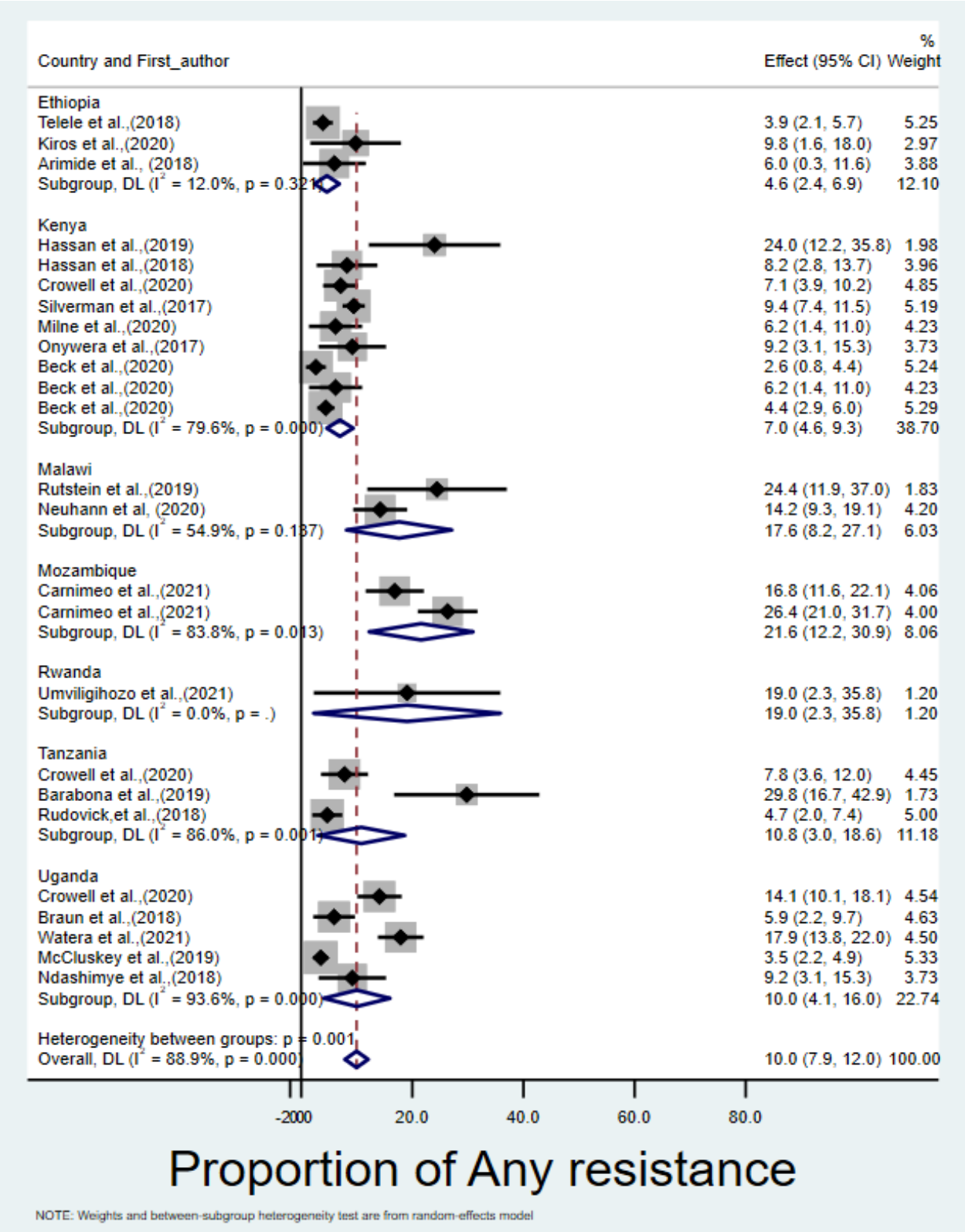


FIGURE 2 | Overall prevalence of PDR. Note: the red dotted line indicates the overall prevalence of PDR.

PDR of RT inhibitors

The prevalence of mutations conferring resistance to NNRTI was 9.4% (95% CI: 7.0%–11.9%, I^2=90.4%) in 21 of the included studies (*Figure 3*). NNRTI resistance reached highest level among different HIV-drugs categories. The prevalence of PDR to NRTI is 2.6% (95% CI: 1.8%–3.4%, I^2=69.2%, in 18 studies (*Figure 4*). Nonetheless, prevalence of PDR to NRTI above 5% have been reported in several countries, including 8.5% in Tanzania(29); 5.2%-6% in Uganda(122) and 6.7% in Malawi(117). Also, population with a high risk of PDR to NRTI are young adults (2.9% in a study from Uganda)(130), and people injecting drugs (15.5% in a study from Kenya)(43). *Supplementary Table S1*.

PDR of PIs

Overall, PI resistance was re-assuring low, 0.7% (95% CI: 0.3%–1.2%, I^2=29.0%) in 8 studies (*Figure 5*). High levels of PDR to PI were reported in studies detecting HIV drug resistant mutations using Next Generation Sequencing (NGS). For-example, PDR to PI of 12%and PDR to PI of 15% was documented by studies using NGS in in Kenya and Malawi respectively, whereas analysis of same samples in the later study by consensus sequencing no mutation associated with PI resistance was detected(113,126)

PDR of INSTIs

We identified four studies that examined HIV-1 INSTI associated resistance in patients prior to ART initiation, which found no participants had mutations associated with high levels of INSTI resistance.

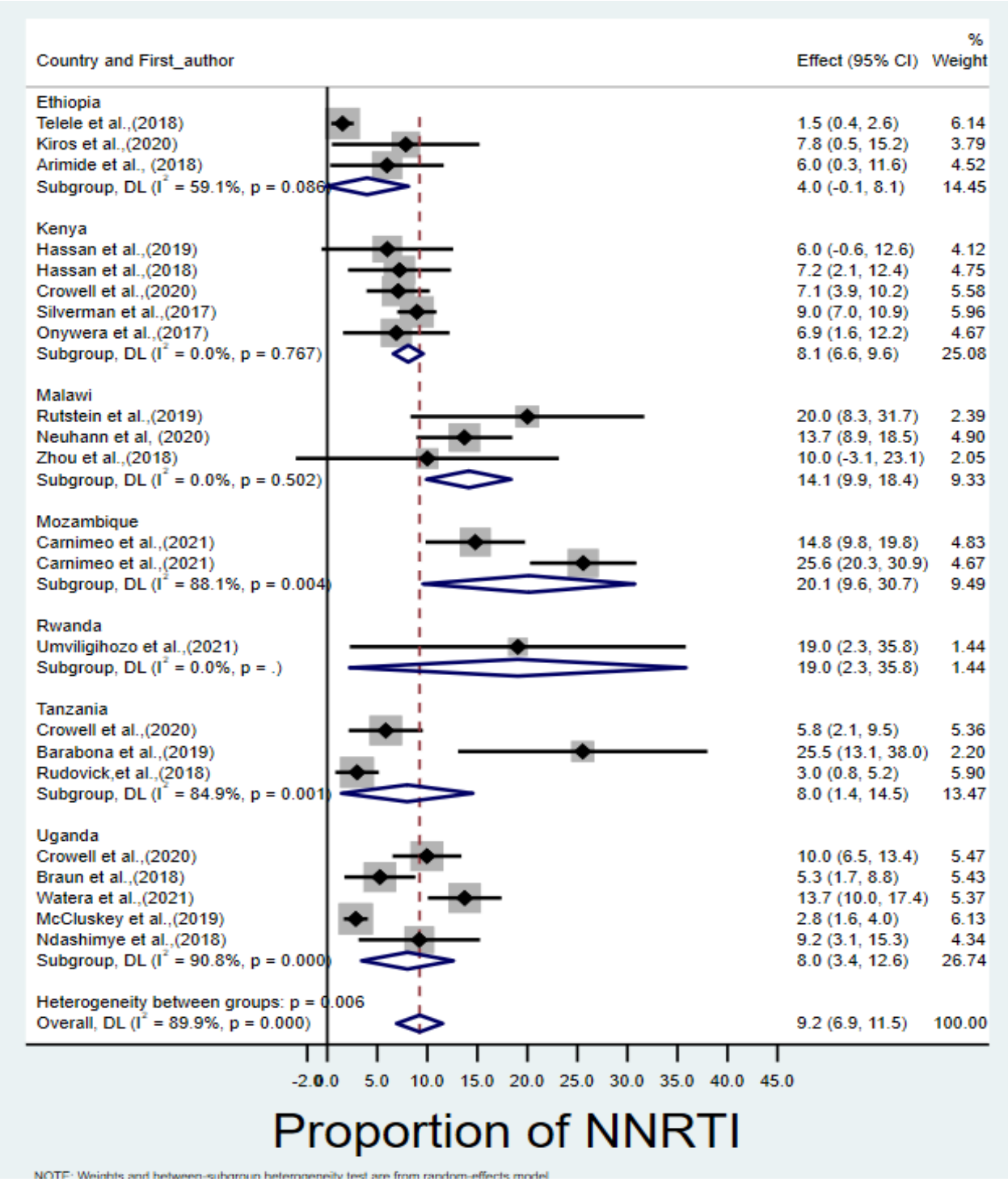


FIGURE 3 | Prevalence of PDR to Non-nucleoside reverse transcriptase inhibitor

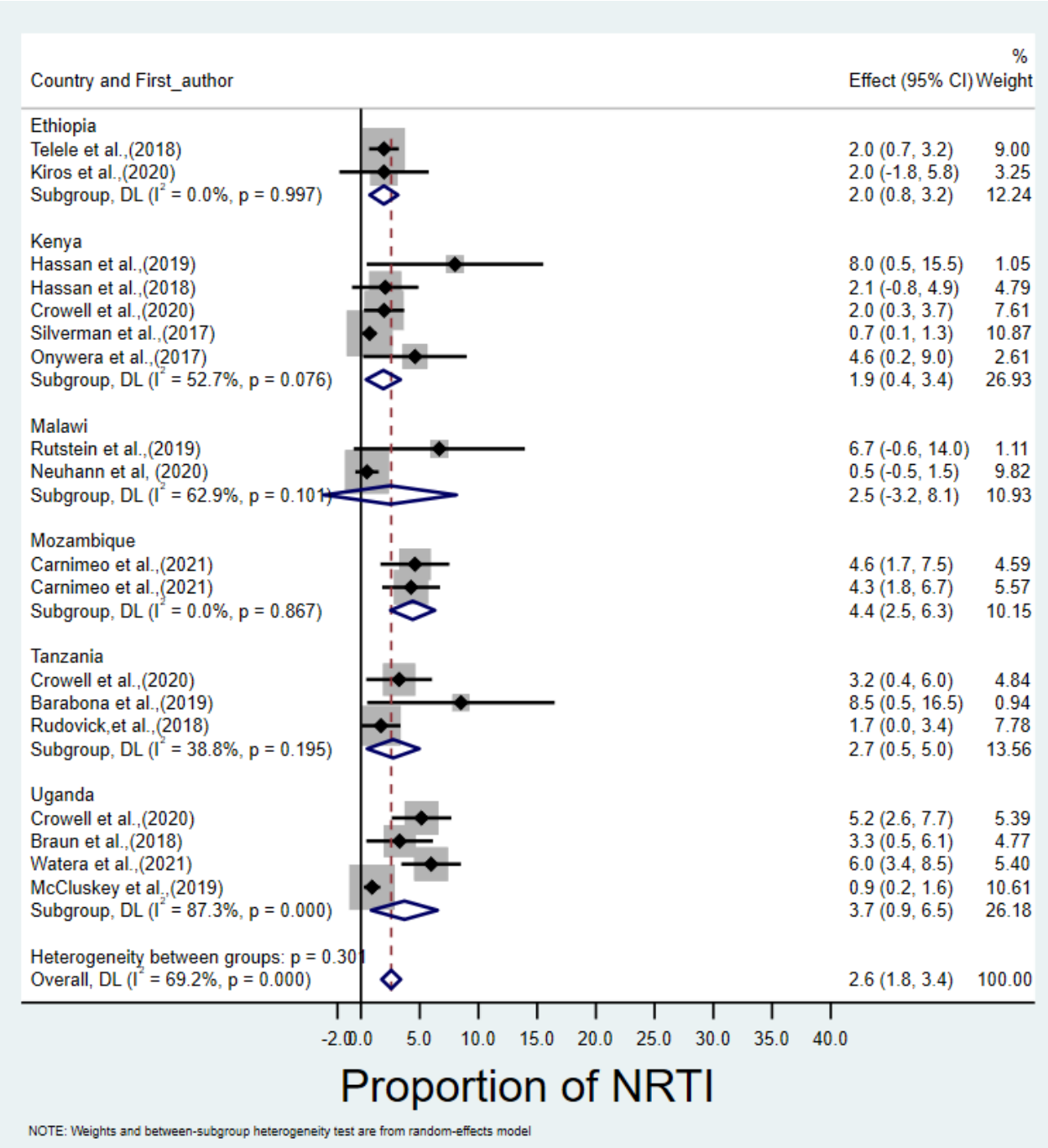


FIGURE 4 | Prevalence of PDR to nucleoside reverse transcriptase inhibitor

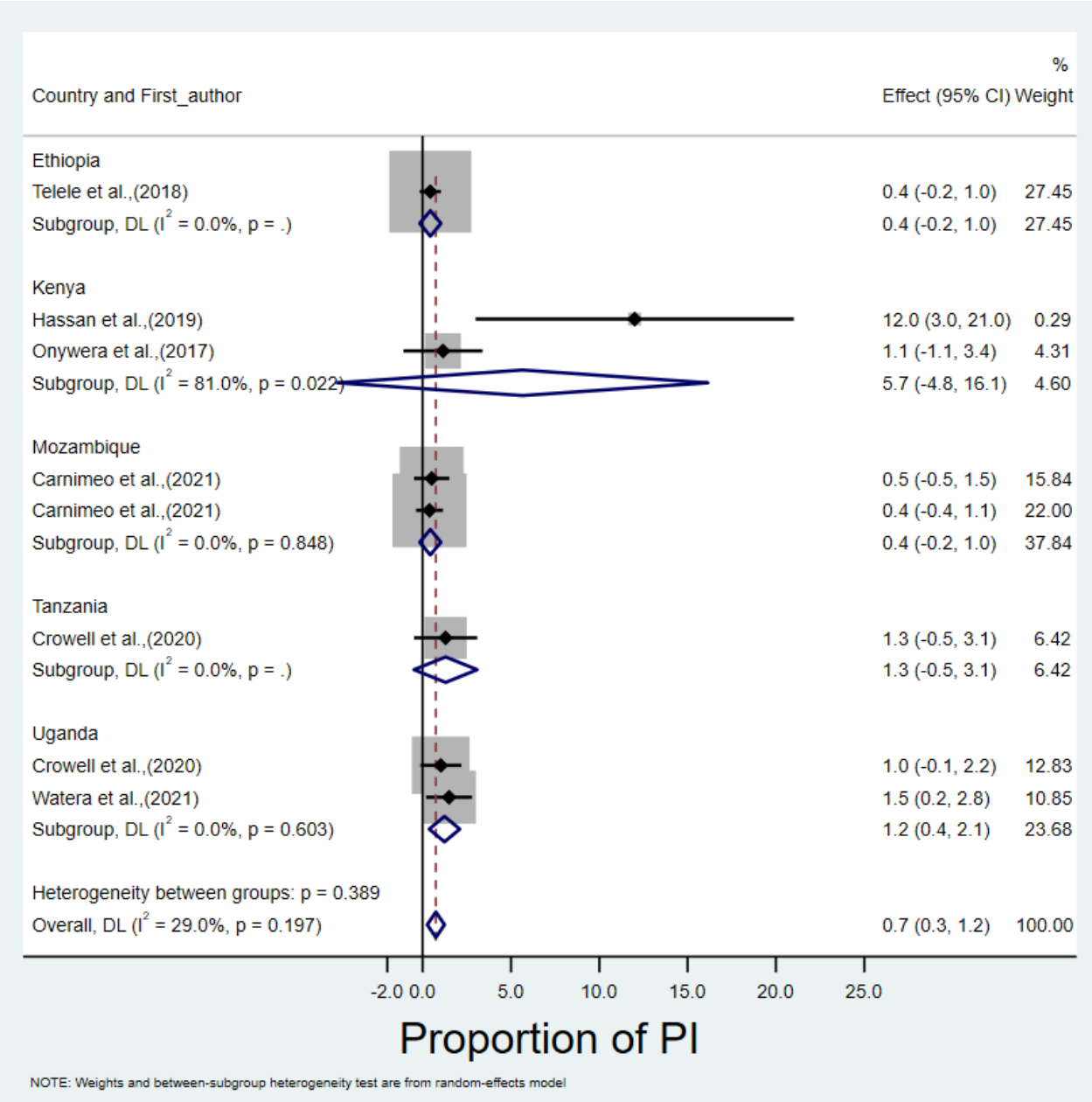


FIGURE 5 | Prevalence of PDR to protease inhibitor

Discussion

In this study, we investigated the overall prevalence of PDR in ART naïve adults living with HIV, in seven eastern African countries for over a period of five years. We observed an overall PDR prevalence of 10.0% (95% CI: 7.9%–12.0%); Whereas previous review study using available data in eastern Africa published until 2016, reported overall PDR prevalence estimates of 8.7%(11). Like our finding PDR was highest to the NNRTI drug class, particularly in more recent studies across the region, which underscores the importance of the current WHO recommendation, to replace NNRTI within the fixed dose combination by dolutegravir for HIV treatment, which has now been introduced in many SSA countries and other LMIC.

Good rates of virological suppression among patients with virological failure on NNRTI based first line ART- possibly as results of suboptimal adherence, have been observed following switching to dolutegravir- based ART(77). Conversely, analysis from the ADVANCE trial indicates that PDR to NNRTI is associated with lower viral suppression rates in patients initiating the dolutegravir based ART(131). Even though, the mechanism by which NNRTI-associated mutations affects dolutegravir-based therapy is still unclear, the widely spread of NNRTI-associated mutations among newly ART initiators in low- and middle-income countries raises concern over the long-term efficacy of dolutegravir-based therapies in resources limited settings, where access to viral load and HIV drug-resistance testing is still limited, and long delays in the management of uncontrolled viremia have been documented.

We observed low however notable prevalence of PDR to NRTI, which may pose risk for the currently preferred first-line ART regimen for HIV treatment in LMIC, which is the fixed dose combination of dolutegravir with tenofovir/lamivudine. HIV drug resistant mutations to NRTI may lead to de facto dolutegravir monotherapy with a risk of acquisition of dolutegravir resistance. Existing evidence, such as from the NADIA and DAWNING trial shows that no evident positive effect of inactive NRTI backbone used in combination with dolutegravir; Although observed emerging intermediate -or -high level dolutegravir resistance within the 48 months follow-up raises concern(77,78). Similarly, available data mainly from randomized clinical trials (RCT) have shown, that virologic efficacy of dolutegravir monotherapy has a sub-optimal potency, with virologic failures increasing significantly over time (37,38,127–129). Hence, there is need for continuous surveillance of pre-existing resistance to the co-administered NRTI. Of-note, prevalence of PDR to NRTI was slightly higher in studies reporting PDR in populations at high risk of HIV transmission, e.g., young-adults and people with intravenous drug use compared to the general adults' population(43,124,130). This observation warrants continuous monitoring of PDR to NRTI in populations at high risk of HIV transmission.

The overall PI resistance rate was re-assuring low, however notable PDR to PI were reported in studies detecting HIV drug resistant mutations using NGS(113,119), which underscores the importance of more sensitive technologies of PDR surveillance, as the minority variants are transmissible and associated with virologic failure(114) Surprisingly, the M46I/L mutation was detected in 3(7%) of the 45 f in Tanzania in 2005 (Supplementary *Table S1*), during the time ART, including PI was not widely available. The observed PI associated HIV drug resistant mutations are likely to be results of naturally occurring polymorphism, rather than transmitted resistance, though PDR or acquired resistance because of undisclosed pre-ART exposure cannot be ruled out completely(49). PDR prevalence data to HIV-1 INSTI in eastern Africa, as in other LMIC is rare(132).

In this study, Intergrase mutations were not found. However, as DTG is relatively new and is being widely rolled-out in eastern Africa, continuous surveillance studies are important to monitor emergence of resistance to DTG at the population level(124,132)

The current analysis extends the sampling period of currently available meta-analysis evidence to 2019(11). Nonetheless, our study has limitations. The variability of methods applied for PDR detection in different studies, may have resulted in underestimation of PDR prevalence rates. Many of the studies included used Sanger based sequencing (PBSS), which is limited to the presence of abundant HIV quasispecies of least 20%. However, HIV variants thresholds as low as 5% are common(114,126). Notably, in the absence of selective drug pressure, other mutations such M184V may quickly disappear from the dominant quasispecies(133). Thus, HIV variants existing at very low levels, may go undetected, especially in patients who have never been exposed to ART. Similarly, the use of different algorithms for HIV drug resistant mutations interpretation among selected studies e.g., HIV drug resistance based on the Stanford HIV drug resistance algorithm, and International Antiviral Society-(IAS)-USA mutations list, which might have resulted in the inclusion of mutation signatures that are excluded in the other algorithm(133). Even though we included studies that reported PDR in ART-naïve, we cannot completely rule out the undisclosed previous exposure to ART. PDR prevalence rates are generally higher in the population with prior exposure to ART as compared to those who have never been exposed to ART(11,120). A small proportion of included studies screened patients for recent HIV infection. Higher levels of PDR may be observed when targeting the acutely/recently HIV infected population, as documented in studies in Malawi and Rwanda(117,129). The possibility is that drug-resistant HIV may revert to wild-type or may be overgrown by the wild viral population(134).

Conclusion

We observed a moderate PDR prevalence among ART-naïve patients in this study. Resistance to NNRTI appears to be more prevalent compared to other ART classes, particularly in more recent studies across the region; Thus, underscoring the importance of the current WHO guideline recommending replacement of NNRTI by the integrase inhibitor dolutegravir, as a fixed combination with tenofovir disoproxil fumarate and lamivudine, as preferred first-line ART regimen. Similarly, the observed PDR to NRTI warrants continuous surveillance of pre-existing resistance to the backbone of the dolutegravir-based regimen in LMIC.

Acknowledgements

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Transparency declarations

None to declare.

Authors' contributions

AJN conceptualized the study, performed the literature search, data extraction, data analysis, and wrote the first draft of the manuscript. JFM participated in the conceptualization of the study, aided in the literature search, data review and data analysis, MW and JK participated in the conceptualization of the study, interpretation of the findings and the writing of the final paper. All authors reviewed and approved the final version of the paper.

Supplementary Data

Table S1. Characteristics of datasets with Reverse Transcriptase (RT)/AND OR Protease (PR) sequences of ART-naïve adults in eight Eastern Africa Countries (2005-2022).

Author, publication year	*Year of Sampling*	*Country origin*	*Study population*	*Females*	*A point prevalence estimate (%)*			
					Any	**NNRTI**	**NRTI**	**PI**
Somi et al., (2008)	2005-2006	Tanzania	39	100%	0.0	0.0	0.0	0.0
Nyombi et al., (2008)	2005	Tanzania	100	100%	4.0	4.0	3.0	0.0
Kiwelu et al.,(2014)	2004-2007	Tanzania	45	100%	7.0	4.0	4.0	7.0
Masimba et al.,(2013)	2005-2007	Tanzania	120	80%	8.4	7.6	3.4	0.0
	2009	Tanzania	119	74%	3.3	3.3	0.8	0.0
Hassan et al.,(2019)	2008	Kenya	50	72%	24	6.0	8.0	12
Rusine et al.,(2012)	2007-2009	Rwanda	138	63%	2.9	2.9	1.4	0.0
Rusine et al.,(2013)	2007-2010	Rwanda	109	52.3%	3.6	3.6	1.8	0.0
Vairo, et al.,(2013)	2010	Tanzania	67	100%	11.9	7.5	1.5	0.0
Telele et al.,(2018)	2009-2011	Ethiopia	461	58%	3.9	1.5	2.0	0.4
Lee et al.,(2014)	2002-2004	Uganda	81	62%	7.0	2.5	5.1	1.2
	2005-2010	Uganda	491	62%	3.0	2.2	1.2	0.0
Hassan et al.,(2018)	2005-2017	Kenya	97	0 %	8.2	7.2	2.1	0.0
Reynolds et al.,(2012)	2004–2009	Uganda	31	45%	13	9.6	3.2	6.4
Onsongo et al.,(2016)	.	Kenya	63	62%	0.0	.	.	0.0
Bartolo et al.,(2009)	2002-2004	Mozambique	104	43%	5.9	1.5	6.0	0.0
Eshleman et al.,(2009)	1998-2003	Uganda	104	55.8%	5.8	0.0	2.9	2.9
Bila et al.,(2013)	2007	Mozambique	47	100%	6.3	0.0	4.3	2.1
	2009	Mozambique	82	100%	8.2	8.2	0.0	0.0
Kamoto et al.,(2008)	2006	Malawi	34	100%	0.0	0.0	0.0	0.0
Abegaz et al.,(2008)	2005	Ethiopia	39	100%	0.0	0.0	0.0	0.0
Ndembi et al.,(2011)	2009-2010	Uganda	70	70.1%	8.6	4.2	2.9	1.4

Ndembi et al.,(2008)	2006- 2007	Uganda	37	100%	0.0	0.0	0.0	0.0
Hamers et al., (2011)	2007-2009	Uganda	570	57%	11.6	.	.	.
		Kenya	404	57%	4.7	.	.	.
Price et al., (2011)	2006-2009	Uganda	26	57%	19	.	.	.
		Rwanda	78	57%	8	.	.	.
		Kenya	49	**15.6**	4	.	.	.
Kantor et al.,(2014)	2006	Kenya	55	61%	1.8	0.0	1.8	1.8
Kantor et al.,(2015)	2005–2007	Malawi	29	44%	6.9	27	20.	3.4
Chung et al.,(2016)	2006	Kenya	386	60%	3.8	3.4	0.8	0.0
	2014	Kenya	567	66%	10.9	10.2	3.2	0.0
Zeh et al., (2016)	2003-2005	Kenya	258	52%	1.2	0.0	1.2	0.0
Mosha et al.,(2011)	2004-2005	Tanzania	44	77%	9.0	9.0	7.0	0.0
Kassu et al., (2007)	2003	Ethiopia	92	60.9%	3.2	2.2	1.1	0.0
Kasang et al.,(2011)	.	Tanzania	88	78%	14.8	10.2	8.0	5.7
Nazziwa et al.,(2013)	2009-2011	Uganda	47	47%	6.0	6.0	0.0	0.0
Hassan et al., (2013)	2008-2010	Kenya	182	75.8%	1.1	0.0	0.5	0.5
Ssemwanga et al.,(2012)-(a)	2004-2010	Uganda	72	.	1.4	1.4	0.0	0.0
Ssemwanga et al.,(2012)-(b)	2008–2010	Uganda	40	100%	2.6	2.5	0.0	0.0
Sigaloff et al., (2012)	2009-2010	Kenya	68	.	13	7.4	1.4	4.4
Kabondo et al.,(2012)	2009	Malawi	109	100%	5.5	4.6	1.8	0.0
Reepalu et al., (2021)	2011-2015	Ethiopia	99	61%	9.1	9.1	1.0	0.0
Mutagoma et al.,(2016)	2011	Rwanda	57	86%	3.5	3.5	0.0	1.8
Crowell et al.,(2020)	2013- 2019	Tanzania	154	59.6%	8.0	6 .0	3.0	1.0
	2013-2019	Kenya	254	59.6%	7.0	7.0	2.0	0.0
	2013-2019	Uganda	291	59.6%	14	10.0	5.0	1.0
Barabona et al.,(2019)	2017	Tanzania	47	75.7%	29.8	25.5	8.5	0.0
Rudovick,et al.,(2018)	2013-2015	Tanzania	235	75.7%	4.7	3%	1.7	0.0
Braun et al.,(2018)	2015	Uganda	152	62.8%	5.9	5.3	3.3	0.0

Rutstein et al.,(2019)	2012–2014.	Malawi	45	39%	24.4	20	6.7	0.0
Silverman et al.,(2017)	2013–2014	Kenya	815	65.1%	9.4	9	0.7	0.0
Milne et al.,(2020)	2006-2010	Kenya	97	100%	6.2	.	.	.
Reynolds et al.,(2016)	2012 –2013	Uganda	75	42%	4	2.7	0.0	1.3
Abdissa et al., (2014)	2010–2012	Ethiopia	12*	.	50	50	0.0	00
Onywera et al.,(2017)	2012	Kenya	87	72%	9.2	6.9	4.6	1.2
Carnimeo et al.,(202)	2017–2018	Mozambique	196	58.7%	16.8	14.8	4.6	0.5
			258	66.7%	26.4	25.6	4.3	0.4
Kiros et al.,(2020)	2018	Ethiopia	51	54.9%	9.8	7.8	2.0	0.0
Watera et al.,(2021)	2016	Uganda	335	61.5%*	18.0	13.7	6.0	1.5
Kaleebu et al.,(2015)	2012-2013	Uganda	413	66.4%	4.5	4.0	2.1	0.0
Beck et al., (2020)	2006	Kenya	303	66.7%	2.6	.	.	.
	2010	Kenya	97	100%	6.2	.	.	.
	2013–2014	Kenya	679	65.7%	4.4	.	.	.
McCluskey et al.,(2019)	2005-2013	Uganda	738	69%	3.5	2.8	1.0	0.0
Neuhann et al, (2020)	2014-2015	Malawi	197	61.9%	14.2	13.7	0.5	0.0
Zhou et al.,(2018)	2008	Malawi	20	.	.	9	2	0
Petch LA et al., (2005)	<2005	Malawi	21	.	0.0	0.0	0.0	0.0
Nyamache et al., (2011)	2005	Kenya	78	60.2%	1.3	1.3	0.0	0.0
Boender et al.,2015	2007–2009	Kenya	424	.	3	.	.	.
		Uganda	606	.	10	.	.	.
Kiptoo et al., (2013)	2005-2006	Kenya	188	100%	3.2	1.6	1.1	0.5
Lihana et al.,(2009)	2005	Kenya	53	66%	7.5	7.5	5.6	0.0
Cheriro et al., (2015)	2009	Kenya	41	68%	7.3	1.0	2.0	1.0
Budambula et al.,(2015)	.	Kenya	32	56.3%	3.1	.	.	3.1
			21	76.2	0.0	.	.	0.0
Arimide et al., (2018)	2011–2013	Ethiopia	67	87%	6.0	6.0	0.0	0.0
Mulu et al., (2014)	2008-2009	Ethiopia	155	54.1%	5.6	5.0	4.0	0.0

Mulu et al., (2015)	2008	Ethiopia	45	53 %	0.0	.	.	.
Huruy et al., (2015)	2010	Ethiopia	83	38.6%	7.2	3.6	1.2	2.4
Amogne et al.,(2016)	2008	Ethiopia	127	31%	3.1	1.6	0.8	0.8
Vidal et al.,(2007)	2002	Burundi	119	59%	0.8	0.8	0.0	0.0
Ndashimye et al (2018)	2007-2011	Uganda	87	100%	8.7	8.7	.	.
Galluzzo et al., (2007)	<2007	Uganda	97	100%	7.2	3.1	4.1	0.0
Osman et al., (2013)	2010	Kenya	58	.	13.8	1.7	15.5	0.0
Parreira et al., (2006)	2003	Mozambique	131	.	12	.	.	0.8
Abreu et al., (2008)	2002	Mozambique	75	100%	0.0	0.0	0.0	0.0
Gale et al., (2006)	<2005	Uganda	187	.	0.0	0.0	0.0	0.0
Bila et al.,(2015)	209-2010	Mozambique	68	72%	.	4.4	0.0	1.5
Vubil et al.,(2016)	2009-2010	Mozambique	95	0.0%	5.3	4.2	1.1	0.0
Otecko et al.,(2016)	2007-2009	Kenya	100	73.7%	0.05	0.03	0	0.03
Umviligihozo et al., (2021)	2005-2011	Rwanda	21	.	19	19	.	.
Sangeda et al.,(2019)	2010	Tanzania	18	.	0.0	0.0	0.0	0.0
Bellochi et al.,(2005)	<2005	Mozambique	58	.	.	5.2	6.9	10.3

CHAPTER 4

Recent HIV-1 infection and pre-treatment drug-resistance in recently infected, treatment-naïve persons in rural Tanzania.

Introduction

This chapter describes the proportion of recent HIV-1 infection among newly diagnosed ART naïve people living with HIV and its predictors, and the prevalence of PDR in recently infected persons in rural Tanzania. This's a cross-sectional analysis for recent HIV Infection among newly diagnosed adults enrolled in the Kilombero and Ulanga Antiretroviral cohort between March 2019 and March 2022. Results are presented in research ***paper II***, entitled "Recent HIV-1 infection and pre-treatment HIV drug-resistance in recently infected adults initiating antiretroviral therapy in rural Tanzania.", this manuscript is under review at the *Journal of Public Health*.

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Recent HIV-1 infection and pre-treatment HIV drug-resistance in recently infected adults initiating antiretroviral therapy in rural Tanzania.

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Abstract

Background: Recently HIV-1 infected patients remain an important threat to ending the HIV epidemic, contributing to high number of transmissions, and possibly spread of viral resistance. We determined the proportion of recent HIV-1 infection (RHI) among newly diagnosed people living with HIV (PLHIV). Additionally, we assessed the prevalence of pre-treatment HIV-1 drug resistance (PDR) in recently infected patients in rural Tanzania.

Methods: We conducted a cross-sectional analysis for RHI among newly diagnosed adults enrolled in the Kilombero and Ulanga Antiretroviral cohort between March 2019 and March 2022. RHI was tested using Asanté™ HIV-1 Rapid Recency® Assay, an immune-chromatographic, lateral-flow, rapid diagnostic test. Risk factors of RHI were determined using logistic regression. Genotypic resistance testing (GRT) was performed for RHI cases.

Results: Among 610 PLHIV, 24 (4%) had RHI. We found no factors associated with RHI. GRT was successful in 16/24 (67%) participants, and 5/16 (31%) had mutations associated with HIV-1 drug resistance: 4/16 (25%) for non-nucleoside reverse transcriptase inhibitors, 2/16 (13%) for nucleoside reverse transcriptase inhibitors and 1/16 (6%) for protease inhibitors.

Conclusion: The low observed prevalence of RHI in this referral hospital-based study, indicates the high number of late diagnoses. Since the observed proportion of recently HIV infected patients with PDR is based on a small sample size, we can't really make any conclusion on this.

Background

Patients who are recently infected with HIV, defined as new infection within the last six months(69), continue to be an important threat to ending the AIDS epidemic, as early and undiagnosed HIV leads to the highest number of transmission and possibly spread of viral resistance(72). Previous studies estimate that 25-50% of new HIV transmissions are due to persons with recent HIV infections (RHI)(135). During the first six months of infection, the high uncontrolled viremia in blood and genital secretions, the absence of HIV-specific neutralizing antibodies and a small viral quasi-species diversity contribute to a high transmissibility of HIV(70). Thus, strategies for early identification, linkage into care and treatment of recently HIV infected patients will contribute to a major extent to the interruption of HIV transmission and hence reductions in new infections.

Integrase inhibitor (INSTI)-based first-line regimens, through their rapid viral suppression and high genetic barrier to developing resistance(1), have been predicted to curb the number of new HIV infections and the spread of resistance – leading the way to reach the UNAIDS target of reducing the number of adults acquiring HIV infection to fewer than 200,000 in 2030(136). Further, dolutegravir has fewer side effects compared to non-nucleoside reverse transcriptase inhibitors (NNRTI) in treatment-naïve and experienced patients(137). However, we do not yet know the potential impact of the massive rollout of dolutegravir in settings with a substantial prevalence of pre-treatment HIV-1 drug resistance (PDR) against nucleoside reverse transcriptase inhibitor (NRTI) backbone drugs that could lead to dolutegravir monotherapy increasing the risk of virological failure and acquisition of new HIV drug resistant mutations against INSTI(110).

Interestingly, comparative evidence shows that PDR is more prevalent in patients with RHI compared to those with long-term HIV infection(71–74,138). During the chronic phase of infection some mutant viral strains may be overgrown by the wild-type population, and thus exist at very low levels, remaining undetected, especially in patients who have never been exposed to antiretroviral therapy (ART)(139). In Tanzania, as in many sub-Saharan African (SSA) countries, these important data on PDR among the recent HIV infected populations are missing, as routine resistance testing at diagnosis of HIV infection is not implemented. We determined the proportion of RHI among newly diagnosed ART naïve PLHIV and its predictors, and the prevalence of PDR in recently infected persons in rural Tanzania.

Methods

Study design and population

This is a cross-sectional study of newly diagnosed PLHIV enrolled in the Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) between March 2019 and March 2022.

Study area

The study was carried out within the Chronic Disease Clinic of Ifakara (CDCI), the HIV Care and Treatment Centre (CTC) of the St. Francis Referral Hospital in Ifakara, Southwestern Tanzania. CDCI is a joint project of the hospital, the Ifakara Health Institute (IHI), the Swiss Tropical and Public Health Institute and the University Hospital Basel, Switzerland. CDCI was among the first rural CTCs in Tanzania started in 2005 to provide HIV care and free ART within the Tanzanian National AIDS Control Care Programme framework. CDCI runs a PLHIV cohort, the KIULARCO, which has been previously described in detail elsewhere(94,96).

Inclusion criteria

For this study, we included newly HIV-1 diagnosed adult PLHIV (aged ≥ 15 years), who were enrolled in KIULARCO, have a signed informed consent, and had baseline plasma sample stored in a -80°C freezer.

Study procedures

At enrolment into KIULARCO, participants receive a clinical baseline evaluation and blood samples are drawn before ART initiation. ART use at enrolment is assessed through a systematic questionnaire. No viral load is routinely done at enrolment. Six months after start of ART and 12 months thereafter, HIV viral load (HVL) is monitored according to Tanzania national guidelines. Besides routine analyses, plasma is cryopreserved at -80^{0}C for research purposes. From 934 eligible participants, 610 biobanked baseline samples were randomly selected due to budget constraints and available recent HIV assay kits. Demographic, clinical and laboratory data was extracted from the KIULARCO electronic database.

Laboratory procedures

Plasma specimens were identified from the KIULARCO biobank, unfrozen and tested using Asanté™ HIV-1 Rapid Recency® Assay, an immune-chromatographic, lateral-flow, rapid diagnostic test(140), according to the manufacturer protocol (Sedia Biosciences, Portland, OR,

USA). HVL was measured for all specimens with probable recent infection (tested positive using Asanté™ HIV-1 Rapid Recency® Assay) by quantifying HIV-1 RNA using the, Roche Cobas Ampliprep/Cobas TaqMan (Roche Diagnostics USA, Indianapolis, IN, USA).

HIV-1 genotypic resistance testing (GRT) was performed on specimens with RHI. GRT was performed using a validated in-house PCR protocol.[18–20] Briefly, RNA was extracted from 150 µl of plasma using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol(141). RNA extracts were retrotranscribed and amplified using the HIV-1 Genotyping Kit Amplification Module (Applied Biosystems™, Thermo Fisher Scientific, USA). A direct sequencing reaction was done using 6 overlapping primers, and assembly program (BioEdit 7.2) was used for sequence analysis. Mutations were interpreted according to the Stanford University's HIV Drug Resistance Database Program version 9.2 (http://hivdb.stanford.edu). HIV drug resistant mutations conferring low, intermediate, or high-level resistance were considered. The reported protease and reverse transcriptase sequences are available in GenBank accession OQ696322-OQ696337.

Definitions and covariates

Recent HIV infection is defined as new HIV infection within the last six months(69)

PLHIV that tested positive for recent HIV infection and had a HVL >1,000 copies/ml were classified as RHI while those with an HVL<1,000 copies/ml were classified as long-term infections (no RHI)(142,143).

Covariates measured were age, gender, marital status, disclosure status, highest education level, distance of residence from the clinic (estimated based on centre of ward of residence), tuberculosis status (positive if positive microscopy with acid-fast bacilli, positive XpertMTB/RIF® assay (Cepheid, Sunnyvale, CA, USA) in sputum or other extra-pulmonary sample, or chest radiograph suggestive of tuberculosis plus at least one symptom, physician diagnosis by ICD-10 code and prescription of anti-tuberculosis medication), body mass index (BMI), HIV WHO stage and CD4 cell count. The nearest recorded measurement of BMI, CD4 cell count, HIV WHO stage up to one month after enrolment was used.

Statistical analysis

Demographic characteristics were summarized using median and interquantile range (IQR) for continuous data, and frequencies and percentages for categorical data. The following variables were considered a priori as potentially associated with RHI: age, gender, marital status, disclosure status, highest education level, distance of residence to clinic, tuberculosis status, BMI, HIV WHO stage and CD4 cell count(144–146). Logistic regression models were used to assess the association between the above variables and RHI. Univariable and multivariable analysis with no model selection were performed. PDR analyses were descriptive. Analyses were performed using Stata version 15.0.

Ethical Considerations

Written informed consent of PLHIV willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714. Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378).

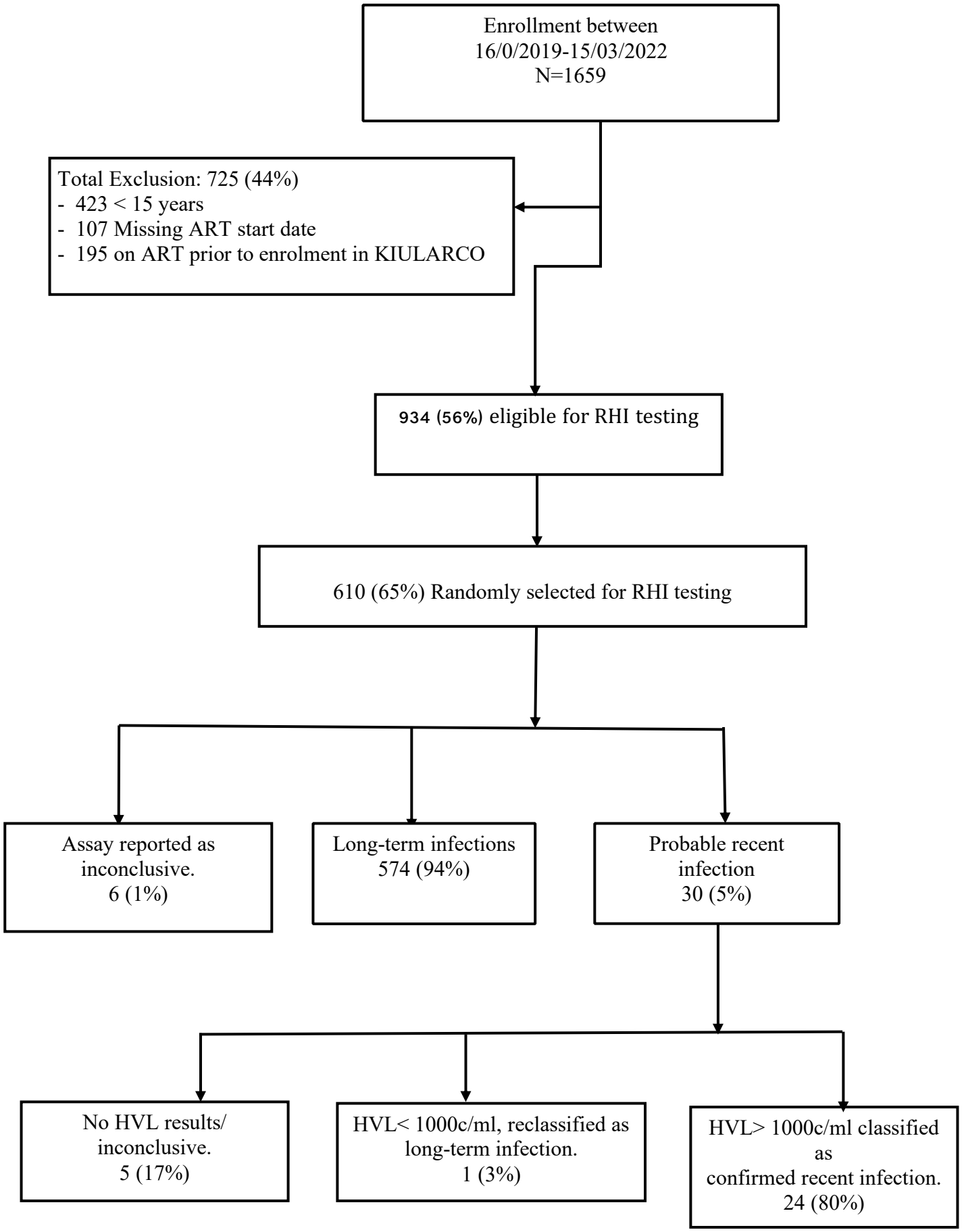
Results

Baseline characteristics

A total of 1659 PLHIV were enrolled into KIULARCO during the study period. We excluded 725 (44%) participants: 423 (58%) were <15 years old, 195 (27%) were previously on ART, and 107 (15%) had no ART start date, leaving 934 (56%) eligible PLHIV. We randomly selected 610 (65%) of the eligible PLHIV for this study.

Among the 610 PLHIV with a recent HIV infection test, 6 had an invalid result, bringing the total number of study participants to 604 (Figure 1). Of these, the median age was 38 years (IQR 30-47), 395 (65%) were female, 377 (62%) were married/cohabiting, 400 (66%) had disclosed their HIV status and 73 (12%) had no formal education. Clinically, 49 (8%) participants suffered from a tuberculosis co-infection, 70 (12%) were undernourished (BMI<18.5), 138 (23%) had a HIV WHO stage III/IV, and 226 (41%) had a CD4 cell count of <350 cells/µl (Table 1).

FIGURE 1: Study participants flowchart



Recent HIV infection and associated factors

Probable RHI was detected in 30 (5%) participants; Among those, 1(3%) participant had a HVL test result of <1000 copies/ml, thus reclassified as chronic infection, and 5(17%) participants had no HVL test result due to inadequate specimen, thus reclassified as inconclusive RHI test results; Whereas, 24 (80%) participants had HVL ≥1000 copies/ml, hence confirmed RHI, corresponding to an RHI prevalence of 4% (24/599). (Figure 1.).

Baseline characteristics among those with RHI were comparable to all study participants with exception of those with RHI having a lower HIV WHO stages I/II (4(17%) vs 138(23%)) and a higher CD4 cell count of ≥350 cells/µl (11(55%) vs 208 (38%)) (Table 1). We found no evidence of an association between risk factors and RHI (Table 2).

Table 1. Characteristics of study participants

Socio-demographic	**Recent HIV Infection (RHI) (N=24)**	**No RHI (N=575)**	**ALL (N=599)**
Age, years (median (IQR)	37 (30 – 47)	38 (30 – 48)	39 (30 – 47)
Age, years, n (%)			
15 – 24	4 (17%)	70 (12%)	74 (12%)
25 – 34	6 (25%)	154 (27%)	160 (27%)
35 - 44	10 (42%)	166 (29%)	176 (29%)
≥45	4 (17%)	185 (32%)	189 (32%)
Gender, female, n (%)	16 (67%)	375 (65%)	391 (65%)
Marital status, n (%)			
Married/cohabiting	15 (63%)	359 (62%)	374 (62%)
Never married	2 (8%)	38 (7%)	40 (7%)
Separated/divorced/widowed	7 (29%)	178 (31%)	185 (31%)
Disclosed HIV status, n (%)			
No	8 (33%)	186 (33%)	194 (33%)
Yes	16 (67%)	379 (67%)	395 (66%)
Missing	0 (0%)	10 (2%)	10 (2%)
Education, n (%)			
None	3 (13 %)	69 (12 %)	72 (12 %)

Primary	19 (79%)	442 (77%)	461 (77%)
Secondary and above	2 (8%)	64 (11%)	66 (11%)
Distance of residence to clinic, n (%)			
<=1 km	17 (77%)	328 (60%)	345 (60%)
2— <50 km	4 (18%)	158 (29%)	162 (28%)
≥50 km	1 (5%)	63 (12%)	64 (11%)
Missing	2 (8%)	26 (5%)	28 (5%)
		Clinical	
Tuberculosis status, n (%)			
Unlikely	23 (96%)	514 (90%)	537 (92%)
Yes	1 (4%)	48 (9%)	49 (8%)
Missing	0 (0%)	13 (2%)	13 (2%)
Body Mass Index (BMI)[a], Kg/m^2, n (%)			
Underweight, <18.5	4 (18%)	66 (12%)	70 (12%)
Normal, 18.5 - <25	10 (46%)	302 (55%)	312 (52%)
Overweight, ≥25	8 (36%)	178 (33%)	186 (31%)
Missing	2 (8%)	29 (5%)	31 (5%)
HIV WHO stage, n (%)			
I/II	20 (83%)	432 (83%)	452 (77%)
III/IV	4 (17%)	134 (24%)	138 (23%)
Missing	0 (0%)	9 (2%)	9 (2%)
CD4 count, cells/µl[a], n (%)			
<100	1 (5%)	112 (22%)	113 (21%)
100 - 350	8 (40%)	216 (41%)	226 (41%)
≥350	11 (55%)	194 (37%)	208 (38%)
Missing	4 (17%)	53 (9%)	57 (10%)

Results are numbers and column % of those with non-missing data; missing data rows are numbers and column %.
[a]BMI, HIV WHO stage and CD4 measurement closest to enrolment upto one month after.

Table 2. Risk factors associated with recent HIV infection

Characteristics	Unadjusted OR (95% CI) [a]	Adjusted OR (95% CI) [ab]
Age, years		
15 – 24	Reference	Reference
25 – 34	0.68 (0.19 - 2.49)	0.35 (0.05 - 2.37)
35 - 44	1.05 (0.32 - 3.45)	2.09 (0.41 – 10.6)
45 +	0.37 (0.09 - 1.52)	0.55 (0.08 – 3.77)
Gender		
Male	Reference	Reference
Female	1.06 (0.45 - 2.52)	1.53 (0.38 – 6.20)
Marital Status		
Married/Cohabiting	Reference	Reference
Never Married	1.27 (0.28 - 5.77)	2.57 (0.44 – 15.2)
Separated/divorced/widowed	0.93 (0.38 - 2.34)	1.46 (0.44 - 4.85)
Disclosed HIV Status		
No	Reference	Reference
Yes	0.97 (0.41 - 2.30)	1.36 (0.41 – 4.56)
Education		
None	Reference	Reference
Primary	0.99 (0.29 - 3.45)	0.50 (0.11 – 2.19)
Secondary and above	0.73 (0.19 - 4.50)	0.30 (0.02 – 3.67)
Distance of residence to clinic		
≤1km	Reference	Reference
2—<50km	0.48 (0.16 - 1.46)	0.32 (0.08 - 1.32)
≥50km	0.30 (0.04 - 2.31)	0.37 (0.03 - 3.28)
Tuberculosis status		
Unlikely	Reference	Reference
Yes	0.47 (0.62 - 3.56)	0.57 (0.05 – 7.30)
Body Mass Index, Kg/m^2		
Normal 18.5 - <25	Reference	Reference
Underweight, <18.5	1.85 (0.56 - 6.09)	3.80 (0.81 – 18.0)
Overweight, ≥25	1.37 (0.53 - 3.52)	1.23 (0.35 – 4.31)

HIV WHO Stage		
I/II	Reference	Reference
III/IV	0.65 (0.22 - 1.94)	0.92 (0.17 – 4.95)
CD4 count, cells/µl		
<100	Reference	Reference
100 – 349	4.11 (0.51 - 33.3)	4.26 (0.45 – 40.6)
350+	6.25 (0.80 - 49.1)	7.01 (0.71 – 69.1)

[a]Odds Ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression.

[b]Adjusted for baseline covariates shown, participants with missing data for any of the variables excluded, N=435.

Table 3. Distribution of Pretreatment Drug Resistance-Associated Mutations- and HIV-1 subtype(s) among recently HIV-1 infected people living with HIV.

Sample ID	Sample date	Major NRTI HIV resistance mutations	Major NNRTI HIV resistance mutations	Major PI HIV resistance mutations	Sub-type
101	05.07.2019	None	K103N	None	C
102	22.10.2021	None	None	None	C
103	11.06.2022	K219Q	None	None	C+D
104	05.07.2021	None	K103N, K238T	None	A1
105	01.06.2020	None	None	None	D
106	04.06.2020	None	None	None	C+D
107	08.06.2020	None	None	None	C
108	30.07.2020	M41L, K65R, M184V	K103N, Y181C	I54M, L90M	C
109	08.10.2019	None	None	None	C
110	04.08.2020	None	None	None	D
111	06.07.2021	None	None	None	C
112	05.07.2021	None	None	None	C
113	27.11.2020	None	None	None	D
114	23.03.2021	None	None	None	C
115	15.9.2021	None	E138K	None	A1
116	30.12.2021	None	None	None	A1

Pretreatment HIV-1 drug resistance mutations

Among 24 PLHIV with RHI, genotypic results for PDR were available for 16 (67%) participants. Any PDR was observed in 5 (31%) participants. PDR for NNRTI, NRTI and PI were observed in 4 (25%), 2 (13%) and 1 (6%) participant, respectively (Table 3.). HIV-1 subtypes were A1 (n = 3 (19 %), C (n = 8 (50%), D (n = 3 (19%)) and CRF C+D (n = 2 (13%)).

Discussion

Main finding of this study

In this study on the prevalence of RHI among newly diagnosed PLHIV in a rural sub-Saharan setting, we observed a prevalence of 4.0%, which was lower compared to other studies in the region(145,147,148). This observation may imply that majority of PLHIV in this rural SSA setting present late for care and treatment, hence representing a bottleneck in curbing the number of new HIV infections. Additionally, we determined the prevalence of PDR among patients with RHI to be 31%. Though on a limited sample size, the observed high DR rates is concerning.

What is already known on this topic?

In previous studies, female gender, young age, secondary and above education, and marital status have been reported as risk factors for RHI in SSA(145–150). However, in our analysis these factors were not found to be independently associated with RHI, likely due to small sample size of RHI cases. Elsewhere, factors such as commercial sex practice, contact with index case and illicit drug utilization have associated with RHI(147) However, these data are not systematically collected in the KIULARCO.

What this study adds

Our results raise several important public health issues; firstly, the observed low prevalence of RHI, which may be this being a health facility-based study, in a referral hospital, where patients attend due to referral from lower-level health facilities, and or severe illness that may be due to advanced HIV disease. This suggests that the prevalence of RHI might be higher in the community and in patients attending lower-level health facilities. In a recent door-to-door testing among general population sample in rural Lesotho, a prevalence of 7% was documented(146) A comparable prevalence of 7.4% was observed in a study targeting fishing community along the shore of Lake Victoria in Tanzania(148).

Secondly, the observed high PDR rates among recently HIV infected PLHIV, suggest that targeting this population is a key consideration while estimating the burden of PDR(74). A comparable high PDR prevalence of 29.8% has been documented among self-reporting ART-naive urban population in Tanzania(29). Our study, however, characterized PDR among recently HIV infected PLHIV in a rural population. PDR prevalence was highest for NNRTI (25%); the NNRTI drug class is being replaced by the more potent integrase dolutegravir based ART in many countries in SSA. However, the widely spread PDR to NNRTI poses threat to the long-term efficacy of dolutegravir. Even though the mechanism is still unclear, PDR to NNRTI has previously been associated with subsequently long-term virologic failure on dolutergravir based ART(131). Prevalence of PDR to NRTI among recently HIV infected PLHIV was observed at 12.5%, which was higher than overall pooled estimate prevalence of PDR to NRTI of 2.5% that was documented in our recent systematic review on PDR prevalence studies among adults in Eastern African countries(56). High prevalence of PDR to NRTI, which is co-administered with dolutergravir in the current WHO recommended first-line ART, risks some patients remaining on functional monotherapy and potential for emergence of dolutergravir resistance(75). The observed high PDR rate to NRTI among recently HIV infected population poses a threat to public health.

Limitations of this study

To the best of our knowledge, this is the first study reporting PDR among recently HIV infected PLHIV in Tanzania. However, our study has several limitations, including the study being in a referral hospital may have underestimated prevalence of RHI. Secondly, a relatively small sample size of patients presenting with RHI and PDR, which limits further exploration of risk factors associated with PDR in the recent HIV infected population. Thirdly, we used the Asanté™ HIV-1 Rapid Recency® Assay, which – after initial documentation of a high sensitively and specificity(140)– showed in some studies limited accuracy(151). However, in this study we added viral load testing to reduce false-positive recent infections. Likewise visual scoring of faint bands may be subjective and lead to misdiagnosis of RHI. Another limitation is that PCR amplification and genotypic testing was only successful for 67% of patients with RHI, which might be due to poor storage quality. Finally, all genotypic data were obtained through standard Sanger sequencing; rates of HIV associated drug resistance mutations would possibly be higher if ultra-sensitive HIV-1 drug resistance testing by deep sequencing had been used.

Conclusion

To conclude, we observed a prevalence of RHI of 4% in this hospital-based cross-sectional study in a rural setting, thus calling for strengthening strategies for early identification of HIV cases and linkage into care and treatment, to enable meeting of the UNAIDS' 95-95-95 goals. Since the observed proportion of recently HIV infected patients with PDR is based on a small sample size, we can't really make any conclusion on this.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

AJN JMF, JK, MW conceptualized the study and designed the experiments. AJN, DM performed the experiments. AJN, JO, FV analysed the data AJN wrote the manuscript. JMF, DM, RN, PSN, JO, FV, JK, MW reviewed and approved the final manuscript.

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Conflict of interest

All authors report no potential conflicts.

CHAPTER 5

Trends in prevalence of the nucleoside reverse-transcriptase inhibitor associated mutations in newly diagnosed antiretroviral therapy-naive patients in the Kilombero and Ulanga Antiretroviral cohort in Tanzania.

Introduction

This chapter compares the levels of pre-treatment HIV-1 resistance to Nucleoside reverse transcriptase inhibitor (NRTI), among antiretroviral-naïve adults people living with HIV in in the Kilombero and Ulanga Antiretroviral cohort, in the first five-year period (2005-2009) of the ART program in Tanzania, and the most recent period (2019-2022). Results are presented in research ***paper III***, entitled "Trends in prevalence of the NRTI associated mutations in newly diagnosed antiretroviral therapy-naive patients in the Kilombero and Ulanga Antiretroviral Cohort in Tanzania.", this manuscript is to be submitted for publication in the journal AIDS.

Trends in Prevalence of the Nucleoside/ Nucleotide Reverse-Transcriptase Inhibitor Associated Mutations in Newly Diagnosed ART-Naive Patients in the Kilombero and Ulanga Antiretroviral cohort, in Tanzania.

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AIDS

Trends in Prevalence of the pre-treatment HIV-1 drug resistance to NRTI in the Kilombero and Ulanga Antiretroviral cohort, in Tanzania.

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Abstract:	Objective: To measure the change in prevalence of pre-treatment HIV-1 drug resistance (PDR) to NRTI in the Kilombero and Ulanga Antiretroviral cohort (KIULARCO), between the first five-year period (2005-2009) of the ART program in Tanzania, and in the most recent period (2019-2022). Methods: Genotypic resistance testing (GRT) was performed to establish PDR in HIV infected person (aged ³ 15 years old) prior ART initiation. Study period: 2005-2009 (the first five-year period of the ART program in Tanzania) and the most recent period (2019-2022). PLHIV were excluded if they were aged less than 15 years and had prior history of ART exposure. Statistical analyses were descriptively performed to compare the prevalence of PDR between the two study periods. Results: The study population comprised 357 ART-naïve PLHIV; 239 during “2005-2009” and 118 in “2019-2022” period, comprised of 66% and 63% female in “2005-2009” and “2019-2022, respectively. Mean age was 38 years in “2005-2009” and 41 years in “2019-2022. The prevalence of PDR to NRTI was 2.1 % “2005-2009” and 3.4% in “2019-2022” (P=0.063). Whereas the frequency of major NRTI associated mutations was 3.8% in “2009-2009” and 7.6% in “2019-2022” (p=0.063174). Conclusions: The prevalence of PDR to NRTI was higher in the most recent period (2019-2022) compared with the first five-year period (2005-2009) of the ART program in Tanzania. Certainly, there is need for continuous surveillance of PDR to NRTI remain important, to prevent risk of failure of newer antiretroviral agents such as dolutegravir, which would be detrimental to Tanzania and other low- and middle-income countries

Background

The wider antiretroviral therapy (ART) coverage inevitably results in increasing rates of an ART use at uncontrolled levels, and risk of acquired HIV drug resistant mutations. This may in turn lead to an increased risk of transmitted drug resistance in individuals who have never been exposed to ART(152). In Sub Saharan Africa (SSA) available data shows increasing trend of pre-treatment HIV-1 drug resistance (PDR) among general adult population, which is mainly driven by non-nucleoside reverse transcriptase inhibitors (NNRTIs) HIV-1 drug resistance associated mutations(11,26,80). The new first-line treatment recommended by the WHO, the integrase inhibitor dolutegravir based ART which is being rolled-out in many countries in SSA, in Tanzania specifically since March 2019, is predicted to curb the number of new HIV-1 infections and the spread of resistance – owing to its high and rapid viral suppression and a high genetic barrier to developing resistance(9,47,75,153).

However, there has been notable increase in prevalence of PDR to Nucleoside reverse transcriptase inhibitor (NRTI), particularly in Eastern Africa(11,56), which may pose risk for the currently preferred first-line ART regimen for HIV treatment in Low- and middle-income countries, which is the fixed dose combination of dolutegravir with tenofovir/lamivudine. HIV drug resistant mutations to NRTI may lead to de facto dolutegravir monotherapy with a risk of acquisition of dolutegravir resistance(38,40,154). With this study, we therefore, measured the change in prevalence of PDR to NRTIs in the Kilombero and Ulanga Antiretroviral cohort (KIULARCO), during first five-year period (2005-2009) of the ART program in Tanzania, and in the most recent period (2019-2022).

Methods

Study design

We performed a cross-sectional analysis for PDR levels to NRTI among ART -naïve adults initiating ART in the KIULARCO, during the recent period (2019-2022), and compared to the previously published data from the same cohort, during the first five-year period (2005-2009) of the ART program in Tanzania(31).

Setting and population

We included newly HIV-1-diagnosed adult people living with HIV (PLHIV) (aged ≥ 15 years) prior to ART initiation, who are enrolled in the KIULARCO. For this study, we included 239 and

118 patients' plasma samples randomly selected for inclusion in "2005-2009" and in "2019-2022", respectively.

Data collection

Data for patient demographics, and on ART was extracted from OpenMRS, the KIULARCO electronic medical record system. Plasma specimens of eligible patients were retrieved from the KIULARCO biobank, unfrozen and tested.

Laboratory analysis

Genotypic HIV-1 resistance testing was performed using a validated in-house PCR protocol(42,95,99). Briefly, HIV-1 RNA was isolated using NucleoSpin RNA Virus, (MACHEREY-NAGEL, Germany), according to the manufacturer's protocol(100), from an equivalent of 150uL of pre-ART plasma samples enrolled in the KIULARCO. DNase-treated HIV-1 RNA was reverse-transcribed and amplified by two semi-nested PCRs. A direct sequencing reaction was done using 6 overlapping primers, and assembly program (BioEdit 7.2) was used for sequence analysis(103). Mutations were interpreted according to the Stanford University's HIV Drug Resistance Database Program version 9.2 (http://hivdb.stanford.edu)(104).

Statistical analysis

Data were analyzed statistically by STATA 15 using descriptive t-test to compare the prevalence of PDR between groups.

Ethics approval

Written informed consent of patients willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714**.** Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378).

Results and Discussion

Study-eligible participants initiating ART in the KIULARCO were tested for HIV-1 drug resistance to NRTI. A total of 357 patients' samples were analysed: 239 during the first five-year period (2005-2009) of the ART program in Tanzania, and 118 in the most recent period (2019-2022). The median age of those in "2005-2009" was slightly younger than in 2019-2022 (38 vs. 41 years). The proportion of women in "2005-2009" and in "2019-2022", was 66% and 63% respectively.

The overall prevalence of PDR to NRTI was 2.1 % and 3.4% in "2005-2009" and "2019-2022" (p=0.233), respectively. Whereas the overall frequency of major NRTI associated mutations was 3.8% in "2009-2009" and 7.6% in "2019-2022" (p=0.063174). The difference in observed prevalence of PDR to NRTI was not statistically significant, possibly due to small sample size. However, a rising trend of PDR to NRTI has previously been reported in the Eastern Africa regions, in a systematic review and meta-analysis using data available until 2016(11).

In "2005-2009", 9 mutations were detected in 5 participants at baseline (*Table 1*). The proportional of common discriminatory NRTI mutations were (M184V 4(1.7%), K65R 1(0.4%), L74LV 1(0.4%)); Whereas the proportional of the thymidine Analog Mutations, TAMs were (M41L 1(0.4%), K70R 1(0.4%), T215F 1 (0.4%)). In "2019-2022", 9 mutations to NRTI were detected in 4 participants at treatment initiation (Table 1) The proportional of common discriminatory NRTI mutations were (M184V 3 (2.0%), K65R 2(1.7%)); Whereas the proportional of the thymidine Analog Mutations, TAMs in 2005-2009 were (M41L 2(1.7%), K70R 1(0.9%), T215F 1 (0.9%) and (K219Q 1(0.9%)) (*Table 1*). Prevalence of K65R mutation associated with reduced susceptibility to tenofovir, was 0.4% in "2005-2009" and 1.7% in "2019-2022" (p=0.104). Elsewhere, a twice increased in prevalence of K65R mutation between to study period has been reported in across-sectional study neighboring country, Kenya(80).

In "2005-2009", subtype C was the most prevalent HIV-1 subtype with a frequency of 43.7%, followed by A1(29.4%) and D (16%). Likewise, in "2019-2022" subtype C was the most prevalent HIV-1 subtype with a frequency of (34.7%), followed by subtype D (28%)) and A1 (20.3%)) (Table 2.). The frequency of subtype D was significant high in "20019-2022" compared to "20019-2022" (p=0.004); Likewise, the recombinant form A1,01_AE (p=0.001). The significant

of HIV-1 subtype on treatment outcome is largely still an area of active investigation. However, HIV-1 subtypes such as C is known to more prone to developing K65R mutations(81,155)

Limitations

The major limitation of our study is the small sample size due to time and budget constraints. Also, prior exposure to antiretrovirals was based on self-report and could be subject to misclassification. Further studies involving a higher number of participants and more sampling time points are needed to determine the trends of PDR, and identify real predictors associated with changes in prevalence of PDR to NRTI.

Conclusion

In summary, the findings show increasing tendency of PDR to NRTI during the study periods; This increased prevalence of PDR suggests that a considerable number of ART-naïve adults initiating dolutegravir based-ART in fixed dose combination of NRTI backbones, may be at risk of being on dolutegravir monotherapy and acquisition of dolutegravir resistance. These findings highlight a need for continuous surveillance of pre-existing resistance to the dolutegravir co-administered NRTI.

Table 1: Prevalence of pre-antiretroviral treatment HIV drug resistance mutations detected in antiretroviral-naïve adult in "2005-2009"(31) and "2019-2022".

Major NRTI Mutations	2005-09 (N=239) n (%)	20019-22 (N=118) n (%)	P-value (X; H_0: "20019-2022" ≥ "20019-2022")
M184V	4 (1.7)	3(2.5)	0.305
T215F	1 (0.4)	1(0.9)	0.278
K65KR	1 (0.4)	2(1.7)	0.104
K70R	1(0.4)	1(0.9)	0.278
M41L	1(0.4)	2(1.7)	0.104
L74LV	1(0.4)	-	-
K291Q	-	1(0.9)	-
Total major NRTI	9(3.8)	9 (7.6)	0.063
No. of individuals with major NRTI mutations	5 (2.1%)	4 (3.4)	0.233

Table 2: Prevalence of HIV-1 subtypes detected in antiretroviral-naïve adult in "2005-2009"(31) and "2019-2022".

HIV-1 subtypes	**2005-09 (N=231) N (%)**	**20019-2022 (N=118) N (%)**	**P-value (X; H_0: "20019-2022" ≥ "20019-2022")**
A1	68 (29.4)	24(20.3)	0.034
B		2 (1.7)	-
C	101 (43.7)	41(34.7)	0.053
D	37 (16)	33 (28.0)	0.004
A1,01_AE	5 (2.2)	11 (9.3)	0.001
CD	11 (4.8)	3(2.5)	0.150
A1D	2 (0.9)	3 (2.5)	0.118
BC	-	1 (0.8)	-
A1, C	5 (2.2)	-	-
A1, C, D	1 (0.4)	-	-
A1,01_AE, C	1 (0.4)	-	-
All recombinants	25 (10.8)	18 (15.3)	0.113

Notes

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Authors' contributions: AJN, JF, MW and JK conceptualized the study, designed the experiments, AJN, wrote the manuscript. AN, Performed the genotypic HIV-1 resistance testing. AJN analyzed the data and reviewed the manuscript. AN, JO, FV, DP, JF, MW and JK participated in the interpretation of the data, writing, reviewing, and approving the final manuscript. The corresponding author AJN is the guarantor of the paper.

Conflicts of interest There are no conflicts of interest.

CHAPTER 6

Virological-outcomes at one-year, and its predictors after initiating dolutegravir (DGT)-based antiretroviral therapy (ART), and prevalence of pretreatment HIV-1 drug resistance among patients failing on DTG-based ART in a rural cohort, in Tanzania.

Introduction

This chapter compares viral suppression in treatment-naïve patients at 12 months after initiating dolutegravir (DTG)-based versus non-nucleoside reverse transcriptase inhibitor (NNRTI)-based ART. Additionally, we assessed predictors associated with viral suppression and analysed pre-treatment HIV-1 drug resistance (PDR) and acquired resistance, side effects, and pregnancy outcomes in patients initiated on a DTG-based regimen. Results are presented in research ***paper IV***, entitled "Transitioning to dolutegravir in a programmatic setting: Virological outcomes and associated factors among treatment-naïve patients with HIV-1 in the Kilombero and Ulanga antiretroviral cohort in rural Tanzania.", this has been published as an original article in the *Journal of Open Forum Infectious Disease*

Open Forum Infectious Diseases

MAJOR ARTICLE

Transitioning to Dolutegravir in a Programmatic Setting: Virological Outcomes and Associated Factors Among Treatment-Naive Patients With HIV-1 in the Kilombero and Ulanga Antiretroviral Cohort in Rural Tanzania

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Background. Virological outcome data after programmatic transition from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based to dolutegravir (DTG)-based antiretroviral therapy (ART) regimens in sub-Saharan Africa (SSA) outside of clinical trials are scarce. We compared viral suppression and associated factors in treatment-naïve people living with HIV (PLHIV) starting DTG- based versus NNRTI-based ART.

Methods. We compared virological suppression at 12 months, after treatment initiation in the two cohorts of participants aged ≥15 years, initiating DTG- and NNRTI-based ART. Drug resistance was assessed among participants with viremia ≥50 copies/mL on DTG.

Results. Viral suppression was achieved for 165/195 (85%) and 154/211 (73%) participants in the DTG- and NNRTI- cohorts, respectively ($P = 0.003$). DTG-based ART was associated with >2 times the odds of viral suppression versus NNRTI-based ART (adjusted odds ratio, 2.10 [95% confidence interval {CI}, 1.12–3.94]; adjusted risk ratio, 1.11 [95% CI, 1.00–1.24]). HIV-1 genotypic resistance testing (GRT) before ART initiation was done in 14 of 30 viremic participants on DTG, among whom nucleoside reverse transcriptase inhibitor (NRTI), NNRTI, and protease inhibitors resistance was detected in 0 (0%), 2 (14%) and 1 (7%), respectively. No resistance was found in the 2 of 30 participants with available GRT at the time of viremia ≥50 copies/mL.

Conclusions. Virological suppression at 1 year was higher in participants initiating DTG- versus NNRTI-based ART. In those with viremia ≥50 copies/mL on DTG-based ART, there was no pretreatment or acquired resistance to the DTG co-administered NRTIs, although the number of samples tested was small.

Keywords. HIV-1; ART-naive; dolutegravir; drug resistance; sub-Saharan Africa.

Background

Globally, almost 38 million people are living with HIV and two thirds of these reside in sub-Saharan Africa (SSA)(1). To end the HIV epidemic with the global 95-95-95 targets set by UNAIDS for 2025, one key element is access to antiretroviral therapy (ART) for all people with HIV (PWH)(156,157). Until recently, in most low- and middle-income countries (LMIC) first-line ART consisted of a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen containing either nevirapine or efavirenz. However, in the last years a substantial increase of pretreatment drug resistance (PDR) for NNRTIs has been observed in SSA(109), in some countries exceeding the World Health Organization (WHO) recommended threshold of 10%(56), thus, compromising the ambitious target to end the HIV epidemic by 2030(158). PDR resulting in virologic failure (VF) might go undetected for long timespans in LMIC due to limited resources for viral load (VL) and resistance testing, leading to increased morbidity, mortality, and onward transmission of virus (159). Therefore, transition to integrase-inhibitors characterized by a faster viral suppression, a higher barrier to resistance and fewer side effects compared to NNRTIs within randomized controlled trials(160), has been advocated by many national HIV programs.

By the end of 2017, dolutegravir (DTG)-based therapy became available to LMIC as the generic fixed dose combination of tenofovir disoproxil fumarate, lamivudine and dolutegravir (TLD) at the favorable price of 75 USD per person per year(161). Since then, many LMIC have rolled out DTG-based ART. Initially, the WHO advised women of childbearing age to avoid DTG-based ART, when planning a pregnancy or not being on consistent contraception due to a signal for an increased risk of neural tube defects in the offspring of women on DTG-based ART at conception in the Tsepamo study in Botswana(162). Only after more data became available showing a considerably lowered risk of neural tube defects, the WHO recommended DTG-based ART for all adolescents and adults in July 2019.

So far, limited data outside of clinical trials is available on the clinical and virologic outcome after the transit from NNRTI-based to DTG-based regimens in SSA(12,137,153). Evaluation of the rollout of DTG-based ART under programmatic conditions in resource-limited regions is important given the challenges outlined above. With this study, we aimed to compare viral suppression in treatment-naïve patients at 12 months after initiating DGT-based versus NNRTI-based ART. Additionally, we assessed predictors associated with viral suppression and analysed

PDR mutations and acquired resistance, side effects, and pregnancy outcomes in patients initiated on a DTG-based regimen.

Methods

Study design and setting

This is a retrospective, observational study nested within the Kilombero and Ulanga antiretroviral cohort (KIULARCO), a prospective cohort of PWH. The cohort includes patients seen at the Chronic Diseases Clinic of Ifakara (CDCI), the care and treatment center for PWH of the Saint Francis Referral Hospital (SFRH), located in rural southeastern Tanzania. The cohort was established in 2005 and captures comprehensive demographic and clinical data, including ART use and monitoring, co-morbidities and treatment outcomes, with details described previously(94,96).

Study population

Two groups of PWH aged ≥15 years attending KIULARCO were included in the analysis: a) treatment-naïve patients initiating NNRTI-based ART between 16th December 2016 and 15th December 2017 (referred to as the NNRTI cohort) and b) treatment-naïve patients initiating DTG-based ART between 16th March 2019 and 15th September 2020 (referred to as the DTG cohort). The separation of periods ensured that no patient in the NNRTI-cohort would have been switched to a DTG-based regimen by the time of outcome assessment, which could have had an impact on VL results.

We excluded patients initiating protease inhibitor (PI)-based therapy in both periods, patients starting NNRTI-based ART between 16th March 2019 and 15th September 2020, treatment-experienced patients (assessed in the baseline questionnaire by asking if they had ever received ART before), those not starting ART during the given time periods and those without written informed consent to KIULARCO (Figure 1). Reasons for excluding patients starting PI-based ART was that this is not the usual first-line therapy in Tanzania. Similarly, those initiating NNRTI-based ART during DTG roll-out were excluded as they were only few and would have only started on this therapy due to specific criteria.

Data collection

Data for patient demographics, routine clinical information, and laboratory data as well as information on ART was extracted from OpenMRS, the KIULARCO electronic medical record system. As per routine care and according to the Tanzania National Guidelines for the Management of HIV and AIDS, patients receive a VL measurement at 6 and 12 months after treatment start, and once yearly thereafter for those with a VL <1000 copies/ml. At the same time point as VL measurements, blood samples are stored in a biobank at -80°C for research purposes.

Laboratory measurements

VL measurement was done using the Abbott Real-time m2000 HIV-1 Assay (Abbott Laboratories, Chicago, Illinois), with a reportable range of 40-10,000,000 copies/mL for blood plasma. For this study, we performed HIV-1 genotypic resistance testing (GRT) from biobanked samples for those with an HIV VL ≥50 copies/mL. In addition, HIV-1 GRT was performed on samples available prior to treatment initiation for cases with a VL≥50 copies/mLat 12 months using cryopreserved samples, to determine PDR before ART initiation in the DTG cohort.

GRT was performed using a validated in-house PCR protocol to determine the HIV-1 drug resistance-associated mutations for Reverse Transcriptase (RT), Protease (PR) and Integrase Inhibitor (INSTI) – mutations(95,99). Briefly, RNA was be extracted from 150 µl of plasma using the PureLink™ Viral RNA/DNA Mini Kit (Invitrogen™, Thermo Fisher Scientific, USA), according to the manufacturer's protocol. RNA extracts were retrotranscribed and amplified using the HIV-1 Genotyping Kit Amplification Module (Applied Biosystems™, Thermo Fisher Scientific, USA)(101). A direct sequencing reaction was done using 6 overlapping primers, and assembly program (BioEdit 7.2) was used for sequence analysis. Mutations were interpreted according to the Stanford University's HIV Drug Resistance Database Program version 9.2 (http://hivdb.stanford.edu)(104). HIV drug resistant mutations conferring low, intermediate, or high-level resistance were considered. The reported protease and reverse transcriptase sequences are available in GenBank accession OQ627458-OQ627474.

Definitions

Viral suppression was defined as <50 copies/ml and viremia as defined as ≥50 copies /ml, at one time-point measurement, and virological failure was defined as ≥1000 copies /ml after a minimum of 6 months on ART, based on two consecutive viral load measurements in 3 months (with

adherence support following the first viral load test), in line with the most recent WHO guidelines(163). Loss to follow up (LTFU) was defined as being >60 days late for a scheduled visit.

Tuberculosis was recorded if within 3 months from enrolment, acid-fast bacilli, or a positive Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) from sputum or an extrapulmonary site were documented, or if anti-tuberculosis drugs with an ICD-10 code or clinical signs suggestive of tuberculosis were present. Unlikely tuberculosis was defined as no prescription of anti-tuberculosis drugs and no diagnosis of tuberculosis by ICD-10. For other cases, an indeterminate tuberculosis status was stated and treated as missing data.

Study Outcomes

The primary outcome was viral suppression at 12- months, allowing for a time window of 5-15 months with a preference for the measurement closest to 12 months after treatment start. The secondary outcomes of this study were prevalence of PDR and acquired resistance among patients presenting with viremia on DTG-based ART, defined as the presence of resistance associated mutations; plus, side effects and pregnancy outcomes in the DTG-cohort.

Statistical methods

Demographic characteristics were summarized using frequencies and percentages. We compared descriptively baseline characteristics between participants with and without a viral load result at 12 months. The proportion of participants with viral suppression in the DTG and NNRTI cohorts was compared using a chi square test. Factors associated with 12-month viral suppression were determined using logistic regression models, reporting odds ratios (OR) and 95% confidence intervals (CI). We also calculated adjusted risk ratios (aRR) after fitting the multivariable model with standard errors estimated using the delta method(164). The multivariable model incorporated the following covariates which were selected a priori: age, gender, marital status, disclosure of HIV status, education level, distance in kilometers of residence from the clinic, body mass index (BMI), HIV WHO stage, CD4 cell count, tuberculosis status and cohort (DTG-based or NNRTI-based ART). No model selection was done. We performed a sensitivity analysis restricting VL measurement to a time window of 9-15 months from treatment start. The analysis was repeated under the assumption that those LTFU were not virally suppressed, as such participants are likely to be off treatment and therefore not suppressed.

The prevalence of HIV-1 drug resistance, the proportion of patients who discontinued DTG-based ART and the proportion of women who conceived while on DTG-based ART and their pregnancy outcomes were described descriptively. All analyses were performed using Stata version 15 (StataCorp LP, College Station, TX, USA).

Ethical considerations

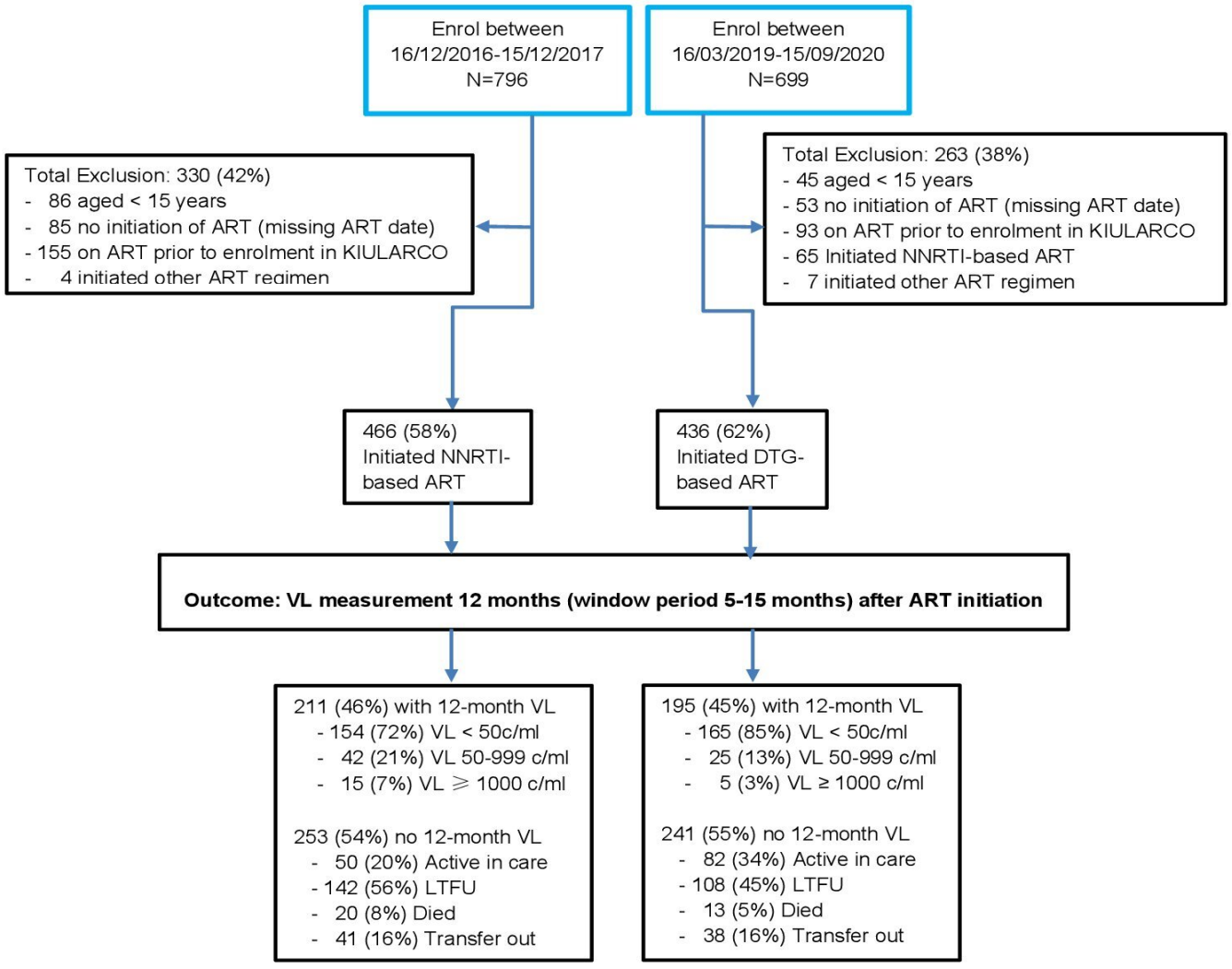
Written informed consent of patients willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714**.** Yearly ethical approval for data and sample collection as well as analysis are sought from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378).

Results

Study Population and Baseline Characteristics

There were 436 and 466 patients in the DTG- and NNRTI- cohorts, respectively (Figure 1.) The combined median age in both cohorts was 38 years (IQR 30-45). In the DTG- and NNRTI-cohorts, 280 (64%) and 300 (65%) patients were female, 292 (67%) and 263 (57%) were married/cohabiting, and 290 (72%) and 318 (79%) had disclosed their HIV status, and 51 (12%) and 106 (25%) were resident ≥50 km from clinic, respectively. The clinical parameters were broadly comparable within the two groups, with a normal BMI (18.5-25 Kg/m^2) in 230 (57%) and 259 (62%), a WHO stage III/IV in 107 (25%) and 166 (39%), a CD4 cell count of ≥350 cells/ml in 142 (37%) and 134 (34%), and tuberculosis coinfection in 33 (8%) and 52 (11%) participants, respectively (Table 1).

FIGURE 1: Patient flowchart for those initiating DTG or NNRTI-based regimens



We compared patients with and those without VL tests at 12 months and found them to be broadly comparable with exception of those with 12 months VL more likely to have disclosed their HIV status (309 (81%) vs 301(70%), be resident <1 km from clinic (210 (54%) vs 213 (45%)) and less likely to be HIV WHO stage III/IV ((115 (29%) vs 159 (35%) and CD4 count ≥350 cells/µl ((118 (31%) vs 151 (38%). Those without 12-month VL were more likely to have missed baseline characteristics measurements compared to those with VL (Table S1).

Table 1: Patients' characteristics at initiation of NNRTI- or DTG-based ART regimens

Patient characteristics	Initiated NNRTI-based ART 2016-2017 N=466	Initiated DTG based ART 2019-2021 N=436	*P-value*
Socio-demographics			
Age, years, n (%)			
15 – 24	52 (11%)	53 (12%)	0.870
25 – 34	136 (29%)	120 (28%)	0.859
35 - 44	164 (35%)	138 (32%)	0.585
≥45	111 (24%)	125 (29%)	0.385
Gender, Female, n (%)	300 (65%)	280 (64%)	0.801
Marital status, n (%)			
Married/Cohabiting	263 (57%)	292 (67%)	0.015
Never married	63 (14%)	28 (6%)	0.270
Separated/Divorced/Widowed	137 (30%)	116 (27%)	0.598
Disclosed HIV status, n (%)			
No	85 (21%)	113 (28%)	0.260
Yes	318 (79%)	290 (72%)	0.044
Missing	60 (13%)	33 (8%)	0.464
Education, n (%)			
None	64 (14%)	28 (6%)	0.269
Primary	347 (75%)	368 (84%)	0.003
Secondary and above	52 (11%)	40 (9%)	0.753
Distance of residence to clinic, n (%)			
<1 km	182 (42%)	240(56%)	0.004
2— <50 km	141 (33%)	135 (32%)	0.859
≥50 km	106 (25%)	51 (12%)	0.062
Missing	34 (7%)	10 (2%)	0.554
Clinical			

ART regimen, n (%)			
DTG-based	0 (0%)	436 (100%)	-
EFV-based	456 (98%)	0 (0%)	-
NVP-based	9 (2%)	0 (0%)	-
Other	1 (0%)	0 (0%)	-
Tuberculosis status, n (%)			
Unlikely	405 (89%)	395 (92%)	0.148
Yes	52 (11%)	33 (8%)	0.650
Missing	6 (1%)	8 (2%)	0.881
Body Mass Index (BMI)[b], Kg/m^2, n (%)			
Underweight (<18.5)			
Normal (18.5 - <25)	66 (16%)	52 (13%)	0.647
Overweight (≥25)	259 (62%)	230 (57%)	0.260
Missing	63 (15%)	123 (30%)	0.025
	43 (9%)	31 (7%)	0.756
HIV WHO stage[a], n (%)			
I/II	260 (61%)	316 (75%)	0.001
III/IV	166 (39%)	107 (25%)	0.017
Missing	37 (8%)	13 (3%)	0.536
CD4 count, cells/µl[a], n (%)			
<100	92 (24%)	62 (16%)	0.230
100 - 350	166 (42%)	184 (47%)	0.347
≥350	134 (34%)	142 (37%)	0.603
Missing	71 (15%)	48 (11%)	0.529

*Results are number and column % of those with non-missing data; missing data rows are number and column %.

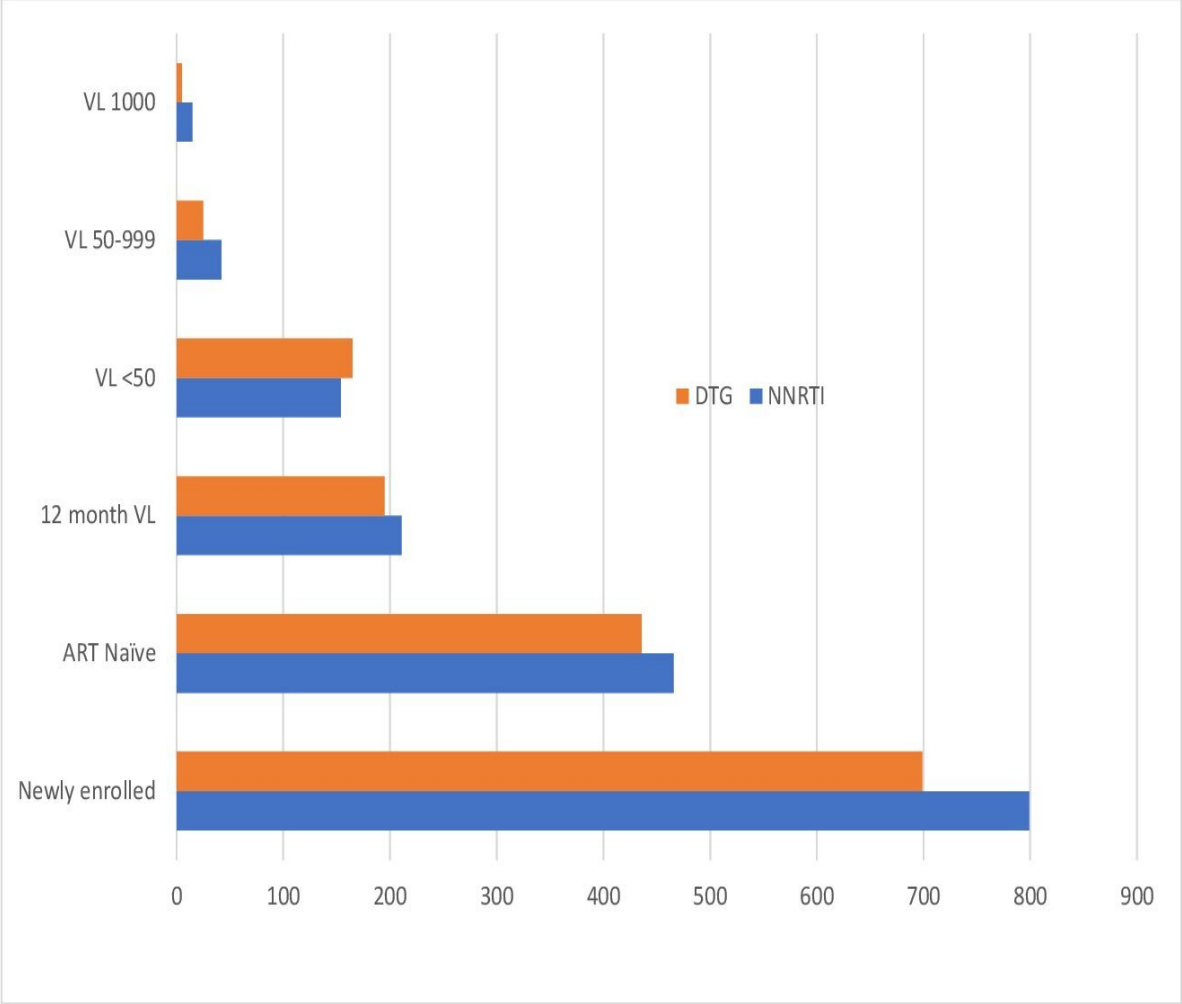
[a]Tuberculosis, BMI, HIV WHO stage and CD4 measurements closest to ART initiation within 6 months before and 3 months after.

Virological Outcome

VL data at 12 months after ART initiation were available for 195/436 (45%) and 211/466 (45%) participants in the DTG- and NNRTI-cohorts, respectively (Figure 2). Among those with VL results available, viral suppression was achieved for 165/195 (85%) and 154/211 (73%) participants in the DTG- and NNRTI- cohorts, respectively (p=0.003). For those with viremia in the DTG- and NNRTI-cohorts, 25/30 (83%) and 42/57 (74%) had a VL 50-999 copies/mL and

5/30 (17%) and 15/57 (26%) had VL ≥1000 copies/ml respectively. At one year following ART initiation, 108/436 (25%) patients in the DTG- cohort and 143/466 (31%) in the NNRTI-cohort were LTFU. (Figure 1). Assuming those LTFU were not virally suppressed, 165/303 (55%) and 154/353 (44%) achieved viral suppression in the DTG- and NNRTI-cohorts, respectively ($p<0.001$).

FIGURE 2: Virologic outcome at 12 months after initiation of reverse transcriptase inhibitor (NNRTI)- and dolutegravir (DTG)-based antiretroviral therapy (ART) in treatment naïve.



Factors associated with virological suppression

Results of the univariable and multivariable analyses are shown in Table 2. DTG-based versus NNRTI-based ART was independently associated with improved viral suppression (aOR 2.10, 95% CI 1.12-3.94 and aRR 1.11, 95% CI (1.00-1.24). In addition, the following factors were independently associated with viral suppression (Table 2): being separated/divorced/widowed (and to some extent married/cohabiting) versus never married, higher education level, and higher (worse) HIV WHO stage.

In sensitivity analysis restricting VL measurements to a time window of 9-15 months including 59/436 (14%) and 133/466 (29%) patients in the DTG- and NNRTI-cohorts, ART regimen is not associated with viral suppression, (aRR) 2.12 95% CI (0.82 – 5.49), compared to using 5-15 months VL windows above (Table S2).

Table 2: Factors associated with viral suppression (HIV-1 RNA <50 copies/ml) at 12 months (5-15 month) among treatment-naïve patients initiating NNRT-based or DTG-based ART.

Characteristics	Unadjusted OR (95% CI) [a]	Adjusted OR (95% CI) [ab]	Adjusted RR (95% CI) [c]
ART regimen			
NNRTI-based	Reference	Reference	Reference
DTG-based	2.16 (1.29 – 3.62)	2.10 (1.12 – 3.94)	1.11 (1.00 – 1.24)
Age, years			
15 – 24	Reference	Reference	Reference
25 – 34	1.56 (0.62 – 3.89)	1.81 (0.57 – 5.69)	1.09 (0.87 – 1.37)
35 - 44	1.05 (0.45 – 2.45)	1.55 (0.51 – 4.73)	1.03 (0.82 – 1.29)
45 and above	1.23 (0.51 – 3.00)	1.36 (0.43 – 4.31)	0.99 (0.78 – 1.26)
Gender			
Male	Reference	Reference	Reference
Female	0.65 (0.38 – 1.12)	0.62 (0.32 – 1.21)	0.96 (0.85 – 1.07)

Marital Status			
Never	Reference	Reference	Reference
Married/Cohabiting	3.19 (1.51 – 6.74)	2.03 (0.70 – 5.86)	0.80 (0.58 – 1.10)
Separated/divorced/widowed	2.94 (1.29– 6.71)	3.32 (1.03– 10.7)	1.04 (0.93 – 1.17)
Disclosed HIV Status			
No	Reference	Reference	Reference
Yes	1.49 (0.81 – 2.71)	1.58 (0.74 – 3.36)	1.12 (0.95– 1.32)
Education			
None	Reference	Reference	Reference
Primary	1.42 (0.65 – 3.09)	1.79 (0.72 – 4.49)	1.14 (0.90 – 1.45)
Secondary and above	1.65 (0.57 – 4.79)	4.72 (1.09– 20.4)	1.31 (1.02– 1.70)
Distance of residence to clinic			
≤1km	Reference	Reference	Reference
2—<50km	1.07 (0.60 – 1.89)	1.27 (0.64 – 2.54)	1.02 (0.91 – 1.15)
≥50km	0.66 (0.33– 1.31)	0.66 (0.30– 1.46)	0.93 (0.78– 1.11)
Tuberculosis status			
Unlikely	Reference	Reference	Reference
Yes	0.73 (0.32 – 1.64)	1.21 (0.44 – 3.33)	0.98 (0.81 – 1.19)
Body Mass Index, Kg/m^2			
Underweight, <18.5	0.70 (0.35 – 1.41)	0.86 (0.37 – 2.01)	0.96 (0.81 – 1.15)
Normal, 18.5 - <25	Reference	Reference	Reference
Overweight, ≥25	1.10 (0.62 – 1.95)	1.46 (0.68 – 3.10)	1.06 (0.93 – 1.20)

HIV WHO Stage			
I/II	Reference	Reference	Reference
III/IV	2.06 (1.24 – 3.41)	2.33 (1.17 – 4.65)	1.14 (1.00 – 1.34)
CD4 count, cells/µl			
<100	Reference	Reference	Reference
100 – 349	1.03 (0.54 – 1.96)	0.70 (0.32 – 1.54)	0.93 (0.81 – 1.06)
350	2.13 (0.99 – 4.56)	0.98 (0.39 – 2.45)	1.00 (0.87 – 1.16)

[a]Odds Ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression.

[b]Adjusted for all variables shown in the table; patients with missing data excluded; N=330

[b]Adjusted risk ratios estimated from the multivariable logistic regression model with standard errors estimated using the delta method, adjusted for all variables shown in the table; patients with missing data excluded; N=330

HIV-1 drug resistance associated mutations

For patients with viremia at 12 months after starting DTG-based ART, blood plasma samples prior to treatment initiation were available for 25/30 (83%) patients. Of those, 14/25 (56%) samples were successfully PCR amplified and sequenced for PDR determination. In 2/14 (14%) samples we detected one mutation each, which were V108I and K103N mutations, known to be associated with NNRTI resistance. Additionally, 1/14 (7%) patient harbored the Q58E mutation, which is a PI-associated HIV drug resistant mutations. No mutations associated with NRTI were detected (Table 3).

Blood plasma samples at VL ≥ 50 copies/mL were available for 13/30 (43%) patients. PCR amplification and sequencing for determination of acquired and/or persisting HIV-1 drug resistance associated mutations was successful for only 2/13 (15%) patients. No mutations associated with HIV-1 drug resistance were detected (Table 3).

TABLE 3: Patterns of mutations detected in the reverse transcriptase and protease region of the HIV-1 pol -sequences in **participants on DTG -based ART with VL>=50 at 12 months.**

Description	At baseline (n = 14)[a]	At 12 months (n = 2)[b]
Major NRTI HIV drug resistant mutations	0 (0%)	0 (0%)
Major NNRTI HIV drug resistant mutations	V108I 1 (7%) K103N 1 (7%)	0 (0%)
Major PI HIV drug resistant mutations	Q58E 1 (7%)	0 (0%)

[a] 14/25 (56%) samples were tested for pretreatment HIV-1 drug resistance associated mutations.

[b] Acquired HIV-1 drug resistance associated mutations was tested for only 2/13 (15%) patients at viremia VL ≥ 50 copies/mL

Side effects and pregnancy outcomes

Seven (of 436, 2%) patients discontinued DTG-based due to side-effects. Of the women initiating DTG-based ART, thirty-three (12%) women had a documented pregnancy either at treatment initiation or during the follow-up period; 25 (76%) of those delivered a live born with no obvious birth defect, 3 (9%) had a reported abortion, 1 (3%) mother had a stillbirth, and 4 (12%) were LTFU before giving birth.

Discussion

In this prospective real-world study from a rural setting in Eastern Africa, we compared the virological outcome of DTG- and NNRTI-based ART in treatment-naïve PWH. We found higher virological suppression rates on DTG-based ART compared to NNRTI-based ART at 12 months after initiation. The documented difference in viral suppression in our cohort was even higher than in the first trials evaluating DTG-containing first-line regimens in African settings(165,166). The cohorts are comparable as there were no major changes regarding the clinical practice guidelines during the timeframes, specifically ART initiation was recommended for all, regardless of CD4 count since October 2016 in Tanzania, and VL testing was recommended to monitor treatment success.

Furthermore, we assessed for the presence of PDR, among patients with viremia ≥50 copies/mL at months after ART initiation in the DTG-cohort. No PDR to NRTI was detected; PDR to NRTI and PIs was rare, albeit the subgroup for available sequencing data was small. Thus, the observed elevated VL might be due to delayed suppression after treatment start or poor adherence with treatment interruption(167). Analyses from randomized controlled trials (RCTs) and real-life data have shown good virological suppression of DTG-based ART among patients with PDR to NNRTI(77,78,168). In our study we did not systematically screen for PDR to DTG, as resistances to INSTI would not be expected during the beginning of DTG-based ART roll-out, as reported in recent studies in similar settings(128,132,169). Moreover, of 30 patients with detectable VL in the DTG-based cohort 25(83%) had low viremia (i.e., 50-999 copies/ mL), which may be a result of momentarily subtherapeutic drug levels due to suboptimal adherence(167).

The fact that we found no HIV-1 associated drug resistance mutations in PWH with a detectable VL on a DTG-based ART is reassuring. However, these results are mitigated by the fact that genotypic results were only available for a small subgroup of participants.

While the high proportion of patients with low viremia at 12 months in the DTG-based cohort might be due to early measurement with an expected further decrease, it remains worrisome, as most patients are expected to suppress viral load within 3 months and low viremia due to poor adherence may subsequently lead to VF and emergence of resistance(170). Even though resistance to DTG is still uncommon in this setting, evidence from other studies now shows that resistance to DTG may emerge over time, particularly following accumulation of resistance to the NRTIs back-bone(38–40,171). Hence vigilance and monitoring of low viremia among PWH on DTG-based therapy remain important.

More than half of the patients in both cohorts had no VL results in the given time span. One reason is the high rate of LTFU, which accounted for 56% and 44% of those without VL results in the NNRTI and the DTG-based cohorts, respectively. This rate is comparable to a previous report from 2005-2016 from the same clinic with LTFU rates of 41% in the first year after enrolment (97). Though some of these patients may have silently transferred and collected medication from another clinic, and therefore have a suppressed VL, others are likely to have suboptimal adherence, or stopped medication altogether. Importantly, viral suppression rate in the sensitivity analysis was still higher in the DTG-based cohort compared to the NNRTI-based cohort. Regardless, the high

rate of LTFU in this rural setting in SSA remains a major concern of the treatment cascade and urgently needs a better understanding of patients' needs and adequate interventions(172).

Being on DTG-based ART was an important factor associated with improved viral suppression, which is in line with existing data from RCTs, that show excellent pharmacokinetic profile and tolerability of DTG, rapid viral suppression, and fewer side effects compared to NNRTIs in treatment-naïve and experienced patients(160). Similarly, in this study, we observed very few patients who discontinued DTG due to side effects. However more data on an extended time span is required and is the aim of future studies. Other factors associated with viral suppression were a higher education level and being separated/divorced/widowed. While other studies have indicated high odds of VF in clients who did not disclose their HIV status(173), we found no evidence of such an association. The association of virological suppression with advanced WHO clinical stages in our study might be due to patients feeling worse and therefore more likely to adhere to medication, also closer care and monitoring, and therefore better adherence(12). However, this outcome might also be affected by a reporting bias, as these patients are attending the clinic and are tested more frequently.

As in other settings, in the first year of DTG rollout in Tanzania, women of childbearing potential were given the choice to start either DTG-based or NNRTI-based ART, based on informed consent according to the WHO guidelines(174). Nevertheless, a number of women became pregnant while on DTG. Reassuringly, we did not document obvious birth defects in the 33 women with live births during the study period. One stillbirth and three abortions of unknown reasons were reported. While the numbers of this study are too low to draw firm conclusions, it is important to observe uptake among women and pregnancy outcomes as a large observational study from eleven LMICs has shown substantial disparities in the uptake of DTG affecting females of childbearing age (175). The benefit of rapid viral suppression in pregnant women is of uttermost importance to avoid viral transmission to their newborns.

To our knowledge, this is the first study addressing virological outcomes among treatment-naïve patients initiating DTG-based ART in Tanzania under programmatic conditions. Our study has several limitations. Most importantly, a large proportion of patients in both cohorts had no VL result in the given time span due to a high rate of LTFU. Another major limitation is unmeasured confounding as this is observational data. Further, many patients who were in active care also had

no VL result. Most likely reasons were stockout of reagents or procedural challenges during VL testing implementation. In both timespans National guidelines recommended VL testing to monitor treatment success. The lack of available VL results might have led to overestimating the rates of viral suppression. Another limitation is that that many blood samples were not available for drug resistance testing. Furthermore, there was significant PCR amplification failure, especially in those with a low viral load, which might be due to poor samples storage quality. Finally, all genotypic data were obtained through standard Sanger sequencing; rates of HIV associated drug resistance mutations would possibly be higher if ultra-sensitive HIV-1 drug resistance testing by Next-Generation sequencing had been used.

Conclusion

Our results underline the benefit of programmatic uptake of DTG-based ART in LMICs. We did not find pretreatment resistance to the DTG co-administered NRTIs nor acquired resistance among viremic patients on DTG-based ART, although the number of samples tested was small. Continuous monitoring of pre-treatment and acquired resistance under programmatic condition during the roll out of DTG-based first-line is of utmost importance. LTFU remains high and needs further attention as it jeopardizes control of the HIV epidemic.

Notes

Authors' contributions: AJN, AE and MW conceptualized the study, designed the experiments, and wrote the manuscript. AN, NK performed the viral load and resistance testing. AN, AE, JO, FV analysed the data and reviewed the manuscript. AN, AE, JO, FV, RN, NK, JF, JK, MJ participated in the interpretation of the data, writing, reviewing, and approving the final manuscript. The corresponding author AN is the guarantor of the paper.

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Patient Consent Statement: Written informed consent of patients willing to participate in KIULARCO are obtained at registration. This study received ethical approval from the University of the Witwatersrand-Human Research Ethics Committee (Medical) Clearance Certificate No.M210714**.** Yearly ethical approval for data and sample collection as well as analysis are sought

from the Ifakara Health Institute institutional review board (IHI/IRB/No16-2006) and the Health Review Committee of the National Institute for Medical Research of Tanzania (NIMR/HQ/R.8c/Vol.I/378)

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Potential Conflicts of Interest: All authors report no potential conflicts.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Table S1: Patients' characteristics at initiation for patients without and with 12-month viral measurement

Patient characteristics	Without 12-month VL N=496	With 12-month VL N=406
Socio-demographics		
Age, years, n (%)		
15 – 24	67 (14%)	39 (10%)
25 – 34	150 (30%)	106 (26%)
35 - 44	155 (31%)	147 (36%)
≥45	125 (25%)	113 (28%)
Gender, Female, n (%)	311 (63%)	271 (67%)
Marital status, n (%)		
Married/Cohabiting	301 (61%)	256 (63%)
Never married	56 (11%)	36 (9%)
Separated/Divorced/Widowed	140 (28%)	113 (28%)
Disclosed HIV status, n (%)		
No	127 (30%)	71 (19%)
Yes	301 (70%)	309 (81%)
Missing	69 (14%)	25 (6%)
Education, n (%)		
None	56 (11%)	37 (9%)
Primary	391 (79%)	326 (81%)
Secondary and above	50 (10%)	42 (10%)
Distance of residence to clinic, n (%)		
<1 km	213 (45%)	210 (54%)
2— <50 km	150 (32%)	126 (33%)

≥50 km	107 (23%)	52 (13%)
Missing	27 (5%)	17 (4%)
Clinical		
Tuberculosis status, n (%)		
Unlikely	436 (90%)	369 (93%)
Yes	51 (11%)	30 (8%)
Missing	10 (2%)	6 (2%)
Body Mass Index (BMI)[b], Kg/m^2, n (%)		
Underweight (<18.5)	66 (15%)	53 (13%)
Normal (18.5 - <25)	254 (59%)	236 (60%)
Overweight (≥25)	112 (26%)	106 (27%)
Missing	65 (13%)	10 (3%)
HIV WHO stage[b], n (%)		
I/II	290 (66%)	286 (71%)
III/IV	159 (35%)	115 (29%)
Missing	48 (10%)	4 (1%)
CD4 count, cells/µl[b], n (%)		
<100	90 (22%)	72 (19%)
100 - 350	161 (40%)	191 (50%)
≥350	151 (38%)	118 (31%)
Missing	95 (19%)	24 (6%)

Results are number and column % of those with non-missing data; missing data rows are number and column %.

[b]Tuberculosis, BMI, HIV WHO stage and CD4 measurements closest to ART initiation within 6 months before and 3 months after.

Table S2: Factors associated with viral suppression (HIV-1 RNA <50 copies/ml) at 12 months (+/- 3 months) among patients initiating NNRT-based or DTG-based ART.

Characteristics	Unadjusted OR (95% CI) [a]	Adjusted OR (95% CI) [ab]
ART regimen		
NNRTI-based	Reference	Reference
DTG-based	2.07 (0.93 – 4.65)	2.12 (0.82 – 5.49)
Age, years		
15 – –4	Reference	Reference
25 – 34	1.11 (0.33 – 3.77)	1.39 (0.32 – 5.93)
35 - 44	0.75 (0.24 – 2.29)	0.81 (0.20 – 3.28)
45 and above	0.51 (0.16 – 1.62)	0.33 (0.06 – 1.43)
Gender		
Male	Reference	Reference
Female	0.72 (0.34 – 1.52)	0.36 (0.13 – 0.98)
Marital Status		
Never	Reference	Reference
Married/Cohabiting	3.04 (1.14 – 8.15)	2.78 (0.71 – 10.9)
Separated/divorced/widowed	2.05 (0.70– 6.00)	2.68 (0.61– 11.7)
Disclosed HIV Status		
No	Reference	Reference
Yes	1.87 (0.81 – 4.32)	1.90 (0.69 – 5.19)
Education		
None	Reference	
Primary	4.76 (1.58 – 14.30)	
Secondary and above	8.25 (1.87 – 36.4)	
Distance of residence to clinic		
≤1km	Reference	Reference
2—<50km	1.08 (0.50 – 2.36)	1.29 (0.52 – 3.21)
≥50km	0.43 (0.67– 1.10)	0.46 (0.16– 1.38)
Tuberculosis status		
Unlikely	Reference	Reference
Yes	0.87 (0.26 – 2.90)	1.42 (0.32 – 6.41)

Body Mass Index, Kg/m^2		
Underweight, <18.5	0.61 (0.22 – 1.68)	0.74 (0.22 – 2.48)
Normal, 18.5 - <25	Reference	Reference
Overweight, ≥25	1.01 (0.46 – 2.26)	1.10 (0.40 – 3.04)
HIV WHO Stage		
I/II	Reference	Reference
III/IV	2.54 (1.29 – 5.01)	2.19 (0.72 – 6.67)
CD4 count, cells/µl		
<100	Reference	
100 – 349	1.42 (0.59 – 3.41)	
350	5.11 (1.66 – 15.7)	

[a]Odds Ratios (OR) and 95% confidence intervals (CI) obtained from logistic regression.
[b]Adjusted for all variables shown in the table except education and CD4 count due to model instability; patients with missing data excluded; N=160

PART 3

GENERAL DISCUSSION

CHAPTER 7

Summary, Overall discussion, and Conclusions

Highlights of the key findings

1. The overall prevalence of pre-treatment HIV-1 drug resistance in Eastern Africa, using data available from 2017-2022, has surpassed 10%.
2. The prevalence of RHI in this referral hospital-based cross-sectional study in a rural setting is 4%
3. The proportional of pre-treatment HIV-1 drug resistance among RHI cases is alarmingly high.
4. There is increasing tendency of pre-treatment HIV-1 drug resistance to NRTI in the Kilombero and Ulanga antiretroviral cohort.
5. Virological suppression rate on DTG-based ART is high compared to NNRTI-based ART at 12 months after initiation.

This thesis is dedicated to epidemiological and public health studies related to pre-treatment HIV-1 drug resistance (PDR) in Eastern Africa, with the focus in under-sampled area of rural Tanzania. To measure the change in PDR as a key component in assessing and addressing the threat of HIV drug resistance in Tanzania as in other Sub-Saharan African (SSA) countries.

We assessed the levels of PDR among ART- naïve adults living with HIV in seven Eastern Africa countries (*In paper I*). The overall pooled prevalence estimate of any PDR was higher than previous reported in the region, that included PDR data available until 2016(11). The high PDR prevalence was mainly driven by non-nucleoside reverse transcriptase inhibitors (NNRTIs) resistance, which underlines the importance of the current WHO recommended programmatic shift to dolutegravir based- triple ART for HIV treatment initiation (176). Tanzania started the rollout of dolutegravir-based ART in March 2019, by switching treatment experienced patients and initiating newly HIV-1 diagnosed patients on dolutegravir based- triple ART(176).

Remarkably, though on a limited sample size, prevalence estimate of any PDR was as high as 31% in the sub-population with recent HIV-infection (RHI) (*In Paper II*). The observed high PDR prevalence in recent versus longer-standing infected individuals could possibly be explained by the fact, that a proportion of drug resistance associated mutations are overgrown by more

replication-competent wild-type virus over time in chronically infected patients(177), especially in individuals who have never been exposed to ART, and thus not detected by standard genotypic viral resistance detection methods, e.g., population-based Sanger sequencing methods. This underlines the importance of intensifying strategies for PDR surveillance among recently HIV-infected patients in Tanzania.

We observed a low but notable prevalence of PDR to nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) below 5%, during a five-year period (2017-20220), in East-Africa (*In paper I*). However, prevalence of PDR to NRTIs > 5% was observed in a few individual studies, including those on PDR to NRTIs in HIV high-risk population groups, e.g., young adults and people with intravenous drug use(43,124,130). Notably, PDR to NRTI was up to 4 times more common among HIV recently infected sub-population (*In paper II*) than observed in the general population (*In paper I*)(56). Moreover, in the general ART-naïve adults' population in the Kilombero and Ulanga antiretroviral cohort (KIULARCO) (*In paper III*), there was a tendency increase in prevalence of PDR to NRTI over time; 2.1% in the first five-year period (2005-2009) of the ART program in Tanzania, 3.4% in the most recent period (2019-2022). Certainly, surveillance of PDR to NRTIs remains particularly important, as these backbone drugs remained the same throughout the change to dolutegravir first line treatment.

Of-note, no major PDR to dolutegravir (DTG)-associated mutation was observed during a five-year period (2017-2022) in Eastern Africa (*In paper I*). Similar observation has also been reported in West Africa(178,179),which further suggests effectiveness of dolutegravir-based regimens, and thus re-assuring for the ongoing DTG- based triple ART rollout in many Low- & middle-income countries(180). Nevertheless, accessory mutations associated with DTG resistance are common (128,180)(48), which - when accumulating with other mutations - may significantly reduce viral susceptibility to integrase inhibitors(180). Even though no major PDR to DTG was reported, DTG is relatively new in Eastern Africa, hence continuous surveillance is warranted.

In an observational study (*In paper IV*), virological suppression at 12 months was significantly higher in patients initiating DTG-based ART compared to NNRTI-based ART under a programmatic condition in a rural Tanzanian setting. DTG is known for its interesting pharmacokinetic profile, including rapid viremia suppression rates and high genetic barrier to developing resistance. However, existing weakness in the ART program functioning, coupled with patients' factors leading to inadequate adherence to ART, inevitably results in an increased risk of acquiring HIV-1 drug resistance mutations, which may in turn lead to an increased risk of PDR in individuals who have never been exposed to ART(152). Even though, we did not assess the emergence of resistance to DTG- in this study, DTG HIV resistance mutation have recently been reported among patients experiencing virologic failure in Tanzania (181), and neighbour country-

Malawi(168). Monitoring of emergence and spread of resistance to DTG remains important, particularly in resource-limited settings.

Moreover, PDR to NRTI have been previously reported as major risk factor for virological failure on DTG based ART, and subsequent emergence of DTG resistance, due to functional monotherapy in triple combination with inactive NRTIs(76,137,168,182). However, in this study (*In paper IV*), there were no HIV drug resistant mutations associated with PDR to DTG co-administered NRTI, among viraemic patients at one year on dolutegravir-based first-line ART. Though firm inference is difficult due to the small number of samples tested.

PDR to protease inhibitors (PI) was below 1% (*In paper I*). This is re-assuring as PI (mainly LPV/r plus a two-NRTI backbone) is still recommended as a preferred first-line for HIV treatment initiation in infants and children in Tanzania as in many other LMICs(176), and remains the second line treatment in adults upon treatment failure. Though on a small sample size, prevalence of PDR to PIs >5% was documented among ART-naïve recently HIV infected adult patients (*In paper II*), also among patients failing on DTG based triple ART with 3TC/TDF, at the time of ART initiation (*In paper IV*). Notably, prevalence of PDR to PIs >5% was reported in individual studies using high throughput deep genotypic resistance testing methods (*In paper I*)(113,119). These observations warrant continuous surveillance of PDR to PI, especially now that the costs for deep sequencing technologies are going down and accessibility is increasing in Tanzania.

The major conclusions from this PhD research.

1. There is a notable high prevalence of PDR in Eastern Africa, and tendency to increasing prevalence of PDR to NRTI particularly in the KIULARCO, as a representative rural SSA setting.
2. There is a high prevalence of PDR to NRTI among recently HIV-1 infected PLHIV, even though inference with small numbers is challenging.
3. High proportion of newly HIV-1 diagnosed PLHIV in this rural Tanzanian setting have chronic HIV-1 infection.
4. Evidence from individual studies in a systematic review shows high PDR to NRTI in populations at high risk of HIV transmission.
5. Finally, being on DTG-based ART was an important factor associated with improved viral suppression; No PDR to INSTI was found.

RECOMMEDANTION

1. Routine surveillance of pre-existing resistance to the DTG co-administered NRTI remains particularly important, in resource-limited settings, to prevent risk of failure of newer antiretroviral agents such as dolutegravir, which would be detrimental for Tanzania and other low- and middle-income countries for the aim to “end AIDS by 2030”.
2. Given that our data show low prevalence of recent HIV-1 infection among newly HIV-1 diagnosed PLHIV, implying that diagnosis of HIV is delayed in this rural Tanzanian setting. It’s important to strengthening strategies for early HIV cases identification and linkage into care and treatment.
3. Particular attention should be paid to people with recent HIV infection, given that our data show high prevalence of not only PDR to NNRTI but also NRTI- backbone mutations in this group. Also, PDR among recently HIV-1 infected population needs to be a consideration while estimating the burden of PDR in a population.
4. Strengthening surveillance of PDR in populations at high risk of HIV transmission (e.g., youth and young adults and people with intravenous drug use) is certainly important.
5. Since DTG is relatively new in Eastern Africa. Long-term continuous surveillance and monitoring of PDR to DTG is warranted.

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Appendix

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Mr A Ntamatungiro

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210714

NAME: (Principal Investigator)	Mr A Ntamatungiro
DEPARTMENT:	School of Public Health Medical School University
PROJECT TITLE:	*Trend of pre-antiretroviral therapy HIV-1 drug resistance in Kilombero and Ulanga antiretroviral cohort, South-Western Tanzania, for over 15 years (2005-2020)*
DATE CONSIDERED:	2021/07/30
DECISION:	Approved unconditionally
CONDITIONS:	Prior condition satisfied 2021/09/22
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Drs J Kagura, J Francis and M Weisser
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/08/26

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **July** and therefore reports and re-certification will be due in the month of **July** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

2021/09/22
Date

EACS, London, October 27-30, 2021

Transitioning to Dolutegravir: Virological outcomes in an HIV cohort in rural Tanzania

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Background
Dolutegravir-based antiretroviral therapy (ART) is being rolled out in many lower- and middle-income countries (LMICs) due to its better tolerability, efficacy and higher resistance barrier than non-nucleoside reserve transcriptase inhibitors (NNRTI). Real-life data are crucial to monitor the success and risks of transitioning to new regimens. We assessed virological outcomes after dolutegravir rollout in rural Tanzania.

Methods
The Kilombero and Ulanga Antiretroviral Cohort (KIULARCO) enrols consenting patients attending the Chronic Diseases Clinic of Ifakara, a rural care and treatment centre for HIV-infected people in south-eastern Tanzania. We compared viral suppression (HIV-1 RNA <50 copies/mL) 12 months after treatment start (window 5-15 months) between adults (≥15 years) initiating dolutegravir-based ART in March 2019 - March 2020 (dolutegravir cohort) versus those initiating NNRTI-based ART in December 2016 - December 2017 (NNRTI cohort). Patients >60 days late for a scheduled visit were defined as loss to follow-up (LTFU). We assessed factors associated with 12-months viral suppression using logistic regression models.

Results
Viral load (VL) measurements were available for 170/297 (57%) participants in the dolutegravir cohort and 210/463 (45%) in the NNRTI cohort, with patients' characteristics shown in table 1. Viral suppression was achieved for 144 (85%) and 151 (72%) in the dolutegravir and NNRTI cohorts, respectively (chi2 p=0.003). 73/297 (25%) in the dolutegravir and 142/463 (31%) in the NNRTI cohorts were LTFU. Assuming those LTFU were not virally suppressed, 144/243 (59%) and 151/352 (43%) achieved viral suppression in the dolutegravir and NNRTI cohorts, respectively (chi2 p<0.001). Adjusted for confounders, DTG-based ART was associated with >2 times the odds of viral suppression versus NNRTI-based ART (table 2).

Conclusion
Virological suppression at one year after treatment start was significantly higher in patients initiating DTG-based ART compared to NNRTI-based regimens. This underlines the importance of programmatic uptake of DTG-based ART in LMICs. LTFU remains high and needs further attention as it potentially jeopardizes control of the HIV epidemic.

9th Annual Joint Advanced Seminars Conference 29th July 2022, Kampala, Uganda (Oral presentation).

Transitioning to Dolutegravir in a programmatic setting: Virological outcomes in the Kilombero and Ulanga Antiretroviral Cohort in rural Tanzania

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Background: Clinical and virologic outcome data after programmatic transition from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based to dolutegravir (DTG)-based antiretroviral treatment (ART) regimens in sub-Saharan Africa outside of clinical trials is scarce. Here we evaluate virological outcome of DTG-based ART after the first year of rollout in Ifakara, rural Tanzania.

Method: Within the prospective Kilombero and Ulanga Antiretroviral Cohort (KIULARCO), we analyzed in a nested study virological suppression, defined as HIV-1 RNA <50 copies/mL at 5-15 months after treatment initiation in treatment-naive people living with HIV aged ≥ 15 years, initiating NNRTI-based ART from 16th December 2016 to 15th December 2017 and DTG-based ART from 16th March 2019 to 15th September 2020. Factors associated with viral suppression were assessed using logistic regression.

Results: Viral suppression was achieved for 165/195 (85%) and 154/211 (73%) in the DTG-based and NNRTI-based cohorts, respectively (p=0.003). 108/436 (25%) in the DTG-based ART and 143/466 (31%) in the NNRTI-based cohorts were LTFU within this first year. Assuming those LTFU were not virally suppressed, 144/243 (59%) and 108/241 (44%) achieved viral suppression in the DTG-based ART and NNRTI-based cohorts, respectively (p<0.001). DTG-based ART was associated with >2 times the odds of viral suppression versus NNRTI-based ART.

Conclusion: Virological suppression at one year after treatment start was significantly higher in patients initiating DTG-based ART compared to NNRTI-based regimens. This underlines the benefit of programmatic uptake of DTG-based ART in rural sub-Saharan Africa.

Wits School of Public Health Research Day and CARTA Conference 14-15 September 2023, Johannesburg, South Africa (Poster presentation).

Recent HIV-1 infection and pre-treatment HIV drug-resistance in recently infected adults initiating antiretroviral therapy in rural Tanzania.

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Background: Recently HIV-1 infected patients remain an important threat to ending the HIV epidemic, as early and undiagnosed HIV leads to the highest number of transmissions and possibly spread of viral resistance. We determined the proportion of recent HIV-1 infection (RHI) among newly diagnosed people living with HIV (PLHIV). Additionally, we assessed the prevalence of pre-treatment HIV-1 drug resistance (PDR) in recently infected PLHIV in rural Tanzania.

Methods: We conducted a cross-sectional analysis for RHI among newly diagnosed adults enrolled in the Kilombero and Ulanga Antiretroviral Cohort between March 2019 and March 2022. Risk factors of RHI were determined using logistic regression. Genotypic resistance testing (GRT) was performed for RHI cases.

Results: Among 604 PLHIV, 24 (4%) had RHI. We found no factors associated with RHI. GRT was successful in 16/24 (67%) participants, and 5/16 (31%) had mutations associated with HIV-1 drug resistance: 4/16 (25%) for non-nucleoside reverse transcriptase inhibitors, 2/16 (13%) for nucleoside reverse transcriptase inhibitors and 1/16 (6%) for protease inhibitors.

Conclusion: The low observed prevalence of RHI in this referral hospital-based study, indicates the high number of late diagnoses., and highlights the need to further strengthen strategies for early HIV diagnosis and linkage to care. Although on a limited sample-size, the observed high proportion of recently HIV infected patients with PDR is a serious public health concern.