Original Article

Antiviral Therapy
June 2022: 1–16

DOI: 10.1177/13596535221102690
journals.sagepub.com/home/avt
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Acquired HIV drug resistance among adults living with HIV receiving first-line antiretroviral therapy in Rwanda: A cross-sectional nationally representative survey

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Abstract

Background: We assessed the prevalence of acquired HIV drug resistance (HIVDR) and associated factors among patients receiving first-line antiretroviral therapy (ART) in Rwanda.

Methods: This cross-sectional study included 702 patients receiving first-line ART for at least 6 months with last viral load (VL) results ≥1000 copies/mL. Blood plasma samples were subjected to VL testing; specimens with unsuppressed VL were genotyped to identify HIVDR-associated mutations. Data were analysed using STATA/SE.

Results: Median time on ART was 86.4 months (interquartile range [IQR], 44.8–130.2 months), and median CD4 count at ART initiation was 311 cells/mm^3 (IQR, 197–484 cells/mm^3). Of 414 (68.2%) samples with unsuppressed VL, 378 (88.3%) were genotyped. HIVDR included 347 (90.4%) non-nucleoside reverse transcriptase inhibitor- (NNRTI), 291 (75.5%) nucleoside reverse transcriptase inhibitor- (NRTI) and 13 (3.5%) protease inhibitor (PI) resistance-associated mutations. The most common HIVDR mutations were K65R (22.7%), M184V (15.4%) and D67N (9.8%) for NRTIs and K103N (34.4%) and Y181C/I/V/YC (7%) for NNRTIs. Independent predictors of acquired HIVDR included current ART regimen of zidovudine + lamivudine + nevirapine (adjusted odds ratio [aOR], 3.333 [95% confidence interval (CI): 1.022–10.870]; p =

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0.046) for NRTI resistance and current ART regimen of tenofovir + emtricitabine + nevirapine (aOR, 0.148 [95% CI: 0.028–0.779]; p = 0.025), zidovudine + lamivudine + efavirenz (aOR, 0.105 [95% CI: 0.016–0.693]; p = 0.020) and zidovudine + lamivudine + nevirapine (aOR, 0.259 [95% CI: 0.084–0.793]; p = 0.019) for NNRTI resistance. History of ever switching ART regimen was associated with NRTI resistance (aOR, 2.53 [95% CI: 1.198–5.356]; *p* = 0.016) and NNRTI resistance (aOR, 3.23 [95% CI: 1.435–7.278], p = 0.005).

Conclusion: The prevalence of acquired HIV drug resistance (HIVDR) was high among patient failing to re-suppress VL and was associated with current ART regimen and ever switching ART regimen. The findings of this study support the current WHO guidelines recommending that patients on an NNRTI-based regimen should be switched based on a single viral load test and suggests that national HIV VL monitoring of patients receiving ART has prevented long-term treatment failure that would result in the accumulation of TAMs and potential loss of efficacy of all NRTI used in second-line ART as the backbone in combination with either dolutegravir or boosted PIs.

Keywords

HIV, acquired drug resistance, first-line ART, unsuppressed viral load, Rwanda

Introduction

HIV prevention and monitoring HIV drug resistance (HIVDR) maximize the long-term effectiveness of first-line antiretroviral therapy (ART), optimize patient outcomes, minimize transmission of drug-resistant HIV and ensure sustainability of ART programs.[1,2] However, resource-limited settings often have limited ART regimens and limited access to routine viral load (VL) testing to monitor treatment outcomes.[1] Even in settings with optimal ART program management, HIVDR is expected to emerge in populations receiving ART, which could affect the response to second-line ART and contribute to transmission of drug-resistant HIV.[3]

In July 2016, the Rwanda ART program initiated the *Treat All* policy, in which all people living with HIV (PLHIV) initiate ART within the same week (preferably on the same day) as diagnosis and undergo VL testing at 6 months following ART initiation. Subsequently, patients receiving ART undergo VL testing every 12 months. Patients with potential virologic failure receive 3 months of enhanced adherence counselling and close monitoring to address adherence-related factors and undergo VL testing after potential adherence issues are addressed.[5]

The standard first-line ART regimens for adults in Rwanda were tenofovir/lamivudine in combination with one non-nucleoside reverse transcriptase inhibitor (NNRTI; efavirenz as the first option or nevirapine) or abacavir/lamivudine plus one NNRTI (efavirenz as the first option or nevirapine). In July 2018, dolutegravir was introduced as first-line ART for ART-naive adults and adolescents who weigh >35 kg. The standard second-line ART regimen is zidovudine, lamivudine and atazanavir/ritonavir or lopinavir/ritonavir according to national ART guidelines adopted in 2016 or a dolutegravir-based regimen after HIV genotyping.[5]

At the end of 2018 in Rwanda, of the >200,000 PLHIV, 83.1% were receiving ART in 539 health facilities.[4] The extent of ART coverage in Rwanda increases the likelihood of HIVDR among PLHIV receiving first-line regimens. Previous studies in Rwanda have characterized transmitted drug resistance[6] and acquired drug resistance to first-line[7] and second-line regimens.[8] However, previous studies on HIVDR or ART failure in Rwanda are outdated, had small sample sizes or included only patients with a short treatment duration[7,9,10]; no nationally representative estimate is available for HIVDR in patients for whom first-line ART has failed. Although the World Health Organization (WHO) recommends that HIV treatment scale-up be accompanied by a robust assessment of drug resistance emergence and transmission, in Rwanda, these factors are monitored only when second-line ART has failed and patients need to switch to a third-line ART regimen. The most recent study on acquired HIVDR for patients receiving first-line regimens in Rwanda was conducted in 2011. We conducted a survey to estimate national prevalence of HIVDR and VL re-suppression among adults living with HIV who had received first-line ART for at least 6 months and who had failed to re-suppress their VL (≥1000 copies/mL).

Methods

Study setting, design and sampling

We conducted a cross-sectional national study and collected blood specimens and minimal information, including sociodemographic and clinical characteristics of patients. Study design followed a two-stage sampling survey design without neither clustering nor stratification (referred as the standard complex survey design).[11] In the first stage, 66 health facilities were selected from the total national sites dispensing ART in the country using

probability proportional to health facility size. In the second stage, eligible patients were selected randomly based on health facility's allocated minimum sample size. The sample size was calculated using formula assumptions and conditions adapted from the WHO generic protocol, which was adjusted to the specific context of Rwanda[11]: The WHO assumed prevalence of viral suppression of 91% which was replaced with a viral re-suppression of 70%,[12–14]. drug resistance prevalence of 50%,[15] assumed percentage of patients on first-line therapy at time of survey of 95.8%, assumed polymerase chain reaction amplification rate of 80%, desired precision of ±5% reduced to ±3.75, design effect for sampling of 1.5 and response rate percent of 75%[13,14] calculated using the following formula.

Assumed chain reaction amplification rate × (1-% of LTFU) × (1-% of deaths) × (1-% of net transfers) using lost to follow-up (LTFU) per year: 2.56%, death per year: 0.91%, transfer in per year; 4.7%, net transfers per year: 1.1%, which is equal to (% of transfer outs − % of transfer ins).

The survey was conducted in 67 health facilities, selected from more than 539 health facilities providing ART in Rwanda. Based on these sample size estimation assumptions and an anticipated NNRTI drug resistance prevalence of 50%, a sample of 750 individuals will provide an effective sample size of 203 individuals across 67 health facilities with a precision that corresponds with an alpha of ±7% and a 95% confidence interval (CI) level. An effective sample size of 377 individuals used in this study provided an estimate with a precision that corresponds with an alpha of ±5%.

The survey included all PLHIV aged ≥18 years who had received HIV first-line ART for at least 6 months, had VL ≥1000 copies/mL from the most recent test at the time of the survey inception and whose latest VL test results were obtained not more than 3 months before the date of sample collection. The study did not include individuals aged <18 years, patients reinitiating ART because of treatment failure, patients receiving second-line or third-line regimens and patients who did not provide written informed consent for participation in the survey.

Data collection

The list of patients receiving first-line ART who met the eligibility criteria in each health facility was obtained from the ART patient register. All eligible patients were contacted and invited to participate in the study during an appointment at the health facilities. We used a structured questionnaire programmed before in Personal Digital Assistant devices to collect patients' demographic and clinical information from patient charts/registers. We followed WHO recommendations to collect blood samples from study participants for HIVDR testing.[16]

Patient flow

Eligible patients with latest VL results ≥1000 copies/mL (recorded in patient file) were listed and contacted for blood draw at the health facilities, and samples were stored at the national reference laboratory. Collected samples were subjected to a second VL test, after which all samples with VL≥1000 copies/mL were genotyped to identify potential HIVDR mutations (Figure 1).

HIVDR genotyping

Whole-blood samples were processed at the Rwanda National Reference Laboratory for VL testing and HIVDR genotyping. VL testing was conducted using the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 for plasma samples. Specimens with unsuppressed VL were subjected to Thermo Fisher Scientific HIVDR genotyping test to detect ART-resistant mutations via sequencing reverse transcriptase and protease genes. Additionally, 65.6% (248) of the HIVDR sequences' raw data and Fasta files were shared with the WHO-designated Specialized Drug Resistance Laboratory (SDRL) at the U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA for quality control and to ensure correct interpretation of individual patients' HIVDR-genotyping results used for this study. The analysis of the HIVDR genotyping results and interpretation for the drug resistance profiles was done using Stanford University HIV Drug Resistance Database. Based on the Stanford HIVdb algorithm the following scoring criteria were used: There are 5 drug resistance levels: 1 indicates susceptible, 2 indicates potential low-level resistance, 3 indicates low-level resistance, 4 indicates intermediate resistance, 5 indicates high-level resistance. The scores are the sum of each mutation penalty score for a drug. Scores less than 10 indicate susceptible; scores between 10 and 14 indicate potential low-level resistance; scores between 15 and 29 indicate low-level resistance; scores between 30 and 59 indicate intermediate resistance. Scores of 60 or greater indicate high-level resistance.

Data analysis

Univariate descriptive statistics, specifically frequency counts and corresponding percentages, were used to describe sample characteristics, social demographics and clinical characteristics, acquired HIVDR testing cascade and various types of mutations present in the sample. Additionally, bivariate analysis with chi-squared tests was used to identify factors associated with failure to re-

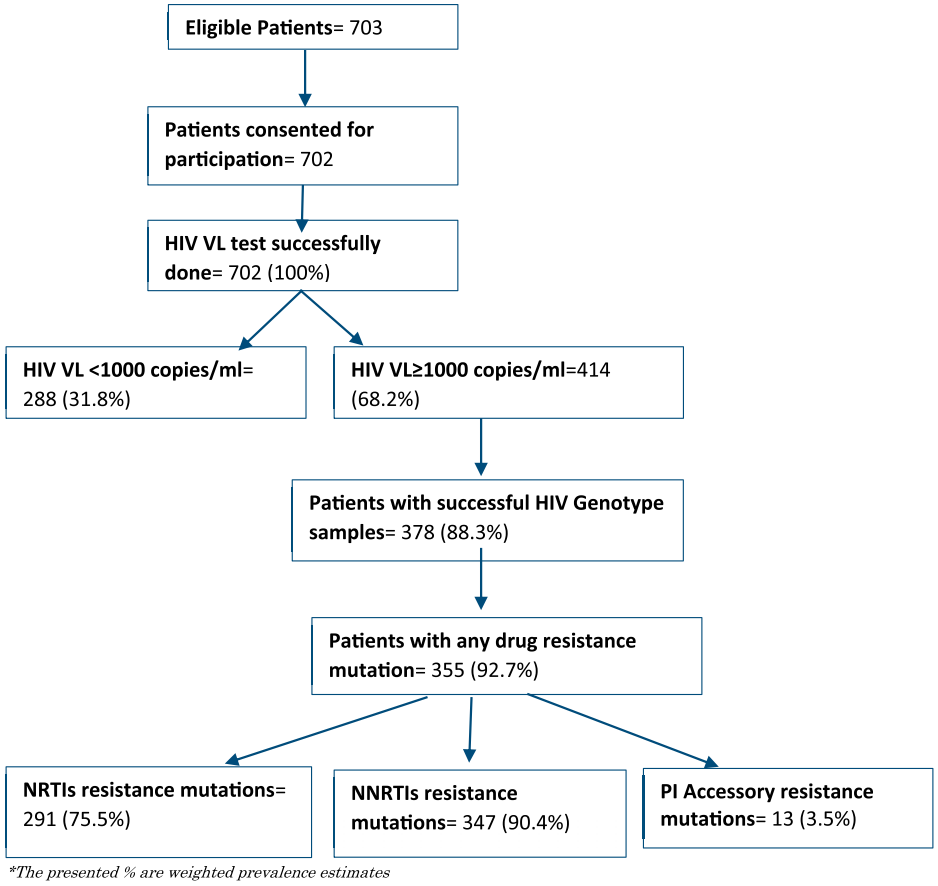

Figure 1. Cascade of patient enrolment based on eligibility criteria, blood samples with unsuppressed VL (VL ≥1000 copies/mL) for genotyping and number of patients with drug-resistant mutation types (based on weighted %). * The presented % are weighted prevalence estimates.

suppress VL and each acquired drug-resistant mutation class (nucleoside reverse transcriptase inhibitors [NRTIs], NNRTIs and protease inhibitors [PIs]), with 0.05 threshold of alpha level associated with 95% CI level (*p*-value). Multivariate logistic regression models were fit for all significant predictors, with *p*-value <0.05 considered statistically significant for each independent outcome (failure to re-suppress and each acquired drug-resistant mutation class). Stepwise backward logistic regression modelling was used to produce adjusted odds ratios and corresponding 95% CI. Per WHO guidelines,[11] the analyses accounted for the standard complex survey design. The standard complex survey design was employed because neither clustering nor stratification was done during sampling.[11] STATA/SE software (version 16, Stata Corp LLC) was used for data analysis.

Ethical consideration

This survey was reviewed and approved by the Rwanda National Ethics Committee and was reviewed in accordance with the US Centers for Disease Control and Prevention (CDC) human research protection procedures and was determined to be research, but CDC investigators did not interact with human subjects or have access to identifiable data or specimens for research purposes. All participants signed the informed consent form before enrolling in the study.

Results

Of the 702 patients included in this survey, 469 (66.8%) were women and most (334 [47.6%]) were aged 35–49 years. Median CD4 count at ART initiation was 311 cells/mm^3 (interquartile range [IQR], 197–484 cells/mm^3), and median time on ART was 86.4 months (IQR, 44.8–130.2 months). Of the participants, 68.3% had received treatment for ≥5 years and 37.3% of those receiving ART had a history of switching ART regimens at least once. The most common regimens at the time of specimen collection were tenofovir + lamivudine + efavirenz (39.3%), tenofovir + lamivudine + nevirapine

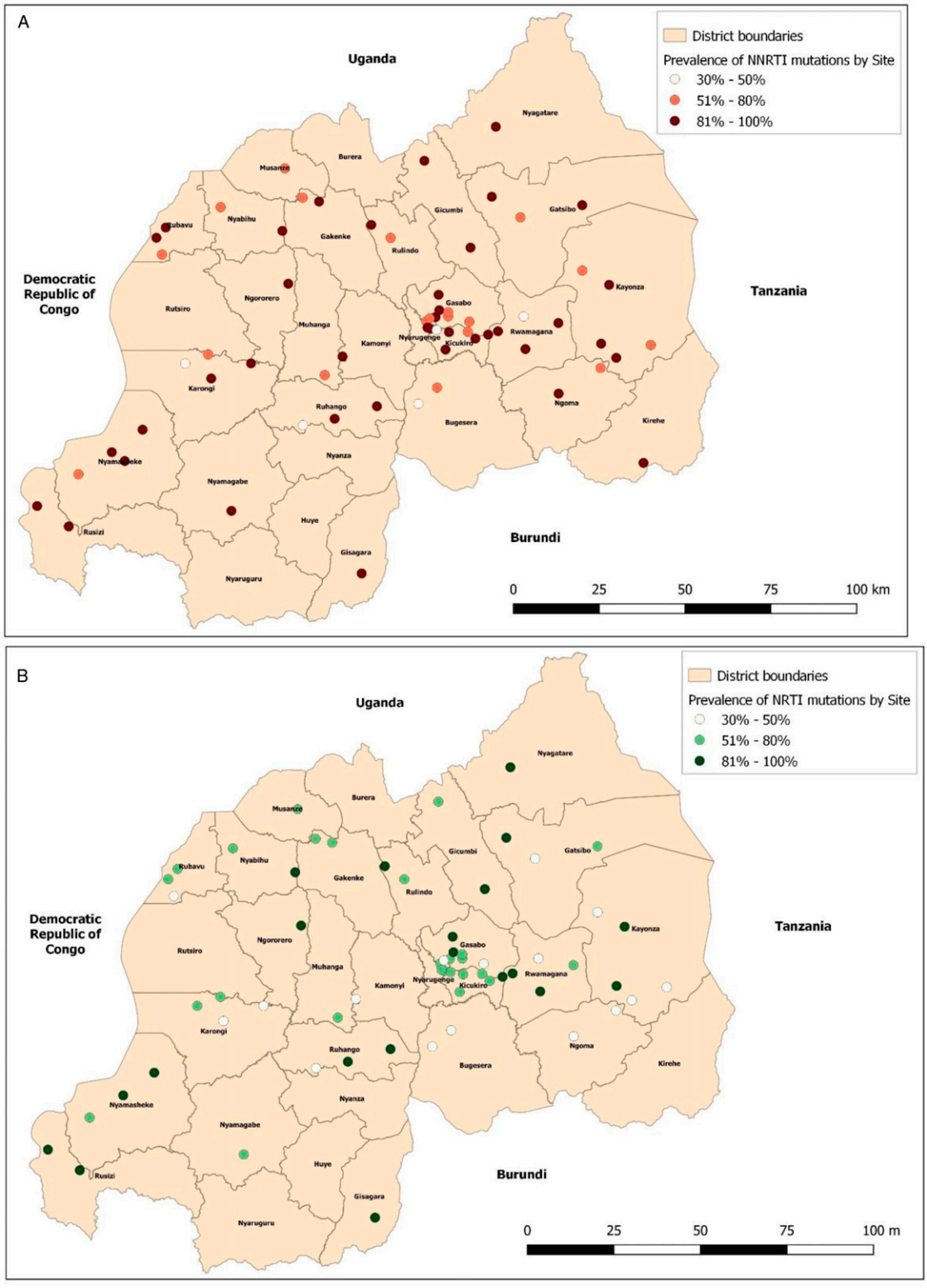

Figure 2. Maps of Rwanda showing the geographic distribution and prevalence of NNRTI-resistant (panel A) and NRTI-resistant (panel B) mutations in samples tested per site with VL >1000 copies/mL.

(17.2%) and zidovudine + lamivudine + nevirapine (16.8%).

HIVDR-testing cascade and geographical distribution of HIVDR mutations

Nucleotide sequencing in the reverse transcriptase gene identified NNRTIs and NRTIs. The number and weighted prevalence of any drug-resistant mutations was 355 [92.7%, 95% CI: 86.2–96.3], of NRTIs was 291 [75.5%, 95% CI: 66.7–82.5], of NNRTIs was 347 [90.4%, 95% CI: 81.5–95.2), of PIs was 13 [3.5%, 95% CI: 1.4–8.7] (Figure 1). Figure 2 presents the geographical distribution by proportion of NRTI-resistant and NNRTI-resistant mutations with corresponding patient number (N) enrolled in study health facilities. Both NRTI-resistant and NNRTI-resistant mutations were concentrated in the City of Kigali and in the Western province.

Quality Control of HIVDR genotyping

The quality control (QC) and quality assurance process used in this study examined the sequence data at the batch and survey level according to recommended WHO HIVResNet HIVDR laboratory operational framework.[17] At the batch level, sequences were analysed by the checking the raw chromatogram quality, sequence length, single-stranded coverage, presence of stop codons, out-of-frame insertions or deletions, missing or gaps in the sequences, highly unusual mutations, excessive ambiguity, excessive number of mixed bases and APOBEC mutations. However, at survey level, all sequences were examined to identify expected and unexpected sequence similarity between pairs of sequences that were analysed in different batches to generate a sequence identity matrix with confirmation done by using the online drug resistance quality control tool developed by WHO and British Columbia Centre for Excellence in HIV/AIDS. The sequences that passed QC were submitted to Stanford's HIV drug resistance database (HIVdb) where the same parameters were checked again in addition to obtaining the drug resistance profile for this study. A total of 1572 raw data (ab1 files) and 262 assembled sequences (Fasta files) were submitted from NRL and examined by the CDC International Laboratory Branch through workflow process described above for the final drug resistance profiles, of which 158 sequences (60%) were 100% identical, 80 sequences (31%) with 1–3 nucleotides and 24 sequences (9%) with up to 15 nucleotides differed mostly because of nucleotide mixture calling. The overall greater proportion of identical sequences and some with a few differing nucleotides (>90%) indicates that NRL staff are well trained and competent in the genotyping process to generate highly reliable HIVDR results reported in this study.

Drug-resistant mutations by drug class and current ART regimen

The prevalence of any drug-resistant mutations was 92.7% (355), 75.5% (291) for NRTIs, 90.4% (347) for NNRTIs and 3.5% for PI (Figure 1). We classified NRTI and NNRTI mutations by ART regimen. K65R was the leading NRTI mutation 22.7% (65), followed by M184V 15.4% (44) and D67N 9.8% (28) (Table 1).

The leading NNRTI mutation was K103N 34.4% (118), which has been linked to efavirenz and nevirapine-based regimens (Table 2). Additionally, we found Y181C (5.8%), Y181I (0.6%), Y181V (0.3%), Y181YC (0.3%) and Y188L (1.5%) mutations, which are selected by nevirapine and substantially affect HIV susceptibility to etravirine used in third-line combination ART (Table 2). K101E mutation was observed in 7.3% of samples and reduces susceptibility of HIV to all NNRTI (efavirenz, nevirapine and etravirine) used in Rwanda.

Predictors of failure to re-suppress VL

In the univariate analysis, latest CD4 categories and current ART regimen were significantly associated with failure to re-suppress (VL ≥1000 copies/mL). Those with latest CD4 ranging between 200 and 349 cells/ mm^3 were significantly less likely not to re-suppress compared to those with latest CD4 less than 200 cells/mm^3 (adjusted odds ratio [aOR]=0.26, [95% CI: 0.13–0.52]; p < 0.001). Participants on 'TDF + FTC + NVP' as the current ART regimen were at a higher risk of not re-suppressing (aOR = 1.84, [95% CI: 1.03–3.28]; p = 0.040), whereas participants on other EFV or NVP non-based regimens were less likely not to re-suppress (aOR=0.19, [95% CI: 0.07–0.49]; p <0.001) as compared to participants who were on 'TDF + 3TC + EFV' as a reference group (Table 3).

Predictors of HIVDR mutations

Univariate and multivariable analyses were conducted to evaluate variables associated with HIVDR mutations.

Patients currently receiving zidovudine + lamivudine + nevirapine were significantly more likely to have any NRTI-resistant mutation (adjusted odds ratio [aOR], 3.33 [95% CI: 1.022–10.870]; p = 0.046) than those receiving tenofovir disoproxil fumarate + lamivudine + efavirenz. Patients with a history of ever switching ART regimens were 2.53 times more likely to develop NRTI-resistant mutations ([95% CI: 1.198–5.356]; $p = 0.016$) compared

Table 1. NRTI-resistant mutations associated with first-line ART regimens among adults living with HIV (n = 286) in Rwanda.

	Patients' current ART regimen								
NRTI resistance mutations only	AZT + 3TC + EFV, n (%)	AZT + 3TC + NVP, n (%)	TDF + 3TC + EFV, n (%)	TDF + FTC + NVP, n (%)	ABC + 3TC + EFV, n (%)	ABC + TC + NVP, n (%)	ABC + 3TC + DTG or TDF + 3TC + DTG, n (%)	Other, n (%)	Total, n (%)
A62AV	1 (14.3)	1 (2.3)	1 (0.8)	2 (3.0)	0	0	0	1 (20.0)	6 (2.1)
A62V	1 (14.3)	0	7 (5.4)	5 (7.5)	0	2 (14.3)	0	0	15 (5.2)
D67A	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
D67DN	1 (14.3)	2 (4.7)	0	0	0	0	0	0	3 (1.0)
D67DN, K70KE, M184V, K219KQR	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
D67Deletion	0	0	0	2 (3.0)	0	0	0	0	2 (0.7)
D67G	0	0	4 (3.1)	0	0	0	0	0	4 (1.4)
D67H/D67N/D67P/D67T	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
D67N	0	9 (20.9)	13 (10)	5 (7.5)	0	0	0	1 (20.0)	28 (9.8)
D67NT	0	0	0	0	0	0	0	1 (20.0)	1 (0.3)
D67T	0	0	0	1 (1.5)	0	0	0	0	1 (0.3)
E44D	0	2 (4.7)	8 (6.2)	1 (1.5)	1 (5.6)	0	1 (50.0)	0	13 (4.5)
E44D, K65R, M184I	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
E44ED	0	0	1 (0.8)	1 (1.5)	0	0	0	0	2 (0.7)
K65KR	0	0	2 (1.5)	1 (1.5)	0	0	0	0	3 (1.0)
K65KR, K70KE, M184V	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K65N	0	0	0	2 (3.0)	1 (5.6)	0	0	0	3 (1.0)
K65R	0	0	36 (27.7)	26 (38.8)	1 (5.6)	1 (7.1)	1 (50.0)	0	65 (22.7)
K65R, M184I	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K65R, M184V	0	0	0	1 (1.5)	0	0	0	0	1 (0.3)
K65R, M184V, K219KQ	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K70E	0	0	6 (4.6)	1 (1.5)	0	0	0	0	7 (2.4)
K70E, M184V, K219R	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K70EG	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K70G	0	0	0	1 (1.5)	0	0	0	0	1 (0.3)
K70GR	0	0	0	0	1 (5.6)	0	0	0	1 (0.3)
K70KE	0	0	0	1 (1.5)	0	0	0	0	1 (0.3)
K70KQ	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K70KR	1 (14.3)	2 (4.7)	1 (0.8)	0	0	0	0	0	4 (1.4)
K70NS	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
K70Q	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)

(continued)

Table 1. (continued)

NRTI resistance mutations only	Patients' current ART regimen								Total, n (%)
	AZT + 3TC + EFV, n (%)	AZT + 3TC + NVP, n (%)	TDF + 3TC + EFV, n (%)	TDF + FTC + NVP, n (%)	ABC + 3TC + EFV, n (%)	ABC + TC + NVP, n (%)	ABC + 3TC + DTG or TDF + 3TC + DTG, n (%)	Other, n (%)	
K70R	1 (14.3)	1 (2.3)	2 (1.5)	1 (1.5)	0	0	0	0	5 (1.7)
L74I	0	0	1 (0.8)	0	0	2 (14.3)	0	0	3 (1.0)
L74LI, M184V	0	0	0	0	1 (5.6)	0	0	0	1 (0.3)
L74V	0	0	3 (2.3)	1 (1.5)	9 (50.0)	6 (42.9)	0	0	19 (6.6)
L74V, V75T, Y115F, M184V	0	0	0	0	1 (5.6)	0	0	0	1 (0.3)
M184I	0	0	1 (0.8)	1 (1.5)	0	0	0	0	2 (0.7)
M184MIV	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
M184MV	0	0	1 (0.8)	1 (1.5)	2 (11.1)	0	0	0	4 (1.4)
M184V	2 (28.6)	18 (41.9)	16 (12.3)	4 (6.0)	1 (5.6)	1 (7.1)	0	2 (40.0)	44 (15.4)
M41L	0	4 (9.3)	10 (7.7)	3 (4.5)	0	0	0	0	17 (5.9)
M41L, E44D, K70R, M184V, K219Q	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
M41ML	0	3 (7.0)	1 (0.8)	5 (7.5)	0	0	0	0	9 (3.1)
T69G	0	1 (2.3)	0	0	0	0	0	0	1 (0.3)
V75M	0	0	1 (0.8)	0	0	1 (7.1)	0	0	2 (0.7)
V75VI	0	0	0	0	0	1 (7.1)	0	0	1 (0.3)
V75VIM	0	0	1 (0.8)	0	0	0	0	0	1 (0.3)
Y115F	0	0	0	1 (1.5)	0	0	0	0	1 (0.3)
Total	**7**	**43**	**130**	**67**	**18**	**14**	**2**	**5**	**286**

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitors; ART, antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; ABC, abacavir; DTG, dolutegravir.

Table 2. NNRTI-resistant mutations associated with first-line ART regimens among adults living with HIV (n = 342) in Rwanda.

	Patient current ART								
NNRTI resistance mutations	AZT + 3TC + EFV, n (%)	AZT + 3TC + NVP, n (%)	TDF + 3TC + EFV, n (%)	TDF + FTC + NVP, n (%)	ABC + 3TC + EFV, n (%)	ABC + 3TC + NVP, n (%)	ABC + 3TC + DTG or TDF + 3TC + DTG, n (%)	Other, n (%)	Total
A89G	0	0	0	0	1 (4.8)	0	0	0	1 (0.3)
A98AG	0	4 (9.1)	1 (0.6)	2 (2.6)	0	0	0	0	7 (2.0)
A98G	0	6 (13.6)	26 (15.7)	11 (14.1)	1 (4.8)	2 (12.5)	0	0	46 (13.4)
A98G, K101E, G190A	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
A98G, K103N, V108I	0	0	0	0	1 (4.8)	0	0	0	1 (0.3)
A98G, V108VI, G190A	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
E138A	0	0	1 (0.6)	1 (1.3)	0	0	0	0	2 (0.6)
E138EA	0	0	0	0	0	1 (6.3)	0	0	1 (0.3)
G190A	1 (11.1)	2 (4.5)	1 (0.6)	3 (3.8)	0	2 (12.5)	0	1 (20.0)	10 (2.9)
G190A, P225H	0	1 (2.3)	0	0	0	0	0	0	1 (0.3)
G190GA	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
K101DEHQ	0	0	0	0	0	0	1 (25.0)	0	1 (0.3)
K101E	1 (11.1)	4 (9.1)	11 (6.6)	4 (5.1)	2 (9.5)	0	0	0	22 (6.4)
K101E, G190A	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K101E, V106M, Y181YC, G190A	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K101E, Y181C	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
K101H	0	0	3 (1.8)	3 (3.8)	0	0	0	0	6 (1.7)
K101H, K103NS, Y181C, G190A	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K101KE	0	0	1 (0.6)	1 (1.3)	1 (4.8)	1 (6.3)	0	1 (20.0)	5 (1.5)
K101KE, K103KN, E138A, V179T	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K101KE, K103KN, V179IL	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
K101KPQT	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K101P	1 (11.1)	0	4 (2.4)	0	1 (4.8)	0	0	0	6 (1.7)
K103E	0	1 (2.3)	0	0	0	0	0	0	1 (0.3)
K103KM	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
K103KN	1 (11.1)	1 (2.3)	7 (4.2)	1 (1.3)	0	0	1 (25.0)	0	11 (3.2)
K103N	3 (33.3)	12 (27.3)	65 (39.2)	22 (28.2)	9 (42.9)	4 (25.0)	1 (25.0)	2 (40.0)	118 (34.4)
K103N, E138G, V179L, P225H, BK238T	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K103N, P225H	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)

(continued)

Table 2. (continued)

NNRTI resistance mutations	Patient current ART								
	AZT + 3TC + EFV, n (%)	AZT + 3TC + NVP, n (%)	TDF + 3TC + EFV, n (%)	TDF + FTC + NVP, n (%)	ABC + 3TC + EFV, n (%)	ABC + 3TC + NVP, n (%)	ABC + 3TC + DTG or TDF + 3TC + DTG, n (%)	Other, n (%)	Total
K103N, P225PH, K238T, K20R, V21I, V35T, T39A, E40D, S68SKNR, K122E, D123S, K173S, Q174K, D177E, G196GR, T200S, Q207A, R211S, V245E, D250E, S251ST	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K103N, Y181C, P225H	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K103NS	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
K103S	1 (11.1)	2 (4.5)	3 (1.8)	2 (2.6)	0	1 (6.3)	0	0	9 (2.6)
K108I	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
L100I	0	0	10 (6.0)	0	2 (9.5)	0	0	0	12 (3.5)
L100I, K103N, P225H	0	0	0	0	1 (4.8)	0	0	0	1 (0.3)
L100LI	0	0	3 (1.8)	0	0	0	0	0	3 (0.9)
L100I	0	0	2 (1.2)	0	0	0	0	0	2 (0.6)
M41L	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
V106	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
V106A	0	1 (2.3)	0	1 (1.3)	0	0	0	0	2 (0.6)
V106I	0	0	4 (2.4)	2 (2.6)	0	0	1 (25.0)	0	7 (2.0)
V106M	1 (11.1)	0	2 (1.2)	1 (1.3)	0	0	0	0	4 (1.2)
V106VI	0	1 (2.3)	0	2 (2.6)	0	0	0	0	3 (0.9)
V108I	0	0	3 (1.8)	3 (3.8)	0	0	0	1 (20.0)	7 (2.0)
V108I, F227L	0	1 (2.3)	0	0	0	0	0	0	1 (0.3)
V108VI	0	0	0	0	0	1 (6.3)	0	0	1 (0.3)
V179E	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
V179T	0	0	0	0	1 (4.8)	0	0	0	1 (0.3)
V188L	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
V35T	0	0	1 (0.6)	0	0	0	0	0	1 (0.3)
Y181C	0	5 (11.4)	1 (0.6)	9 (11.5)	1 (4.8)	4 (25.0)	0	0	20 (5.8)
Y181I	0	1 (2.3)	0	1 (1.3)	0	0	0	0	2 (0.6)
Y181V	0	0	0	1 (1.3)	0	0	0	0	1 (0.3)
Y181YC	0	1 (2.3)	0	0	0	0	0	0	1 (0.3)
Y188L	0	1 (2.3)	2 (1.2)	2 (2.6)	0	0	0	0	5 (1.5)
Total	**9 (100.0)**	44 (100.0)	166 (100.0)	**78 (100.0)**	**21 (100.0)**	**16 (100.0)**	**4 (100.0)**	**5 (100.0)**	**343 (100.0)**

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitors; ART, antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; ABC, abacavir; DTG, dolutegravir.

Table 3. Multivariable logistic regression to determine factors associated with failure to re-suppress the VL among adults living with HIV in Rwanda.

	Failure to re-suppress (VL ≥ 1000 copies/mL							
	N	n	Crude odds ratio	P < |z|	95% CI	Adjusted odds ratio	P < |z|	95% CI
Age categories								
<25	84	57	1.00	—	—	—	—	—
25–34	132	79	0.87	0.735	0.38–1.98	—	—	—
35–49	334	215	0.72	0.477	0.29–1.79	—	—	—
50–59	108	46	0.55	0.358	0.55–1.98	—	—	—
60+	44	18	0.46	0.340	0.09–2.31	—	—	—
Latest CD4 categories								
<200	270	191	1.00	—	—	—	—	—
200–349	217	107	0.30	0.001	0.17–0.52	0.26	0.001	0.13–0.52
350–500	142	82	0.67	0.442	0.24–1.88	0.62	0.402	0.20–1.91
Ever shifted ART regimen								
Yes	262	142	1.00	—	—	—	—	—
No	440	273	1.08	0.805	0.56–2.11	—	—	—
Time on ART								
≤1 Year	47	30	1.00	—	—	—	—	—
]1–5 Years	203	103	1.13	0.838	0.34–3.72	—	—	—
]5–10 Years	240	144	1.05	0.899	0.48–2.28	—	—	—
]10+ years	212	138	1.64	0.395	0.52–5.17	—	—	—
Patient current ART								
TDF + 3TC + EFV	316	194	1.00	—	—	—	—	—
TDF + FTC + NVP	136	95	1.92	0.094	0.89–4.14	1.84	0.040	1.03–3.28
AZT + 3TC + EFV	20	11	0.91	0.858	0.30–2.71	1.02	0.976	0.29–3.61
AZT + 3TC + NVP	86	58	1.62	0.504	0.38–6.81	1.44	0.591	0.37–5.62
ABC + 3TC + EFV	49	25	0.67	0.581	0.16–2.83	1.67	0.559	0.29–9.61
ABC + 3TC + NVP	29	17	1.04	0.954	0.29–3.71	1.21	0.790	0.29–5.10
Other regimen not based on EFV or NVP	57	10	0.14	0.001	0.05–0.39	0.19	0.001	0.07–0.48

to those with no history of switching ART regimens (Table 4).

Individuals with current ART regimen tenofovir disoproxil fumarate + emtricitabine + nevirapine (aOR, 0.148 [95% CI: 0.028–0.779]; p = 0.025), zidovudine + lamivudine + efavirenz (aOR, 0.105 [95% CI: 0.028–0.779]; p = 0.020) and zidovudine + lamivudine + nevirapine (aOR, 0.259 [95% CI: 0.016–0.693]; p = 0.019) were less likely to have any NNRTIs mutations than those on the reference regimen tenofovir disoproxil fumarate + lamivudine + efavirenz (Table 4). Patients who ever switched ART regimens were 3.23 times more likely to have NNRTI-resistant mutations (aOR, 3.23 [95% CI: 1.435–7.278]; p = 0.005; Table 5).

Discussion

This is the first national cross-sectional study examining the prevalence and distribution of HIVDR mutations associated with first-line regimen failure in the Rwanda national HIV prevention, care and treatment program. Of all patients with unsuppressed VL at study inception using VL collected 3 months prior to the study, 31.8% had suppressed VL at the second VL measurement (VL test done using samples collected in the study), indicating that most patients enrolled had failed to re-suppress their VL. These patients once on an NNRTI-based regimen should be switched to a different ART regimen based on a single viral load test and switched based on a second VL test after enhanced adherence counselling once on a non-NNRTI based regimen as recommended by the 2021 WHO guideline.[18]

In this study, 92.7% of patients had at least one drug-resistant mutation against NRTIs, NNRTIs or PIs used in the national program. The resistant mutations identified against different drug classes were 90.4% for NNRTIs, 75.5% for NRTIs and 3.5% for PIs. Other studies conducted elsewhere in adults aged ≥18 years have found varying results for prevalence of NRTI-resistant and NNRTI-resistant mutations. Studies have found 93.11% of patients with NNRTI-resistant mutations, 74.40% of patients with NRTI-resistant mutations in Hunan South China and 93% and 96% of patients with NRTI-resistant and NNRTI-resistant mutations, respectively, in resource-limited settings (i.e. Thailand, South Africa, India, Malawi and Tanzania).[19,20] Another study found substantially lower drug-resistant mutation rates: 11% of patients had NRTI-associated drug-resistant mutations and 17% of patients had no known mutations, depending on the VL monitoring practice in place.[21]

The most common HIVDR mutations identified in our study were K65R, M184V and D67N for NRTIs and K103N, A98G and Y181C/I/V for NNRTIs. It is likely that some of the patients on TDF-based first-line therapy previously acquired the D67N mutation while receiving zidovudine. Overall, the NRTI-resistant mutations observed in our study allow effective NRTIs to remain as backbone options for second-line ART. Tenofovir disoproxil fumarate genotypic resistance, defined by K65R/N and/or K70E/Q/G, occurs in 20%–60% of individuals with unsuppressed VL receiving WHO-recommended tenofovir-containing first-line regimens.[22]

In our study, 22% of patients had the K65R/N mutation and 8% had K70E/G/Q. This suggests that one of three NRTI-resistant mutations developed against tenofovir. This high prevalence could be influenced by the fact that most patients in our study were receiving tenofovir-based ART. K65R/N and K70E/G/Q are rarely combined because of the reduced virus replication capacity when the two mutations occur together. The second most prevalent NRTI-resistant mutation was M184V/I (15%), which is selected by lamivudine or emtricitabine and is reported as the most common NRTI-resistant mutation after first-line ART failure.[23,24] Although M184V causes high-level resistance to lamivudine and emtricitabine, the M184V/I mutation also is associated with reduced viral fitness and increased virus susceptibility to zidovudine and tenofovir. For this reason, lamivudine/emtricitabine is usually maintained in the backbone of second-line combination ART.[25] Our finding that K65R was more common than M184V is unique compared to other studies which were conducted in prior years and merits further exploration and validation as part of drug resistance surveillance in other countries.[26]

In addition to common NRTI-resistant mutations, our study identified A62AV mutations (2.1%), which is also a tenofovir accessory resistant mutation that often occurs in combination with the multi-NRTI-resistant mutations K65R or Q151M. A62V is widespread in subtype A. However, we also found D67D/N and K219K/Q/R (0.3%), which are thymidine analogues mutations (TAMs) and not tenofovir induced. M41l, E44D, K70R, M184V, K219Q (0.3%), M41Ml (0.3.1%) and V75M (0.7%) are TAMs and are not selected by tenofovir. These findings suggest that first-line ART failed or that there might have been some patients transferred to different health facilities and initiated tenofovir-based ART after a zidovudine/stavudine-based regimen failed, but the patients did not disclose their ART history.

Furthermore, TAMS/multiple TAMS in the study were rare in our study, though some of the patients had received zidovudine for a long period. This suggests that national HIV VL monitoring of patients receiving ART has prevented long-term treatment failure that would result in the accumulation of TAMs and potential loss of efficacy of all NRTI. In contrast to our NRTI-resistant mutations findings, our NNRTIs results would exclude any further use of first-generation NNRTIs (efavirenz and nevirapine)

Table 4. Multivariable logistic regression to determine factors associated with any NRTI-resistant mutations among adults living with HIV (*n* = 378) in Rwanda.

Any NRTI-resistant mutations	N	N	Crude OR	P<|z|	95% CI	Adjusted OR	P<|z|	95% CI
Gender								
Females	255	193	1.000	—	—	—	—	—
Males	123	98	2.634	0.142	0.717–9.671	—	—	—
Age-groups (years)								
<25	56	43	1.000	—	—	—	—	—
25–34	74	57	0.364	0.310	0.051–2.617	—	—	—
35–49	190	148	0.798	0.580	0.355–1.793	—	—	—
50–59	42	33	0.646	0.299	0.281–1.485	—	—	—
60+	16	10	0.322	0.226	0.050–2.053	—	—	—
Current ART regimen								
TDF + 3TC + EFV	178	130	1.000	—	—	1.000	—	—
TDF + FTC + NVP	85	67	0.890	0.829	0.304–2.604	0.685	0.517	0.214–2.187
AZT + 3TC + EFV	11	7	1.142	0.868	0.231–5.662	1.066	0.941	0.190–5.978
AZT + 3TC + NVP	49	43	3.016	0.079	0.878–10.362	3.333	0.046	1.022–10.870
ABC + 3TC + EFV	23	18	2.287	0.343	0.406–12.885	1.394	0.657	0.315–6.172
ABC + 3TC + NVP	17	14	6.011	0.048	1.018–35.495	4.010	0.094	0.785–20.483
Other regimen not based on EFV or NVP	10	7	0.847	0.870	0.112–6.411	0.565	0.496	0.107–2.996
Ever switched ART regimen								
No	243	181	1.000	—	—	1.000	—	—
Yes	135	110	2.051	0.066	0.953–4.415	2.533	0.016	1.198–5.356
Time on ART (years)								
<1	30	24	1.000	—	—	—	—	—
1–4	95	63	0.655	0.660	0.097–4.418	—	—	—
5–9	124	94	1.574	0.645	0.222–11.153	—	—	—
10+	129	110	1.731	0.511	0.329–9.099	—	—	—
CD4 categories at ART initiation								
<200	96	85	1.000	—	—	—	—	—
200–350	125	95	0.360	0.084	0.112–1.151	—	—	—
350–500	73	54	0.483	0.232	0.144–1.612	—	—	—
500+	65	40	0.401	0.090	0.139–1.159	—	—	—
Latest CD4 categories								
<200	175	142	1.000	—	—	—	—	—
200–349	95	68	0.807	0.625	0.337–1.932	—	—	—
350–500	74	55	0.464	0.114	0.178–1.210	—	—	—

Abbreviations: NRTI, nucleoside reverse transcriptase inhibitors; aOR, adjusted odds ratio; CI, confidence interval; ART, antiretroviral therapy, AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; ABC, abacavir.

because of the cross-resistance of NNRTI mutations. The emergence of Y181C/I/V, mostly selected by nevirapine, would also limit the use of etravirine, which is reserved as a rescue drug for patients for whom second-line regimens have failed. K101E decreases HIV susceptibility to all NNRTIs (efavirenz, nevirapine and etravirine). This suggests that national HIV VL monitoring of patients receiving ART has prevented long-term treatment failure that would result in the accumulation of TAMs and potential loss of efficacy of all NRTI used in second-line ART as the backbone in combination with either dolutegravir or boosted PIs.

We found that switching the initial ART regimen was significantly associated with developing NRTI-resistant or NNRTI-resistant mutations. These results could be because of the fact that patients switched their regimens either because of side effects or ART toxicity. The low barrier to resistance of NNRTIs used in first-line ART might lead to resistant mutations from the time of clinical side effects/toxicity to the time of switching the initial ART regimen. In

Table 5. Multivariable logistic regression to determine factors associated with any NNRTI-resistance mutations among adults living with HIV (n = 378) in Rwanda.

Any NNRTI-resistant mutations	N	n	Crude OR	P<|z|	95% CI	Adjusted OR	P<|z|	95% CI
Gender								
Females	255	232	1.000	—	—	—	—	—
Males	123	115	2.634	0.142	0.717–9.671	—	—	—
Age-groups (years)								
<25	56	54	1.000	—	—	—	—	—
25–34	74	64	0.133	0.037	0.202–0.878	—	—	—
35–49	190	174	0.108	0.035	0.014–0.850	—	—	—
50–59	42	40	0.357	0.388	0.034–3.804	—	—	—
60+	16	15	1.764	0.700	0.094–33.051	—	—	—
Current ART regimen								
TDF + 3TC + EFV	178	166	1.000	—	—	1.000	—	—
TDF + FTC + NVP	85	78	0.205	0.061	0.039–1.077	0.148	0.025	0.028–0.779
AZT + 3TC + EFV	11	9	0.120	0.030	0.018–0.812	0.105	0.020	0.016–0.693
AZT + 3TC + NVP	49	44	0.240	0.014	0.078–0.742	0.259	0.019	0.084–0.793
ABC + 3TC + EFV	23	21	0.399	0.311	0.066–2.406	0.207	0.093	0.033–1.306
ABC + 3TC + NVP	17	16	2.123	0.541	0.183–24.587	1.250	0.854	0.111–14.029
Other regimen not based on EFV or NVP	10	8	0.119	0.063	0.013–1.122	0.069	0.011	0.009–0.531
Ever switched ART regimen								
No	243	218	1.000	—	—	1.000	—	—
Yes	135	129	2.036	0.064	0.959–4.323	3.231	0.005	1.435–7.278
Time on ART (years)								
<1	30	28	1.000	—	—	—	—	—
1–4	95	85	1.734	0.591	0.226–13.302	—	—	—
5–9	124	116	1.234	0.847	0.140–10.882	—	—	—
10+	129	118	0.868	0.860	0.174–4.324	—	—	—
CD4 categories at ART initiation								
<200	96	93	1.000	—	—	—	—	—
200–350	125	116	0.461	0.420	0.068–3.107	—	—	—
350–500	73	65	0.652	0.439	0.217–1.952	—	—	—
500+	65	54	0.322	0.267	0.042–2.436	—	—	—
Latest CD4 categories								
<200	175	163	1.000	—	—	—	—	—
200–349	95	83	0.576	0.347	0.180–1.842	—	—	—
350–500	74	68	1.366	0.584	0.441–4.235	—	—	—

Abbreviations: NNRTI, non-nucleoside reverse transcriptase inhibitors; aOR, adjusted odds ratio; CI, confidence interval; ART, antiretroviral therapy; AZT, zidovudine; 3TC, lamivudine; EFV, efavirenz; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; ABC, abacavir.

addition, we found that patients receiving tenofovir, lamivudine and efavirenz were more likely to develop NNRTI-resistant mutations than patients on other regimens. In 2009, the tenofovir-based regimen was introduced as the preferred first-line ART in Rwanda. During our study, patients received zidovudine-based ART if they had contraindications to use tenofovir or nevirapine. It is possible that patients stopped their initial ART before the treatment was changed and that NNRTI-resistant mutations developed during that period.

Our findings are subject to several limitations. Some of the assumptions used for sample size determination were posteriorly determined, and therefore, conservative assumption was used to maximize sample size. The sample size estimation was therefore based on more stringent assumptions derived from credible and relevant references in order not to under-estimate the required sample size. We included only patients with unsuppressed VL, for whom first-line ART had failed. This VL threshold cannot capture drug-resistant mutations that occur at lower load viraemia

and that are associated with subsequent virologic failure. Because our study was conducted at the time dolutegravir was first introduced in Rwanda, it was not possible to characterize potential dolutegravir-resistant mutations. Our logistic regression analysis only included variables that were available. Because the data were collected from patient charts (files), which are associated with missing variable bias, we might have missed or not captured variables that might have changed our results.

NRTI-resistant mutations preserve effective second-line ART with selected NRTIs as the backbone in combination with either dolutegravir or boosted PI.[22] The prevalence of acquired HIV drug resistance (HIVDR) was high among patient failing to re-suppress VL and was associated with current ART regimen and ever switching ART regimen. The findings of this study support the current WHO guidelines recommending that patients on an NNRTI-based regimen should be switched based on a single viral load test and suggests that national HIV VL monitoring of patients receiving ART has prevented long-term treatment failure that would result in the accumulation of TAMs and potential loss of efficacy of all NRTI used in second-line ART as the backbone in combination with either dolutegravir or boosted PIs. This study further supports the bi-annual revisions and updates to the national HIV treatment guidelines.

Acknowledgements

We would like to thank the Government of Rwanda, particularly the Rwanda Biomedical Center (RBC) survey team, for their dedication to this study and the participating health facilities and patients for their collaboration. In addition, we would also like to acknowledge technical support received from our partners at CDC and WHO.

Authors' contributions

GM contributed to study conception, study design, data collection and analysis and led manuscript writing. MR, EK, SSM, RCNM, JMS, ABS and ER contributed to study conception and study design, drafted and edited the manuscript and interpreted the results. ET, SSM, RCNM, AK, GM, GNR and ER were responsible for conducting statistical analysis, interpreting results and drafting and editing the manuscript. GM, MRH, JD, CB, MR, SDH and ER contributed to collecting data, managing field activities, manuscript editing and interpreting results. MR, GNR and ER supervised the study and protocol implementation and contributed to manuscript development and interpretation of results. All authors read and approved the final submitted version of the manuscript.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: We declare a conflict of interest. Josh DeVos is a co-inventor in U.S. patent US10053741B2.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study is supported by the President's Emergency Plan for AIDS Relief (PEPFAR) through the Centers for Disease Control and Prevention (CDC) under the terms of cooperative agreement #GH001612-04. The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official position of the funding agencies.

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