IOSR Journal Of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 20, Issue 5 Ser. 1 (Sept. – Oct. 2025), PP 09-16
www.Iosrjournals.Org

Molecular Characterization Of Mutation Patterns In HIV-1 Among Patients In The Kenyan Coast

Don Kennedy Omutiti[1], Stephen Ger Nyanjom[1], Humphrey Kimani Wanjiku[2], Alex Kattam Maiyo[2], Matilu Mwau[2], Eddy Okoth Odari[1]
(Jomo Kenyatta University Of Agriculture & Technology, P.O. Box 62000 Nairobi, 00200, Kenya)
(Kenya Medical Research Institute, P.O. Box 50400 Nairobi, 00200, Kenya)

Abstract:
Background*: Drug resistance in HIV-1 is caused by changes (mutations) in the virus's genetic structure. Mutations are very common in HIV since it replicates at an extremely rapid rate and does not contain the proteins needed to correct the mistakes made during this process. This study aimed to determine the molecular characteristics and mutation patterns of HIV-1 subtypes circulating in patients failing first-line antiretroviral therapy within selected facilities in Kilifi County, Kenya.*
Materials and Methods*: In this cross-sectional laboratory-based study, a total of 1393 participants were recruited all aged 18 years and above and on first line ART at the time of sample collection with no history of defaulting treatment. Plasma samples were used for HIV-1 viral load testing through the Abbott m2000sp and Abbott m2000rt platform and those with a viral load ≥1000 copies/mL (69 participants) were subjected to HIV-1 genotyping or sequencing analysis through the 3730XL DNA Analyzer. Determination of drug resistance was done through the Stanford University HIV Database. Summaries on socio-demographics were presented using descriptive statistics. Fisher-Freeman-Halton Exact Test was used to determine the association between the HIV-1 subtypes and variables gender, age, CD4 count, WHO staging, levels of drug resistance, regimen combinations, and relevant mutations (P< 0.05 was considered significant).*
Results*: The gender demographics involved 982 females (70.5%) and 411 males (29.5%). The average age in the sampled population was 46.16 years with majority of the participants in the age range between 41 and 50 years. A total of 68 samples with viral loads ≥1000 copies/mL were sequenced. Two main subtypes were identified in this study namely HIV-1 clade A (sixty-seven participants) and HIV-1 clade C (one participant). For the high level drug resistance (NRTI), notable resistance patterns were observed in clade A highlighting either resistance in single regimen or multiple regimens such as Abacavir, Tenofovir, and other NRTI backbones. For the low level resistance (NNRTI) category, the p-value of 0.015 indicated a statistical significance of the mutation patterns versus the HIV-1 subtypes (A and C). Low level mutations can accumulate whenever there are treatment switch delays as a result of gaps in viral load monitoring.*
Conclusion: *There is the need to incorporate routine drug resistance monitoring particularly when it comes to making regimen switch coupled with an efficient viral load monitoring system. Accessibility to drug resistance testing is crucial in early detection of treatment failure. There is also the need to avoid delayed switching of regimens which might contribute to early mortality.*
Key Word: *Drug resistance; Mutations; Subtypes.Genotyping*

Date of Submission: 27-08-2025 Date of Acceptance: 07-09-2025

I. Introduction

Human Immunodeficiency Virus (HIV) typically attacks the CD4 cells (T cells) which play a critical role in boosting the body's natural immune system providing protection against infections. A declined immune status is likely to result in the viral progression towards Acquired Immunodeficiency Syndrome (AIDS) [1,2]. HIV drug resistance refers to the ability of the virus to replicate in the presence of antiretroviral therapy as a result of mutations in the viral genetic structure caused by the rapid replication rate and lack of crucial proteins needed to make an independent replication cycle [3]. Combination antiretroviral therapy for HIV infection has saved millions of lives since it was introduced. As coverage of antiretroviral therapy continues to grow, some degree of emergence and transmission of HIV drug resistance is inevitable. Significant population-level HIV drug resistance could potentially restrict future therapeutic options and increase treatment costs by requiring new and more expensive antiretroviral regimens. However, as the experiences of many countries demonstrate, HIV drug resistance can be monitored, and steps can be taken to minimize its emergence. Some degree of HIV drug resistance is anticipated to occur among people receiving treatment even when appropriate regimens are

provided, and optimal adherence is achieved [4,5]. Understanding the emergence and transmission of population-level HIV drug resistance and the interaction between its various determinants require routine surveillance, monitoring and evaluation, and operational research. Numerous studies in Kenya have documented the circulating HIV-1 subtypes and the corresponding mutation patterns [6–10]. The current study expounds on the existing knowledge by evaluating the mutation patterns and subtype analysis of patients failing first line ART regimen in selected facilities in Kilifi County, Kenya.

II. Material And Methods

This cross-sectional laboratory-based study was carried out on patients within Kilifi County spread across three facilities namely Kilifi County Referral Hospital, Ngerenya Dispensary, and Takaungu Dispensary from July 2019 to February 2024. A total of 1393 adult subjects (982 females & 411 males) aged ≥ 18 were recruited through consent in this study.

Study Design: Cross-sectional laboratory-based study

Study Location: This was a hospital-based study carried out Comprehensive Care Clinics of Kilifi County Referral Hospital, Ngerenya Dispensary, and Takaungu Dispensary, Kilifi County, Kenya.

Study Duration: July 2019 to February 2024.

Sample size: 1393 patients.

Sample size calculation: The Cochran's Sample Size Formula was used to estimate sample size using a prevalence of 10.6% (referenced from a previous study on HIV transmitted drug resistance in Mombasa, Kenya), 95% confidence interval and final adjusted sample size ($N_{adjusted}$) to ensure enough participants are captured in the study**.** The sample size obtained for this study was 1393 patients.

Subjects & selection method: The study population was drawn from patients visiting the comprehensive care clinics in the three facilities Kilifi County Referral Hospital, Ngerenya Dispensary, and Takaungu Dispensary, Kilifi County, Kenya. Cluster random sampling was used with each selected facility considered as a cluster and patients were randomly selected within the facilities based on the inclusion criteria. Kilifi County Referral Hospital being the referral hospital accounted for a significant proportion (96.6%) of the recruited study participants, Ngerenya Dispensary (0.9%), and Takaungu Dispensary (2.4%).

Inclusion criteria:
1. Either sex
2. Aged ≥ 18 years,
3. Patients on first line ART regimens

Exclusion criteria:
1. Patients on second or third line ART regimes
2. Patients with a recorded history of ART defaulting
3. Patients with a previous history of ART exposure prior to diagnosis with HIV (pre-exposure prophylaxis or post-exposure prophylaxis)

Procedure methodology

After screening and acquisition of written informed consent from patients, retrospective data was collected including socio-demographic characteristics such as age, gender, religion, occupation status, marital status, current ART regimen, previous ART regimen, total months on ART, WHO staging and CD4 count on ART enrolment, average BMI, previous viral load (if available), and current viral load.

HIV-1 viral RNA Quantitation: The samples were stored at −80 °C prior to thawing at room temperature before they are processed. The extractions were processed as per the manufacturer's instruction indicated in the Abbott sample preparation system (*m*2000sp) and *m*2000rt (real-time PCR) protocols with reportable range of 40 to 10,000,000 copies/ml for plasma.

HIV-1 Viral RNA Extraction: For the samples with viral load 1000 copies/mL and above, viral RNA extraction was initiated through the use of QIAamp Viral RNA Mini Kit following the manufacturer's instructions.

RNA Denaturation and Primer Annealing: The SuperScript™ III One-Step RT-PCR with Platinum™ Taq Kit was used in this step (Invitrogen, Carlsbad, CA, USA). The first step commenced with the RNA denaturation using the Veriti™ Thermal Cycler (Applied Biosystems) whereby the RNA mix was heated at 65°C for 10 minutes. This was followed by immediate cooling on an ice set for a duration of 1 minute which aimed to eliminate any new formations of RNA secondary structures. Lastly, the RT-PCR Master Mix, enzymes, and buffer were added before proceeding to reverse transcription.

Reverse Transcription (cDNA Synthesis): This process involved conversion of the HIV-1 RNA to complementary DNA (cDNA) using the Veriti™ Thermal Cycler (Applied Biosystems) with the steps involving Reverse Transcription (50°C for 45 minutes- 1 cycle), enzyme inactivation (94°C for 2 minutes-1 cycle), denaturation (94°C for 15 seconds-40 cycles), annealing (50°C for 20 seconds-40 cycles), extension (72°C for 2 minutes-40 cycles), final extension (72°C for 10 minutes-1 cycle) and hold (4°C for 18 hours' maximum).

Nested PCR: AmpliTaq Gold™ LD DNA Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the nested PCR master mix were used with the preparations involving nested PCR master mix (47.5 µL) and the AmpliTaq Gold™ LD DNA Polymerase (0.5 µL) with the final total volume of 48 µL for a single reaction.

ExoSAP-IT™ PCR Product Cleanup: Following completion of the nested PCR, the products were cleaned. Briefly, the new plate holding the nested PCR products was placed on ice together with the ExoSAP-IT™ PCR product cleanup reagent. 4µL of ExoSAP-IT™ PCR product cleanup reagent was added to the nested PCR products well (each containing 10µL). Steps in PCR Product Cleanup included digest (37°C for 15 minutes), heat deactivation (80°C for 15 minutes) and hold (4°C).

Gel Electrophoresis: The nested PCR products were verified using gel electrophoresis with the running conditions were set at 100V for 45 minutes.

Cycle Sequencing Reactions: Six sequencing mixes were provided with the HIV 1 Genotyping Kit (F1, F2, F3, R1, R2, R3) which includes three forward primers and three reverse primers. They were vortexed briefly and centrifuged for 2-3 seconds to ensure all contents were fixated at the bottom. The pGEM sequencing control on ice was left to thaw on ice, vortexed briefly, centrifuged for 2−3 seconds to collect contents at the bottom. 18µL of each of the six sequencing mixes were added to the appropriate wells of a chilled 96 well plate followed by the addition of 2µL of purified nested PCR products. One well was used to hold 20µL of pGEM Sequencing Control. Steps in cycle sequencing involved denaturation (96°C for 10 seconds-25 cycles), annealing (50°C for 5 seconds-25 cycles), extension (60°C for 4 minutes-25 cycles), and hold (4°C- Maximum of 18 hours).

Purification of Sequencing Reactions: This process involved using the BigDye XTerminator™ Purification Kit. The X-Terminator solution was removed from 4°C storage and allowed to thaw at room temperature. It was then vortexed for ten seconds and thereafter mixed with SAM™ solution. The SAM™/BigDye X-Terminator™ bead working solution was prepared as follows: SAM™ solution (90µL), X-Terminator™ Solution (20µL) with the total volume being 110µL.

Loading onto the 3730XL genetic analyzer (Applied Biosystems). The protocol involved selection of the 50 cm capillary length, the number of capillaries, and the POP 7™ polymer type. The instrument protocol was HIV_DR_OPT_2 while the analysis protocol was HIVDR_Analysis.

Statistical analysis

MS Excel 2016 was used collect all data captured in the study including quantitative and categorical variables. The data generated was initially subjected to exploratory data analysis on the MS Excel platform before exportation to IBM SPSS Statistics version 30 (SPSS Inc., Chicago, IL) and R version 4.4.2 (GNU Project/GNU General Public License) for further analyses. This included frequencies and percentages. Chi-Square Test of Independence was used to determine the association of significance between categorical variables and those that failed to meet the frequency of five (violating chi-square rule) were subjected to Fisher's Exact test to evaluate the significance of the p-values. The level $P < 0.05$ was considered as the cutoff value or significance. Tables and figures were also in visualization of the data.

III. Result

A total of 1393 patients were recruited for the study and blood samples collected successfully for viral load testing. Of these, 982 (70.5%) were female while 411 (29.5%) were male. The average age in the sampled population was 46.16 years with majority of the participants in the age range between 41 and 50 years totaling to 528 (37.9%). Extreme age groups of below 20 and above 81 had the least representation of between 21 (1.5%) and 1(0.1%) respectively. In the marital categorization, 706 (50.7%) identified as being in a monogamous marriage, 132 (9.5%) in a polygamous marriage, 230 (16.5%) divorced, 191 (13.7%) identified as being widowed, 123 (8.8%) identifying as single, and 11(8%) being undefined. The occupational category was classified into three, employed, self-employed, and unemployed. Majority of the sampled population 1361 (97.7%) reported as being self-employed, followed by the employed at 14 (1.0%) and lastly, the unemployed 18 (1.3%).

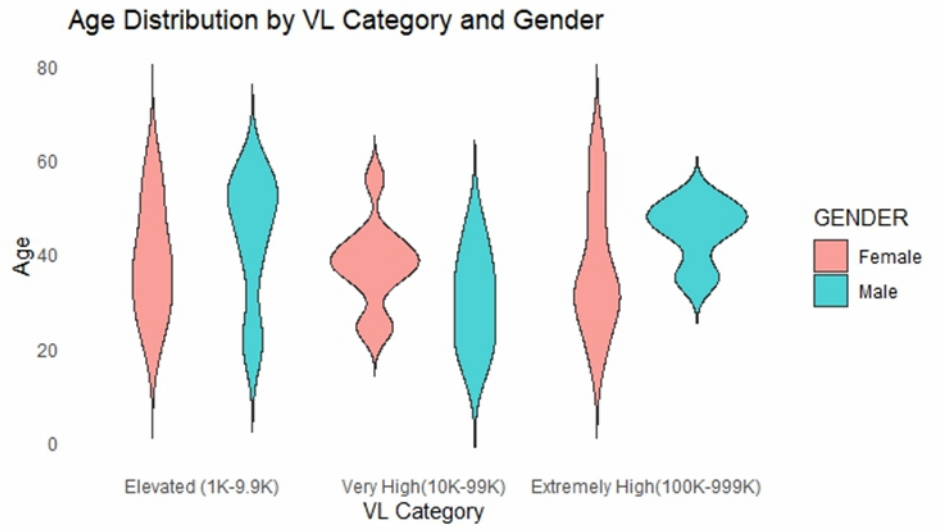
The WHO staging pre-ART included 462 (33.2%) individuals were categorized under stage one, 543 (39.0%) were enrolled in stage two, stage three had a total of 350 (25.1%), and stage four had the least number at 38 (2.7%).

The CD4 count pre-ART was captured in this study. Patients with CD4 greater than 500cells/µL accounted for 5.9% (82 patients) of the sampled population. Those with less than 200 cells/µL accounted for 42.6% (593 patients). Those with CD4 count of 200-349cells/µL accounted for 27.2% (378 patients) and lastly, 350-499 cells/µLl incorporated 130 patients (9.3%).

All the participants in the study were on first line regimens. The highest proportion of participants sampled 859 (61.7%) were on TDF/3TC/DTG with the least proportion being on ABC/3TC/NVP combination 1 (0.1%). The second highest majority were on TDF/3TC/EFV 478 (34.3%). Other categories included ABC/3TC/DTG 8 (0.6%), ABC/3TC/EFV 2 (0.1%), AZT/3TC/NVP 33 (2.4%), TDF/3TC/NVP 5 (0.4%), and AZT/3TC/EFV 4 (0.3%).

For the viral load outcomes, 1267 patients (91%) had an undetectable viral load while those with low risk low level viremia (21-199 cp/mL) were 29 patients (2.1%). High risk low level viremia (200-999 cp/mL) accounted for 28 patients (2.0%). Those with a viral load outcome ≥1000 copies/mL totaled to 69 patients (5.0%). Out of the 69 patients (≥1000 copies/mL), 68 were successfully sequenced for HIV drug resistance (HIVDR). Fig. 1 shows the viral load distribution across three categories for both genders.

Fig. 1: Violin Plot Showing Viral Load (≥1000cp/mL) Distribution by Age and Gender



Two main subtypes were identified in this study namely HIV-1 clade A and HIV-1 clade C accounting for 67 and 1 patients respectively. For HIV-1 clade A, 18(1.29%) were male while female accounted for 49(3.52%) while clade C had only one female (0.07%). In the age categorization, 31-40 years accounted for the highest proportion of patients with HIV-1 clade A 20(1.43%) while 61-70 accounting for 2(0.14%) with clade C patient falling in the category 31-40 years (0.07%). For the CD4 count, the highest proportion of patients fell within <200 cells/µL for HIV-1 clade A (39 patients,2.80%) and HIV-1 clade C had 1(0.07%). For WHO clinical staging, stage two accounted for the highest proportion of clients 29(2.08%) for clade A while stages three 1(0.07%) accounted for the highest proportion in clade C. There was no statistical significance between the HIV-1 clades (A&C) and the variables gender, age, CD4 count, and WHO clinical staging (Table no. 1).

Table no. 1: Correlation between HIV-1 Subtypes and Gender Identity, Age, CD4 Count, and WHO Clinical Staging

Variable	Clade A n (%)	Clade C n (%)	P value
Gender Identity			
Male	18(1.29)	0	1.000
Female	49(3.52)	1(0.07)	
Age (Years)			
<20	4(0.29)	0	1.000
21 – 30	13(0.93)	0	
31 – 40	20(1.43)	1(0.07)	
41 – 50	17(1.22)	0	
51 – 60	11(0.79)	0	
61 – 70	2(0.14)	0	
CD4 Count (cells/μL)			
>500	4(0.29)	0	1.000
350 – 499	7(0.50)	0	
200 – 349	17(1.22)	0	
<200	39(2.80)	1(0.07)	
WHO Clinical Staging			
Stage 1	20(1.43)	0	0.279
Stage 2	29(2.08)	0	
Stage 3	12(0.86)	1(0.07)	
Stage 4	6(0.43)	0	

The resistance patterns were classified into three categories namely high, intermediate, and low level resistance across the NRTI/NtRTI and NNRTI as depicted in Table no 2. For the high-level drug resistance patterns between HIV-1 Clade A and Clade C, the NRTI resistance shows varied resistance across Clade A (0.14–0.57%) while for Clade C, the focus was only on one sample (only 1 case of triple resistance). There was no statistical significance (p-value 0.397) particularly given the incomparable sample size between the two Clades. In the NNRTI resistance**,** Clade A exhibited minimal resistance (0.07–0.57% per combination), while Clade C had no incidence of resistance. The p-value (1.000) indicated no significant difference.

In the intermediate-level drug resistance patterns between HIV-1 Clade A and Clade C for NRTI, low resistance rates are observed in Clade A (0.07–1.44%) with notable regimens being Emtricitabine and Lamivudine 20 (1.44%) whereas Clade C has 1 (0.07%) for the same combination. The p-value (0.456) indicated no statistically significance. For the NNRTI resistance, Clade A resistance ranged within 0.07–0.79% with notable regimen being Etravirine 11 (0.79%). Clade C had resistance on Nevirapine and Rilpivirine 1 (0.07%). The p-value (0.103) suggested no significant difference.

For the low-level drug resistance between HIV-1 Clade A and Clade C, in the NRTI Resistance category, Clade A showed minimal resistance (0.07–0.29%). The p-value (1.000) suggested no significant difference. For NNRTI Resistance, Doravirine 23 (1.65%) showed high resistance levels in Clade A while for Clade C, the levels were minimal 1 (0.07%) cutting across Doravirine, Efavirenz, Etravirine. The p-value (0.015) suggested a statistically significant difference even though the overall resistance remains very low (Table no. 2).

Table no 2: Resistance Prevalence (High, Intermediate, & Low Level Resistance)

Variables	HIV-1 Clade A	HIV-1 Clade C	p-value
High Level Drug Resistance (NRTI/NtRTI)	**n(%)**	**n(%)**	
No Resistance	41 (2.9%)	0 (0%)	0.397
Abacavir	2 (0.14%)	0 (0%)	
Tenofovir	2 (0.14%)	0 (0%)	
Abacavir, Stavudine	4 (0.29%)	0 (0%)	
Stavudine,Tenofovir	3 (0.22%)	0 (0%)	
Abacavir, Tenofovir	2 (0.14%)	0 (0%)	
Abacavir, Stavudine, Tenofovir	8 (0.57%)	1 (0.07%)	
Abacavir, Emtricitabine, Lamivudine	5 (0.39%)	0 (0%)	
High Level Drug Resistance (NNRTI)			
No Resistance	44 (3.16%)	1 (0.07%)	1.000
Efavirenz	1 (0.07%)	0 (0%)	
Nevirapine	4 (0.29%)	0 (0%)	
Rilpivirine	1 (0.07%)	0 (0%)	
Efavirenz, Nevirapine	8 (0.57%)	0 (0%)	
Efavirenz, Nevirapine, Rilpivirine	8 (0.57%)	0 (0%)	

Doravirine, Efavirenz, Nevirapine, Rilpivirine	1 (0.07%)	0 (0%)	
Intermediate Level Drug Resistance (NRTI/NtRTI)			
No Resistance	37 (2.66%)	0 (0%)	0.456
Abacavir	1 (0.07%)	0 (0%)	
Lamivudine	1 (0.07%)	0 (0%)	
Tenofovir	4 (0.29%)	0 (0%)	
Emtricitabine	2 (0.14%)	0 (0%)	
Emtricitabine, Lamivudine	20 (1.44%)	1 (0.07%)	
Didanosine, Tenofovir	1 (0.07%)	0 (0%)	
Stavudine, Didanosine	1 (0.07%)	0 (0%)	
Intermediate Level Drug Resistance (NNRTI)			
No Resistance	50 (3.59%)	0 (0%)	0.103
Etravirine	11 (0.79%)	0 (0%)	
Nevirapine	1 (0.07%)	0 (0%)	
Rilpivirine	1 (0.07%)	0 (0%)	
Nevirapine, Rilpivirine	1 (0.07%)	1 (0.07%)	
Doravirine, Etravirine	1 (0.07%)	0 (0%)	
Efavarenz, Nevirapine, Rilpivirine	2 (0.14%)	0 (0%)	
Low Level Resistance (NRTI/NtRTI)			
No Resistance	62 (4.5%)	1 (0.07%)	1.000
Stavudine	1 (0.07%)	0 (0%)	
Stavudine + Didanosine	4 (0.29%)	0 (0%)	
Low Level Resistance (NNRTI)			
No Resistance	44 (3.16%)	0	0.015
Doravirine, Efavirenz, Etravirine	0	1 (0.07%)	
Doravirine	23 (1.65%)	0 (0%)	

Table no. 3 shows the distribution of mutation patterns across NRTI, NtRTI, and NNRTI categories. Kilifi County Hospital had the highest prevalence of mutations across categories. 30.9% of cases had K65R, 568G, K70I, K103N, Y181C, G190A mutations**,** indicating significant resistance accumulation. Smaller percentages showed other mutations. Takaungu Dispensary had only 2.9% of cases exhibited mutations, with all belonging to the K65R, 568G, K70I, K103N, Y181C, G190A category.

For the age categories, 31-40 years had the highest mutation diversity, with 7.4% carrying K65R, 568G, K70I, K103N, Y181C, G190A, 1.5% with K70E, Y115F, M184V, Y188C, G190A, and 1.5% in all remaining categories. 41-50 years (13.2%- K65R, 568G, K70I, K103N, Y181C, G190A) and 21-30 years (10.3%- K65R, 568G, K70I, K103N, Y181C, G190A) also showed notable resistance. <20 years (2.9%- K65R, 568G, K70I, K103N, Y181C, G190A) and 61-70 years had the lowest resistance, likely due to less exposure to ART or smaller sample sizes.

Females had a higher mutation rate (35.3%) compared to males (19.1%). The K65R, 568G, K70I, K103N, Y181C, G190A category was more common in females (27.9%) than in males (5.9%). Other mutations were mostly observed in females.

TDF/3TC/EFV had the highest mutation rates, particularly in K65R, 568G, K70I, K103N, Y181C, G190A (23.5%), followed by K70E, Y115F, M184V, Y188C, G190A (4.4%) and A62GV, K65R, S68G, K70Q, K101E (2.9%). AZT/3TC/NVP also showed notable resistance, mainly under K65R, 568G, K70I, K103N, Y181C, G190A (7.5%). TDF/3TC/DTG had no major mutations recorded.

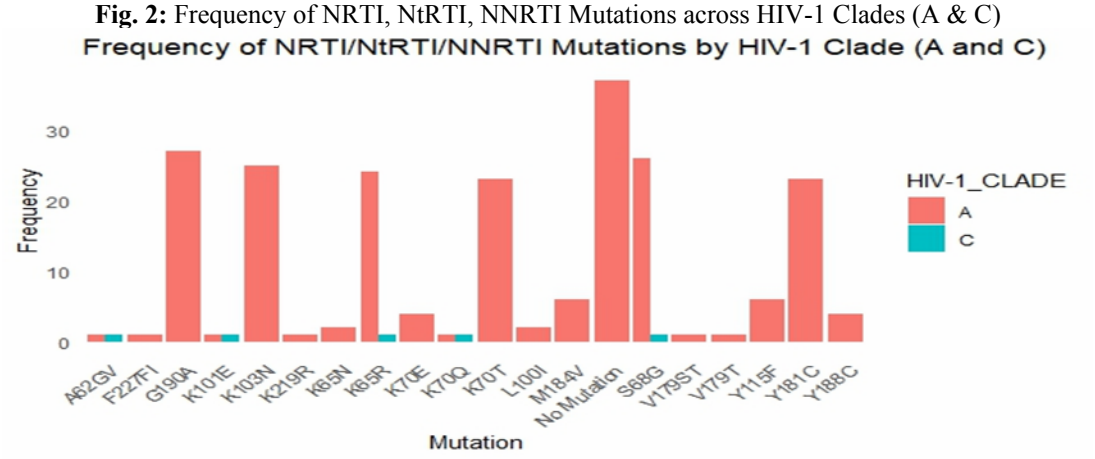
Patients with VL ≥1000cp/mL (52.9%) showed the highest mutations, especially among K65R, 568G, K70I, K103N, Y181C, G190A (26.5%), A62GV, K65R, S68G, K70Q, K101E (2.9%), K70E, Y115F, M184V, Y188C, G190A (5.9%), and 1.5% for other remaining mutations. Fig. 2 shows the frequency of NRTI, NtRTI, NNRTI Mutations across HIV-1 Clades (A & C).

Table no. 3: Cross Tabulation of NRTI/NtRTI/NNRTI Mutations

Variable	Variable group	No.&% of Relevant NRTI/NtRTI/NNRTI Mutations:					
		None	A62GV, K65R, S68G, K70Q, K101E	K65R, 568G, K70I, K103N, Y181C, G190A	K70E, Y115F, M184V, Y188C, G190A	K65N, 568G, Y115F, M184V, L1001, K103N, V1795T, F227FI	K65N, 568G, Y115F, M184V, L1001, K103N, V1795T
FACILITY NAME	Kilifi C.H	37(54.4%)	2(2.9%)	21(30.9)	4(5.9%)	1(1.5%)	1(1.5%)
	Takaungu.D.	0	0	2(2.9%)	0	0	0
Age	<20 Yrs	2(2.9%)	0	2(2.9%)	0	0	0
	21-30Yrs	4(5.9%)	0	7(10.3%)	2(2.9%)	0	0
	31-40Yrs	11(16.2%)	2(2.9%)	5(7.4%)	1(1.5%)	1(1.5%)	1(1.5%)

	41-50Yrs	7(10.3%)	0	9(13.2%)	1(1.5%)	0	0
	51-60Yrs	11(16.2%)	0	0	0	0	0
	61-70Yrs	2(2.9%)	0	0	0	0	0
Gender	Female	24(35.3%)	2(2.9%)	19(27.9%)	3(4.4%)	1(1.5%)	1(1.5%)
	Male	13(19.1%)	0	4(5.9%)	1(1.5%)	0	0
Drug Combination	ABC/3TC/DTG	1(1.5%)	0	0	1(1.5%)	0	0
	ABC/3TC/EFV	1(1.5%)	0	0	0	0	0
	AZT/3TC/NVP	10(14.7%)	0	5(7.5%)	0	0	0
	TDF/3TC/DTG	16(23.5%)	0	0	0	0	0
	TDF/3TC/EFV	8(11.8%)	2(2.9%)	16(23.5%)	3(4.4%)	1(1.5%)	1(1.5%)
	AZT/3TC/EFV	1(1.5%)	0	2(2.9%)	0	0	0
WHO Stage	Stage 1	13(19.1%)	0	5(7.4%)	2(2.9%)	0	0
	Stage 2	17(25.0%)	0	11(16.2%)	1(1.5%)	0	0
	Stage 3	5(7.4%)	2(2.9%)	5(7.4%)	1(1.5%)	0	0
	Stage 4	2(2.9%)	0	2(2.9%)	0	1(1.5%)	1(1.5%)
CD4 (Cells/UL)	No Record	9(13.2%)	0	1(1.5%)	1(1.5%)	0	0
	> 500 C/UL	1(1.5%)	0	2(2.9%)	1(1.5%)	0	0
	350-499C/UL	4(5.9%)	0	2(2.9%)	0	0	0
	200-349C/UL	12(17.6)	0	6(8.8%)	0	0	0
	< 200 C/UL	11(16.2%)	2(2.9%)	12(17.6%)	2(2.9%)	1(1.5%)	1(1.5%)
Viral Load	≥1000CP/ML	17(25.0%)	2(2.9%)	18(26.5%)	4(5.9%)	1(1.5%)	1(1.5%)

Fig. 2: Frequency of NRTI, NtRTI, NNRTI Mutations across HIV-1 Clades (A & C)



IV. Discussion

Two main subtypes were identified in this study namely HIV-1 Clade A which was the most prevalent and HIV-1 Clade C. This is in accordance with previously published data in the coastal region that highlighted subtype A as the most prevalent clade in circulation, A (66%), 46 subtype C (7%), 69 subtype D (10%), 2 subtype G (>1%), and 110 CRF and unique recombinant form (URFs, 17%) [11]. The diversity in strain further complicates management of HIV as it raises the concern of drug resistance. Susceptibility to antiviral drugs declines following emergence of new strains. Other studies have found connection between resistance to Tenofovir and the mutation K65R which is elevated among individuals with subtype C HIV-1 strains.

The study indicated that majority of the study participants with high viral load did not depict any form of resistance (high, low or intermediate). This was a clear indication of the effectiveness of their respective ART regimens with the spike in viral loads likely occurring as a result of adherent factors. The efficacy of ART regimens is majorly derived from the good adherence practices among patients. Key outcomes of good adherence include a boosted immune function, prolonged viral suppression and non-development of drug resistance.

For the high level drug resistance (NRTI), notable resistance patterns were observed in clade A highlighting either resistance in single regimens or multiple drug combinations, for instance, Abacavir, Tenofovir, and other NRTI backbones or involving a combination of either two in this category Abacavir, Emtricitabine, Lamivudine. For the NNRTI, high level resistance was noted in Efavirenz, Nevirapine, Rilpivirine, and Doravirine in Clade A where no notable resistance was witnessed in Clade C. Intermediate-Level Drug Resistance included mutations against Emtricitabine, Lamivudine, and Tenofovir.

For the low level resistance (NNRTI), the p-value of 0.015 indicated a statistical significance of the resistance levels between Clade A and Clade C. There are numerous factors that could provide an insight on the possibility of developing low level drug resistance. From this study, the dominance of clade A was evident justifying other studies with a similar outcome [7,11]. This higher prevalence indicates that selective drug pressure

is much higher in Clade A compared to other HIV-1 strains contributing to the emergence of resistance mutations.

Historically, the national regimen combination has included two nucleoside reverse transcriptase inhibitors and a non-nucleoside reverse transcriptase inhibitor. Efavirenz and Nevirapine, both NNRTI, are typical ART regimens that have been linked to development of drug resistance. In this study, Efavirenz indicated the probability of high level drug resistance (0.07%) or in other instances, participants were resistant to more than one NNRTI inclusive of Efavirenz, for instance, 0.57% (Efavirenz and Nevirapine), 0.57% (Efavirenz, Nevirapine, Rilpivirine), and 0.07% (Doravirine, Efavirenz, Nevirapine, Rilpivirine). This study conforms to other studies which have established a linked in Efavirenz and Nevirapine contributing to the development of resistance in HIV-1 Clade A. Two notable mutations were identified in this study, K103N and Y181C, both of which have linked to development of virological failure in Efavirenz and Nevirapine. In one study, comparing patients on Efavirenz and Nevirapine, K103N was identified in 83% and 28% of the sampled population respectively while Y181C was identified in 56% and 20% respectively [12]. Other notable mutations in the NNRTI category included K101E which confers resistance to Efavirenz and Nevirapine, L1001 which largely occurs together with K103N conferring resistance to Efavirenz, V179S/T & F227FI which on their own have minimal impact in terms of conferring resistance, but contributed significantly to NNRTI-related mutations, Y188C responsible for lowering susceptibility to Nevirapine, and lastly, G190A which causes resistance to Efavirenz and Nevirapine.

The study highlighted the high mutation patterns associated with NNRTIs, more specifically Efavirenz and Nevirapine, due to their low genetic barrier when it comes to development of resistance. The effectiveness of these regimens drastically reduces even in the presence of a single mutation[13,14]. In contrast with the nucleoside reverse transcriptase inhibitors where high level drug resistance is dependent on presence of multiple mutations, the non-nucleoside reverse transcriptase inhibitors bind to the reverse transcriptase through a hydrophobic pocket and therefore, minor structural alterations such as Y181C and K103N can result in non-binding of drugs [15]. Furthermore, Nevirapine and Efavirenz are widely used in low and middle income countries such as Kenya leading to selective pressure in the drugs, a key trigger for resistance development. Notably, treatment interruption or sub-therapeutic drug levels also trigger resistance development. Also identified in this study pertains to resistance patterns of Doravirine. Considered to have a higher genetic barrier compared to other NNRTIs such as Nevirapine and Efavirenz, the outcomes of this study highlight the increased probability of Doravirine associated mutations which are exacerbated by the existence of other major mutations notably K103N, L100I, V179S/T, Y188C**,** and F227FI. The existence of these mutations are likely to decrease the efficacy of Doravirine.

V. Conclusion

This study highlights the need to incorporate drug resistance testing to detect early signals for regimen failure, particularly for patients with a trend of regular unsuppressed viral loads. Detection of regimen failure in both NRTIs and NNRTIs may necessitate switching to regimens with higher genetic barrier. However, monitoring and prevention of the development of drug resistance is crucial to ensure patients have access to other regimen options. The identification of two subtypes, A and C in this study was a clear indication of multiple introductory events with the possibility of the presence of additional subtypes in circulation. Subtype variability could also provide clues on circulating recombinant forms in the population. Different subtypes have the possibility of developing unique resistance patterns that compromise the treatment efficacy.

VI. Acknowledgement

The authors would like to acknowledge the Department of Health Services, Kilifi County Government, Kenya, all the select health facilities in Kilifi County and their staff spearheaded by Kilifi County Referral Hospital (Comprehensive Care & Research Clinic – CCRC & the Laboratory Department) and study participants who contributed immensely to the outcomes of this research. This was an independent project enabled by the technical support from the Kenya Medical Research Institute (KEMRI) through the Centre for Infectious and Parasitic Diseases Control Research (CIPDCR) for permitting access to the state-of-the-art HIV viral load testing laboratory at KEMRI, Nairobi. This work was partially funded by the National Research Fund (NRF) grants, Government of Kenya.

VII. Ethical Statement

Approval for the study was obtained from the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Committee (KEMRI/SERU/CIPDCR/017/3457).

References

[1]. Weiss, R. A., Dalgleish, A. G., Loveday, C. & Pillay, D. Human Immunodeficiency Viruses. In Principles And Practice Of Clinical Virology 721–757 (John Wiley & Sons, Ltd, 2004). Doi:10.1002/0470020970.Ch25.

[2]. Seitz, R. Human Immunodeficiency Virus (HIV). Transfus Med Hemother 43, 203–222 (2016).

[3]. Domingo, E., García-Crespo, C., Lobo-Vega, R. & Perales, C. Mutation Rates, Mutation Frequencies, And Proofreading-Repair Activities In RNA Virus Genetics. Viruses 13, 1882 (2021).
[4]. Carr, A., Mackie, N. E., Paredes, R. & Ruxrungtham, K. HIV Drug Resistance In The Era Of Contemporary Antiretroviral Therapy: A Clinical Perspective. Antiviral Therapy 28, 13596535231201162 (2023).
[5]. Wainberg, M. A. HIV-1 Subtype Distribution And The Problem Of Drug Resistance. AIDS 18 Suppl 3, S63-68 (2004).
[6]. Adhiambo, M. Et Al. Human Immunodeficiency Virus (HIV) Type 1 Genetic Diversity In HIV Positive Individuals On Antiretroviral Therapy In A Cross-Sectional Study Conducted In Teso, Western Kenya. The Pan African Medical Journal 38, (2021).
[7]. Adungo, F. O. Et Al. Diversity Of Human Immunodeficiency Virus Type-1 Subtypes In Western Kenya. World Journal Of AIDS 4, 365–372 (2014).
[8]. Hué, S. Et Al. HIV Type 1 In A Rural Coastal Town In Kenya Shows Multiple Introductions With Many Subtypes And Much Recombination. AIDS Res Hum Retroviruses 28, 220–224 (2012).
[9]. Kageha, S. Et Al. HIV Type 1 Subtype Surveillance In Central Kenya. AIDS Research And Human Retroviruses 28, 228–231 (2012).
[10]. Nduva, G. M. Et Al. Phylogeographic Assessment Reveals Geographic Sources Of HIV-1 Dissemination Among Men Who Have Sex With Men In Kenya. Front. Microbiol. 13, (2022).
[11]. Nduva, G. M. Et Al. HIV-1 Transmission Patterns Within And Between Risk Groups In Coastal Kenya. Sci Rep 10, 6775 (2020).
[12]. Ngo-Giang-Huong, N. Et Al. Resistance Patterns Selected By Nevirapine Vs. Efavirenz In HIV-Infected Patients Failing First-Line Antiretroviral Treatment: A Bayesian Analysis. Plos ONE 6, E27427 (2011).
[13]. Nastri, B. M. Et Al. HIV And Drug-Resistant Subtypes. Microorganisms 11, 221 (2023).
[14]. Wensing, A. M. Et Al. 2022 Update Of The Drug Resistance Mutations In HIV-1. Topics In Antiviral Medicine 30, 559 (2022).
[15]. Yardley, W. Nucleotide Reverse Transcriptase Inhibitors For Antiretroviral Therapy. J Antivir Antiretrovir 14, 238 (2022).