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Drug resistance mutations after the first 12 months on antiretroviral therapy and determinants of virological failure in Rwanda

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Abstract

OBJECTIVE To evaluate HIV drug resistance (HIVDR) and determinants of virological failure in a large cohort of patients receiving first-line tenofovir-based antiretroviral therapy (ART) regimens.
METHODS A nationwide retrospective cohort from 42 health facilities was assessed for virological failure and development of HIVDR mutations. Data were collected at ART initiation and at 12 months of ART on patients with available HIV-1 viral load (VL) and ART adherence measurements. HIV resistance genotyping was performed on patients with VL ≥1000 copies/ml. Multiple logistic regression was used to determine factors associated with treatment failure.
RESULTS Of 828 patients, 66% were women, and the median age was 37 years. Of the 597 patients from whom blood samples were collected, 86.9% were virologically suppressed, while 11.9% were not. Virological failure was strongly associated with age <25 years (adjusted odds ratio [aOR]: 6.4; 95% confidence interval [CI]: 3.2–12.9), low adherence (aOR: 2.87; 95% CI: 1.5–5.0) and baseline CD4 counts <200 cells/μl (aOR 3.4; 95% CI: 1.9–6.2). Overall, 9.1% of all patients on ART had drug resistance mutations after 1 year of ART; 27% of the patients who failed treatment had no evidence of HIVDR mutations. HIVDR mutations were not observed in patients on the recommended second-line ART regimen in Rwanda.
CONCLUSIONS The last step of the UNAIDS 90-90-90 target appears within grasp, with some viral failures still due to non-adherence. Nonetheless, youth and late initiators are at higher risk of virological failure. Youth-focused programmes could help prevent further drug HIVDR development.

keywords HIV, drug resistance, tenofovir, Rwanda, viral failure

Introduction

The increasingly widespread use of antiretroviral therapy (ART) has substantially improved the prognosis of people living with HIV. Mortality among persons on ART has fallen and may now be no higher than for other chronic diseases, even in resource-limited settings (RLS) [1, 2]. Rwanda achieved universal ART coverage in 2012, when more than 90% of people in need of ART (according to national guidelines) received drug therapy [3]. The success of the national HIV programme translates to a variety of favourable indicators including an increase in life expectancy of HIV-infected Rwandans that is near the life expectancy of uninfected individuals [2]. Despite these successes, HIV drug resistance poses a growing threat to the ongoing success and durability of ART regimens during the continued ART scale-up in many countries [4].

According to a systematic review in 2011, the overall transmitted HIV drug resistance in RLS was 6.6% and negatively impacted outcomes of first-line ART [5, 6]. Genotyping tests performed on eight patients in a Rwandan study evaluating drug resistance mutations (DRMs) among patients on first-line ART found two cases with thymidine analogue mutations (TAMs) [7]. The mutations included D67N, K70R and K219Q in one case and D67N and K70R in the other [7]. Likewise, Mutwa *et al.* found TAMs in 31% of 57 children and adolescents who had virological failure on first-line ART.[8] However, all DRM studies conducted in Rwanda took place when the guidelines recommended thymidine analogues (i.e. zidovudine and stavudine) for first-line ART [9]. Since 2009, the Rwanda ART guidelines have recommended tenofovir (TDF)-based regimens for ART-naïve patients [10]; TDF selects the K65R mutation rather than TAMs.[11, 12]

A number of studies that evaluated drug resistance after 12 months of ART in RLS have been conducted, but most of these had small numbers of patients. Studies on DRMs within Rwanda have also been based on small numbers of patients and were focused on mutations associated with thymidine analogues rather than TDF-based regimens. This study aimed to address both gaps by evaluating DRMs after 12 months on ART among a large national sample of patients, the majority of whom started TDF-based regimens as their first-line therapy.

Methods

Study setting and study design

At the end of 2010, 83 041 Rwandans were on ART in 295 health facilities. ART coverage was at 75.5% [13, 14]. Eligibility criteria to start ART in 2010 were WHO clinical stage IV, or a CD4 cell count <350 cells/μl. Exceptions were patients with TB/HIV co-infection and pregnant women, whose ART treatment was initiated for life when CD4 cell counts were <500 cells/μl. First-line ART consisted of TDF and lamivudine (3TC) in combination with one NNRTI (either nevirapine or efavirenz). Patients received individual counselling at each clinic visit, both before and during ART. Biological follow-up of outcomes of ART consisted of CD4 monitoring every 6 months and HIV-1 viral load monitoring once per year.

We conducted a nationwide retrospective cohort study based on the WHO generic protocol for evaluating the emergence of HIV-1 drug resistance [15]. A blood specimen and related demographic and clinical information was collected from ART patients starting their first year of treatment. Using an electronic monitoring and evaluation database (TRACnet) [16], we selected 42 health facilities among the 269 that were ART sites by the end of 2009. Eligibility criteria for inclusion of health facilities were that the facilities had functioned as an ART site for at least 1 year, so that programme functioning was well established and enrolment of at least one patient per month between November 2010 and January 2011. Health facilities with larger numbers of patients on ART were also enrolling larger numbers of patients initiating ART.

Inclusion criteria applied to patients in these 42 health facilities were as follows: age 15 years and older) and on first-line ART for 12 months ($\pm$1 month). Patients who were known to have received single-dose nevirapine as prevention of mother-to-child HIV transmission prior to starting ART and patients who received other ART prophylaxis for other reasons were also included. We excluded patients who were transferred from other health facilities to avoid missing information preceding enrolment, children (below 15 years of age) and patients who had stopped ART and were restarting in the study period.

Data Collection

Initially, 837 patients who started ART between November 2010 and January 2011 and completed 12 months of treatment by December 2011 met the inclusion criteria. However, due to missing medical files, we removed nine patients and were left with 828 within our retrospective cohort. This cohort was composed of patients still alive and active in the programme, those lost to follow-up and those who had died and/or transferred out. Of these, 711 patients were still active in the programme and were invited for blood collection. Through healthcare providers, we invited them for a fixed data collection day; 597 patients (84% of invited) presented themselves on data collection day and consented to give a blood sample for HIV-1 viral load and resistance genotyping. We conducted verbal interviews using personal digital assistant and chart reviews by trained data collectors to record information on all 828 patients.

Blood samples for the 597 consenting patients were obtained using standard methods for blood sample processing. HIV-1 viral load testing was performed at the National Reference Laboratory in Kigali, Rwanda, using the Roche system CAP/CTM (COBAS AmpliPrep and COBAS TaqMan 96) with Kit HI2CAP. One millilitre of plasma from each blood draw was stored at -80 °C for use in future analysis. Seventy-one samples having a viral load of $\geq$1000 copies/ml were analysed for the presence of ART resistance mutations using the TRUGENE HIV-1 Genotyping Assay on the OpenGene DNA System (Siemens HealthCare, USA) at the College of American Pathologist-accredited HIV Diagnostics and Reference Laboratory at the Walter Reed Army Institute of

Research in Maryland, USA. Seventy samples were successfully sequenced; one sample failed to amplify three times and another sample after a failure yielded a sequence having high background and of poor quality. ART resistance mutations and subsequent susceptibility profiles were generated with Siemens FDA-approved GeneObjects 4.1 software and Guidelines 17.0. HIV-1 subtype was determined using phylogenetic tree analysis of the sequences against selected reference sequences using MegAlign (DNASTAR, Inc., Madison WI, USA), Stanford HIV Drug Resistance Database (http://hivdb.stanford.edu), HIV-1 BLAST analysis of each region (www.hiv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html) and the HIV sequence database subtyping tool (www.hiv.lanl.gov). Protease and reverse transcriptase sequences for each specimen were submitted to GenBank (Accession numbers KU922763 – KU922832 and KU922833 – KU922902, respectively).

Variable Definition

The primary outcome of interest in this study was HIV drug resistance (HIVDR). HIVDR outcomes were defined according to WHO protocol [1] as (i) prevention of HIVDR when an HIV RNA viral load was <1000 copies/ml; (ii) potential HIVDR (virological failure) when HIV RNA was ≥1000 copies/ml with no observation of HIVDR mutations in the genotype test; and (iii) HIVDR when HIV RNA was ≥1000 copies/ml with HIVDR mutations observed in genotypic testing. HIV RNA viral load suppression was defined as viral load <400 copies/ml.

For this research, 'retention' was a multicategory variable that established the proportion of patients known to be alive and receiving ART, that transferred out, that were known and documented to have died 12 months after ART initiation and that were lost to follow-up according to the national definition. Patients were scheduled to have monthly clinic visits and drug pickups. They were deemed to be lost to follow-up if they failed to present themselves for three consecutive visits/pickups.

We collected information on sociodemographic characteristics, previous ART exposure, clinical baseline information, initial ART prescription and any prescription following a switch within 12 months, and immunological evolution. Adherence was evaluated by visual analogue scale during the 30 days prior to data collection and by appointment keeping records from pharmacies.

Statistical analysis

We used descriptive statistics to describe the sample with respect to sociodemographic and clinical characteristics and to describe the various types of mutations present in the sample. Using univariate and multivariate logistic regression, a number of variables were examined as independent predictors of virological failure and drug resistance. For each potential predictor, including age, sex, marital status, type of health facility, ever attended school, active tuberculosis when starting ART and self-reported adherence during the last 30 days, we first calculated the odds ratio with 95% confidence intervals using univariate logistic regression. Model selection within the multivariate analyses was completed by identifying the model that minimised the Akaike's information criterion (AIC). Data analysis was performed using SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

Ethics

Written informed consent for conducting an interview and taking a blood sample was obtained from all patients prior to any procedure. The protocol for this study was approved by the National Research Committee on HIV/AIDS and by the Rwanda National Ethics Committee.

Results

Patients' characteristics and retention after 12 months of ART

A total of 828 adult patients were included, of whom 552 (66%) were female. Table 1 presents the baseline characteristics of the cohort. The median age was 37 years (IQR: 30–45), and youth (≤25 years) comprised 10% of participants. Overall, 62% had ever attended school and among them 75% had only ever attended primary school; 60% of the study population were in a partner relationship with 39% married and 21% cohabitating.

Of 828 adult patients who initiated first-line ART and remained on therapy for 12 months, 711 (85.9%) were still taking their first-line ART regimen from the same site of ART initiation (i.e. that they did not change their original prescribed ARVs and were not transferred out), 66 patients (8%) were transferred out to other ART health facilities, 27 patients (3.2%) had died, and 23 patients (2.7%) were lost to follow-up at 12 months. Only one patient stopped ART and one person switched ART regimens.

Clinical, immunology and virological outcomes

The median CD4 count was 273 cells/μl (IQR: 175–331 cells/μl) at ART initiation, 392 cells/μl (IQR: 256–533 cells/μl) at 6 months and 420 cells/μl (IQR: 269–587

cells/μl) at 12 months. At baseline, 29% of patients had advanced immunosuppression (CD4 < 200 cells/μl); half of patients (51%) were in WHO clinical stage I, 28% in stage II, 17% in stage III and 4% in stage IV. At ART initiation, 6% of all participants were also taking antituberculosis drugs and 3% developed tuberculosis between ART initiation and 12-month follow-up. The majority of patients (96%) initiated TDF-based regimens. Median adherence using visual analogue scale was 97% over the past 30 days.

Virological and drug resistance outcomes

Among 597 samples collected, 587 underwent analysis, and among those, 507 (86.4%) had suppressed their VL (VL < 400 copies/ml), 71 (12.1%) had virological failure (VL ≥1000 copies/ml), and 9 (1.5%) were between 400 and 999 copies.

Table 2 presents the results of the logistic regression analyses for virological failure as an outcome. Virological failure was strongly associated with age below 25 years (adjusted odds ratio [aOR]: 6.4; 95% confidence interval [CI]: 3.2–12.9; $P < 0.001$), low adherence (aOR: 2.87; 95% CI: 1.5–5.0; $P < 0.001$) and CD4 count <200 cells/μl (aOR 3.4; 95% CI: 1.9–6.2; $P < 0.001$) at ART initiation. There was no significant association between virological failure, sex, education level, type of health facility, previous ART exposure or active tuberculosis at ART initiation.

Genotype sequencing was performed on the 71 samples with HIV-1 RNA VL ≥1,000 c/ml. Seventy samples were successfully sequenced; one sample did not amplify (three times) and no sequence was obtained. The predominant HIV subtype was A1 at 77.1% followed by C at 12.9% and D at 5.7% (Table 1). HIVDR mutations were observed in 9.1% of the patients (54/590), while DR mutations were observed in 2.7% (16/590). Table 3 lists the mutations identified along with their frequencies. The predominant nucleoside reverse transcriptase inhibitor (NRTI) mutations were M184V (55.7%) and K65R (41.4%), but thymidine analogue mutations were few, and no complete pathway was found. For non-nucleoside reverse transcriptase inhibitors (NNRTI), the most common mutations were Y181C, K103N and G190A with frequencies of 41%, 20% and 17%, respectively. Finally, minor resistance mutations at amino acid positions L10 and K20 were observed for protease inhibitors (PI) except for the predominance of the polymorphism M36I.

Interpretation of these mutations showed that 16 (22.9%) patients who had virological failure did not harbour any resistance mutations and 54 (77.1%) had at least one resistance mutation. The frequencies of resistance to at least one ARV medication were 62.1% for NRTIs, 74.7% for NNRTIs and 12.9% for PIs. With respect to resistance to an entire drug class, 27% of patients were resistant to NNRTIs, but no patients were completely resistant to NRTIs or PIs. Among 70 successful sequenced samples, 42.9% had virus resistant to 5 NRTIs used in Rwanda (ABC, ddI, 3TC, d4T, TDF) except AZT. Moreover, 38.5% of patients resistant to

Table 1 Baseline patient characteristics

Variable	Count (percentage) or Median (IQR)
Age (in years) (n = 828)	37 (30–45)
<25 years	83 (10.0)
≥25 years	745 (90.0)
Sex (n = 828)	
Female	547 (66.1)
Male	281 (33.9)
Marital Status (n = 754)	
Single	105 (13.9)
Now Married	296 (39.3)
Cohabitant	157 (20.8)
Divorced	48 (6.4)
Widow	117 (15.5)
Others	31 (4.1)
Ever to school (n = 650)	
No	133 (20.5)
Yes	517 (79.5)
WHO stage baseline (n = 819)	
WHO I	422 (51.5)
WHO II	228 (27.8)
WHO III	137 (16.7)
WHO IV	32 (3.9)
CD4 at baseline (n = 799)	
≤200 CD4	228 (28.5)
>200 CD4	571 (71.5)
ART exposure before HAART (n = 719)	
Yes	63 (8.8)
No	656 (91.2)
Reason of ART exposure (n = 58)	
PMTCT prophylaxis	51 (87.9)
PMTCT and PEP	3 (5.2)
PEP only	4 (7.0)
TB at baseline (n = 783)	
Yes	45 (5.7)
No	738 (94.3)
Level of health facility (=828)	
Health Centre	341 (41.2)
District Hospital	470 (56.8)
Reference hospital	16 (1.9)
HIV subtypes	
A1	54 (77.1)
C	9 (12.9)
D	4 (5.7)
A/D	1 (1.4)
18_cpx	1 (1.4)
1 B/A	1 (1.4)
Total	70 (100)

these 5 NRTIs were also resistant to all NNRTIs used in Rwanda (EFV, NVP and ETR). Patients who were on an NVP-based regimen were at six times greater risk of developing at least one drug resistance mutation than patients on an efavirenz-based regimen (OR 6.6; *P*-value 0.01). Among patients with virological failure, the majority (54; 77.1%) shifted from first-line ART to second-line ART due to the resistance mutations that developed. There was no significant association between developing drug resistance mutation and age <25 years.

Table 4 presents the frequency of resistance to specific ARVs. Among 70 samples with successful sequencing, 58% had virus resistant to 3TC, 44% to TDF and 43% to ABC, DDI and D4T. For NNRTIs, 76% had virus resistant to NVP and sensitivity to EFV and ETR at 24% and 44%, respectively. All available protease inhibitors and zidovudine were 100% sensitive.

Discussion

Our study provides a novel assessment of the drug resistance mutations among patients who started on TDF-based regimens in Rwanda. This study was required as previous studies on HIVDR in Rwanda were conducted on patients who received thymidine analogue-based first-line treatments, which are no longer the primary first-line therapies in Rwanda [17]. We found that after 12 months of ART, 88.1% of patients suppressed their VL and virological failure was 12%. Among those with virological failures, 22.9% (16/70) were failing ART without a drug resistance mutation (potential drug resistance). The remaining 77.1% (54/70) had drug resistance mutations and were shifted to second-line ART regimens. The predominant mutations identified were M184V and K65R for NRTI, Y181C for NNRTIs, K103N and G190A for NNRTIs and minor mutations for PIs. Virological failures were associated with young age, suboptimal adherence and low CD4 at ART initiation.

The success of HIV drug treatment met the WHO recommendation of >85% of patients on ART suppressing HIV-1 VL after 12 months of treatment. At 88.1% viral suppression after 12 months on treatment, Rwanda seems within reach of the third step of the 90-90-90 target recently set by UNAIDS [18]. Viral suppression results

Table 2 Predictors of virological failure

Patients' characteristics	VL > 1000 copies/ml *n* (%)	VL < 1000 copies/ml *n* (%)	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)
Age				
<25 years	22 (33.8)	43 (66.2)	**5.0 (2.7–9.1)**	**6.4 (3.2–12.9)**
≥25 years	48 (9.2)	472 (90.8)		
Sex				
Male	19 (10.0)	170 (90.0)	0.7 (0.4–1.3)	
Female	51 (12.9)	345 (87.1)		
Marital status				
Not union	35 (15.6)	189 (84.4)	1.7 (1.0–2.8)	1.3 (0.7–2.3)
In union	35 (9.8)	322 (90.2)		
Self-report adherence				
Adherence by VAS < 95%	23 (23.7)	74 (76.3)	**2.9 (1.6–5.0)**	**2.7 (1.5–5.0)**
Adherence by VAS ≥ 95%	47 (9.6)	515 (90.4)		
Ever to school				
No	13 (11.5)	100 (88.5)	0.9 (0.5–1.8)	
Yes	57 (12.3)	406 (87.7)		
CD4 at ART initiation				
≤200 CD4	29 (19.6)	119 (80.4)	**2.4 (1.4–4.0)**	**3.4 (1.9–6.2)**
>200 CD4	39 (9.2)	383 (90.8)		
ART exposure before				
Yes	8 (16)	42 (84)	1.4 (0.6–3.2)	
No	61 (11.5)	468 (88.5)		
TB at ART initiation				
Yes	1 (4.0)	24 (96.0)	0.3 (0.04–2.2)	
No	68 (12.2)	491 (87.8)		
Type of heath facility				
Public	59 (12.8)	402 (87.2)		
Mission	11 (8.8)	114 (91.2)	1.5 (0.8–3.0)	

OR: odds ratio; CI: confidence interval. Values in bold are statistically significant at the 0.05 significance level.

Table 3 Resistance mutations by drug class

NRTIs (*N* = 70)			NNRTIs (*N* = 70)			PIS (*N* = 70)		
Mutation	Frequency	Per cent	Mutation	Frequency	Per cent	Mutation	Frequency	Per cent
M184V	39	55.7	Y181C	29	41.4	L10I	24	34.3
K65R	29	41.4	K103N	14	20.0	L10V	9	12.9
A62V	8	11.4	G190A	12	17.1	L33F	2	2.9
K70E	6	8.6	A98G	8	11.4	V11I	1	1.4
Y115F	5	7.1	V90I	8	11.4	L10I/M	1	1.4
K219E	5	7.1	V108I	7	10.0	L33V	1	1.4
K219R	1	1.4	K101E	6	8.6	A71T	1	1.4
K70E/R	1	1.4	Y181I	3	4.3			
V75I	1	1.4	G190S	2	2.9			
V75M	1	1.4	K101N	1	1.4			
D67N	1	1.4	F227L	1	1.4			
K70R	1	1.4	Y188L	1	1.4			
M184I	1	1.4	Y181C*/V	1	1.4			
M41L	1	1.4	Y181I/V	1	1.4			
			M230L	1	1.4			
			E138G	1	1.4			
			L100I	1	1.4			
			K101Q	1	1.4			
			K103T	1	1.4			

were comparable to those found by Rusine *et al.* in a smaller prospective cohort of 213 patients on ART in Kigali where 86% achieved HIV viral suppression [7, 19]. Another retrospective study conducted in Rwanda by Frank *et al.* found 85% of patients virally suppressed after 12 months on ART [20]. Likewise, other countries in RLS such as Malawi, Mali, Burkina Faso and South Africa reported that HIV-1 suppression with ART was between 85% and 88% [17, 21, 22].

In our study, treatment failure was associated with age being <25 years. A similar result was reported in a Kenyan cross-sectional study of 238 patients on ART where patients aged 15–35 years were more likely to experience virological failure [23]. Qualitative studies have suggested that low adherence to treatment particularly in adolescents may explain the higher risk of ART failure [24]; however, our model suggests that even when adjusting for adherence, young patients remain at higher risk of virological failure. This is of particular concern given that ART is a lifelong engagement and that they will have many years of treatment ahead of them. Requiring second-line treatment early on could lead to important complications in the future. There are two possible explanations for youth being associated with viral failure independently of adherence. First, it may be indicative of transmitted HIV drug resistance. Second, it may be due to measurement bias given that adherence is measured in the past 30 days. Thus, poor adherence in the first few months of ART could go unnoticed and certainly could lead to drug resistance. The association between virological failure and late initiation has been reported by others, such as Matthew *et al.* who followed a cohort of 820 patients in South Africa and found that the risk of failure was nearly double among those with CD4 counts ≤200 cells [25]. The findings emphasise the reason why early treatment initiation is good, not only in preventing morbidity, mortality and decreasing transmission but also in preventing HIV drug resistance. Low adherence has long been known to lead to HIV drug resistance, so observing this in our data was expected. The Rwanda national HIV programme provides basic adherence counselling and has community outreach to minimise loss to follow-up. These findings highlight a need for enhanced adherence interventions among the youth in HIV care.

Among patients with virological failure, 77.1% harboured at least one drug resistance mutation and as such were indicated to shift to second-line ART. This proportion is similar to a smaller Nigerian study that found 77.7% (14/18) of its participants had major drug resistance mutation to either NRTIs or NNRTIs [26], and in the study conducted by Ziada El-Khatib, 78% of failing patients presented at least one major resistance mutation [17]. However, 22.9% of patients with VL failure presented only wild-type virus (no drug resistance mutation found), indicating the failure is likely due to poor use of ART rather than failure of the ART itself. These patients were kept on first-line ART, and adherence was

Table 4 Resistance and sensitivity of individual antiretroviral drugs

Class	ARV	% Resistant	Probable resistance	Sensitive
NRTIs	3TC	58.6	4.3	37.1
	TDF	44.3	4.3	51.4
	ABC	42.9	0.0	57.1
	DDI	42.9	0.0	57.1
	D4T	42.9	0.0	57.1
	AZT	0.0	0.0	100.0
NNRTIs	NVP	75.7	0.0	24.3
	EFV	31.4	44.3	24.3
	RLP	28.6	1.4	70.0
	ETR	1.4	54.3	44.3
PIs	SQV	8.6	4.3	87.1
	TPV	1.4	0.0	98.6
	ATV	0.0	0.0	100.0
	RIT	0.0	0.0	100.0
	LPV	0.0	0.0	100.0
	FPV	0.0	0.0	100.0
	IDV	0.0	0.0	100.0
	NFV	0.0	0.0	100.0
	DRV	0.0	0.0	100.0

ABC, Abacavir; AZT, Zidovudine; DDI, Didanosine; 3TC, Lamivudine; D4T, Stavudine; TDF, Tenofovir; NVP, Nevirapine; EFV, Efavirenz; ETR, Etravirine; ATV, Atazanavir; RIT, Ritonavir; DRV, Darunavir; LPV, Lopinavir; SQV, Saquinavir; NFV, Nelfinavir; TPV, Tipranavir, IDV, Indinavir; FPV, Fosamprenavir; RLP, Rilpivirine.

reinforced by the study team. Thus, drug resistance monitoring prevented the premature switching from first line to second line in patients failing ART after 12 months.

The predominant drug resistance mutations for NRTIs were K65R and M184V and for NNRTIs were Y181C and K103N. These mutation sites were corroborated by other studies where Hassan *et al.* found that M184V was predominant (43.6%) in Kenya [23], and the predominance of M184V and K103N was found by Rusine *et al.* and Ziada El-Khatib [7, 17]. However, the predominance of K65R in our study differs from other studies mentioned due to use of TDF instead of thymidine analogue-based regimen. Indeed, a large portion of patients had virus resistant to all NRTIs used in Rwanda except the thymidine analogue zidovudine, for which there was negligible resistance. Given that lamivudine has residual anti-HIV activity despite the presence of resistance [27], the use of zidovudine in combination with lamivudine and boosted lopinavir or atazanavir as the choice second-line regimen is viable despite the large number of individuals with a large number of HIV drug resistances. As the K65R is a dead-end mutation (in contrast to the TAM pathways), use of a TDF-based first-line regimen allows for a viable second-line regimen using zidovudine with a boosted PI (particularly as K65R increases zidovudine susceptibility). This differs from failure on a thymidine analogue (zidovudine or stavudine) first-line regimen where full TAM pathway resistance mutations eliminate the viability of standard second-line regimens, allowing only for monotherapy with boosted PIs.

Although this study succeeded in updating the Rwandan HIVDR profile using a cohort of patients initiating TDF-based regimens, doing so with a much larger sample than comparable studies, it has several limitations. As we collected information from health facilities' documents, the data were not always available. Attrition bias was possible due to the patients who were not present to provide blood on data collection day; however, comparisons of patient characteristics between those who missed and were present on blood collection day did not reveal any meaningful differences. Another probable bias is usage of visual analogue scale in 30 previous days for the assessment of adherence, which would include recall and social desirability biases. In this study, we did not trace patients to confirm whether they were still alive or not and the rate of transfers was high. This might reduce the rate of patients still alive on ART.

In conclusion, the monitoring of HIV drug resistance in Rwanda showed that HIV viral suppression (88.8%) which contributes to prevention of HIV drug resistance was greater than the WHO target at >85%. Prevalence of HIV drug resistance was 9%, and potential drug resistance was 2.7%. Among patients who are failing first-line ART in Rwanda 22.2% had wild-type virus (no drug-associated mutations), which means that they are likely failing due to poor ART adherence. The national HIV programme should focus on improving HIV services to adolescents and youth and to reinforce adherence among patients on ART in order to reduce the proportion of patients developing HIVDR.

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