HIV-1 Subtype–Specific Distribution of Genotypic Drug Resistance Mutations Among Patients with Advanced HIV Disease Failing First-Line ART in Homa Bay, Kenya

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Article

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Abstract

HIV-1 genetic diversity may influence the selection of antiretroviral drug resistance mutations (DRMs); however, subtype-specific resistance patterns remain incompletely characterized in East Africa, where subtype A1 predominates. We examined the distribution of genotypic DRMs across HIV-1 subtypes among patients with advanced HIV disease failing NNRTI-based first-line therapy in western Kenya. In this cross-sectional molecular sub-analysis, 65 hospitalized individuals (≥15 years) with virological failure (HIV-1 RNA ≥1000 copies/mL) had a 1,084-bp pol gene fragment sequenced. Subtypes were assigned using REGA. DRMs were identified using the Stanford HIV Drug Resistance Database. Associations between subtype and specific mutations were evaluated using Fisher's exact test. Subtype A1 dominated (70.8%), followed by D (13.8%), recombinants (9.2%), and A2 (6.2%). We detected dual-class resistance in 73.8% of participants. The most frequent mutations were M184V (64.6%) and K103N/S (60.0%). Subtype A1 sequences were less frequently observed to have K101E/H (8.7%, p=0.011) and Y181C/I/V (17.4%, p=0.027) than non-A1 subtypes. No significant associations were observed between subtype and NRTI mutations. These findings suggest possible subtype-associated differences in NNRTI resistance patterns that warrant validation in larger studies, underscoring the importance of continued molecular surveillance to guide resistance monitoring and optimize long-term treatment strategies in the era of integrase inhibitor–based therapy.

Keywords: HIV-1 subtypes; Subtype-specific mutations; genotypic drug resistance; NNRTI resistance; K101E; Y181C; advanced HIV disease; first-line ART; molecular epidemiology; viral evolution

1. Introduction

HIV has persisted as a serious threat to public health with an estimated 40.8 million people living with the virus worldwide [1]. In Kenya, the epidemic remains substantial, with an estimated 1.3 million people living with HIV and approximately 22,000 deaths annually, with the highest burden concentrated in Western Kenya [2]. The use of antiretroviral therapy (ART) has greatly improved the quality and length of life of people living with HIV, while significantly reducing both HIV-related deaths and the risk of further transmission [3].

However, increased prevalence of drug resistance mutations (DRM) has been reported, attributed to the wide and long use of ART [3]. The emergence of antiretroviral drug resistance is a key impediment to achieving viral suppression. Late detection of virological failure and

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accumulation of drug-resistant mutations, especially in resource-limited settings like Kenya, worsens the situation [4].

High genetic variability of HIV affects the response to antiretroviral treatment and the emergence of drug resistance [5]. HIV-1 subtype distribution varies across regions and exhibits considerable sequence variability in structural and regulatory genes, which may influence viral fitness, transmission dynamics, and patterns of resistance emergence. Globally, subtype C is the most common (46·6%), followed by subtype B (12·1%), then subtype A (10·3%), and with lower frequencies for the other subtypes and Circulating Recombinant Forms (CRFs) [6]. Subtype A has been identified as the most common subtype in Kenya, with subtypes D, C, G, and CRFs being reported in low prevalence [7,8].

Variations in the rate of HIV-1 transmission, disease progression, treatment failure, accuracy of viral load measurements, and development of DRMs can be observed in different subtypes [9–11]. Though the current antiretroviral regimen has proven effective against all HIV-1group M subtypes, evidence of differences in the development of drug resistance mutations and polymorphisms across these subtypes is emerging across different parts of the globe.

In a study conducted in the United Kingdom, the K65R resistance mutation was found to be twice as likely in patients with subtype C compared with other subtypes [12]. Higher K65R and Q151M selection was reported in subtype C first-line failure [13]. Additionally, differences in the development of DRMs have been observed, particularly within the subclades of Subtype G in infants on first-line antiretroviral therapy (ART) in Nigeria [14]. Despite the successful nationwide transition to integrase strand transfer inhibitor (INSTI)-based regimens (Dolutegravir), characterizing the resistance landscape of the circulating subtypes reservoir is essential for monitoring transmitted drug resistance (TDR).

Whereas subtype–resistance associations have been well described for subtype B and C, data from subtype A1 predominant settings like western Kenya remain limited, particularly among ART-experienced individuals with advanced disease. Emerging evidence suggests that viral genetic background may influence the probability of selecting specific resistance mutations under drug pressure. A study conducted in sub-Saran Africa found mutations E138A, V106M, Y181C more common in subtype C than in subtype A and D, and Mutation K101E more common in subtype C and D than in subtype A [13].

Characterizing subtype-associated patterns of drug resistance mutations may enhance long-term resistance surveillance and improve understanding of resistance evolution. In the context of Kenya's transition to integrase inhibitor–based regimens, continued monitoring of NNRTI resistance remains important, as mutations affecting drugs such as etravirine can influence options for third-line therapy. While the high prevalence of virological failure among hospitalized individuals in Homa Bay County has been documented, the molecular drivers behind these resistance profiles, specifically the role of HIV-1 subtypes, require further investigation. We therefore set out to explore whether the distribution of individual resistance mutations differs by subtype among patients with virological failure in a high-burden Kenyan setting.

2. Material and Methods

2.1. Study Design, Site, and Population

This study was a molecular sub-analysis nested within a larger facility-based cross-sectional survey conducted at Homa Bay County Referral Hospital, western Kenya. The parent study assessed the prevalence of virological failure and antiretroviral drug resistance among hospitalized patients with advanced HIV disease (CD4+ T-cell count <350 cells/μL) [15].

The parent study enrolled 187 HIV-1–infected individuals aged ≥15 years who were hospitalized in the medical ward during the study period and had been receiving NNRTI-based first-line ART for at least six months prior to admission.

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Of the 187 participants, 69 had virological failure, defined as HIV-1 viral load ≥1000 copies/mL, and were eligible for genotypic drug resistance testing and HIV-1 subtype determination at the Kenya Medical Research Institute (KEMRI)/HIV laboratory in Kisumu. Four samples failed amplification, leaving 65 participants with successful sequencing results.

The present molecular sub-analysis examined the subtype-specific distribution of genotypic drug resistance mutations among these 65 participants with confirmed virological failure and available sequence data.

2.2. Laboratory and Analytical Procedures

2.2.1. Samples Collection

The sample collection methods for this cohort have been previously described. Briefly, whole blood was collected from each participant; 100 µL was used to prepare Dried Blood Spots (DBS) for genotyping, and the remainder was processed to obtain plasma for viral load (VL) testing. Demographic and treatment histories were obtained via standardized questionnaires during hospitalization.

2.2.2. HIV-1 Viral Load Quantification

HIV-1 Viral load RNA quantification was done at KEMRI HIV Research Laboratory by an automated real-time PCR, COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0, as per manufacturer's instructions and reagents (Roche Diagnostic System Branchburg, NL, USA). The detectable range of this real-time PCR is 20 copies/mL to10,000,000 copies/mL.

2.2.3. Genotyping

A WHO-validated in-house assay [16] was used to amplify a 1,084-bp fragment of the HIV-1 *pol* gene, encompassing protease (PR) codons 6–99 and reverse transcriptase (RT) codons 1–251. Total nucleic acid (TNA) was extracted from dried blood spots (DBS) using the NucliSENS® silica-based Boom method (bioMérieux, Inc., Durham, NC, USA) according to the manufacturer's instructions. Primary RT-PCR was performed using the SuperScript™ III One-Step RT-PCR System (Invitrogen, CA, USA) with outer primers PrtM-F1 and RT-R1. The 50 µL reaction involved RNA denaturation at 65 °C for 10 min, cDNA synthesis at 50 °C for 45 min, and initial denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, 50 °C for 20 s, and 72 °C for 2 min.

Nested PCR was performed using Taq DNA Polymerase (Applied Biosystems, CA, USA) and inner primers Prt-F2 and RT-R2. Thermocycling conditions consisted of an initial denaturation at 94 °C for 4 min, followed by 40 cycles of 94 °C for 15 s, 55 °C for 20 s, and 72 °C for 2 min. Amplified products were visualized via 1% agarose gel electrophoresis with SYBR™ Green staining.

Cycle sequencing was performed on purified nested PCR products using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with six overlapping sequencing primers: Prt-F2, A35V, AV36V, AV44, 90V1, and RT-R2. The sequencing profile included 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Final products were analyzed on an ABI 3130xl Genetic Analyzer (Applied Biosystems) following the manufacturer's protocol.

The raw sequence data were assembled using RECall v.3.1 software [17]. The quality of the sequences was assessed following WHO recommendations using RECall, the WHO HIVDR QC Tool, and Stanford University HIVdb tools.

2.3. Subtype and Mutation Determination

Subtypes were determined using the REGA v.3.0 HIV subtyping tool.

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Drug resistance-associated mutations and polymorphisms were identified using the Stanford University HIV drug resistance database (HIVdb v.9.5) and interpreted using the Stanford HIVdb algorithm and the IAS-USA drug resistance list [18].

2.4. Statistical Analysis

Descriptive statistics were used to summarize the baseline demographic and clinical characteristics among the participants.

The association between HIV-1 subtypes and major NRTI and NNRTI DRMs and polymorphisms obtained from the Stanford HIV database, was first analyzed using Fisher's exact test to compare the distribution of each mutation across all individual HIV-1 subtypes with categorical values converted to binary values. Due to the targeted nature of this molecular study and the frequency of specific mutational events, associations were primarily interpreted through exact p-values to ensure robustness.

For mutations showing significant distributional variance ($p < 0.05$), a targeted analysis was performed to calculate measures of association. For these specific mutations, subtypes were dichotomized into the predominant subtype (A1) and a combined comparison group (Non-A1 subtypes). Odds Ratios (OR) with 95% Confidence Intervals (CI) were calculated using the Risk Estimate function to quantify the likelihood of mutation selection relative to the viral genetic background.

A significance level of $p < 0.05$ was used for all interpretations. Given the mutation-level nature of these comparisons and the modest sample size, these analyses were interpreted cautiously. All data analyses were conducted using IBM SPSS Statistics (Version 25)

Results

A total of 187 patients were included in the primary study, out of which 69 had viral load >1,000 copies/mL and were eligible for drug resistance testing. Four samples failed amplification, and thus 65 sequences were successfully generated for analysis. The demographic and clinical characteristics of the sequenced cohort are summarized in Table 1.

Table 1. Descriptive characteristics of the study participants.

Characteristic	**N= 65**
Females, n (%)	31[47.7)
Age, years, median [IQR]	33 [28-39]
Current first line ART,	n (%)
TDF/3TC/EFV	46 (70.8)
AZT/3TC/NVP	9 (13.8)
TDF/3TC/NVP	2 (3.1)
ABC/3TC/EFV	1 (1.5)
AZT/3TC/EFV	6 (9.2)
TDF/3TC/DTG	1 (1.5)
Subtypes, n (%)	
A1	46 (70.8)
D	9 (13.8)
A2	4 (6.2)
Recombinants	
A1D	5 (7.7)
CD	1 (1.5)

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1.1. Subtype Characterization

Subtype determination using the REGA subtyping tool identified subtype A1 as the most prevalent, detected in 46 patients (70.8%), with lower prevalence rates for subtype D (n=9, 13.8%), recombinants (n=6, 9.2%), and A2 (n=4, 6.2%) (Table 1).

1.2. Distribution of Drug Resistance Mutations

Dual-class resistance to both NRTIs and NNRTIs was observed in 48 participants (73.8%). The most frequent NRTI resistance mutation was M184V, detected in 42 participants (64.6%). Other NRTI mutations included K65R and thymidine analogue mutations (TAMs).

No statistically significant associations were observed between NRTI mutations and HIV-1 subtype using Fisher's exact test (all $p > 0.05$) (Table 2).

Table 2. Association between HIV-1 subtypes and NRTI mutations by Fisher's exact test.

Subtype	**A1 (N-46)**		**A2 (N-4)**		**Recombinants (N-6)**		**D(N-9)**	
	n (%)	**p-value**	**n (%)**	**p-value**	**n (%)**	**p-value**	**n (%)**	**p-value**
NRTI mutations								
E44A/D	6 (13.0)	1.000	1 (25.0)	0.417	0 (0.0)	0.594	1 (11.1)	1.000
K65R	11 (23.9)	1.000	1 (25.0)	1.000	2 (33.3)	0.615	1 (11.1)	0.672
70E/G/Q/T/R	11 (23.9)	0.547	2 (50.0)	0.278	2 (33.3)	0.648	2 (22.2)	1.000
L74I/V	9 (19.6)	1.000	1 (25.0)	1.000	1 (16.7)	1.000	2 (22.2)	1.000
M184V	29 (63.0)	0.780	3 (75.0)	1.000	4 (66.7)	1.000	6 (66.7)	1.000
TAM1	14 (30.4)	0.550	1 (25.0)	1.000	1 (16.7)	1.000	2 (22.2)	1.000
TAM2	12 (26.1)	1.000	1 (25.0)	1.000	2 (33.3)	0.648	2 (22.2)	1.000
V75M	4 (8.7)	1.000	0 (0.0)	1.000	0 (0.0)	1.000	1 (11.1)	0.538
Y115F	5 (10.9)	0.662	0 (0.0)	1.000	0 (0.0)	1.000	1 (11.1)	1.000
Any NRTI DRM	33 (71.7)	0.758	3 (75.0)	1.000	6 (100.0)	0.327	6 (66.7)	0.687

1.3. Subtype-Specific Mutation Associations

The most frequent NNRTI mutation overall was K103N/S, identified in 39 participants (60.0%). Differences in the distribution of specific NNRTI mutations were observed between subtype A1 and non-A1 subtypes. Subtype A1 sequences were observed less frequently to harbour K101E/H ($p = 0.011$) and Y181C/I/V ($p = 0.027$) compared with non-subtype A1 sequences (Table 3).

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Table 3. Association between HIV-1 subtypes and NNRTI mutations by Fisher's exact test.

Subtype	A1 (N-46)		A2 (N-4)		Recombinants (N-6)		D(N-9)	
	n (%)	p-value	n (%)	p-value	n (%)	p-value	n (%)	p-value
NNRTI mutations								
A98G	9 (19.6)	0.258	1 (25.0)	0.496	0 (0.0)	0.579	0 (0.0)	0.333
E138Q	3 (6.5)	0.347	0 (0.0)	1.000	2 (33.3)	0.091	1 (11.1)	1.000
G190A/S	14 (30.4)	0.256	2 (50.0)	0.610	2 (33.3)	1.000	5 (55.6)	0.260
H221Y	4 (8.7)	1.000	1 (25.0)	0.328	1 (16.7)	0.455	0 (0.0)	0.584
K101E/H	4 (8.7)	**0.011**	2 (50.0)	0.130	3 (50.0)	0.056	2 (22.9)	0.642
K103N/S	28 (60.9)	1.000	2 (50.0)	1.000	4 (66.7)	1.000	5 (55.6)	1.000
L100I	8 (17.4)	1.000	1 (25.0)	0.533	2 (33.3)	0.266	0 (0.0)	0.337
P225H	8 (17.4)	0.093	0 (0.0)	1.000	0 (0.0)	1.000	0 (0.0)	0.586
V108I	9 (19.6)	1.000	1 (25.0)	0.567	0 (0.0)	0.583	2 (22.9)	0.667
V179I/T	6 (13.0)	0.169	0 (0.0)	1.000	0 (0.0)	1.000	0 (0.0)	0.584
Y181C/I/V	8 (17.4)	**0.027**	3 (75.0)	0.052	3 (50.0)	0.179	3 (33.3)	0.687
Y188L	3 (6.5)	1.000	0 (0.0)	1.000	1 (16.)	0.328	0 (0.0)	1.000
Any NNRTI DRM	38 (82.6)	0.735	3 (75.0)	0.567	6 (100.0)	0.583	6 (66.7)	0.349

Analysis of accessory polymorphisms showed a variation in the distribution of V179D/I (p = 0.048). This polymorphism was detected in 17 of 46 (37.0%) subtype A1 sequences and was not observed in subtype D sequences. In the protease region, common polymorphisms including K20R and L10I/V were identified; however, no significant subtype-specific associations were observed (Table 4).

Table 4. Association between HIV-1 subtypes and Polymorphisms by Fisher's exact test.

Subtype	A1 (N-46)		A2 (N-4)		Recombinants (N-6)		D(N-9)	
	n (%)	p-value	n (%)	p-value	n (%)	p-value	n (%)	p-value
Polymorphisms								
K20R	16 (34.8)	1.000	3 (75.0)	0.109	2 (33.3)	1.000	1 (11.1)	0.152
L10I/V	16 (34.8)	0.148	0 (0.0)	0.313	1 (16.7)	0.662	2 (22.2)	1.000
K101A	4 (8.7)	1.000	0 (0.0)	1.000	0 (0.0)	1.000	1 (11.1)	0.538
S68G	36 (78.3)	0.484	0 (0.0)	1.000	1 (16.7)	1.000	1 (11.1)	1.000
T69I/N	5 (10.9)	1.000	0 (0.0)	1.000	1 (16.7)	1.000	1 (11.1)	1.000
V118/I	2 (4.3)	0.574	0 (0.0)	1.000	1 (16.7)	0.328	1 (11.1)	0.458
V179D/I	17 (37.0)	0.140	2 (50.0)	0.581	1 (16.7)	0.657	0 (0.0)	**0.048**
V90I	5 (10.9)	1.000	0 (0.0)	1.000	0 (0.0)	1.000	2 (22.2)	0.247
Dual Class DRM	33 (71.7)	0.758	3 (75.0)	1.000	6 (100.0)	0.327	6 (66.7)	0.687

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Any major DRM	39 (84.8)	0.718	3 (75.0)	0.533	6 (100.0)	0.579	6 (66.7)	0.171

Analysis of the dichotomized groups revealed that subtype A1 demonstrated lower odds of harbouring the K101E/H mutation (OR = 0.163; 95% CI: 0.041–0.653; p = 0.011) and the Y181C/I/V mutation (OR = 0.234; 95% CI: 0.072–0.761; p = 0.027) compared to non-A1 subtypes (Table 5).

Table 5. Subtype-specific Distribution and Odds Ratios of Significant NNRTI Resistance Mutations among A1 and Non-A1.

Subtype	**A1 (N-46), Non-A1 (N-19)**			
	n (%)	**p-value**	**OR**	**95% CI**
K101E/H	4 (8.7)	**0.011**	0.163	0.041-0.653
Y181C/I/V	8 (17.4)	**0.027**	0.234	0.072-0.761
V179D/I	17 (37.0)	**0.140**	0.320	0.081-1.260

Discussion

In this molecular study of patients with virological failure in Homa Bay, we observed differences in the distribution of specific NNRTI resistance mutations across HIV-1 subtypes. While NNRTI resistance was high across all subtypes, and subtype A1 harboured the majority of NNRTI resistance due to its high local prevalence, this subtype demonstrated significantly lower frequencies of K101E/H and Y181C/I/V mutations. These associations persisted when effect sizes were quantified using odds ratios. This pattern may reflect differences in mutational probabilities across viral genetic backgrounds rather than differences in overall resistance burden. No significant associations were observed between subtype and NRTI resistance mutations. We also identified a borderline significant subtype-associated distribution of the accessory polymorphism V179D/I, which was majorly present in subtype A1 but absent in subtype D sequences. These findings are consistent with other studies, which suggest that HIV-1 genetic background may influence the selection and accumulation of resistance mutations [13,19–21]. In sub-Saharan Africa, K101E and Y181C have been reported to occur more frequently in subtypes C and D than in A1 [13]. Subtype-specific selection patterns have also been described globally, including a higher likelihood of K65R in subtype C [12], and associations between CRF02_AG and NNRTI mutations V90I, A98G, and V106I [22]. Although [6] did not observe NNRTI–subtype associations in Nigeria, they reported subtype-specific differences in NRTI mutations, suggesting that subtype–resistance relationships may vary depending on treatment exposure and analytical approach.

Unlike our findings, a study conducted in Kenya, focusing on individuals initiating therapy, did not find a significant association between HIV-1 subtypes and resistance mutations [23]. Differences in treatment exposure may partly explain this contrast, as our cohort consisted exclusively of individuals with virological failure after sustained NNRTI-based therapy. Similarly, a study conducted in Uganda comparing resistance profiles between subtype A and D in individuals on Tenofovir-based and Zidovudine-based regimens did not find significant subtype-level differences in overall resistance profiles [24]. Notably, our analysis evaluated associations at the level of individual mutations rather than aggregated resistance patterns, which may allow detection of subtype-specific differences that are not apparent when resistance is analysed as a composite outcome.

The NNRTI mutations identified in this study have well-characterized phenotypic consequences that continue to drive virological failure in contemporary cohorts. K101E remains a critical non-polymorphic mutation selected by first- and second-generation NNRTIs; it reduces susceptibility to Nevirapine (NVP) by 3- to 10-fold and to Efavirenz (EFV) by approximately 2-fold [25]. Recent

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surveillance data confirms that K101E and K101H often co-occur with other mutations to significantly compromise the efficacy of NNRTI-based regimens [26].

Similarly, Y181C remains one of the most clinically significant mutations, particularly in the context of the Kenyan epidemic. It confers high-level resistance (>50-fold) to NVP and approximately 2-fold reduced susceptibility to EFV [3,25]. Crucially, recent evidence emphasizes that Y181C/I/V variants selected during first-line failure significantly reduce the durability of second-generation NNRTIs like Etravirine (ETR) and Rilpivirine (RPV), with Y181C identified as a major contributor to ETR resistance in recent weighted genotypic scores [25,27]

The lower frequency of Y181C/I/V in subtype A1 is particularly noteworthy, as this substitution confers significant cross-resistance to second-generation NNRTIs like Etravirine, which is currently utilized in Kenya's third-line salvage regimens [28]. EFV is still recommended in Kenya as an alternative first-line drug for those who cannot tolerate Dolutegravir (DTG), the anchor drug of the current preferred first-line regimen [28]. NVP is also an alternative first-line drug for infants [28]. The lower frequency of these mutations in subtype A1 within this cohort suggests potential differences in mutational pathways across subtypes under NNRTI pressure, although larger studies are required to determine the reproducibility and clinical significance of this observation.

Consistent with previous studies from Kenya [8,29–31], subtype A1 remained predominant in this cohort, followed by subtype D and recombinant forms. This is an indicator that these subtypes have persisted as the subtypes being spread in Kenya. Contrary to our findings, subtypes C and G have been observed in Kenya before [30,32].

The persistent presence of recombinant A1D is indicative of the continued recombination between the dominant subtypes in the locality. Recombinant forms have been increasingly reported in regions with established subtype diversity [7,33]. However, because sequencing was limited to the partial pol region, additional recombination outside this fragment may have been missed, as studies using near-full-length genome sequencing have demonstrated greater recombinant complexity [34,35]. There is a need to fully define the main reason driving the epidemiology of recombinants, especially in high HIV burdened areas like Homabay, as this could guide policies to curb the increase in recombinants that poses a challenge in the management of HIV-1.

This study was not without limitations. First, the sample size was relatively small, and findings must be interpreted cautiously. However, the cohort is representative of the high-risk, hospitalized population in Homa Bay experiencing virological failure, providing critical insights into the subtype-specific resistance landscape of a high-burden region. Secondly, phenotypic resistance testing was not performed; therefore, the functional impact of the observed mutations on drug susceptibility would not be directly quantified. Third, subtype assignment was based on partial pol sequencing, which may underestimate recombinant complexity. Finally, this analysis was conducted among individuals with advanced disease and virologic failure and may not be generalizable to treatment-naïve populations.

In conclusion, our analysis suggests possible differences in the frequency of selected NNRTI resistance mutations across HIV-1 subtypes in this high-burden setting, with K101 and Y181 mutations occurring at a lower frequency in subtype A1. While Kenya has successfully transitioned to DTG-based regimens, these findings remain critical for long-term surveillance and for defining the durability of alternative and third-line ART options. These results contribute to the growing body of evidence examining potential subtype-associated resistance patterns under drug pressure. Given the exploratory nature of this study, these findings require confirmation in larger, longitudinal cohorts.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Médecins Sans Frontières (MSF) Ethics Review Board (Protocol ID: 1743, approved 10 Sep 2017) and the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (Protocol NON-KEMRI 586, approved 09 Oct 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. In the case of participants under the age of 18, informed consent was obtained from a parent or legal guardian.

Data Availability Statement: The HIV-1 pol gene sequences generated and analysed during the current study have been deposited in the GenBank database under accession numbers PZ148509–PZ148573. Other study data are available from the corresponding authors upon reasonable request and with the permission of the Kenya Medical Research Institute (KEMRI) and Médecins Sans Frontières (MSF).

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Abbreviations

The following abbreviations are used in this manuscript:

3TC	Lamivudine
ABC	Abacavir
APC	Article Processing Charge
ART	Antiretroviral Therapy
AZT	Zidovudine
DRM	Drug Resistance Mutation
EFV	Efavirenz
HIV-1	Human Immunodeficiency Virus Type 1
KEMRI	Kenya Medical Research Institute
MSF	Médecins Sans Frontières
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PLHIV	People Living with HIV
TDF	Tenofovir Disoproxil Fumarate
VF	Virological Failure

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VL Viral Load

Appendix A

Appendix A.1 Supplementary Table 1 : HIV-1 pol Primers for PCR Amplification and Cycle Sequencing

Primer Name	Use	Direction	Sequence (5′ → 3′)	HXB2 Position[1]
PrtM-F1	RT-PCR	Forward	CCT CAA ATC ACT CTT TGG CAR CG	2253–2275
RT-R1	RT-PCR	Reverse	ATC CCT GCA TAA ATC TGA CTT GC	3370–3348
Prt-F2[2]	Nested PCR / Seq	Forward	CTT TGG CAA CGA CCC CTY GTC WCA	2265–2288
RT-R2[2]	Nested PCR / Seq	Reverse	CTT CTG TAT GTC ATT GAC AGT CC	3326–3304
A35V	Sequencing	Forward	AGT CCT ATT GAR ACT GTR CCA G	2556–2577
AV36V	Sequencing	Forward	CAG TAC TGG ATG TGG GRG AYG	2869–2889
AV44	Sequencing	Reverse	TTT YTC TTC TGT CAA TGG CCA	2639–2619
90V1	Sequencing	Reverse	TAC TAG GTA TGG TAA ATG CAG T	2952–2931

Notes: [1] Nucleotide positions are based on the HIV-1 HXB2 reference strain (Accession: K03455). [2] Primers Prt-F2 and RT-R2 served as both inner primers for the nested PCR and as sequencing primers.

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