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ORIGINAL RESEARCH

Characterization of HIV-1 drug resistance among patients with failure of second-line combined antiretroviral therapy in central Ethiopia

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Abstract

Background: As a consequence of the improved availability of combined antiretroviral therapy (cART) in resource-limited countries, an emergence of HIV drug resistance (HIVDR) has been observed. We assessed the prevalence and spectrum of HIVDR in patients with failure of second-line cART at two HIV clinics in central Ethiopia.

Methods: HIV drug resistance was analysed in HIV-1-infected patients with virological failure of second-line cART using the geno2pheno application.

Results: Among 714 patients receiving second-line cART, 44 (6.2%) fulfilled the criteria for treatment failure and 37 were eligible for study inclusion. Median age was 42 years [interquartile range (IQR): 20–45] and 62.2% were male. At initiation of first-line cART, 23 (62.2%) were WHO stage III, mean CD4 cell count was 170.6 (range: 16–496) cells/μL and median (IQR) HIV-1 viral load was 30 220 (7963–82 598) copies/mL. Most common second-line cART regimens at the time of failure were tenofovir disoproxil fumarate (TDF)-lamivudine (3TC)-ritonavir-boosted atazanavir (ATV/r) (19/37, 51.4%) and zidovudine (ZDV)-3TC-ATV/r (9/37, 24.3%).

Genotypic HIV-1 resistance testing was successful in 35 (94.6%) participants. We found at least one resistance mutation in 80% of patients and 40% carried a protease inhibitor (PI)-associated mutation. Most common mutations were M184V (57.1%), Y188C (25.7%), M46I/L (25.7%) and V82A/M (25.7%). High-level resistance against the PI ATV (10/35, 28.6%) and lopinavir (LPV) (5/35, 14.3%) was reported. As expected, no resistance mutations conferring integrase inhibitor resistance were detected.

Conclusions: We found a high prevalence of resistance mutations, also against PIs (40%), as the national standard second-line cART components. Resistance testing before switching to second- or third-line cART is warranted.

KEYWORDS

Africa, cART, eastern Africa, genotypic resistance testing, HIV, resistance mutations, second-line cART

INTRODUCTION

Certain resistance mutations of HIV impair the efficacy of drugs used for HIV treatment within different combined antiretroviral therapy (cART) regimens. Following the significantly increased availability of cART in resource-limited settings (RLS) over the past decade, HIV drug resistance (HIVDR) has now emerged to become a significant problem [1], leading to a high rate of patients experiencing treatment failure. More than 30% of HIV-positive individuals receiving protease inhibitor (PI)-based second-line cART in combination with nucleoside reverse transcriptase inhibitors (NRTIs) in sub-Saharan Africa (SSA) had a viral load (VL) > 400 copies/mL after 48 weeks of treatment [2], indicating a success rate of second-line cART of only *c.* 70%, measured by sustained virological suppression (SVS). It has been estimated that, by 2030, 0.8–4.6 million patients (6.6–19.6% of patients receiving cART) may need second-line cART in SSA [3]. Consequently, it is expected that new strategies with replacement of currently used standard cART regimens by integrase inhibitor (INI)-based regimens [such as a fixed dose of tenofovir disoproxil fumarate (TDF) + lamivudine (3TC) + dolutegravir (DTG)] will be necessary to achieve the United Nations programme on HIV/AIDS (UNAIDS) goal of viral suppression in 90% of patients with cART [4].

According to the latest Ethiopian 'national consolidated guideline for comprehensive HIV prevention, care and treatment' published in August 2018, a triple therapy comprising 3TC, TDF and DTG or efavirenz (EFV) should be administered as preferred first-line cART regimens for HIV-infected adult patients, where available [5]. As second-line cART, a combination of TDF, 3TC and ritonavir-boosted lopinavir (LPV/r) or atazanavir (ATV/r) is primarily recommended, if zidovudine (ZDV) was used in first-line therapy. If TDF was used in first-line therapy, AZT-3TC in combination with LPV/r or ATV/r is recommended. A regime comprising ritonavir-boosted darunavir (DRV/r), abacavir (ABC) and 3TC in combination with EFV or nevirapine (NVP) is recommended as third-line cART, although not yet widely implemented [5].

Regarding surveillance of HIV plasma VL during cART and thus recognition of virological failure (VF), the testing capacities are insufficient for routine healthcare service in Ethiopia and many other settings in SSA [6]. In Ethiopia, despite the wide availability of cART across the country, VL testing services are still limited to a few reference laboratories, hampering HIV treatment surveillance and early detection of treatment failure. Only limited data on the efficacy of second-line cART and the impact of HIV-1 resistance mutations are available from the country, with great variability in the results. One study conducted in southwestern Ethiopia indicates that SVS is achieved only in 66% of patients receiving cART [7], which falls well short of the UNAIDS target of virological control [8]. A recently published systematic review described a cART failure rate of 16% in Ethiopia, even though specific contributing factors were not well addressed [9]. In a similar multisite analysis from SSA it was demonstrated that 12% of patients receiving second-line cART faced VF [10]. As HIVDR testing is not routinely performed prior to switching from failing cART regimens to second- or third-line treatment due to lack of resources, the frequency and distribution of specific resistance mutations are not well studied.

Overall, neither the prevalence of VF in patients receiving second-line cART in Ethiopia nor the frequency and distribution of underlying resistance mutations have been adequately studied, although this information is needed for guidance of treatment strategies. Therefore, this cross-sectional study was conducted to assess the prevalence and resistance profiles of HIVDR in patients with failure of second-line cART treated at two large HIV clinics with over 10 000 patients under care in Adama and Asella, two cities in central Ethiopia.

METHODS

Between April and May 2019, HIV-1-infected patients treated with second-line cART at one of the two HIV outpatient clinics in the Ethiopian cities Adama and Asella were screened for second-line treatment failure according to patient record, which was defined as HIV load > 1000 copies/mL (based on two consecutive measurements at 3-month intervals during clinical routine investigations) after initiation of second-line cART. The term

second-line cART is only used in intended switches of the to PI-containing regimens due to treatment failure of a first-line ART regimen. Patients meeting these inclusion criteria were contacted and asked to participate in this study. After informed consent was obtained from eligible patients, whole-blood samples (10 mL) were collected and plasma was separated. Data about age, sex, WHO stage of HIV disease, CD4 cell nadir, current and previous VL, time of first initiation of cART, first-line cART regimens and cART history, reason for switching to second-line cART, time of second-line cART initiation, type of second-line cART, and concomitant rifampicin-containing tuberculosis treatment were taken from the patient record.

Frozen plasma samples were transported to the Institute of Virology in Cologne, Germany, for resistance testing. The genotypic HIV-1 resistance testing was performed as described by Lübke et al. [11], the clinical outcome was predicted according to Sierra et al. [12] and interpretation using the web-based geno2pheno application, a genotypic interpretation system for identifying viral drug resistance using next-generation sequencing data, following the protocol described previously [13] and with HIV-data base described by Liu and Shafer 2006 [14]. All results from genotyping and resistance testing were transmitted to the treating physicians for guidance of third-line cART.

The statistical analysis was performed using IBM SPSS Statistics for Windows v.25.0 (IBM Corp., Armonk, NY, USA). Data were summarized using descriptive statistics, i.e. frequency (percentage), mean (standard deviation, SD) and median (interquartile range, IQR). Differences were considered statistically significant at $p < 0.05$.

Ethical considerations

Ethical approval was obtained from the institutional review board (IRB) of the College of Health Sciences of Arsi University (reference no. AU/HSC/120/27-28/11) and from the IRB of the University Hospital Düsseldorf, Heinrich Heine University (reference no. 2019–403-kFogU). All study-related procedures were performed after ethical approval and written informed consent was obtained from each participant.

RESULTS

At the time of data collection, from a total of 11 092 adult HIV-infected patients (3770 in Asella and 7322 in Adama), cART treatment was managed in the two participating HIV clinics. Of these, 60.0% were female and 6.5% of them had been receiving a second-line cART regimen for a period of > 6 months at the two clinics (Figure 1). According to documentation in the patient records, 6.2% (44/714) had a confirmed HIV plasma VL > 1000 copies/mL. Informed consent to participate was obtained from 37 of these patients and plasma samples were provided. The median (IQR) age was 42 (20–45) years and 62.2% were male. At first initiation of cART, 23 (62.2%) were WHO stage III. The mean CD4 count at cART initiation was 170.6 (range: 16–496) cells/μL. The median (IQR) total duration of cART was 119 (101–136) months and the median duration of treatment with a second-line regime was 54 (42–78) months. At the time of sampling, median HIV-1 VL was 30 220 (7963–82 598) copies/mL (see Table 1). All patients were infected with HIV-1 subtype C.

cART history of study participants

In this study the most common initial first-line cART regimens were stavudine (D4T)-3TC-NVP in 15 patients (40.5%) and TDF-3TC-EFV in eight (21.6%) patients. The most common first-line cART regimens prior to switching to the current second-line cART regimens were TDF-3TC-EFV, D4T-3TC-NVP and AZT-3TC-NVP in 11 (29.7%), seven (18.9%) and seven (18.9%) patients, respectively. (Table 2). The majority of patients (29/37, 78.4%) were switched to second-line cART due to virological or immunological failures. The remainder were switched for other reasons, such as drug toxicity and lack of availability of prior cART. The median duration of cART was almost 10 years, with a median of 4.5 years on a second-line regimen.

During sample collection, all regimens used as second-line in this cohort were PI-based. The most frequently used second-line cART regimens were TDF-3TC-ATV/r in 20/37 patients (54.1%) and AZT-3TC-ATV/r in 10/37 patients (27.0%) (Table 2). Almost a quarter of these patients were simultaneously treated for tuberculosis at the time of the failure of the second-line cART. During sampling, the mean absolute lymphocyte count and the mean (± SD) haemoglobin level were 1932 ± 633 cells/μL and 13.5 ± 2 g/dL, respectively.

Genotypic analysis of resistance-related mutations

Genotypic resistance analysis for HIVDR mutations was successful in 35/37 (94.6%) of the participants. Here, the M184V mutation (20/35; 57.1%) was the most prevalent

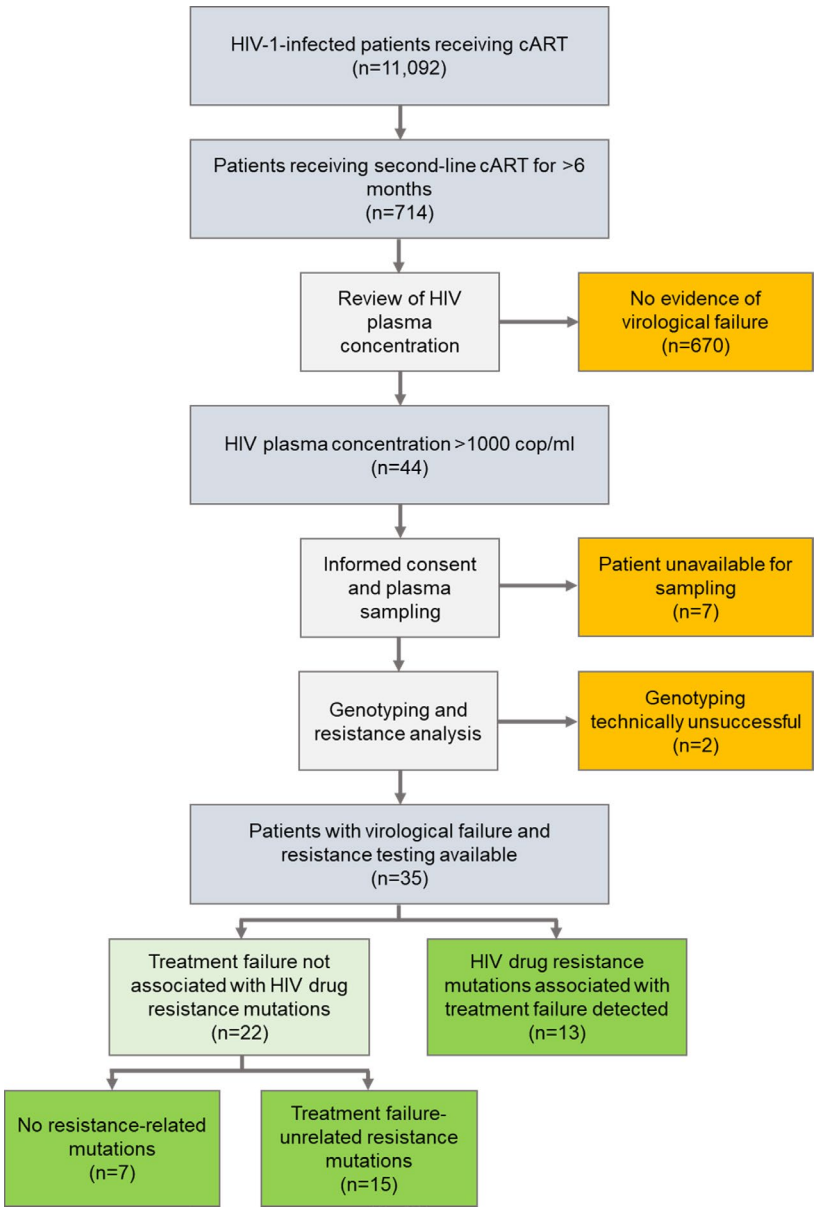

FIGURE 1 Study flow diagram

NRTI-related HIVDR mutation, followed by the thymidine analogue mutations T215Y/F/I (9/35; 25.7%), D67G/N (6/35; 17.1%), and K219Q/E (5/35; 14.3%). The mutation K65R, which can be selected by TDF, occurred in only 14.3% (5/35) of the genotyped HIV strains (Figure 2a). In total, 80% of ($n = 28$) of patients carried any NRTI resistance-associated mutation.

Regarding non-NRTI (NNRTI)-related resistance mutations, the Y188C was the predominantly detected mutation (9/35; 26.7%), followed by the G190A/E (8/35; 22.8%), the K103N, and the Y181C (6/35; 17.1% each) (Figure 2b). In total, 80% of ($n = 28$) of patients carried any NNRTI resistance-associated mutation.

The most common PI-related HIVDR mutations were the V82A/M and the M46I/L (9/35, 25.7% each), and a total of 40% ($n = 14$) carried any PI-associated mutation, as indicated in Figure 2c. Even though all participants had a VL >1000 copies/ml at least 6 months after initiation of PI-based second-line cART, in 60% ($n = 21$) no PI-associated drug resistance mutations were detected. The median duration of the second-line regimen based on ATV or LPV was significantly associated with the presence of PI resistance mutations (72 months with vs. 46 months without PI resistance mutations; $p = 0.005$).

No mutations related to high-level resistance (HLR) against integrase inhibitors (INIs) were detected in this cohort. Genotyping for HIVDR-associated mutations did not reveal any INI-related mutations in 91.7% of the samples (33/36). The only detected INI-accessory mutations were G163MRV and E157Q.

TABLE 1 Characteristics of study participants ($n = 37$)

General and demographic data	
Age (years) [median (IQR)]	42 (20–45)
Included at study site [*n* (%)]	
Asella	18 (48.6%)
Adama	19 (51.4%)
Female sex [*n* (%)]	14 (38%)
Duration of cART (months) [median (IQR)]	
First-line cART	61 (33–81)
Second-line cART	54 (42–78)
Overall	119 (101–136)
Receiving tuberculosis treatment at the time of data acquisition [*n* (%)]	9 (24.3%)
Initial WHO stage of HIV infection [*n* (%)]	
Stage IV	2 (5.4%)
Stage III	23 (62.2%)
Stage II	7 (18.9%)
Stage I	5 (13.5%)
Laboratory data summary	
Baseline CD4 count (cells/μL) [mean (range)]	170.6 (16–496)
CD4 count at start of second-line cART regime (cells/μL) [mean (range)]	200.7 (4–609)
HIV plasma viral load at initiation of second-line cART (copies/mL) [median (IQR)]	70 164 (11 724–120 346)
HIV plasma viral load at sample collection (copies/mL) [median (IQR)]	30 220 (7963–82 598)
Lymphocyte count at sample collection (cells/μL) [mean (SD)]	1932 (± 633)
Haemoglobin level at sample collection (g/dL) [mean (SD)]	13.5 (± 2)

Abbreviations: cART, combined antiretroviral therapy; IQR, interquartile range; WHO, World Health Organization.

Resistance interpretation according to geno2pheno

Overall, second-line treatment failure was associated and probably caused by an underlying HIVDR in 37.1% (13/35) of cases. In 42.9% of patients (15/35), mutations causing HIVDR were detected, but there was no association with the currently failing second-line cART. There was no evidence of reduced sensitivity to any antiretroviral drug in 20.0% (7/35) of the analysed samples. There was no significant difference in median plasma VL between 'patients with no evidence of reduced susceptibility' and 'patients with drug resistance mutations' (30 220 copies/mL vs. 30 835 copies/mL; $p = 0.937$).

In particular, in the NRTI group, HLR against FTC and 3TC [57.1% (20/35) each] were most common, followed by didanosine (DDI, 31.4% [11/35]) and ABC [28.6% (10/35)]. Intermediate- and low-level resistance were less common. Concerning NNRTIs, HLR against NVP (62.9%, $n = 22$), EFV (48.6%, $n = 17$) and rilpivirine (RPV, 28.6%, $n = 10$) but not against etravirine (ETR, 5.7%, $n = 2$) were common. Among the PI group, a drug class almost exclusively used for second-line treatment in Ethiopia, HLR was less common (LPV, 14.3%, $n = 5$) compared with NRTIs and NNRTIs. The highest rate of HLR was detected against ATV (28.6%, $n = 10$), followed by indinavir and nelfinavir (22.9%, $n = 8$). The presence of the I50L mutation in 11.4% ($n = 4$, Figure 2c), which can be considered the signature resistance mutation of ATV, explains that ATV has the highest percentage of resistance in the PI group. There were virtually no restrictions for the use of DRV, as it was the most susceptible among the PI class. All INIs tested showed susceptibility > 90% and detected resistance was rated as low or intermediate at most (see Table 3).

DISCUSSION

To our knowledge, this is the first study analysing HIVDR mutations in patients with second-line treatment failure in Ethiopia. We found an overall moderate frequency of treatment failure of about 5% in more than 700 patients on second-line cART treated at two large HIV clinics in central Ethiopia. We found HIVDR-associated mutations in 80% of patients with virological failure (Figure 2). This finding confirms the concerning results of a study

TABLE 2 Combined antiretroviral therapy (cART) history of study participants ($n = 37$)

Initial first-line cART regimens	
D4T−3TC-NVP	15 (40.5%)
TDF−3TC-EFV	8 (21.6%)
AZT−3TC-NVP	5 (13.5%)
D4T−3TC-EFV	4 (10.8%)
AZT−3TC-EFV	3 (8.1%)
TDF−3TC-NVP	1 (2.7%)
AZT−3TC-LPV/r	1 (2.7%)
First-line cART regimens before switch to second-line	
TDF−3TC-EFV	11 (29.7%)
D4T−3TC-NVP	7 (18.9%)
AZT−3TC-NVP	7 (18.9%)
AZT−3TC-EFV	5 (13.5%)
TDF−3TC-NVP	3 (8.1%)
Others[a]	4 (10.8%)
Second-line cART regimens at data collection	
TDF−3TC-ATV/r	20 (54.1%)
AZT−3TC-ATV/r	10 (27.0%)
ABC−3TC-ATV/r	4 (10.8%)
ABC−3TC-LPV/r	2 (5.4%)
AZT−3TC-LPV/r	1 (2.7%)

Abbreviations: 3TC, lamivudine; ABC, abacavir; ATV/r, atazanavir/ritonavir; AZT, zidovudine; D4T, stavudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir disoproxil fumarate.

[a]Others: ABC-3TC-EFV ($n = 1$); D4T-3TC-EFV ($n = 1$); AZT-3TC-LPV/r ($n = 1$) and ABC-3TC-NVP ($n = 1$).

conducted by Fox et al. [15] in South Africa, reporting the presence of HIVDR in 85% of patients with second-line cART failure.

According to the Ethiopian 'National HIV Prevention, Care and Treatment Guidelines' [5], monitoring of VL in patients receiving cART is recommended 6 and 12 months after initiation of cART and every 12 months afterwards. If VL is > 1000 copies/mL, enhanced adherence support and retesting after 3 months are advised. If the VL is again > 1000 copies/mL at retesting despite adherence support, the cART should be switched to second- or third-line regimens. In our cohort of patients with second-line cART failure, HIVDR-associated mutations are detected at a high rate, with many of those probably being acquired during previous first- or second-line cART regimens. Thus, close monitoring of treatment response by more frequent determination of VL for early detection of VF and testing for HIVDR in the event of VF in patients receiving second-line regimens for optimal choice of third-line cART seem to be beneficial to increase treatment safety and success rate, as recommended in different HIV treatment guidelines [16,17].

The need for HIVDR testing is also illustrated by the emergence of resistance mutations with longer cART durations. As shown in a Nigerian cohort, longer duration of therapy is associated with the occurrence of HIVDR and VF [18]. In our cohort, the median duration on second-line PI-based regimens was significantly associated with the occurrence of PI resistance. First-line regimens were administered for > 5 years on average. In the current era of recommended initiation of cART irrespective of the CD4 cell count, improving access to cART for HIV-infected patients in SSA and increasing duration of therapy, it is to be expected that resistance-associated mutations will significantly increase, especially in low and middle-income countries with limited options of therapy monitoring.

The high proportion of almost one-quarter of patients taking anti-tuberculous therapy among those with second-line treatment failure in our study was striking. It is known that anti-tuberculous therapy and the associated drug interactions may contribute to cART treatment failure [19], for example due to a reduction in the drug levels of LPV/r by simultaneous rifampicin treatment. Different reports from northern Ethiopia suggest that

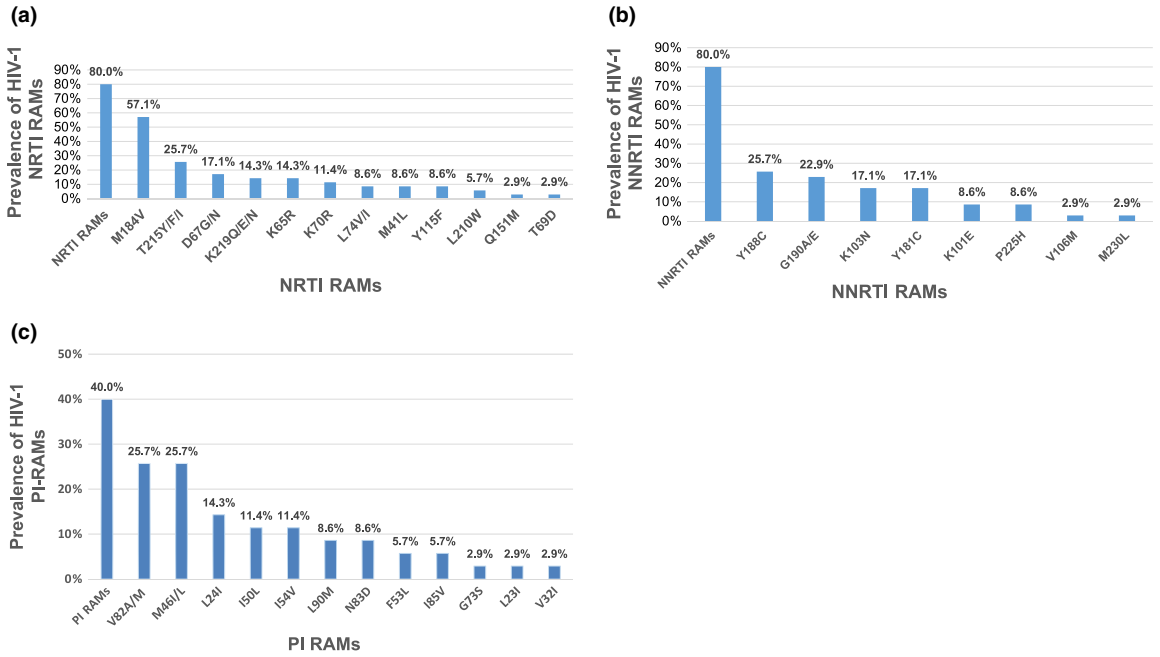

FIGURE 2 Indicates the prevalence of resistance mutations among patients with second-line treatment failure ($n = 35$). (a) Prevalence of different nucleoside reverse transcriptase inhibitor (NRTI)-related HIV-1 resistance mutations. (b) Prevalence of different non-NRTI-related HIV-1 resistance mutations. (c) Prevalence of different protease inhibitor (PI)-related HIV-1 resistance mutations. RAMs, resistance-associated mutations

the tuberculosis incidence and HIV treatment failure are linked [20,21]. According to these reports, co-medication with cART and occurrence of tuberculosis are independent predictors of immunological failure and a reason for regimen changes. Our data confirm that patients receiving tuberculosis treatment and cART at the same time require particularly close therapy monitoring.

With regard to the different resistance mutations, it was noticed that more than 50% of the study participants received TDF-3TC-ATV/r as second-line cART. Despite the high number of TDF-containing regimens, HLR against TDF was detected in only 17.1% of cases. The HLR rate against 3TC and FTC was considerably higher at 57.1% each. This finding is in line with a report by Mulu 2017 [22] from Ethiopia, which described a relatively low HLR rate against TDF but a higher HLR rate against 3TC [22]. The M184V was the most frequently detected resistance mutation in our study cohort. This observation is consistent with previous study results from Ethiopia and other countries in SSA [22–24]. However, in South Africa the K65R was the second most prevalent resistance mutation [23], whereas in our study it was the T215Y/F/I. This finding is explained by the much higher percentage of thymidine-analogue-containing regimens in our cohort and the fact that TDF has only been recently introduced to Ethiopia.

Regarding NNRTI resistance mutations, HLR was most prevalent against NVP and EFV. This finding is in line with two reports from South Africa by Etta et al. [25] and Steegen et al. [23]. It is noteworthy that HLR against RPV (28.6%) and ETR (5.7%), the latest-generation NNRTIs not available in this country, were also observed in our cohort, which could be explained by mutations conferring cross-resistance. Higher rates of new-generation NNRTI resistance have also been reported from South Africa, where high resistance rates of 33% against ETR and 42% against RPV were striking [25]. Nevertheless, according to our findings, ETR-based regimens seem to be a possible choice for third-line cART in the local setting, and similar to the South African context [26], INI-based cART regimens could be a better treatment option in Ethiopia.

The most frequently detected NNRTI-associated mutation in our cohort were the Y188C (25.7%) and the G190A/E (22.9%) (Figure 2b), both conferring NVP and EFV resistance. Of note, the Y188C is also leading to a reduced RPV sensitivity but selection pressure is most likely caused by NVP-based cART treatment. Compared with a South African cohort, the prevalence of Y188C was lower (12%), whereas the prevalence of G190A/E (24%) was about the same in our cohort [23]. In HIV-1 subtype B, the most frequent resistance mutation associated with NVP is Y181C, while in our study, Y188C was predominantly

TABLE 3 Phenotypic resistance pattern against antiretroviral drugs among the study participants ($n = 35$)

	Resistance level [*n* (%)]			
Antiretroviral drug	**Susceptible**	**Low**	**Intermediate**	**High**
Nucleoside reverse transcriptase inhibitors				
Abacavir (ABC)	14 (40.0%)	8 (22.9%)	3 (8.6%)	10 (28.6%)
Zidovudine (AZT)	21 (60.0%)	2 (5.7%)	7 (20.0%)	5 (14.3%)
Stavudine (D4T)	18 (51.4%)	0	10 (28.6%)	7 (20.0%)
Didanosine (DDI)	14 (40.0%)	8 (22.9%)	2 (5.7%)	11 (31.4%)
Emtricitabine (FTC)	14 (40.0%)	0	1 (2.9%)	20 (57.1%)
Lamivudine (3TC)	14 (40.0%)	0	1 (2.9%)	20 (57.1%)
Tenofovir (TDF)	23 (65.7%)	3 (8.6%)	3 (8.6%)	6 (17.1%)
Non-nucleoside reverse transcriptase inhibitors				
Efavirenz (EFV)	10 (28.6%)	2 (5.7%)	6 (17.1%)	17 (48.6%)
Etravirine (ETR)	15 (42.9%)	7 (20.0%)	11 (31.4%)	2 (5.7%)
Nevirapine (NVP)	10 (28.6%)	2 (5.7%)	1 (2.9%)	22 (62.9%)
Rilpivirine (RPV)	15 (42.9%)	5 (14.3%)	5 (14.3%)	10 (28.6%)
Protease inhibitors				
Atazanavir (ATV)	20 (57.1%)	5 (14.3%)	0	10 (28.6%)
Darunavir (DRV)	34 (97.1%)	1 (2.9%)	0	0
Fosamprenavir (FPV)	21 (60.0%)	5 (14.3%)	4 (11.4%)	5 (14.3%)
Indinavir (IDV)	21 (60.0%)	4 (11.4%)	2 (5.7%)	8 (22.9%)
Lopinavir (LPV)	23 (65.7%)	3 (8.6%)	4 (11.4%)	5 (14.3%)
Nelfinavir (NFV)	20 (57.1%)	3 (8.6%)	4 (11.4%)	8 (22.9%)
Saquinavir (SQV)	22 (62.9%)	5 (14.3%)	1 (2.9%)	7 (20.0%)
Tipranavir (TPV)	27 (77.1%)	3 (8.6%)	4 (11.4%)	1 (2.9%)
Integrase inhibitors				
Dolutegravir (DTG)	34 (97.1%)	1 (2.9%)	0	0
Elvitegravir (EVG)	32 (91.4%)	2 (5.7%)	1 (2.9%)	0
Raltegravir (RAL)	32 (91.4%)	2 (5.7%)	1 (2.9%)	0

detected in line with a report by Mulu et al. [22]. This finding was possibly related to the prevailing HIV-1 subtype C in our study population. By contrast, the most commonly detected NNRTI mutation in South Africa was the K103N/S (53%), which was less common in our study cohort (17.1%). The V106A/I/M was the second most prevalent NNRTI mutation in South Africa (40%) but the least commonly detected mutation in Ethiopia.

The cART regimens were changed due to side effects or availability issues at the treatment centre. The term second-line cART is only used in intended switches of the cART regimen due to treatment failure of the first-line cART regimen to PI-based regimen [5]. At 6 months after initiation of PI-based second-line treatment in our cohort of patients with VF, we found PI-associated mutations in about 40% of the investigated samples. The most common major HIV-1 mutations leading to PI resistance were the V82A/M and the M46I/L (Figure 2c). This finding is comparable to a previous investigation from South Africa [27]. However, in our cohort we detected a higher frequency of these mutations. In general, the frequency of PI resistance seems to be variable in comparably designed studies. In a report from Vietnam, 64% of the patients who developed VF while being treated with PI-based cART regimens had at least one major PI mutation [28], but in a report from Rwanda, major PI-associated mutations were detected in only 17% of the participants [29]. In our cohort, according to resistance testing, the majority of the participants could continue with PI-based cART with appropriate NRTI backbone, but resistance testing is useful before selection of PI-based third-line options.

In this study, we observed the I50L protease mutation, which confers clinically significant ATV-specific resistance, in 11.4% of patients with second-line treatment failure. Notably, this ATV signature mutation leads to hypersensitivity to many other PIs, including third-line options such as DRV [30,31].

As expected, there was little evidence of circulating low-level INI resistance in our cohort. Thus, the entire

class of compounds appears promising for second- and third-line cART regimens, but appropriate selection of a backbone must be ensured. Second-generation INIs such as dolutegravir or bictegravir should be preferred because of their higher genetic barrier to resistance.

The small sample and extraction of clinical data from patient records are limitations of this study. Patients who were receiving second-line cART without available VL test result were excluded from the study, and thus there might be selection bias concerning the prevalence of VF and HIVDR mutations. Also, therapeutic drug measurement was not performed in order to further investigate and validate the suspected drug interactions between tuberculosis therapy with rifampicin and cART, resulting in VF due to low drug levels. As only patients with the prevailing HIV-1 subtype C were included, the reported resistance patterns may not be generalizable to other settings in SSA with different predominant subtypes.

In conclusion, we detected a high prevalence of HIV-1 resistance mutations threatening the efficacy of standard first- and second-line cART components in our study population. The M184V, Y188C, M46I/L and V82A/M were the most frequently detected HIV-1 mutations, conveying resistance against NRTIs, NNRTIs and PIs, respectively. Our findings suggest that HIV-1 subtype C might have a preferred resistance pathway in regard to NVP, as indicated by the predominant selection of Y188C, in contrast to the Y181C in subtype B. The susceptibility against INIs was not impaired, making this drug class a valuable choice for second- as well as third-line therapy. Initiation of antituberculous therapy was associated with treatment failure and requires special attention in patients on second-line cART. Resistance testing is warranted before switching to second- or third-line cART and more therapeutic cART options, in particular INIs, should be made available.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTION

TBT and TF developed the study design, performed analysis and interpretation of the data, and helped to write the first draft of the manuscript. EK and EH carried out resistance testing and interpretation, and helped to write the first draft of the manuscript. AF, BEOJ, HMO and NL interpreted the data and helped to revise the manuscript. GJ, ZH and YE developed the study design, collected samples and helped to write the manuscript. TL, DH and RK critically revised the manuscript. All authors approved the manuscript for publication.

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