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Rilpivirine and Etravirine resistance among HIV-1 infected patients failing first generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) in Busia, Western Kenya

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Rilpivirine (RPV) and Etravirine (ETR) are second-generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are not used for HIV-1 treatment in Kenya. In this cross-sectional study, we sequenced and analyzed the reverse transcriptase and pol regions of HIV-1 genome from 140 HIV infected individuals from Busia County Referral Hospital, Western Kenya, who were on anti-HIV treatment with confirmed virologic failure. All the participants were on first-generation NRTI's and NNRTI's for more than 12 months at the time of the study. Briefly, HIV RNA was extracted from plasma samples and sequenced to analyze for the presence of HIV-1 drug resistance mutations. The study findings showed that approximately 46% of the population had genotypic drug resistance against both Etravirine and Rilpivirine which were classified as ranging from potentially low level resistance to high level resistance despite being exposed to first-generation NNRTIs only. The study thus reveals that cross-resistance was demonstrated between primary and secondary NNRTI drugs. The development of cross resistance for RPV and ETR in patients on EFV and NVP poses a challenge in the use of these drugs as second generation NNRTI drugs.

Key words: Non-nucleoside reverse transcriptase inhibitors (NNRTIs), NRTIs, Kenya, cross-resistance, Etravirine (ETR), Rilpivirine (RPV), HIV-1.

INTRODUCTION

As of 2019, 38 million people were estimated to be living with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) globally (Joint United Nations Programme on HIV/AIDS, 2019; Vardell, 2020).

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Sub-Saharan Africa bears the largest global HIV/AIDS burden with 70% of the infections (Kharsany and Karim, 2016). South Africa leads in the number of HIV/AIDS cases in Africa and globally (Mabaso et al., 2019). Kenya on the other hand, is among the top five countries with the highest HIV burden globally (Kimanga et al., 2014) and has made considerable progress in the fight against HIV/AIDS over the years (Kenya AIDS Response Progress Report, 2016; Mwau et al., 2018; Joint United Nations Programme on HIV/AIDS, 2018b). By the end of 2018, an estimated 1.6 million people were living with HIV, 46,000 people being newly infected with HIV, and 26,000 dying from AIDS-related in Kenya (Joint United Nations Programme on HIV/AIDS, 2018b). Approximately 1.5 million people between 15-49 years were living with HIV/AIDS in Kenya by the end of 2017 (National AIDS Control Council, 2018). The number of new HIV-1 infections in Kenya was estimated to be 77,647 in 2015 alone (National AIDS Control Council (NACC), 2016; Joint United Nations Programme on HIV/AIDS, 2018c), with the numbers dropping to 52,800 in 2017 (National AIDS Control Council, 2018). There was a significant decline in the total number of HIV/AIDS orphans from 959,334 children in 2010 to 661,119 in 2015 (National AIDS Control Council, 2018).

Globally, there has been a concerted effort to scale up the provision of antiretroviral therapy (ART) (Aung et al., 2018; Dokubo et al., 2014) especially following the recent findings showing that ART is important in decreasing HIV transmission rates under the framework of HIV Treatment as Prevention (TasP) in different settings (Brault et al., 2019, 2020; Girum et al., 2018; Montaner et al., 2014; Osler et al., 2018). There has been a massive scale-up of antiretroviral therapy (ART) treatment and the associated services in sub-Saharan Africa in the past decade (Kharsany and Karim, 2016; Vandormael et al., 2019) to reduce AIDS-related mortality and morbidity as well as prevent HIV transmission and hence reducing its prevalence (Kharsany and Karim, 2016; Montaner et al., 2014; Osler et al., 2018). Antiretroviral drug regimens against HIV/AIDS available globally are formulated against HIV-1 subtype B, the most common subtype in the Americas and Western Europe (Crawford et al., 2014; Junqueira and de Matos Almeida, 2016; Kantor et al., 2014; Taylor et al., 2008). However, HIV-1 subtype B accounts for a small fraction (~12%) of the global HIV epidemic with subtypes A1, C, and D accounting for most of the epidemic (~88%) currently witnessed especially in sub-Saharan Africa (Faria et al., 2019; Lessells et al., 2012; Shao and Williamson, 2012; Venner et al., 2016).

There has been a steady increase in the number of HIV infected individuals in Kenya (National AIDS Control Council, 2018). By the end of 2018, 89% of those infected with HIV in Kenya had been diagnosed and knew their HIV status while 68% of those infected were already on medication (Arts and Hazuda, 2012; Marsh et al., 2019; Joint United Nations Programme on HIV/AIDS, 2018a). The introduction of the “test and treat” strategy in Kenya under the 90-90-90 United Nations declared target where everyone who tests HIV seropositive is immediately initiated on ART and pre-exposure prophylaxis (PrEP) to populations at risk of HIV infection in many high HIV burden countries as a prevention strategy has contributed to increased number of Kenyans under ART (Eakle et al., 2018).

The recommended first-line non-nucleoside reverse transcriptase inhibitors (NNRTI) medications for adult and adolescent HIV-1 treatment in Kenya are Tenofovir disoproxil fumarate (TDF), lamivudine (3TC) (or emtricitabine, FTC), Dolutegravir (DTG) or efavirenz (EFV) 400/600 mg (National AIDS and STI Control Programme, 2018) ; Vitoria et al., 2019; World Health Organisation, 2019). Whereas FTC is not commonly used in Kenya, DTG has since been approved as a first line drug of choice replacing EFV in program settings due to its tolerability, durability, effectiveness, simplicity, cost-savings and high resistance barrier (de Waal et al., 2018; Lahuerta et al., 2020; Paul and Ugwu, 2020; World Health Organisation, 2019). Etravirine (ETR) and Rilpivirine (RPV) are second generation NNRTIs suitable for people failing the first generation NNRTIs (Diphoko et al., 2018; Teeranaipong et al., 2016). One of the future options is to ratify the use of ETR and RPV which have a higher genetic barrier and hence demonstrate lower resistance profiles than their first-generation counterparts. However, several studies have demonstrated the cross-resistance between first and second-generation NNRTIs, thereby compromising the use of ETR and RPV as salvage drugs (Anta et al., 2013; Svärd et al., 2017; Vitoria et al., 2019). There is limited data on ETR and RPV resistance patterns in sub-Saharan Africa, in Kenya and also in Busia County which was our study site.

This study aimed at evaluating the prevalence of resistance against second-generation non-nucleoside reverse transcriptase inhibitors (ETR and RPV) in patients who were on first-generation NNRTIs (EFV and NVP) for more than 12 months in Western Kenya. The results from this study are aimed at advising on the use of second-generation NNRTIs as alternatives in HIV treatment programs hence influencing policy.

METHODOLOGY

Study site setting

The study was conducted at the Busia County Referral Hospital, Busia County, Western Kenya. All patients were on first-generation NRTI and NNRTI regimens at the hospital’s HIV comprehensive care center (CCC) which provides HIV treatment and care services to patients from Kenya’s Western region.

Study participants

The study participants were HIV-1 positive adult patients (aged between 18 years and 60 years), receiving a triple ARV therapy

Table 1. RT, nested and sequencing PCR Primers used for the amplification of the specific target gene region (Pol-Reverse transcriptase region).

Primer Code	Direction	Sequence
RT18	Forward 1	5'GGAAACCAAAAATGATAGGGGGAATTGGAGG 3'
KS104	Reverse 1	5' TGACTTGCCCAATTTAGTTTTCCCACTAA 3'
KS101	Forward 2	5'GTAGGACCTACACCTGTTCAACATAATTGGAAG 3'
KS102	Reverse 2	5'CCCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG 3'

regimen classified as standard first-line (like TDF or AZT, 3TC, and either NVP or EFV) from Busia county hospital for twelve months or more. The patients must have attended the clinic at least once within the previous six months and given informed consent to participate in the study. Study participants who demonstrated virologic failure as per the Kenyan guidelines with viral loads of >1,000 copies/mL had their blood tested for HIV drug resistance.

Ethical considerations

This study was approved by the KEMRI/National Scientific and Ethical Review Committees. Written informed consent was obtained from each participant before conducting the study procedures. Participants not willing to provide informed consent were excluded from the study.

Laboratory testing

HIV-1 RNA viral load testing

Plasma samples for HIV-1 viral RNA and drug resistance testing stored at -70°C were retrieved and thawed at room temperature. A total of 925 patients qualified for viral load testing based on the above inclusion criteria. Blood was drawn from these participants and viral RNA was extracted from blood plasma using Qiagen RNA MiniAmp kit (Qiagen, 2020). Viral load testing was performed using Abbott M2000SP/RT (Abbott Molecular, Inc., Des Plaines, IL, USA) viral load testing assay, whose lower detection limit was 40 copies/mL. Three levels of controls (high positive, low positive and negative controls) were included in each run as per good clinical laboratory guidelines for quantitative testing to ensure accuracy and reliability of results.

HIV-1 drug resistance testing

Reverse transcriptase (RT) genotyping for resistance identification was performed for all participants having a viral load of >1,000 copies/ml (n=146). Reverse transcription of the RNA was performed by priming with UNINEF7 primer (5'-GCACTCAAGGCAAGCTTTATTGAGGCTT-3') close to the 3′ end of the viral RNA. The extracted RNA (3 µl) was reverse transcribed in a total volume of 20 µl with 500 µM dNTP, 2.5 µM primer, 1X RT buffer, 5 mM $MgCl_2$, 10 mM DTT, 40 U RnaseOUT, and 400 U SuperScriptTM III RNase H− RT. The first-round of PCR had 25 µl reaction volume with a mixture containing 3 µl of 5 U Expand Long Template (Roche Diagnostics, Indianapolis, IN), 2.5 µl of 5X buffer, 0.3 µl of each RT18 and KS104 primers (Table 1), 2.0 µl dNTP, 2.0 µl $MgCl_2$, 14.7 µl of distilled water and 0.2 µl of Taq polymerase. The cycling conditions were 1 cycle of 95°C for 10 min and 35 cycles of 95°C for 30 s, annealing at 30°C for 60 s, and 72°C for 1 min, and final extension of 72°C for 10 min. From the first-round PCR products, 3 µl was used as a template for the second reaction volume with the second set of primers in Table 1 (KS101 and KS102). The products were then directly sequenced using the second set of primers (KS101 and KS102) (Table 1). This sequence PCR was carried in a reaction mixture of 20 µl with a dilution of 1:10. These contained 3 µl of DNA, 5X sequence buffer, 2.0 µl BigDye®, 10.5 µl of distilled water, and 1.5 µl of forward and reverse primers. Amplification was carried out using a thermal cycler at following PCR conditions: denaturation for 5 min at 96°C, and again for 10 s at 96°C, annealing at 50°C for 5 s, and final extension 60°C 4 min for 25 cycles. The HIV-1 genotyping assay, which sequences the HIV-1 pol gene (base pairs covering PR region: codons 4 - 99 and RT region: codons 41 - 247), was performed directly using an automated ABI 3100 Genetic Analyze. Sequence quality control was performed using the Los Alamos HIV-1 sequence quality assurance tool on https://www.hiv.lanl.gov/content/sequence/QC/index.html?sample_input=1. HIV-1 resistance-associated mutations and phenotypic drug resistance profiles were obtained from the Stanford University HIV drug resistance database on https://hivdb.stanford.edu/hivdb/by-sequences/. For statistical analysis, number of NNRTI DRAMs per person and the differences of their distribution in either EFV- or NVP-based regimen were analyzed by Pearson's chi-square test. Susceptibility/resistance analysis within EFV- or NVP-based regimen (paired samples: susceptibility of ETR vs RPV in failed EFV- or NVP-based regimen) was analyzed by using Wilcoxon signed-rank test. Pearson's correlation coefficients were calculated. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Participant demographics

A total of 925 participants met the study's inclusion criteria with 548 (59.24%) females and 377 (40.76%) males (Table 2). The participants' ages ranged between 21-67 years (IQR= 14.25), the mean age was 38.79 years, a median of 44 years, and a mode of 38 years. The participants were either on EFV or NVP based regimens with 73.9% (n=684) being on EFV-containing regimens and 26.1% (n=241) of study participants being on NVP containing drug combination (Table 2).

Viral load results

Ten (10) samples failed HIV viral load (VL) testing with

Table 2. Socio-demographic and clinical characteristics for HIV infected participants enrolled at the Busia County Referral Hospital Comprehensive Care Centre in 2019.

Variable	n (%)
Gender	n=925 (%)
Male	377 (40.76%)
Female	548 (59.24%)
Age (years)	**n=925 (%)**
<30	177
31-40	380
41-50	226
>50	142
Range	23-67
Mode	38
Mean	38.79
STDEV	10.03
Treatment regimen	**n=925 (%)**
NVP based regimen	241 (26.1)
EFV based regimen	684 (73.9)
Treatment combinations	
AZT+3TC+NVP	190 (20.5)
TDF+3TC+EFV	578 (62.5)
AZT+3TC+EFV	157 (17)
Viral load counts (copies/ml)	**n=915 (%)**
Male	371 (40.5%)
Female	544 (59.5%)
Minimum	Not Detected
Maximum	1,234,454
Detectable viral load counts	**n= 218 (%)**
Range	54 -1,234,454
Mean	62,542
Virologic treatment failure	**n= 146 (%)**
Female	101 (69.2%)
Male	45 (30.8%)
VL Range	1472 - 1,234,454 copies/ml
Mean	93,300.30 copies/ml

915 successfully testing for HIV VL counts. Of the 915 eligible participants with successful HIV VL test results, 224 (24.5%) had detectable HIV VL (>40 copies/ml) while 691 (75.5%) had HIV VL counts below detection levels (<40 copies/ml). Virologic treatment failure (described as HIV VL counts > 1000 copies/ml) was reported in 16% (146/915) of the participants (Table 2), although only 140 were successfully sequenced and tested for drug resistance (GenBank accession numbers MW618176-MW618315). HIV-1 drug resistance-associated mutations (DRAMs) were reported in 62.1% (87/140) of the participants successfully sequenced and tested for drug resistance.

NNRTI drug resistance associated mutations (DRAMs)

A total of 197 NNRTI DRAMs conferring resistance to first generation (EFV and NVP), and second generation (ETR and RPV) NNRTIs were present and distributed among 82/140 (58.6%) of the participants in the study population. K103N/S family of NNRTI DRAMs had the highest frequency at n=39(19.8%) followed closely by G190A/S family DRAMs at n=33(16.8%) of the total NNRTI DRAMs (Table 3).

While fifty-eight (participants did not possess any NNRTI-DRAM, 21 participants had one NNRTI-DRAM each, 27 participants had 2 NNRTI-DRAMs each, 22 participants had 3 NNRTI-DRAMs each, 7 participants had 4 NNRTI-DRAMs each and 2 participants had 5 NNRTI-DRAMs each. The maximal number of NNRTI-DRAMs present in one participant was 6, present in 3 participants (Figure 1).

Comparison between different NNRTI DRAMs between EFV and NVP groups showed that there were significantly higher frequencies of mutations in the EFV group compared to the NVP group [p=<0.001] (Figure 2).

ETR/RPV resistance causing mutations

Overall,76 (54.3%) of participants successfully sequenced and tested for resistance did not possess any ETR/RPV resistance causing mutations and hence contained the wild-type virus in regards to ETR and RPV DRAMs. Sixty-four (64) participants in the study had at least one or more mutations conferring varying degrees of phenotypic drug resistance profiles to both ETR and RPV. Interestingly, there was comparatively equal number of participants with ETR and RPV DRAMs (64 for each).

Despite these similarities, the resultant phenotypic resistance profiles and especially high-level resistance were higher for RPV than for ETR. The most common ETR/RPV DRAM was G190A/S (35%) followed by K101E/P and Y181C/S/H DRAMs at 21 and 15% respectively (Figure 3).

G190A mutation presents alone conferred potential low-level resistance to ETR and low-level resistance to RPV. Y181C/S/H mutation alone conferred intermediate resistance to EFV, ETR, and RPV but high-level resistance to NVP while Y188L mutation in isolation conferred high-level resistance to EFV, NVP, and RPV and potential low-level resistance to ETR. On the other

Table 3. Frequency of NNRTI DRAMs present in HIV infected participants enrolled at the Busia County Referral Hospital Comprehensive Care Centre in 2019.

NNRTI DRAM	Number of DRAMs	Percent
K103N/S	39	19.8
G190A/S	33	16.8
K101E/P	20	10.2
H221Y	18	9.1
Y181C/S/H	17	8.6
V108I	16	8.1
K238T	16	8.1
A98G	9	4.6
E138A/K/G	6	3.0
P225H	6	3.0
V179D/T/E	5	2.5
L100P/I	4	2.0
Y188N/F	4	2.0
M230L	4	2.0
Total	**197**	**100**

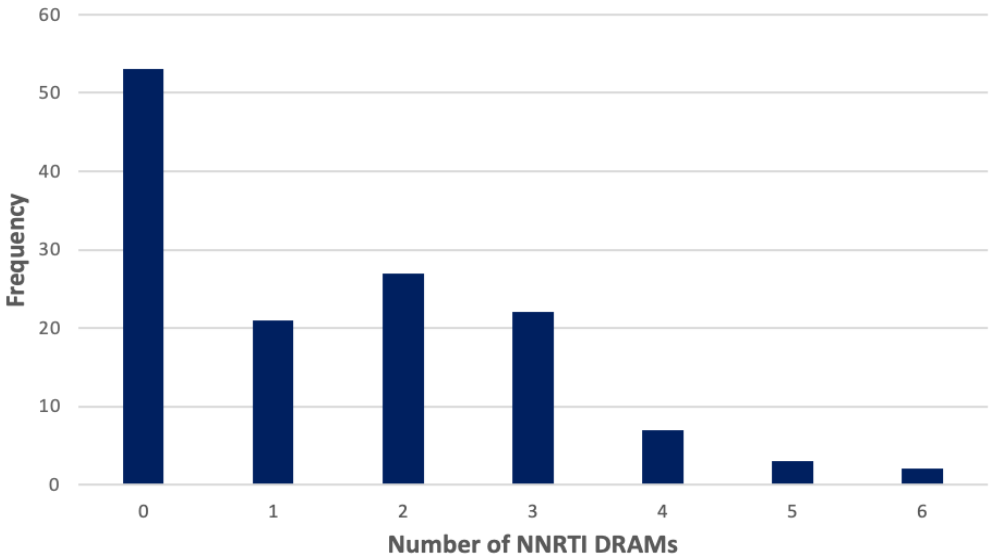

Figure 1. Frequency of cumulative number of NNRTI DRAMs for HIV infected participants enrolled at the Busia County Referral Hospital Comprehensive Care Centre in 2019 (n=82).

hand, E138 series DRAMs (E138K, E138A, E138Q, and E138G), usually rare mutations in non-B subtypes had effects of potential low-level resistance to ETR and low-level resistance to RPV when found in isolation whereas V179/T/E mutation conferred potential low-level resistance to both ETR and RPV. K103N and K103E did not affect the phenotypic resistance profiles for ETR and RPV.

A comparison between subtypes revealed that subtype A1 was present in 50% of the participants with ETR and RPV resistance profiles, subtype D represented 28%, subtypes A1_D and A1_C represented 5% each, subtypes A1_J, B, and G represented 3% each whereas B_C represented 2% and A1_F1 represented 1% (Figure 4).

Overall, 76 participants were susceptible to both ETR and RPV. On the other hand, 22 participants had potential low-level resistance to ETR with 5 participants

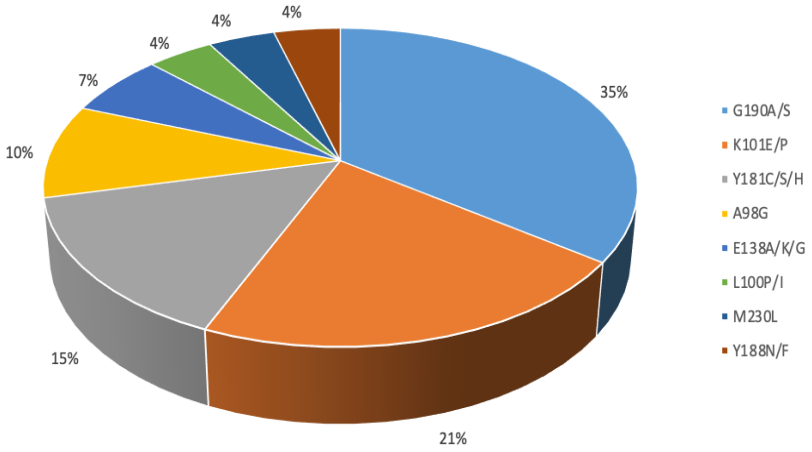

Figure 3. Proportions of ETR/RPV DRAMs for HIV infected participants enrolled at the Busia County Referral Hospital Comprehensive Care Centre in 2019 (n=82).

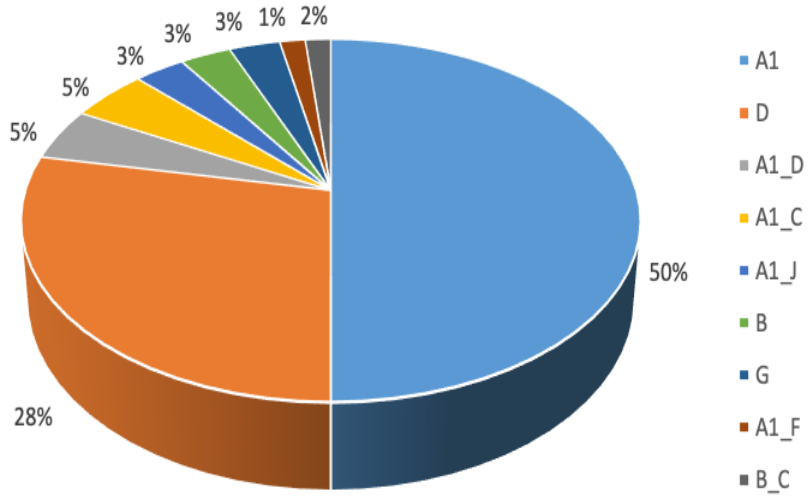

Figure 4. Proportions of ETR/RPV resistance profiles in different subtypes for HIV infected participants enrolled at the Busia County Referral Hospital Comprehensive Care Centre in 2019 (n=82).

demonstrating potential low-level resistance to RPV. Ten (10) participants demonstrated low-level resistance to ETR while 14 participants had low-level resistance to RPV (Table 4). Twenty-three (23) participants demonstrated intermediate resistance to ETR with 22 participants demonstrating intermediate resistance towards RPV. There were 9 participants with high-level resistance to ETR compared to 23 participants with high-level resistance to RPV (Table 4). Of those with high-level resistance to ETR, 2 (22%) were males whereas 7 (78%) were females. Of the 23 participants with high-level resistance to RPV, 14 (60.9%) were females while 9 (39.1%) were males. The differences between ETR and RPV phenotypic resistance profiles were statistically significant (p-value = 0.0015). Of the 76 participants who were susceptible to ETR and RPV (with wild-type virus), 39 (51.3%) were females whereas 36 (48.7%) were males. Of the 64 participants with any level of phenotypic resistance, 39 (60.9%) were females while 25 (39.1%) were males.

Six (6) participants reported the rare E138 series mutations (E138K, E138A, E138Q, and E138G) that confer phenotypic resistance to ETR and RPV. All these participants with E138 series mutations had HIV-1

Table 4. Distribution of the phenotypic drug resistance profiles for ETR and RPV for HIV infected participants enrolled at the Busia County Referral Hospital Comprehensive Care Centre in 2019.

Phenotype	EFV	NVP	ETR	RPV
High-Level Resistance	62	74	9	23
Intermediate Resistance	13	1	23	22
Low-Level Resistance	1	2	10	14
Potential Low-Level Resistance	3	2	22	5
Susceptible	61	61	76	76

subtype A1.

DISCUSSION

Our study confirmed the high level of cross-resistance between first-generation NNRTIs (EFV and NVP) and second-generation NNRTIs (ETR and RPV) as reported in several other studies (Diphoko et al., 2018; Saravanan et al., 2017). Sluis-Cremer (2014), reported the high level of cross-resistance between first and second-generation NNRTIs used for prevention and treatment of infection against HIV as a major source of concern for treatment programs, especially in resource-limited settings. Our study, in agreement with the aforementioned study, showed that 45.7% of our participants had genotypically predicted resistance to both ETR and RPV. Teeranaipong et al. (2016) reported that only 11.1% and 10.9% of patients who failed the NVP-based regimen and 32.2 and 31.6% of patients who failed EFV-based regimen in a Thai population were susceptible to ETR and RPV respectively. Our study, on the other hand, demonstrated that 20% of patients who failed EFV and NVP-based regimens were susceptible to both ETR and RPV. The difference in ETR/RPV susceptibility between the Thai and Busia (current) studies could be attributed to the differences in study sites and populations. However, there was some level of agreement between the two studies concerning the similarity in ETR and RPV susceptibilities.

First-generation NNRTIs (NVP and EFV) have low genetic barriers making them more prone to drug resistance (Echagüen et al., 2005; Ndashimye and Arts, 2019; Saravanan et al., 2017; Usach et al., 2013). Second generation NNRTIs (ETR and RPV) have previously demonstrated higher susceptibility profiles compared to the first generation NRRTIs in a majority of the studies (Diphoko et al., 2018; Echagüen et al., 2005; Usach et al., 2013; Zhou et al., 2016) due to their higher genetic barriers (Saravanan et al., 2017), a concept that was confirmed by our current study.

Till date, there are mixed reports on the differences between ETR and RPV genotypic and phenotypic resistance profiles from different research studies. Saravanan et al. (2017), reported 47% and 65% ETR and RPV resistance profiles respectively, contrary to our current study which reported similar percentages of resistance profiles between ETR and RPV (both with 45.7% resistance). Teeranaipong et al. (2014) reported 32.2 and 31.6% susceptibility in ETR and RPV, respectively which was in concordance with the results of this study. There is therefore a need for more studies to clearly define the prevalence of ETR and RPV-associated mutations in populations receiving first-generation NNRTIs especially sub-Saharan Africa.

This study shows G190A/S mutations were the most prevalent ETR/RPV resistance causing mutations in this population followed by K101E/P and Y181C/S/H with 21.9, 13.7 and 10.2% respectively. This differed slightly with the findings of Saravanan et al. (2017), who showed Y181C/H mutations as the most prevalent, followed by G190A/S and K101E/H/P (45, 32.5, and 26%) respectively. On the other hand, Gallien et al. (2015) study on subtype B predominant population reported a higher prevalence of Y181C/I/V mutations (18%) followed by K101E/P (9%) and interestingly by the rare E138A/G/K/Q/R/S (6%), a sharp contrast with the findings of our study. This implies the possibility of differences in the prevalence of ETR/RPV resistance causing mutations based on the most prevalent HIV subtypes. More studies on cross-resistance between first and second-generation NNRTIs need to be carried out to determine the effect of different subtypes on cross-resistance.

Conclusion

This study has shown that high levels of cross-resistance between NNRTIs were observed; that is, cross-resistance within the first generation NNRTIs (NVP and EFV) and within second-generation NNRTI (ETR and RPV) as well as between first-generation and second-generation NNRTIs. The prevalence of ETR and RPV DRAMs was significantly higher in females than in males. Also, high viral load counts were indicative of genotypic and phenotypic ETR and RPV drug resistance. Drug resistance patterns to ETR and RPV were significantly

affected by the subtype in any given participant.

Limitations of the study

The small sample size, cross-sectional of virologic failure treated HIV patients and inclusion of infected at different times, may not be representative the epidemic drive in the region. Therefore, this data should be interpreted with caution. Additionally, recombination patterns in a heterogeneous epidemic are complex and will require another generation of genome data level to fully understand the changing dynamics of viral genotypes in this region of Kenya.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Anta L, Llibre JM, Poveda E, Blanco JL, Álvarez M, Pérez-Elías MJ, Aguilera A, Caballero E, Soriano V, De Mendoza C, Iribarren JA, Gatell JM, Ribera E, Martínez-Picado J, Clotet B, Jaén A, Dalmau D, Peraire J, Vidal F, Gómez-Sirvent JL (2013). Rilpivirine resistance mutations in HIV patients failing non-nucleoside reverse transcriptase inhibitor-based therapies. AIDS 27(1):81-85.

Arts EJ, Hazuda DJ (2012). HIV-1 antiretroviral drug therapy. Cold Spring Harbor Perspectives in Medicine 2(4):a007161.

Aung ZZ, Oo MM, Tripathy JP, Kyaw NTT, Hone S, Oo HN, Majumdar SS (2018). Are death and loss to follow-up still high in people living with HIV on ART after national scale-up and earlier treatment initiation? A large cohort study in government hospitalbased setting, Myanmar: 2013-2016. PLoS ONE 13(9):1-15.

Brault MA, Spiegelman D, Abdool Karim SS, Vermund SH (2020). Integrating and Interpreting Findings from the Latest Treatment as Prevention Trials. Current HIV/AIDS Reports 17(3):249-258.

Brault MA, Spiegelman D, Hargreaves J, Nash D, Vermund SH (2019). Treatment as Prevention: Concepts and Challenges for Reducing HIV Incidence. Journal of Acquired Immune Deficiency Syndromes 82(2):S104-S112.

Crawford KW, Njeru D, Maswai J, Omondi M, Apollo D, Kimetto J, Gitonga L, Munyao J, Langat R, Aoko A, Tarus J, Khamadi S, Hamm TE (2014). Occurrence of etravirine/rilpivirine-specific resistance mutations selected by efavirenz and nevirapine in Kenyan patients with non-B HIV-1 subtypes failing antiretroviral therapy. AIDS 28(3):442-445.

de Waal R, Lessells R, Hauser A, Kouyos R, Davies MA, Egger M, Wandeler G (2018). HIV drug resistance in sub-Saharan Africa: public health questions and the potential role of real-world data and mathematical modelling. Journal of Virus Eradication 4(Suppl 2):55-58.

Diphoko T, Gaseitsiwe S, Kasvosve I, Moyo S, Okatch H, Musonda R, Wainberg M, Makhema J, Marlink R, Novitsky V, Essex M (2018). Prevalence of rilpivirine and etravirine resistance mutations in HIV-1 subtype C-infected patients failing nevirapine or efavirenz-based combination antiretroviral therapy in botswana. AIDS Research and Human Retroviruses 34(8):667-671.

Dokubo EK, Baddeley A, Pathmanathan I, Coggin W, Firth J, Getahun H, Kaplan J, Date A (2014). Provision of antiretroviral therapy for HIV-positive TB patients — 19 countries, sub-saharan Africa, 2009-2013. Morbidity and Mortality Weekly Report 63(47):1104-1107.

Eakle R, Venter F, Rees H (2018). Pre-exposure prophylaxis (PrEP) in an era of stalled HIV prevention: Can it change the game? Retrovirology 15:29 (2018).

Echagüen, AO, Arnedo M, Xercavins M, Martinez E, Rosón B, Ribera E, Domingo P, González A, Riera M, Llibre JM, Gatell JM, Dalmau D, Nevirapine, Efavirenz and Abacavir (NEFA) Resistance Substudy Team (2005). Genotypic and phenotypic resistance patterns at virological failure in a simplification trial with nevirapine, efavirenz or abacavir. AIDS 19(13):1385-1391.

Faria NR, Vidal N, Lourenco J, Raghwani J, Sigaloff KCE, Tatem AJ, Van De Vijver DAM, Pineda-Peña AC, Rose R, Wallis CL, Ahuka-Mundeke S, Muyembe-Tamfum JJ, Muwonga J, Suchard MA, De Wit TFR, Hamers RL, Ndembi N, Baele G, Peeters M, Dellicour S (2019). Distinct rates and patterns of spread of the major HIV-1 subtypes in Central and East Africa. PLoS Pathogens 15(12):e1007976.

Gallien S, Charreau I, Nere ML, Mahjoub N, Simon F, de Castro N, Aboulker JP, Molina JM, Delaugerre C (2015). Archived HIV-1 DNA resistance mutations to rilpivirine and etravirine in successfully treated HIV-1-infected individuals pre-exposed to efavirenz or nevirapine. Journal of Antimicrobial Chemotherapy 70(2):562-565.

Girum T, Wasie A, Worku A (2018). Trend of HIV/AIDS for the last 26 years and predicting achievement of the 90-90-90 HIV prevention targets by 2020 in Ethiopia: A time series analysis. BMC Infectious Diseases 18(1):1-10.

Junqueira DM, de Matos Almeida SE (2016). HIV-1 subtype B: Traces of a pandemic. Virology 495:173-184.

Kantor R, DeLong A, Balamane M, Schreier L, Lloyd RM, Injera W, Kamle L, Mambo F, Muyonga S, Katzenstein D, Hogan J, Buziba N, Diero L (2014). HIV diversity and drug resistance from plasma and non-plasma analytes in a large treatment programme in western Kenya. Journal of the International AIDS Society 17(1):19262.

Kenya AIDS Response Progress Report (2016). Country progress report-Kenya Global AIDS Monitoring 2017. Available at: http://www.unaids.org/sites/default/files/country/documents/KEN_2017_countryreport.pdf

Kharsany ABM, Karim QA (2016). HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. The Open AIDS Journal 10(1):34-48.

Kimanga DO, Ogola S, Umuro, Ng'ang'a A, Kimondo L, Murithi P, Muttunga J, Waruiru W, Mohammed I, Sharrif S, De Cock KM, Kim AA (2014). Prevalence and incidence of HIV infection, trends, and risk factors among persons aged 15-64 years in Kenya. Journal of Acquired Immune Deficiency Syndromes 66 Suppl 1(Suppl 1):S13-26.

Lahuerta M, Syowai M, Vakil S, Odhiambo J, Gitonga M, Sugandhi N, Odhiambo S, Kimani M, Wamicwe J, Batuka J, Imbuki E, Bartilol K, Abrams EJ (2020). Monitoring the transition to new antiretroviral treatment regimens through an enhanced data system in Kenya. PLoS ONE 15(4):e0232104.

Lessells RJ, Katzenstein DK, De Oliveira T (2012). Are subtype differences important in HIV drug resistance? Current Opinion in Virology 2(5):636-643.

Mabaso M, Makola L, Naidoo I, Mlangeni LL, Jooste S, Simbayi L (2019). HIV prevalence in South Africa through gender and racial lenses: results from the 2012 population-based national household survey. International Journal for Equity in Health 18(1):1-11.

Marsh K, Eaton JW, Mahy M, Sabin K, Autenrieth CS, Wanyeki I, Daher J, Ghys PD (2019). Global, regional and country-level 90-90-90 estimates for 2018. AIDS 15;33 Suppl 3(Suppl 3):S213-S226.

Montaner JSG, Lima VD, Harrigan PR, Lourenço L, Yip B, Nosyk B, Wood E, Kerr T, Shannon K, Moore D, Hogg RS, Barrios R, Gilbert M, Krajden M, Gustafson R, Daly P, Kendall P (2014). Expansion of HAART coverage is associated with sustained decreases in HIV/AIDS morbidity, mortality and hiv transmission: The "HIV treatment as prevention" experience in a Canadian setting. PLoS ONE 9(2):1-10.

Mwau M, Syeunda CA, Adhiambo M, Bwana P, Kithinji L, Mwende J, Oyiengo L, Sirengo M, Boeke CE (2018). Scale-up of Kenya's national HIV viral load program: Findings and lessons learned. PLoS ONE 13(1):e0190659.

National AIDS and STI Control Programme (2018). Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection in Kenya. Available at: http://cquin.icap. columbia.edu/wp-content/uploads/2017/04/ICAP_CQUIN_Kenya-ARV-Guidelines-2018-Final_20thAug2018.pdf

National AIDS Control Council (2018). Kenya HIV Estimates Report 2018. Available at: https://doi.org/10.1111/j.1365-2664.2007.0

National AIDS Control Council (2016). Kenya HIV County Profiles 2016. NACC, Ministry of Health, Government of Kenya. Available at: https://doi.org/10.1017/CBO978110741532.

Ndashimye E, Arts EJ (2019). The urgent need for more potent antiretroviral therapy in low-income countries to achieve UNAIDS 90-90-90 and complete eradication of AIDS by 2030. Infectious Diseases of Poverty 8(1):63.

Osler M, Hilderbrand K, Goemaere E, Ford N, Smith M, Meintjes G, Kruger J, Govender NP, Boulle A (2018). The continuing burden of advanced HIV disease over 10 years of increasing antiretroviral therapy coverage in South Africa. Clinical Infectious Diseases 66:S118-S125.

Paul NI, Ugwu RO (2020). Dolutegravir (DTG) Based Fixed Dose Combination (FDC) of Tenofovir/Lamivudine/Dolutegravir (TLD) and Viral Load Suppression in Children in Port Harcourt, Nigeria. Journal of Scientific Research and Reports 26(2):52-59.

Qiagen (2020). QIAamp® Viral RNA Mini Handbook. Available at: https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-kits/

Saravanan S, Kausalya B, Gomathi S, Sivamalar S, Pachamuthu B, Selvamuthu P, Pradeep A, Sunil S, Mothi SN, Smith DM, Kantor R (2017). Etravirine and rilpivirine drug resistance among HIV-1 subtype C infected children failing non-nucleoside reverse transcriptase inhibitor-based regimens in South India. AIDS Research and Human Retroviruses 26(2):52-59.

Shao Y, Williamson C (2012). The HIV-1 epidemic: Low- to middle-income countries. Cold Spring Harbor Perspectives in Medicine 2(3):a007187.

Sluis-Cremer N (2014). The emerging profile of cross-resistance among the nonnucleoside HIV-1 reverse transcriptase inhibitors. Viruses 6(8):2960-2973.

Svärd J, Mugusi S, Mloka D, Neogi U, Meini G, Mugusi F, Incardona F, Zazzi M, Sönnerborg A (2017). Drug resistance testing through remote genotyping and predicted treatment options in human immunodeficiency virus type 1 infected Tanzanian subjects failing first or second line antiretroviral therapy. PLoS ONE 12(6):e0178942.

Taylor BS, Sobieszczyk ME, McCutchan FE, Hammer SM (2008). The challenge of HIV-1 subtype diversity. New England Journal of Medicine 358(15):1590-1602.

Teeranaipong P, Sirivichayakul S, Mekprasan S, Ohata PJ, Avihingsanon A, Ruxrungtham K, Putcharoen O (2016). Role of Rilpivirine and Etravirine in Efavirenz and Nevirapine-Based Regimens Failure in a Resource-Limited Country: A Cross- Sectional Study. PloS One 11(4):e0154221.

Teeranaipong P, Sirivichayakul S, Mekprasan S, Ruxrungtham K, Putcharoen O (2014). Rilpivirine versus etravirine validity in NNRTI-based treatment failure in Thailand. Journal of the International AIDS Society 17(4 Suppl 3):19740.

Joint United Nations Programme on HIV/AIDS (2018a). 90-90-90: treatment for all | UNAIDS. Miles To Go - UNAIDS Data 2018. Available at: https://www.unaids.org/en/resources /909090.

Joint United Nations Programme on HIV/AIDS (2018b). *KENYA | 2018*. Country Factsheets. Available at: https://www.unaids.org/en/regionscountries/countries/kenya.

Joint United Nations Programme on HIV/AIDS (2018c). *UNAIDS Data 2018*. Available at: https://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf.

Joint United Nations Programme on HIV/AIDS (2019). Fact Sheet - Global AIDS Update 2019, 2018 Global HIV Statistics. Available at: https://www.unaids.org/sites/default/files/media_asset/2019-UNAIDS-data_en.pdf.

Usach I, Melis V, Peris JE (2013). Non-nucleoside reverse transcriptase inhibitors: A review on pharmacokinetics, pharmacodynamics, safety and tolerability. Journal of the International AIDS Society 16(1):1-14.

Vandormael A, Akullian A, Siedner M, de Oliveira T, Bärnighausen T, Tanser F (2019). Declines in HIV incidence among men and women in a South African population-based cohort. Nature Communications. 10(1):1-10.

Vardell E (2020). Global Health Observatory Data Repository. Medical Reference Services Quarterly 39(1):67-74.

Venner CM, Nankya I, Kyeyune F, Demers K, Kwok C, Chen PL, Rwambuya S, Munjoma M, Chipato T, Byamugisha J, Van Der Pol B, Mugyenyi P, Salata RA, Morrison CS, Arts EJ (2016). Infecting HIV-1 Subtype Predicts Disease Progression in Women of Sub-Saharan Africa. EBioMedicine 13:305-314.

Vitoria M, Rangaraj A, Ford N, Doherty M (2019). Current and future priorities for the development of optimal HIV drugs. Current Opinion in HIV and AIDS 14(2):143-149.

World Health Organisation (WHO) (2019). Policy Brief: Update of Recommendations on First-and Second-Line Antiretroviral Regimens. WHO Library Cataloguing-in-Publication Data. Available at: https://apps.who.int/iris/handle/10665/325892

Zhou Y, Lu J, Wang J, Yan H, Li J, Xu X, Zhang Z, Qiu T, Ding P, Fu G, Huan X, Hu H (2016). Prevalence of HIV antiretroviral drug resistance and Its Impacts on HIV-1 virological failures in Jiangsu, China: A Cross-Sectional Study. BioMed Research International 2016:1752437.